ACC&D
Alliance for Contraception in CATS & DOGS

Contraception and Fertility Control in Dogs and Cats

A Report of the Alliance for Contraception in Cats & Dogs (ACC&D)

February 2013

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Dedication

This publication is dedicated to the animals that have inspired us to care so deeply about this topic, and to a future with new tools to improve the welfare of animals, especially those animals beyond our grasp today.

Joyce Briggs

President

Alliance for Contraception in Cats & Dogs

Preface

In 2002, Katherine Moldave, MBA and Linda Rhodes, VMD, PhD compiled and published *Contraception and Fertility Control in Animals*, believed to be the first effort to summarize the technologies and issues related to this topic in a comprehensive format that would be useful to scientists, academicians, the animal health and welfare industries, and interested laypeople alike. The authors subsequently donated the copyright to the Alliance for Contraception in Cats & Dogs (ACC&D).

In the past decade, several contraceptive products for use in dogs have been approved in various markets, research in relevant traditional and emerging technologies has expanded greatly, interest in this area of animal health has increased, and, as a key component of our mission, ACC&D has worked to focus stakeholder attention on the opportunities and issues involved in non-surgical approaches to contraception and fertility control in "owned" and "unowned" cats and dogs. At the end of 2011, ACC&D decided that the time had come to update the portions of the report related to cats and dogs.

The lead author for *Contraception and Fertility Control in Cats and Dogs* is one of the original authors of *Contraception and Fertility Control in Animals* (2002), Katherine Moldave. The regulatory chapter has been updated by its original author, Linda Rhodes. ACC&D commissioned this update with the support of a generous grant from PetSmart Charities®. Happily, this has been a more significant undertaking than originally anticipated since so much has occurred in the intervening years. ACC&D thanks Ms. Moldave for her many hours of work beyond what was contracted to update and expand the original text; her commitment to the project has truly gone above and beyond.

*Contraception and Fertility Control in Dogs and Cat*s is not a scholarly work or exhaustive review article. It is intended to provide a single source of information and foster collaboration among interested parties. We are counting on readers to help us identify information that may not have been included in this compilation but should be covered in future updates. We have tried to be as geographically inclusive as possible but acknowledge that some aspects of the compilation have a United States orientation. Therefore, we are particularly interested in updates, additional information, and perspectives from areas outside the US.

The following ACC&D scientific advisors, members of the board of directors, and field experts have provided information and/or review and ACC&D is grateful to them for their invaluable contributions:

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1.0 Introduction

1.1 Scope of the Report

This report is an update of the canine- and feline-related sections of the 2002 report *Contraception and Fertility Control in Animals* and is a compilation of publicly available material on the subjects of contraception and fertility control in dogs and cats, and dog and cat population statistics and issues. Sources include the published scientific literature, government publications, company websites and other Internet pages, and discussions with leaders in the field. We have included references, not as a comprehensive review of the literature, but to serve as a beginning for those interested in delving further into the subject.

Although we have tried to be thorough in researching and updating publicly available material describing methods of, and issues related to, contraception and fertility control in dogs and cats, it is quite possible that we have overlooked some fact our readers feel should have been included, or included a statistic that's become outdated – for these oversights we offer our apologies in advance. We invite you to notify the Alliance for Contraception in Cats & Dogs (ACC&D) (acc-d.org) of suggested additions for the next update. We trust readers will find that the important sources, topics, and opinions are well covered in this report.

We have included an overview of the basics of control of reproduction and a review of the types of treatments that have either been available commercially over the years or have been investigated by experts.

To complement the scientific and technical material, there is a review of various companies that have done research and development in this field, including a snapshot of the history of product development by animal health companies over the years. Some of the important characteristics of the market that will be helpful to consider for a company interested in developing, commercializing, and/or marketing a contraceptive product are reviewed, and we also include an overview of the particular regulatory considerations that we think apply to these types of products.

We feel strongly that to make the decades of research in this field continue to come to fruition with products that can be used commercially, the science and the business must come together. This report is written for people who are interested in contraception and fertility control in cats and dogs. Some sections are more technical than others, but lay readers who wish to have an overview of the overall landscape in the area of contraception and fertility in cats and dogs will be able to gain a working knowledge, while readers interested in a bit more information on the scientific aspects will be able to review what research approaches have emerged and why. We hope interested lay people, veterinarians, researchers, animal welfare organizations, business people, potential investors in emerging relevant technologies, and animal health professionals will all find the report useful.

1.2 Brief Review of the Historical Context of Contraception and Fertility Control Research in Animals

"The Pill," approved in the United States (US) in 1960, was considered a breakthrough in contraception for women. Daily oral treatment with low dose estrogen or estrogen/progesterone combinations became a widely adopted method for human reproductive control.

Research has been published for more than 40 years indicating that non-surgical contraception for animals is possible. Since daily treatment with steroids is impractical for most species, other methods have been explored. A variety of approaches have been used in many species of animals, including early laboratory work in rodents and studies on dogs, cats, cows, and monkeys. Much of this work was directed towards exploring contraceptive approaches for humans, but some of it was clearly geared towards creating alternatives to surgery for animals.

Given that the research was so promising, why have so few of these approaches made it to the marketplace?

Demonstrating effectiveness (i.e., that a particular treatment can cause suppression of fertility for a period of time) is not enough. To move an idea from the lab into the marketplace, the technology must be efficacious and safe, and a number of other requirements must also be

met. For a company to decide to take a product forward, a technically feasible, stable formulation must be developed and large-scale manufacturing must be possible. The regulatory path must be clear, risks well defined, and the cost of development reasonable. The market must be clearly defined and the cost of the final product must be low enough to make the investment attractive (i.e., generate sufficient profit margins). Money must be available to complete the project and support an initial marketing campaign. Finally, a company or a group of investors must be willing to take the risk of a long and expensive development project.

Other means of developing a contraceptive for cats and dogs can be imagined. For example, a humanitarian, nonprofit organization could subsidize the development and registration costs for a product that may not be commercially feasible, and such a product could be provided at cost to spay/neuter clinics and shelters. Early-stage companies and humanitarian organizations could collaborate to bring a product through the riskier stages of development, and then partner with a larger company for distribution and sales, with the profits of such a venture subsidizing the contraception of needy animals.

Over the years, technical issues and pitfalls have stood between various approaches and the marketplace. In addition, there are sociopolitical factors (see Chapter 5) that have likely slowed the advance of contraception and fertility control in animals.

In the early 1980s, when some methods were being tested in research laboratories, the large pharmaceutical companies were dismantling their reproductive biology research groups. Controversy over the side effects of intrauterine devices and controversy over abortion and contraception in general led companies to shy away from the entire field. Animal health R&D groups working on reproduction were also downsized, a process that does not appear to have been reversed. In animal health companies, many decision makers were also not interested in non-surgical contraception. A common opinion was that spaying and neutering were the drivers that brought clients to the veterinary practice. Providing an alternative, they worried, could have a negative impact on the number of new clients coming in the doors of the veterinary practice and thereby diminish the customer base. Non-surgical contraception also suffered from the fact that when each company looked at what the other industry leaders were doing, nobody seemed to be working in this area, which also reinforced the negative perception – if this is such a good idea, why aren't our competitors doing it? Over the years, some of the animal health companies have supported research on various approaches, but only fairly recently have commercial products emerged for non-surgical contraception for dogs and cats.

1.3 Targets and Historical Approaches to Non-Surgical Sterilization in Dogs and Cats

Note: The majority of the information in this section is contained in a paper entitled Historical Approaches to Non-surgical Contraception in Dogs and Cats *by Beverly J. Purswell and Wolfgang Jöchle and submitted as an abstract in the Proceedings of the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control in 2010. It has been edited slightly and updated in places. Proceedings from all previous symposia are available at acc-d.org.*

For millennia, surgical castration – defined here as removal of the testes or ovaries – has been the only reliable and permanent method of contraception in domestic animals. The oldest evidence of surgical castration of domestic male animals can be traced back to the late neolithicum (7000-6000 BC). Documentation for surgical castration in male dogs goes back to ancient China, Siberia, and Greek and Roman antiquity. In literature throughout the ages, many authors discuss dog breeding, management, and health care. Contraception is not a part of this body of literature. This is in sharp contrast to the amount of literature on male and female castration of horses and mules. One English book on the art of hunting, dating back to 1575, mentions castration of male and female dogs, but does not give any technical details.

From the 15th to the 19th centuries, evidence of European professionals with a special license for castrating male and female farm animals can be found. The professionals were organized into guilds, and had lists of fees that were approved by local authorities or the ruling princes. Modern veterinary medicine in the late 18th century and

19th century slowly took over the castration business, at least in pets and horses, and brought a level of humaneness to the process. In 1975, in a British Small Animal Veterinary Society publication, it was mentioned that anyone older than 18 years of age was legally entitled to perform castration of a cat or a dog at any time until it is 6 months of age, provided that adequate anesthetic was administered. By 2007, the law had changed and castration of dogs and cats could only be provided by veterinarians.

The 20th century saw modern animal welfare legislation in a few Scandinavian countries that forbade any surgery in healthy pets. Once data on the health benefits of gonadectomy (e.g., lessening of mammary tumors in bitches[1] (female dogs) and benign prostatic hyperplasia (BPH) in male dogs) were provided, neutering and spaying of pets became legal again (with at least one exception).[2]

Contraceptive medications in dogs and cats have only had more recent attention in the latter half of the 20th century. Around 1960, due to the availability of orally active and increasingly more effective progestins ("The Pill"), efforts began on a larger scale to control reproduction in dogs and cats. As oral contraception products became widely available for women, the desire to use these products in pets became more mainstream. The status of dogs and cats also changed during this time to true companions, family members, and even child substitutes. It was at this time that animal welfare advocates began to be concerned about the fate of unwanted and unplanned offspring and the horror of increasing numbers of dogs and cats euthanized each year, and launched educational campaigns to inform the public about preventing unwanted litters. Progestin-based "Pills for pets" were developed in Europe, coming to the market in 1963. Large-scale population control for dogs and cats in the US began in the early 1970s.

Progestins (progesterone-like compounds) have been the most common compounds used to address estrous control in the dog. These progestins, administered orally or by injection, have had varying results and acceptance by veterinarians and pet owners. Medroxyprogesterone acetate (MPA) in tablet form was the "Pill" that was marketed first in Europe in 1963. In the US, MPA was marketed as an injectable product and was used in dogs with disastrous results. Introduced in 1964, MPA was produced as a long-acting crystalline suspension (4-5 months duration, Promone-E® or Depo-Provera®). This product was very effective in estrous suppression but caused pronounced cystic endometrial hyperplasia in the uterus, resulting in an epidemic of pyometra. This problem resulted in a withdrawal of the product from the market 2 years later, never to appear again for this use. As a further consequence, veterinarians in the US lost confidence in any hormone-based contraception and the veterinary profession in North America thus went to a strictly surgical means of estrous control.

Because of the veterinary and animal welfare environment in Europe, veterinarians worked with the available pharmaceuticals to develop safe protocols for progestins. During the 1960s and 1970s, other progestins were introduced, such as chlormadinone acetate (CAP), delmadinone acetate (DMA), and proligestone (PRO). Use of the progestins was refined by lowering the dose and adjusting the timing of administration. Emerging insights into the canine reproductive cycle began to allow for strategies uniquely suited for the species. In the bitch, pregnancy and pseudopregnancy are endocrinologically and morphologically very similar. Use of these compounds for treatment for estrous prevention and an interruption of cyclicity was found to be safe when begun 4 months after an estrus, and at least 1 month prior to the next anticipated estrus. The treatment could be repeated every 4-5 months for years of safe estrous prevention.

Megestrol acetate (MGA) was another early progestin that was marketed for use in dogs in Europe, the US, and Canada, beginning in the early 1970s. This product is an

[1] While the term "bitch" is not used commonly in the US to refer to female dogs, it is used in the rest of the world and we hope its use in this document does not offend any of our readers.

[2] According to a December 2011 article on the *Science Nordic* website, "The Norwegian Animal Welfare Act makes it clear that surgical procedures are not to be used to adapt animals to the needs of humans, unless strictly necessary." The article also stated that the Norwegian Food Safety Authority (NFSA) was working on a revision that would permit neutering of dogs "when mandated by utility, or if it helps give the dog a justifiable quality of life, including social contact with other dogs." See sciencenordic.com/should-dogs-be-neutered.

oral tablet that has been the only product approved for use in the breeding bitch in the US. Marketed as Ovaban®, there were two protocols approved for use in the dog. One was at a higher dose and short duration to stop a cycle once begun. The other protocol was at a lower dose and longer duration to prevent the cycle from occurring. Although the marketing of MGA as a specific veterinary product has been discontinued, MGA is available as a generic for human use but used only on a limited basis in the US.

In cats, progestins have proven to be problematic. Progestins have never been as popular as they were in dogs. Their use has been restricted to oral MGA or MPA, once or twice weekly for estrous suppression or prevention. Cats have problems with progestins causing an adverse effect to the adrenals, resulting in diabetes which can be irreversible. Because of this significant, life-altering side effect and other health risks, progestins are not typically used in the cat. Use of progestins in cats is especially rare in the US, while in Europe it is somewhat more common.

In addition to progestins, androgens have been used for estrous control in the dog. Testosterone, either oral or injectable, is used routinely in racing greyhound bitches while training and competing. The side effect of masculinization, while desirable in the racing athlete, limits the use of testosterone in the wider pet population. Another compound with androgenic activity, mibolerone (MIB), was marketed in North America. Introduced in 1978, MIB (Cheque®) was discontinued in 1990. Some veterinarians continue to use MIB for estrous control in the bitch by accessing it from compounding pharmacies. MIB requires daily oral use, begun at least 30 days from the onset of an estrus, to successfully prevent cycling. Use of MIB, aside from the show-dog world, has been limited in the pet population due to its androgenic side effects (clitoral enlargement, musky body odor, and behavioral changes).

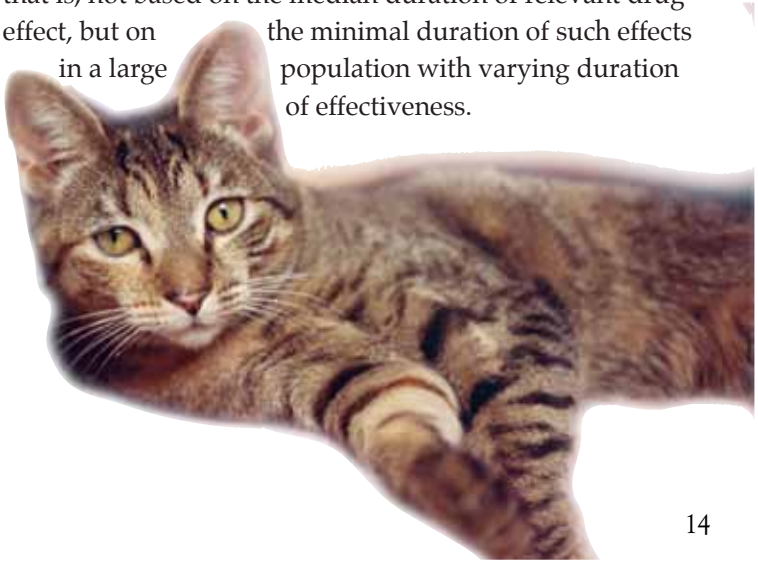
The next hormonal approach to contraception in small animals to be developed was gonadotropin- releasing hormone (GnRH) analogs. The advantage to using GnRH or its analogs is that these compounds are effective in males and females, since GnRH is the master control hormone for reproduction. Also, the GnRH decapeptide has the same amino-acid sequence in all mammals, making any product potentially useful in a variety of species. The development of long-acting preparation of the GnRH agonists has long been sought. Compounds began to emerge as promising candidates. Long-acting powerful analogs have been shown to occupy GnRH receptors at the pituitary and after a short period of stimulation cause the cells to reduce or stop the synthesis of the receptor protein, making the cells insensitive to GnRH. This process is called receptor down-regulation. As down-regulation occurs, production of gonadotrophins by the pituitary (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)) ceases, effectively shutting down spermatogenesis and androgen production in the male and cyclic ovarian function in the female.

This effect is well known and as early as 1989 had been suggested as a potential estrous suppression hormone for the bitch. Three factors have prevented rapid development of a GnRH product for use in estrous suppression in small animals:

1. High cost of these analogs
2. Initial induction of a (sometimes) fertile estrus prior to down regulation
3. Strong variations in the duration of contraception among individual animals

The GnRH agonist leuprolide (Lupron®) came onto the market for human use in the 1990s, but its substantial cost prevented its veterinary use.

Peptech Animal Health in Australia developed two much less expensive implants for either a 6- or 12-month suppression of fertility in male dogs using the GnRH analog deslorelin. Research showed that treatments with these implants, containing down-regulating doses of deslorelin, resulted in cycle control in the bitch and queen (female cat), and in suppression of spermatogenesis and libido in male dogs. Its use has been studied in male cats but the product is not approved for this use. Approval for the sale of the deslorelin implant for use in male dogs under the trade name Suprelorin® was obtained in New Zealand and Australia in 2003 and in the European Union (EU) in 2007. Another GnRH analog, an azagly-nafarelin implant, was approved in Europe in 2006 under the trade name Gonazon® for use in female dogs; its use has also been studied in cats. Suprelorin and Gonazon are examples of a novel approach to defining duration of drug effect: that is, not based on the median duration of relevant drug effect, but on the minimal duration of such effects in a large population with varying duration of effectiveness.



Note that Gonazon, although approved by European regulators, was not introduced to the market, quite likely because of a business decision. The safety of these analogs allows for repeated treatments even if the effect of a prior treatment has not expired. Induction of estrus and ovulation as an initial treatment response can be avoided by either implanting bitches during their luteal (progesterone) phase, 60 days post-estrus, or after a short-term pretreatment with exogenous progestins, such as MGA.

Male dogs have been subject to contraceptive treatments only in the last decade. Surgical castration of male dogs continues to be taboo among many cultures. Beginning several decades ago, efforts were undertaken to find a safe single intratesticular treatment causing the testes to atrophy. A variety of compounds have been tested. The first approved product (Neutersol®, zinc gluconate/arginine) to fulfill both the safety and effectiveness criteria required by the FDA became commercially available in the US in 2003. Distribution was halted in 2005 when the patent-holder and marketing company severed ties. Neutersol is no longer available in the U.S; however the product has been brought back to certain markets re-named as Esterilsol®, sponsored by a company named Ark Sciences, Inc. The product is approved in several Latin American countries. Ark Sciences plans to bring the product back to the US in 2013 under the new name, Zeuterin™. Unlike the GnRH treatment, which in most animals is reversible, intratesticular treatments result in irreversible destruction of germ cells and hormone-producing tissues. Another zinc gluconate-based sterilant for male dogs, Infertile®, was introduced in Brazil in 2009. Work continues on other formulations administered similarly and targeted to males.

Another avenue for contraception is immunocontraceptive vaccines. Porcine zona pellucida (PZP) vaccines derived from porcine oocytes have been used in a variety of species. These vaccines have been shown to cause reversible infertility in ruminants, horses, seals, and elephants. In bitches, an irreversible infertility was induced by destroying the entire ovarian follicle but treatment with a PZP vaccine was ineffective in the queen. These results have led to efforts to identify canine-specific and feline-specific antigens in canine and feline zonae pellucidae and to use suitable candidates for attempting to develop zona pellucida (ZP) immunocontraceptive vaccines for bitches and queens.

Hormonal antigens are another avenue to immunocontraceptive vaccines. The antigenicity of GnRH complexes has been confirmed since the 1970s. Because small peptides make weak antigens, they must be conjugated to large proteins and potent but safe adjuvants are needed. Adjuvants must render the vaccine effective with a practical number of booster injections, and must also cause minimal site reactions. These vaccines would be effective in multiple species and in males and females, due to the fact that they antagonize the effects of GnRH.

In 2004, Pfizer Animal Health acquired the Australian animal health company CSL and its US subsidiary Biocor, which had a gonadotropin-releasing factor (GnRF, another term for GnRH**)** vaccine for use in male dogs for the treatment of benign prostatic hyperplasia. Although Pfizer obtained a conditional license for this product for treatment of canine BPH from the United States Department of Agriculture (USDA) in 2004, no further licensure occurred in the US and the product is no longer available. This vaccine required two injections, 4-6 weeks apart, to be repeated every 6 months, and did result in contraception in the course of treating BPH. Similar vaccines are currently available in swine (Improvac™) and in horses (Equity™). The availability and success of these GnRH(F) vaccines may hold promise for their use in cats and dogs.

Other avenues to non-surgical pet contraception are being explored. They include the use of GnRH peptide and non-peptide antagonists. To make these compounds useful as dog and cat contraceptives, depot preparations will need to be developed. Cost, again, will need to be addressed to gain widespread acceptance for use in companion animals. Depending on the objective (i.e., whether or not permanent sterility is desired), the advantages of these approaches may be quick action, reversibility of effects, and safety. Research is also underway on approaches for providing permanent sterilization.

As discussed in Chapter 5 of this update, there are several general stakeholder groups whose visions of contraception and fertility control in dogs and cats differ. For some, reversibility is important; for others, permanence is essential; for still others, having several options is desirable. This creates challenges as well as opportunities.

1.4 Considerations for Commercializable Approaches to Non-Surgical Contraceptive Methods

What does it take for a contraceptive or sterilant to be "commercializable?" The table below, based on *A Summary of ACC&D's Priorities for Non-surgical Sterilization and Key Challenges in Their Development* (Briggs and Rhodes, ACC&D 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Populations Control 2010), provides a summary:

Table 1-1: Summary of Characteristics Required for Commercialization

Parameter	Comments
Approvable	The ability of a given product to be approved by relevant regulatory agencies is a key practical consideration. The question of potential for regulatory approval needs to be asked repeatedly at different stages of research and development to encourage focus on advances that have potential for commercialization.
Financially feasible	Financing, whether the potential product is corporate- or organization (e.g., nonprofit)-based, is critical. The Michelson Prize & Grants program (see Chapter 4, section 4.3.3.2) is a significant new resource, and various types of public-private partnerships and emerging options such as L3C corporations (Limited Liability Corporations created to support philanthropic investment) are examples of approaches that may be applicable to certain stakeholder groups.
Deliverable	The method of administration needs to fit a given application. For example, a product requiring two or more treatments may have promise for some market segments such as veterinary clinics, but there is likely only one opportunity to treat populations such as feral cats, community/street dogs, and certain pets that are difficult to treat more than once (e.g., hard to handle, or owner who may not to return for follow-up treatment).
Documentable	Traditionally, US-based veterinary practitioners and animal welfare agencies have had a positive opinion of the value of perceived non-reproductive effects of sterilization surgery on dog and cat health and behavior. Currently, there is a "reframing" occurring related to the "gold standard" status of surgical contraception. This "reframing" strives for a more objective comparison between spay/neuter and current or future non-surgical alternatives. In addition, different cultures value these effects (e.g., the effects of castration on some male dogs) differently.
Affordable	While there will always be pet owners to whom cost is not a consideration in the health of their companion animals, affordability is key for many individuals and stakeholder groups, particularly those involved with shelter animals and animals that are feral or free-roaming. Pet owners who are not price sensitive can also benefit from approaches that may happen to be relatively inexpensive.
Able to be manufactured in amounts that meet demand	Unfortunately, in the drug-development world there are many instances in which safe, effective products able to be produced in a laboratory setting have been unable to be approved and commercialized because they were not able to be manufactured on a large scale, either due to inherent product characteristics, manufacturing challenges that could not be addressed successfully, or manufacturing cost.
Acceptable	Stakeholder groups for which a given product is intended must find that product acceptable. This includes veterinary practitioners who may be hesitant to embrace new alternatives. As with any innovation, thought and opinion leaders representing relevant stakeholder groups will be needed to explore how to integrate new methods.

2.0 The Physiology of Reproductive Control in Mammals – Overview of the Major Systems that Control Reproduction

The purpose of this brief overview is to give readers the background necessary to understand how the potential products for contraception and fertility control work. This is an oversimplification of a wonderfully complex system, but the material should serve to orient non-biologists reading this report.

2.1 Brain

The major control center for reproduction is the brain, where specific neurons synthesize gonadotropin-releasing hormone (GnRH) under a number of influences, such as light levels, body condition, age, and the blood levels of various hormones. One of the most interesting things about brain secretion of GnRH is that it is secreted in pulses and not continuously. The pulses are important – if GnRH is not delivered in pulses, it does not have the normal effect on the reproductive system. This small fact becomes important in understanding one of the leading approaches to animal contraception, the GnRH agonist compounds.

GnRH is a decapeptide (small protein made up of 10 amino acids) that acts directly on the pituitary. GnRH is considered the master hormone that controls the release of the major reproductive hormones. Interfere with GnRH, and you interrupt all of reproduction in both males and females. In fact, not only is reproduction interrupted, but the species-specific reproductive behaviors are disrupted as well.

2.2 Pituitary

The pituitary gland has specific cells – the gonadotrophs – that have receptors for GnRH that bind to the peptide. The regulatory system is highly complex, and at the risk of oversimplifying, we can say that once the GnRH binds to its receptor on specific pituitary cells, it causes the release of two larger protein hormones called gonadotropins – luteinizing hormone (LH) and follicle-stimulating hormone (FSH) – which are secreted into the blood. As the brain gives off pulses of GnRH, these pulses reach the pituitary and cause the pituitary to give off pulses of LH and FSH.

2.3 Gonads (Ovaries and Testes)

Once the pituitary secretes LH and FSH, they travel in the blood to the gonads – ovaries in females and testicles in males. These two hormones bind to receptors on the gonads. They coordinate the estrous cycle (heat) or the menstrual cycle of the female and are important in the production of estrogen and progesterone. In the male, LH and FSH are important for sperm maturation and stimulation of the production of testosterone.

When the steroid hormones (estrogen, progesterone and testosterone) are secreted from the female or male gonads, these hormones travel in the blood to the brain, where they turn off the secretion of GnRH. This is called negative feedback. For example, GnRH causes the pituitary to make more LH, which stimulates the testes to make more testosterone, which goes to the brain and causes the brain to make less GnRH. Levels of LH go down, because the pituitary doesn't get the GnRH signal. When the LH in the blood falls, no testosterone is made. As blood levels of testosterone decrease, the brakes are off the GnRH in the brain, and the system kicks in to make more GnRH, and so on. This is how the system is regulated.

The system is more complex in the female, but the essential message is the same. The estrogen and progesterone produced in the ovary are the negative feedback signals for GnRH. It is not important for this report to detail the complex regulation of the estrous and menstrual cycles. However, this negative feedback concept is important in understanding contraceptive technology. Giving progesterone, for example, will shut down production of GnRH, interrupting fertility.

In the ovary, where the eggs are produced, each egg is surrounded by a protective coating called the zona pellucida (ZP). The ZP is made up of several glycoproteins, i.e., complex proteins with various sugar molecules attached. From recent work in molecular biology, we know that in each species there are different – but similar – ZP proteins around the egg. For example, the ZP proteins from a pig (porcine) are similar to – but not exactly the same as – the ZP proteins of a cat. A great deal of work in animal contraception has been undertaken with vaccines using the porcine ZP proteins, including exploration of the potential of use in multiple species.

2.4 Unique Aspects of Canine and Feline Reproductive Biology

During the 2009 Alliance for Contraception in Cats & Dogs (ACC&D) Think Tank on

Immunocontraceptive Approaches for Sterilization of Dogs and Cats, Dr. Beverly Purswell briefly described canine- and feline-specific reproductive characteristics, as summarized below (acc-d.org/ThinkTanks).

Unique aspects of dog reproduction include the fact that diestrus (progesterone phase) occurs after every estrus; if not impregnated, bitches will experience a pseudo-pregnancy lasting the same length of time as an actual pregnancy, unlike most animals which experience a decrease in progesterone as soon as it is determined that the animal is not pregnant. Note that only one-quarter to one-third of these pseudo-pregnancies are evident (Jöchle, personal communication 2012). Dogs also ovulate primary oocytes requiring two meiotic divisions before fertilization, and canine follicles undergo pre-ovulatory luteinization. Dogs are monestrus, meaning they have one estrous cycle per season, which averages one to three cycles per year for domesticated dogs.

In contrast, domestic cats are induced ovulators, stimulated to ovulate in response to mating, though spontaneous ovulation events have been observed. Cats are prolific breeders, able to become pregnant within days after delivering a litter, and have been demonstrated capable of producing five litters in one year. Additionally, a cat can become sexually mature as early as 4 to 5 months of age. Cats are also seasonal breeders, induced to reproduce by long daylight hours, such that equatorial populations breed year-round.

3.0 Overview of Technological Approaches and Their Applications in Dogs and Cats

Chapter 3 summarizes technologies that:

- Have been the basis of the non-surgical products that have been approved in various markets or
- Are being investigated for their potential utility as non-surgical approaches to contraception or sterilization of dogs and cats

Chapter 4 provides information on specific products as well as companies, institutions, and organizations active in the area.

Please see Chapter 5, section 5.8, for descriptions of "ideal products" based on the different segments in the market.

3.1 Surgical Approaches

3.1.1 Overview

Gonadectomy refers to surgical removal of ovaries or testes performed to eliminate reproductive function irreversibly. Reichler (2008) notes that the first written documentation referring to neutering companion animals can be found in the Mosaic laws of approximately 600 BC. Eighteenth- and nineteenth-century writings refer to employing gonadectomy not only to stop reproduction in dogs and cats, but also to suppress sexually-related behaviors.

Terms used in this overview:

- Gonadectomy refers to the surgical removal of the male or female gonads (testes or ovaries)
- Ovariohysterectomy refers to the surgical removal of the ovaries and the uterus (more common in the United States (US) than in Europe)
- Ovariectomy refers to surgical removal of the ovaries (more common in Europe than in the US)
- The term "spay" is used commonly to refer to ovariohysterectomy and ovariectomy
- Orchiectomy (male castration) refers to surgical removal of the testicles and spermatic cords
- The term "neutering" is used commonly to refer to male surgical castration, although it is used to refer to surgical sterilization of female animals as well.
- The term "castration" may refer to sterilization of males or sterilization of males and females, and this is defined for a given example

There is discussion and even controversy in the literature regarding how old dogs and cats should be when gonadectomy is performed, as well as the benefits and risks associated with the procedures. Because companion animals are living longer, unanticipated effects related to gonadectomy are becoming "more visible" (Reichler 2009).

In fact, veterinary practitioners vary regarding their advice about when the surgery should be performed. Generally, American veterinarians encourage their clients to have elective gonadectomy performed in dogs and cats when the animals are between 6 and 9 months old, but "there does not appear to be any scientific evidence to document that this is the optimal age, [and] the age at which pets have traditionally been spayed and neutered has varied through the years and with geographic location" (Root Kustriz 2007).

"It is important to note that pediatric neutering has been campaigned for and popularized by the animal welfare field. By sterilizing puppies and kittens aged over 6 weeks and weighing more than 2 pounds … shelters and breeders can ensure the inability of those animals to be accidentally or intentionally bred by new owners. Because this is considered extremely important for population control, animal welfare and veterinary organizations (including the American Veterinary Medical Association (AVMA)) support pediatric sterilization. Shelters have found that there is poor follow-through by adopters on spaying/neutering, even when a contract with a deposit for the surgery is in place … there is accordingly great interest in non-surgical procedures that can treat juvenile cats and dogs" (Briggs, personal communication 2012).

The Association of Shelter Veterinarians (ASV) medical care guidelines for spay/neuter programs note that "Owned pets may best be served by scheduling surgery at 4 months of age or older to allow time for the development of immunity through vaccination. Neutering prior to sexual maturity is strongly recommended to prevent the birth of unintended litters, which commonly occurs when surgery

is delayed. In situations involving animals that will be placed for adoption, neutering is best performed prior to adoption (as early as 6 weeks of age) to ensure compliance" (Looney et al. 2008).

Further discussion of all the factors that may be involved in making this decision is beyond the scope of this document. Readers may consult sources such as Root Kustritz (2007), Reichler (2009), and other publicly available literature.

Theoretically, the general health- and behavior-related advantages and disadvantages of surgical approaches apply to both dogs and cats, though the relative weights of advantages and disadvantages may vary.

The following table and related notes summarize the current thinking regarding pros and cons of gonadectomy, in general, in dogs and cats, and is derived from Root Kustritz (2007, personal communication 2012), Reichler (2009), and Rhodes (personal communication 2012).

Table 3-1: Pros and Cons of Gonadectomy in Dogs and Cats

	Pros	Cons
Female Dog	▪ Completely effective sterilant ▪ Decreased incidence of mammary neoplasia (depending on timing of gonadectomy) (a) ▪ Decreased incidence of reproductive tract (ovarian/uterine) disease (b) ▪ Decreased incidence of reproductive behaviors (c) ▪ Eliminates the risk of difficult birth (dystocia)	▪ Surgical complications ▪ Increased incidence of urinary incontinence (e) ▪ Increased incidence of hematologic, bone, and bladder tumors (f) ▪ Increased disposition to knee injury (g) ▪ Obesity (h) ▪ Possible breed-related decreased lifespan (d)
Male Dog	▪ Completely effective sterilant ▪ Decreased incidence of reproductive tract (testicular and prostatic) disease (except prostate tumors) (b) ▪ Decreased incidence of reproductive behaviors (c) ▪ Possible increased lifespan (d)	▪ Surgical complications ▪ Increased incidence of hematologic, bone, and prostate tumors (f) ▪ Increased predisposition to knee injury (g) ▪ Obesity (h)
Female Cat	▪ Completely effective sterilant ▪ Decreased incidence of mammary neoplasia (depending on timing of gonadectomy) (a) ▪ Decreased incidence of reproductive tract (ovarian/uterine) disease (b) ▪ Decreased incidence of reproductive behaviors (c) ▪ Eliminates the risk of difficult birth (dystocia)	▪ Surgical complications ▪ Obesity (h) ▪ Possible increase in diabetes mellitus (i)
Male Cat	▪ Completely effective sterilant ▪ Decreased incidence of reproductive behaviors (c)	▪ Obesity (h) ▪ Possible increase in diabetes mellitus (i)

(a) Mammary neoplasia. In dogs, mammary neoplasia represents the most common tumor type. Although the incidence is less in cats, mammary tumors make up 17% of the neoplasms that occur in female cats; 85% of those are cancerous. The risk for development of benign mammary tumors in cats and dogs may be reduced depending on the timing of gonadectomy (Reichler 2009).

(b) Reproductive tract disease. Ovarian tumors and cysts cannot occur after spaying; disease mediated by ovarian hormones (e.g., vaginal hyperplasia) is "virtually nonexistent" after spaying in dogs and cats (Reichler 2009). Owners can be expected to be concerned about pyometra as well, which can be prevented by spaying.

In male dogs, "Bilateral orchiectomy has a prophylactic and therapeutic effect on androgen-dependent diseases such as benign prostatic hyperplasia (BPH), chronic prostatitis, and perineal hernia … Castration also prevents testicular and epididymal disorders, such as neoplasia, torsion of the spermatic cord, orchitis, and epididymitis … these diseases are very rare in tomcats" (Reichler 2009).

(c) Behavior. Typically male dogs are neutered to ameliorate behavioral problems, while female cats [and female dogs] are neutered to prevent reproduction, and tomcats are neutered to limit particular sexually related behaviors such as urine spraying (Reichler 2008). Note that behavioral effects of gonadectomy are poorly defined (Levy, personal communication 2012). It can be assumed that one of the reasons owners choose to have bitches spayed is to eliminate the bleeding that accompanies estrus (Rhodes, personal communication 2012). For more discussion on perception vs. data on behavior change in surgically sterilized dogs and cats, see Chapter 5, section 5.2.2.

(d) Lifespan. The literature refers to increased lifespan in gonadectomized dogs (e.g., Reichler 2009), though Waters et al. (2009) determined that in a group of long-lived Rottweilers "removal of ovaries during the first 4 years of life erased the female survival advantage."

(e) Urinary incontinence. In 3%-21% of spayed bitches, urinary incontinence may occur right after surgery or as long as 10 years later. Approximately 75% of the dogs that do develop urinary incontinence do so within about 3 years of spaying. Factors including weight, breed, and timing of spaying can influence whether or not a given bitch develops incontinence (Reichler 2009).

(f) Cancer. Castrated dogs are at a 2.4 to 4.3 times increased risk of prostatic tumors – most of which are malignant – compared to intact dogs. The nature of a cause-and-effect relationship is unclear (Root Kustritz 2007).

Castrated dogs are at a two-fold greater risk of developing osteosarcoma (reported incidence of 0.2%) than intact dogs. There is a "significant association between gonadal hormone exposure and risk of bone sarcoma … in [male dogs] castrated before 1 year of age (lowest gonadal exposure), the risk for bone sarcoma was almost 4 times greater than in sexually intact males. In females spayed before 1 year of age, bone sarcoma incidence was more than 3 times greater than the rate in sexually intact females. The risk factor of early [gonadectomy] was found to be independent of adult height or body weight" (Reichler 2009). The nature of a cause-and-effect relationship has not been defined (Root Kustritz 2007).

In dogs, incidence of transitional cell carcinoma (TCC) of the bladder is no more than 1% and certain breeds are more at risk than others; however, gonadectomized animals have a 2 to 4 times greater risk of developing TCC than intact animals (Root Kustritz 2007). Reichler (2009) notes "the increased risk for developing TCC in neutered dogs of both sexes is not explained at this time."

The relative risk for cardiac and splenic hemangiosarcoma (reported incidence of 0.2%) in animals that have been spayed or neutered is increased. Spayed females appear to be at 2.2 and 5 times the risk of splenic and cardiac hemangiosarcoma, respectively, than intact females, while castrated males have 2.4 times the risk versus intact males (Root Kustritz 2007).

(g) Cruciate ligament. A study of records of animals treated at an orthopedic veterinary practice over 2 years indicated that the prevalence of anterior cruciate ligament rupture (reported prevalence in the subject population was 3.48%) in neutered male and female dogs was significantly greater than that of intact dogs (Slauterbeck et al. 2004).

(h) Obesity. Obesity is a multifactorial issue in dogs and cats. Veterinarians report spayed or castrated dogs and cats as obese compared to intact dogs and cats but gonadectomy is "the most commonly reported risk factor." Cats have an increased tendency to become obese after surgical sterilization, with no correlation between age at gonadectomy, and weight or body fat. In dogs, age at gonadectomy does appear to be a factor, with an increase in obesity incidence in dogs that underwent gonadectomy at greater than 5 months of age than those gonadectomized at less than 5 months of age. In any case, "in both dogs and cats, obesity is not a mandatory consequence of gonadectomy; instead, it is controllable with an appropriate diet, feeding regimen, and exercise regimen" (Root Kustritz 2007).

(i) Diabetes. Gonadectomized cats have a greater risk of developing diabetes mellitus than intact cats. Root Kustritz (2007) notes an 8.7 times greater risk versus intact cats; Reichler (2009) cites a 2- to 9-fold increased risk. Diabetes risk may be related to (preventable) increased obesity rates in gonadectomized cats and dogs.

3.1.2 Surgical Methodologies

3.1.2.1 Females

For owners of female cats and dogs who do not want their pets to reproduce or who don't want to tolerate estrous behavior, the current method of choice in the US is ovariohysterectomy, although as noted above, ovariectomy is becoming increasingly accepted.

Ovariohysterectomy involves putting the animal under general anesthesia, shaving the abdomen, making an incision in the midline of the abdomen or the flank, and removing the entire uterus and both ovaries. Ligatures are placed to tie off the major blood vessels, ligate the uterine stump, and close the incision. Ovariectomy involves removal only of the ovaries though still under general anesthesia. Some veterinarians may administer fluids and post-operative pain management drugs.

Reichler (2008) notes that "preference [for ovariohysterectomy versus ovariectomy] was most likely based on the presumption that future uterine pathology is prevented by removing the uterus. However, historical reviews of the short-term and long-term complications after ovariohysterectomy and ovariectomy leads [sic] to the conclusion that there is no benefit and thus no indication for removing the uterus during routine neutering in healthy bitches."

Van Goethem et al. (2006) conducted a literature review to assess whether or not ovariectomy can be considered a safe alternative to ovariohysterectomy in dogs. Researchers concluded that the procedures were equivalent in terms of long-term urogenital issues that include endometritis, pyometra, and urinary incontinence, and pointed out that ovariohysterectomy "is technically more complicated, time consuming, and is probably associated with greater morbidity (larger incision, more interoperative trauma, increased discomfort) compared with ovariectomy." It is clear that ovariohysterectomy is a bigger surgery (Jöchle, personal communication 2012).

Studies of laparoscopic ovariohysterectomy have been reported by European and US investigators. Laparoscopic ovariohysterectomy and ovariectomy procedures provide advantages of improved visualization of the genitourinary tract, less tissue trauma, and reduced postoperative pain, recovery time, and risk of infection and other complications compared to traditional ovariohysterectomy. Possible disadvantages include equipment cost, potential morbidity related to bleeding and injury to the viscera (which can also be seen in the open surgical technique), the need for specific training and anesthetic protocols, equipment limitations usually preventing large-scale use in neutering programs, and difficulty taking laparoscopic techniques into remote or mobile applications (Dupré and Fiorbianco 2008, Levy, personal communication 2012). High Quality High Volume Spay Neuter (HQHVSN) veterinarians reportedly perform much more efficient and less traumatic procedures than less experienced veterinarians; these surgical procedures may compare differently to laparoscopic procedures (Levy, personal communication 2012).

3.1.2.2 Males

Male dogs and cats are castrated under general anesthesia. Incisions or an incision is made in the scrotum (pre-scrotal incisions are most common in the US in dogs) and each testicle is exteriorized, the blood supply and spermatic cord are ligated, and the incision is closed in dogs or (commonly) left open in cats. Typically the procedure is completed quickly and risk of infection is low.

Some veterinarians recommend this surgery for dogs that have not yet reached sexual maturity to prevent them from developing aggressive behavior, in the belief that castration eliminates testosterone, and reduction in testosterone will result in a reduction in aggression, but there is controversy on the relationship of aggressive behavior to sex steroids. Castration may not result in decreased aggression.

Although there has been concern that the urethral diameter is decreased in male cats following prepubertal castration, numerous studies have found no correlation between castration and urethral diameter or lower urinary tract disease (Root Kustritz 2010). In general, there seems to be neither an increase nor a decrease in health issues in castrated male cats versus non-neutered males; however, there is a higher risk of diseases such as FIV and FeLV transmitted by fighting among non-neutered males.

One alternative to surgical castration in males is a vasectomy; however, this procedure is not widely performed in part because it does not affect undesirable aggressive behavior (University of California, Davis (UCD) 2012). However, behavior changes expected post-castration may not be realized; the overwhelming issue related to undesirable aggressive behavior is lack of owner satisfaction. Owners who spend money on contraception want the unwelcome behaviors to be rectified (Jöchle, personal communication 2012).

3.2 Gonadotropin-Releasing Hormone (GnRH)

There are three major considerations governing interventions to create fertility suppression at the level of GnRH:

- Potentially effective in males and females
- Potentially effective in canine and feline species, because GnRH is highly conserved (i.e., the gene coding for GnRH results in the translation of the same decapeptide with the same sequence of amino-acids among mammals)
- Suppression of GnRH will result in suppression of the secretion of the reproductive steroid hormones and therefore suppress sexual behavior and hormone-mediated diseases as well as fertility

Concern has been expressed that since GnRH receptors exist outside the pituitary gland and reproductive tract, approaches targeting GnRH may have effects on non-target tissues. However, no such effects have been identified despite more than a decade of treatment with these approaches (Asa, personal communication 2012).

3.2.1 Overview of GnRH Agonists

Effective GnRH agonists, which mimic the effect of native GnRH but have a longer half-life in the blood, work by binding to and causing down-regulation of the GnRH receptors in the pituitary gland. The continuous administration of agonists (as opposed to the normal pulsatile release of endogenous GnRH) results in a complete suppression of GnRH effect, since to be effective, GnRH must be "seen" through the receptors in the pituitary cells.

GnRH agonists have been developed for use in human medicine and are available as generic peptide drugs such as leuprolide, nafarelin, triptorelin, and histerelin. These peptides have to be given by injection or subcutaneous implantation, because if given orally, they are digested and not biologically active. Effective slow-release implants have been developed for humans that are used for 3-12 months to suppress testosterone in the treatment of prostate cancer and to suppress estrogen in the treatment of endometriosis. They have other uses such as treatment of precocious puberty and have also been developed as inhalant formulations. Further discussion of GnRH agonists for use in humans is beyond the scope of this report.

Another GnRH agonist – deslorelin – was developed in an implant formulation for use in dogs and has been used in both domestic animals and wildlife (see section 3.2.1.1. below).

A disadvantage of the GnRH agonist approach to suppress reproductive activity is that initial administration in males and females typically causes an initial temporary increase in follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In females, this increase may result in inducing estrous. In males, the increase in LH causes an increase in testosterone that does not express itself clinically. (When GnRH agonist implants are used for treatment of human prostate cancer, the stimulation of testosterone aggravates the condition, causing increased bone pain from metastatic tumors and a stimulation of tumor growth. This initial stimulation is called a "flare.") It is important to understand that the mechanism of action of GnRH agonists is characterized by flares of varying duration and that GnRH agonists are therefore not effective in situations in which an immediate suppression of fertility is desired. Once the agonist is discontinued, either by removing an implant, or depletion of the active drug or stopping daily administration of the injectable form of the drug, the return-to-fertility timeframe is unpredictable. So, although a minimum duration of effectiveness can be determined, it is difficult to predict when the effects will wear off in a treated individual.

Studies in dogs using these compounds date back to 1984 (Vickery et al. 1984) and have continued to the present day. A number of compounds have been shown to be effective for use in dogs, and many types of formulations have been studied (Gobello 2007).

While delivery systems for animals were one of the

early limiting factors for development of commercial formulations of GnRH agonists, biocompatible formulations have been developed that are cost effective and convenient to use, and provide long-term release of adequate levels of GnRH agonist (Herbert and Trigg 2005). In fact, in the last decade, two GnRH agonists have been approved for use in contraception in dogs in markets outside the US.

In a relatively recent review article, the authors explain that "All the studies [of GnRH agonists reviewed for this paper] reported a simultaneous decrease in testes volume and consistency. In general testicular volume was decreased 2- to 5-fold in the 4-5 weeks following treatment start, and this regardless of the GnRH agonist used. … Although the original stimulating effect causes serum FSH and LH concentrations to rise from 20 m[inutes] after treatment start, with a peak after 40 m[inutes], these values return to normal in the next 5 h[ours] then collapse on average 2-9 days later … The same is observed for testosterone blood levels. … However, the response was very variable from one individual to another" (references cited in Fontaine and Fontbonne 2011).

One product, Suprelorin® (deslorelin implant), is available as a 6-month (4.7 mg) or 12-month (9.4 mg) implant for use in male dogs. Suprelorin was developed, approved by regulatory bodies and launched in Australia and New Zealand by Peptech Animal Health. Peptech also obtained regulatory approval for use in male dogs in the EU and the product was commercialized in Europe by Virbac. (Virbac ultimately acquired Peptech Animal Health in 2011.) Research has shown that Suprelorin is also effective in fertility suppression in bitches and in male and female cats. Suprelorin has been used off-label to contracept several canid and felid species in US zoos and wildlife in South Africa.

The other GnRH agonist approved in the EU is Gonazon™ (azagly-naraferlin), a controlled-release device developed by Intervet prior to the consolidation of Intervet and Schering-Plough Animal Health (now MSD or Merck Animal Health). Gonazon was approved in the European Union (EU) in 2006 for use in female dogs. Research has shown that it is also effective in female cats, although it was never approved for cats. Unfortunately, Gonazon was not commercialized and is therefore unavailable.

Note that in addition to contraception, GnRH agonists also cause significant shrinkage of the prostate gland (Limmanont et al. 2011), which is a clinical advantage for dogs with benign prostatic hyperplasia, a common condition of older intact male dogs. Dogs with clinical signs of prostate disease are typically castrated to shrink the prostate, so treatment with a GnRH agonist could be of benefit for dogs suffering from this condition.

3.2.1.1 Deslorelin

3.2.1.1.1 Deslorelin in Dogs – Males

As noted above, there has been a great deal of research into the use of GnRH agonists in dogs. This section provides some examples of studies on the use of deslorelin in male dogs:

A review article (Kutzler and Wood 2006) describes several studies that have examined the use of deslorelin as a contraceptive in male dogs. Subcutaneous administration of a 6 mg, slow-release deslorelin implant reduced plasma concentrations of LH and testosterone to undetectable values within 4 weeks and caused infertility within 6 weeks. Testosterone and LH concentration and semen quality returned to normal by 60 weeks after implant administration. Histological analysis at the end of the treatment period indicated that the testes and prostate gland of treated dogs were not different from those of untreated controls. Researchers concluded that the implant would be effective for long-term, reversible fertility control in male dogs (Junaidi et al. 2003). Other researchers also concluded that deslorelin-treatment-induced effects on fertility were completely reversible (Trigg et al. 2001, Dubé et al. 1987).

Subsequent work by Junaidi et al. further described deslorelin research in dogs: A study published in 2007 indicated that treatment with a 6 mg deslorelin implant "desensitized the pituitary gonadotrophs to GnRH and also led to a desensitization of the Leydig cells to LH. This explains, at least in part, the profound reduction in the production of androgen and spermatozoa in deslorelin-treated male dogs."

In 2009, Junaidi et al. reported on a dose-response study in which the effects of 3-, 6-, or 12-mg deslorelin implants on pituitary and testicular function were assessed. The researchers concluded that the degree of

suppression of reproduction was not subject to a dose-response relationship; however, the maximum duration of suppression, and therefore the time to resumption of fertility, was dose dependent, with dogs treated with the 12 mg implant taking the longest time to restoration of full ejaculates (Junaidi et al. 2009).

Other work indicated that 8 weeks after treatment with a deslorelin 12-month (9.4 mg) implant, plasma testosterone could not be detected in 23 out of 25 dogs treated. In one dog, plasma testosterone reached zero (below the level of detection of the testosterone assay) at Week 12 and in a second dog, plasma testosterone rose after treatment and subsequently fell to zero at Week 16. Plasma testosterone was reduced for at least 12 months in all but two treated dogs, one of whom was lost to the study on Day 68; in the other dog, suppression was rapid and complete between Week 2 and Week 20 but subsequent surgery suggested that the implant had been "lost." Researchers reported a mean duration of efficacy of 89 weeks, with a range of 56-132 weeks (Trigg and Yeates 2008). It has also been demonstrated that multiple serial implantation in males did not cause adverse effects of diminished efficacy. Dogs that have been re-implanted for four consecutive doses at 6-month intervals with 4.7 mg deslorelin returned to normal steroidogenesis after cessation of treatment (Trigg et al. 2006).

In a discussion of the clinical use of Suprelorin to control fertility in male dogs (von Heimendahl 2010) the author points out that in Europe:

> "Suprelorin is used for different purposes in different countries. This seems to depend on attitudes to surgery for neutering in general and the number of un-neutered pets in the dog population as well as the use in stud dogs. In countries like the UK, where neutering of male puppies at 6 months of age is routine, the implant is used mostly in older non-neutered males where the owner wants to avoid surgery or by breeders with several stud dogs to avoid aggression between males … In Scandinavian countries where neutering of males is not performed routinely as it is against animal welfare legislation, Suprelorin is used more often to pharmacologically castrate males. The implant can be given routinely every six months or when the increase in testicular size indicates that it has stopped working."

3.2.1.1.2 Deslorelin in Dogs – Females

Suprelorin (deslorelin) has been shown to provide effective long-term contraception in bitches (Kutzler and Wood 2006, Gobello 2007). For example, subcutaneous administration of a deslorelin slow-release implant (3, 6, or 12 mg) to bitches "increased the duration of the mean interoestrous interval … at all doses" and suppressed estrus for up to 27 months independent of the stage of the estrous cycle at implantation. When serum progesterone (P4) was greater than 5ng/mL, the initial stimulatory effect caused estrous cycle induction 4-8 days after implantation. Six out of nine bitches that were mated after recovery from treatment became pregnant (Trigg et al. 2001). (See also Kaya et al., Chapter 3, section 3.2.1.1.5.)

To suppress the possibility of inducing an estrus when initiating treatment, the AZA Wildlife Contraception Center (WCC) at the Saint Louis Zoo (US) recommends "supplemental progestin treatment for 2 weeks (7 days prior to and 7 days after implant insertion)," suggesting that megestrol acetate (MGA) (a progestin) is the simplest approach, and cautioning that Depo-Provera® should not be used (stlzoo.org/animals/scienceresearch/contraceptioncenter/contraceptionrecommendatio/contraceptionmethods/suprelorin-deslorelin/).

GnRH agonists also were at one time used as pro-fertility agents in female dogs due to their characteristic "flare" (Gobello 2007); however, for induction of estrus, the agents had to be administered by constant infusion or by injection several times a day for up to 14 days. Therefore, that approach was deemed clinically ineffective for estrous induction. Fontaine and Fontbonne (2011) point out that while the "two-step mechanism" of deslorelin implants (Suprelorin 4.7 mg) "may allow activation and inhibition of the oestrous cycle, which both find clinical applications in our everyday activity [as veterinarians], from oestrous [sic] induction to chemical sterilization," data regarding use in bitches were primarily derived from animals in an experimental rather than a clinical setting. In this study of use of deslorelin to control fertility in the bitch, researchers investigated responses to a 4.7 mg deslorelin implant administered at various stages of the estrous cycle since responses to implantation vary depending on the stage of the estrous cycle in a given animal. Forty-six out of 47 bitches (97.8%) experienced induced estrus

during the week after implantation, and this response was not related to the stage of estrus in any particular animal. By 30 days post-implantation, 43 of the 47 dogs (91.5%) were not exhibiting signs of estrus (Gobello 2007). Discussion of the use of the deslorelin implant to induce estrus is included in the Gobello article but is beyond the scope of this report; readers wishing to learn more about the findings related to estrous induction can access a summary at zoovet.ee/product/docs/2050981722.pdf.

Results reported in 2010 by Fontaine et al. at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control involved documenting the influence of the stage of the estrous cycle on the use of the 4.7 mg deslorelin implant for contraception in bitches. Researchers implanted 60 healthy bitches. Implants were placed in the umbilical region. The stage of the estrous cycle was determined based on history, vaginal smear, progesterone assay, and ovarian ultrasound at study start and on Day 15 and Day 30. Researchers found the following estrous cycle distribution in study animals: 41 were in anestrus when implanted; 14 were in diestrus; 5 were in estrus. The following results were obtained:[3]

- 40 of 41 bitches implanted in anestrous exhibited induced estrus. Thirty days post-implantation, 38 animals no longer exhibited signs of estrus.
- 5 of the 14 bitches implanted in diestrus exhibited estrous induction and researchers removed the implants.
- In 40 bitches exhibiting induced estrus, estrus was observed 4.2 +/- 1.4 days after implantation, and ovulation occurred 12 +/- 2 days later.
- 13 bitches were anovulatory, 11 of which were implanted in early anestrus and 2 were implanted in estrus.
- Ovarian cysts were reported in 2 animals; persistent estrus was reported in 2 animals. These animals were neutered via surgery.

Researchers documented down-regulation in 31 of the 41 animals implanted in anestrus and concluded that "deslorelin implants appear to be a quick and safe way to neuter bitches. Diestrus seems to represent the best period to avoid estrous induction. It is advisable to monitor the implanted bitches in the 30 days following implantation in order to confirm down-regulation."

Herbert and Trigg (2005) note the wide variability among GnRH-agonist-treated bitches in terms of resumption of estrous cycles: "This variability may be the result of genetic variations in the sensitivity of individual animals to GnRH–induced down-regulation. As the variability is often greatest at lower doses it may represent a dose-response relationship where a particular dose may be sub-threshold for some individual animals. An alternative hypothesis is that there may be some variability in the implant manufacture process. There also appears to be a proportion of animals that continue to cycle during GnRH agonist treatment, i.e. 'non-responders.' … The basis of this variability is probably genetic … "

Note that the use of GnRH agonists to treat urinary incontinence in ovariohysterectomized dogs has also been described (e.g., Reichler et al. 2003) but discussion of this application is beyond the scope of this document.

3.2.1.1.3 Deslorelin in Cats – Toms

Work reported in 2010 (Goericke-Pesch et al.) demonstrated that male cats implanted with the 4.7 mg Suprelorin implant displayed significantly reduced mean testosterone concentrations within 28 days. Researchers found that mean T concentrations were below the limit of detection on Day 20. Time to complete suppression of fertility, as measured by T values under the limit of detection of the assay, was 20 days to 11 weeks post-implantation.

An approximately 21% decrease in mean testicular size was observed at Week 4 in treated toms; beginning at Week 12, mean testicular size decreased by greater than 50% and this decrease was maintained as long as fertility was suppressed. Researchers noted that "… penile spines disappeared, as in surgically castrated cats. All castration-related side effects [were] observed following successful down-regulation and ceased T production, [including] a significant increase of food intake … urine marking stopped or at least significantly decreased [and] following an initial increase, sexual behavior, mounting, libido, and mating were significantly reduced in treated toms after 11-16 weeks; however, mounting could be observed after excessive animation by a teaser queen. Toms become temporarily infertile after treatment; however, infertility may be delayed by about 6 weeks after successful down-regulation … all

[3] Please note that the terms anestrus and diestrus seem to be used interchangeably.

effects [were] fully reversible. Duration of efficacy – as observed from clinical experiences – varied between six and 24 months. Return of spermatogenesis to pre-treatment semen quality may take up to five - six months; initial return can be expected in five to nine weeks."

Histological examination of testes has confirmed induction of infertility, with a return to normal parameters after removal of the implants (Novotny et al. 2012).

3.2.1.1.4 Deslorelin in Cats – Queens

Suprelorin (deslorelin) has been shown to effectively suppress ovarian activity in cats, but the duration of suppression reported was variable. The drug was given at 6 mg via a long-acting subcutaneous implant to 10 mature female cats and compared with 10 untreated controls, and animals were observed for 14 months. Treatment with deslorelin, as expected, initially stimulated estradiol release, followed by its decrease. Return to estrus was variable, and ranged from 7.5 to up to 14 months or greater (six animals had not yet returned to estrus at the end of the study). Some cats that demonstrated slightly elevated estrogen levels were given a second implant (Munson et al. 2001).

Goericke-Pesch et al. 2010 reported that queens can be implanted during seasonal anestrus, in estrus or in interestrus. Researchers noted that induction of estrus in queens implanted during seasonal anestrus can be expected to occur. In the study, "following the initial increase of [estradiol] (E2) and also of progesterone (P4) concentrations, hormone concentrations started to decrease 2-4 weeks after implantation in treated queens. A temporary increase of E2 with or without estrus signs may be observed during the effective treatment and is followed by phases without sexual activity, indicating that treatment is still effective." In this study, suppression of estrus varied between 6 and 24 months, and researchers noted that "Interestingly, injection of a second 4.7 mg implant during effective estrous suppression (a temporary increase of E2 was observed in 1/10 cats, therefore 5/10 were implanted again) did not influence the duration of efficacy (one implant: 11.1 ± 2.9 months versus two implants: 11.0 ± 2.3 months)."

As of the date of this 2010 Goericke-Pesch et al. publication, no data regarding reversibility had been published; however, the authors note that "it can be demonstrated that at the end of the efficacious period, ovarian weight and uterine diameter are similar to untreated controls … observations restricted to clinical cases [indicate] that queens mated following treatment in naturally occurring estrus conceived and delivered healthy kitten[s]."

A study published in 2012 was designed to ascertain the safety and efficacy of 4.7 mg deslorelin implants in postponing puberty in 15 domestic queens. Researchers diagnosed puberty by "the presence of the typical oestrous behavior and vaginal cytology finding." An estrous response was seen in one treated queen; in another, clinical signs of pyometra were seen. Upon ovariectomy at puberty, ovaries "appeared small" in treated queens compared to control queens. Researchers concluded that "long-term-release deslorelin, administered at approximately 50% adult body weight, postponed feline puberty without altering [growth] rate" (Risso et al. 2012).

Another recently published study assessed the effectiveness and safety of deslorelin implants for suppression of estrous behavior and mating activity in queens "in a controlled ambient environment in feline queens in the presence of a tomcat." Cats in treatment Group 1 received a deslorelin implant (9.5 mg), cats in Group 2 received a 5 mg MGA tablet and 9.5 mg deslorelin implant, and cats in Group 3 received a placebo implant. At 18.5 months into the study the queens were ovariohysterectomized. Researchers weighed the ovaries and uteri and performed histological examination. Estradiol levels in Groups 1 and 2 were significantly below those in Group 3, and ovarian and uterine weights differed significantly among the groups, and were the lowest in Groups 1 and 2. Groups 1 and 2 had significantly higher numbers of primordial and primary follicles than in Group 3 (placebo group). Endometrial gland, antral follicle, and corpora lutea numbers were highest in the placebo group (Group 3). Researchers concluded "Deslorelin implants successfully suppressed estrous behavior and E(2) secretion in queens for 18.5 mo[nths] of the study period. Further investigations are needed to demonstrate the effects of GnRH agonists on ovarian interstitial tissue" (Toydemir and Kiliçarslan 2012).

At this juncture "there is inadequate understanding of the incidence of induced estrus, ability to conceive during estrus, and – if pregnancy occurs – impact on parturition and lactation" in female cats treated with deslorelin (Briggs, personal communication 2012).

3.2.1.1.5 Recently Reported Research Involving the Use of Deslorelin for Contraception

Research involving the use of deslorelin implants for contraception and other indications in dogs and/or cats is ongoing, with more than 50 studies believed to be underway at the time of this update (Briggs, personal communication 2012).

The following studies or reviews related to contraception and reported at the 7th International Symposium on Canine and Feline Reproduction in July 2012 are summarized below. Proceedings of this symposium are available at ivis.org/proceedings.

Table 3-2: Use of Deslorelin for Contraception in Cats and/or Dogs[4]

Deslorelin acetate (Suprelorin) effects in semen quality of domestic cats (Ackermann et al.)
Investigators found that the use of deslorelin acetate in domestic cats decreased semen quality but did not suppress production of sperm completely, possibly due to inter-animal variation; "the return of spermatic production was not observed in semen collection."
Effect of deslorelin acetate (Suprelorin) in domestic cat testicular morphology (Ackermann et al.)
Investigators found that "deslorelin acetate causes atrophy in seminiferous tubules, lineage sperm depletion and decrease of epididymal content, indicating partial suppression of spermatogenesis. After termination of treatment the recovery of spermatogenesis was observed through the recovery of spermatozoa lineage in the seminiferous tubules and increase of sperm content in epididymal lumen."
Delay of puberty and reproductive performance in male dogs following the implantation of 4.7- and 9.4-mg GNRH-agonist deslorelin at early prepubertal age (Sirivaidyapong et al.)
Investigators assessing the length of effectiveness (i.e., suppression of reproduction) of deslorelin implants in male dogs implanted at 4 months old found that the 4.7 mg implant was effective for less than 2 years in three of four treated dogs. The 9.4 mg implant was effective for 2.5 years in Beagles (n=2) and 3.2 years in mixed breed dogs (n=2). Reproductive characteristics in control dogs developed normally.
Postponement of puberty using GnRH agonists implants in bitches of different breeds (Fontaine et al.)
Researchers investigated the use of 4.7- or 9.4-mg deslorelin implants for puberty postponement in client-owned bitches younger than 6 months old. One bitch implanted with a 4.7 mg implant "was re-implanted with a similar implant 6 months after the first implantation at the request of the owner." Bitches implanted with the 4.7 mg implant showed signs of estrus at 13-24 months post-implantation; six did not display signs of estrus at study end (16-25 months post-implantation). None of the bitches treated with the 9.4 mg implant showed signs of estrus by the end of the study period (8-15 months post-implantation). Researchers concluded that "Although further fertility could not be studied, [the] data seems [sic] to indicate that implantation of bitches of various breeds less than 6 months of age is a valuable and safe way to postpone puberty, without noticeable side effects."
Onset of sterility following administration of a 4.7 mg deslorelin implant in adult male dogs (Romagnoli et al.)
Investigators administered deslorelin implants to six client-owned dogs referred to the University of Padova (Italy) Veterinary Teaching Hospital "with the request to control aggressiveness and/or fertility." All dogs presented with normal clinical and reproductive parameters. The characteristic post-treatment "flare" occurred initially "during the first month … followed by a progressive decline in most of the seminal parameters considered … during the subsequent 3 months." Sterility characterized as "complete" was achieved at 54 +/- 21 days.
Behaviour and the pituitary-testicular axis in dogs before and after surgical or chemical castration with the GnRH agonist deslorelin (de Gier et al.)
Investigators compared specific effects of surgical castration and use of a GnRH implant in owned dogs. Eighteen dogs underwent surgical castration and 24 received a Suprelorin 4.7 mg implant. Endocrinological parameters and aggression, fear/insecurity, play behavior and sexual behavior were assessed and questionnaires were completed prior to and 4-5 months post-procedure. No significant difference was found between the two approaches in terms of plasma testosterone concentration and behavioral parameters. The perceived effect of surgical castration on male sexual behavior in the presence of bitches in estrus was greater than that of the implant and "despite the similarly low basal plasma testosterone concentrations in both groups … [in all implanted dogs] the pituitary testicular axis was not completely down-regulated."

Pharmacodynamics and pharmacokinetics of a sustained-release implant of deslorelin in companion animals (Navarro and Schober)
Investigators confirmed the characteristic "dual mode of action of sustained-release forms of GnRH agonists" and noted that "the individual variations of the duration of action may be explained by the mode of action involving gene regulation."
Clinical use of deslorelin implants for long-term contraception in prepubertal bitches (Kaya et al.)
Investigators assessed the use of 4.7 mg (n=5) and 9.4 mg (n=5) deslorelin implants in prepubertal (4-month-old) bitches in terms of duration of effectiveness, safety, and reversibility "with special regard to the time of epiphyseal closure." The study indicated that both formulations provide effective and safe long-term prevention of estrus in prepubertal bitches implanted at 4 months of age; while height at withers was not affected significantly, delay in epiphyseal closure was seen. As of the reporting, none of the bitches showed any sign of estrus throughout the study (15-17 months after the experiment had started).
GnRH agonist implants result in estrous induction and estrous suppression (Fontbonne et al.)
GnRH agonists are used to induce estrus; however review of this specific use is beyond the scope of this document. The authors cite a relative lack of information regarding estrous prevention and suppression in prepubertal bitches and queens, although some studies have been undertaken (e.g., Trigg et al. 2006, Goericke-Pesch 2011 EVSSAR Congress). References cited in this abstract can be found at ivis.org/proceedings/iscfr/2012/195.pdf?LA=1.

3.2.1.2 Nafarelin

3.2.1.2.1 Nafarelin in Dogs

The GnRH agonist azagly-nafarelin is used in fish to induce and synchronize egg production. In Europe, it was also approved as Gonazon, an 18.5 mg subcutaneous controlled-release implant intended for use as a long-term blockade of gonadotropin function in female dogs to prevent puberty. As noted earlier in this chapter, Gonazon was not commercialized by its developer Intervet or its successor companies, Intervet/Schering-Plough Animal Health and MSD/Merck Animal Health.

The GnRH agonist nafarelin given daily at 2 μg/kg/day decreased testosterone levels within 3 weeks after initiation of treatment, and normal fertility was restored within 8 weeks following cessation of treatment (Vickery 1985).

In one study of young Beagles approximately 5 months of age, puberty was delayed for 8 to 16 months (Rubion et al. 2006). In that study:

"Control dogs received a placebo implant and treated dogs received Gonazon containing 18.5 mg azagly-nafarelin. Throughout the 1-year treatment estrous behavior was monitored weekly and plasma progesterone concentration, body weight, and height were measured monthly. Following implant removal, estrous detection and progesterone measurement were continued until occurrence of puberty in all bitches. None of the Gonazon-treated bitches displayed puberty during the period in which Gonazon was present. Following removal of Gonazon, resumption of estrus and ovulation occurred naturally or was induced approximately 8.5 months later. No clinically detectable side effects were noted in Gonazon-treated bitches. Height at withers and body weight with time were also unaffected. The implants were well tolerated and generally easy to remove. Researchers concluded that Gonazon safely, efficiently, and reversibly prevents

[4] As reported at the 7th International Symposium on Canine and Feline Reproduction (Whistler, BC, Canada 2012).

reproductive function for 1 year in prepubertal bitches. In older bitches (12 months or more), Gonazon implants blocked reproductive function for approximately 11 months."

3.2.1.2.2 Nafarelin in Cats

In a study described at the 6th International Symposium on Canine and Feline Reproduction in Vienna and published in 2009, researchers inserted one azagly-nafarelin (Gonazon) implant, containing 20 mg azagly-nafarelin, subcutaneously in the necks of six treated queens. Ovarian activity of treated queens and six control queens was monitored for 3 years.

"The contraceptive efficacy of Gonazon was assessed by the proportion of queens in which a progesterone rise indicating ovulation was demonstrated following continuous housing with a vasectomized tomcat, which was rotated each week between treated and control queens. The marker of ovulation used was progesterone concentrations exceeding 10 ng/mL for at least 2 weeks. General safety was assessed by veterinary examinations including weight measurements, performed at study initiation, after one year, and then every 6 months. All six control queens ovulated regularly throughout the 3-year treatment period. At treatment initiation three Gonazon-treated queens had high progesterone levels, suggesting that they had ovulated before treatment. During the week following treatment, two other queens displayed a rise in progesterone concentrations. Later on, all treated queens continuously displayed low progesterone concentrations until 2.5 years post implant insertion. At this stage, two queens had an isolated episode of follicular luteinization, but all queens in the treated group again became anovulatory. This research indicated that Gonazon efficiently prevented ovulation in queens for 3 years and was well tolerated. Return to estrus was not observed towards the end of treatment despite low azagly-nafarelin concentrations in some queens." (Prohaczik et al. 2008; Rubion and Driancourt 2009).

3.2.1.3 Other GnRH Agonists

The GnRH agonist leuprolide acetate, given to dogs as a single injected dose at 1 mg/kg, causes spermatozoa abnormalities and significantly decreases ejaculate volume and testosterone and LH concentrations for 6 weeks. In one study, normal spermatogenesis resumed 20 weeks after treatment (Lacoste et al. 1989). Buserelin implants (6.6 mg) decreased testosterone concentrations in male dogs and produced infertility within 3 weeks; the effect persisted for an average of 233 days (Kutzler and Wood 2006).

3.2.1.4 GnRH Agonists: Summary of Advantages and Disadvantages

Advantages	Disadvantages
Proven to suppress fertility in both males and females	Initially may induce estrus in females
Suppress sexual behavior – females will not come into estrus during treatment, males will behave as castrates	Need to be given repeatedly to maintain effects
Active compounds available as generics (manufactured under current good manufacturing practice (cGMP))	No patent protection for common active drugs
Demonstrated to be effective in a variety of formulations, including depot injections, microspheres, and implants	A commercially viable product may need to have at least 6-12 months of efficacy for convenience (longer duration will be desired for many pets and unowned animals)
Reversible – when the drug is discontinued, reproduction should resume within a reasonable period of time (could be used in animals intended for breeding)	If lifetime contraception is desired, repeated treatments will be necessary lifelong
	Slow onset of activity (generally a few weeks) and variable duration of treatment effects

3.2.2 Overview of GnRH Antagonists

GnRH antagonists block GnRH receptors, and suppress fertility by blocking the GnRH receptors on the pituitary cells. Why were GnRH antagonists developed when available GnRH agonists work so well? The answer is that the initial stimulation side effect caused by agonists can be avoided by using antagonists instead. An antagonist would not cause the initial stimulation of sexual behavior. Unlike the GnRH agonists, which can take several weeks to have a suppressive effect, the antagonists have an immediate effect of suppressing the reproductive hormones.

Gobello (2012) contends that while "GnRH antagonists appear to have a promising future in domestic carnivore reproduction there is still scarce information about them and further work is needed before they could be widely recommended."

Another limiting factor at this juncture is the cost of the peptide GnRH antagonists (Jöchle, personal communication 2012).

3.2.2.1 GnRH Antagonist Peptides

The first GnRH antagonists were peptides with a structure similar to GnRH. Most of the small peptides work in all species, due to the highly conserved structure of the GnRH receptor.

"The peptides involved are typically more expensive to manufacture [and] are often only effective at much higher doses [than GnRH agonists] … The high dose requirement in turn limits the potential for combination of the antagonists into … long-term release technologies without involving overly large implants or injection depots. First-generation GnRH antagonists [such as] detirelix had the problem of histamine release activity, most of which has been overcome in many [later-generation] antagonists [such as] azaline, acyline, degarelix, abarelix, cetrorelix, and ganirelix [although unpleasant side effects were reported in conjunction with use] in some human patients" (Concannon 2006).

Examples of small peptide compounds that have been studied are antide (Iturelix), cetrorelix (Cetrotide™, Astra Medica), ganirelix (Antagon™, Organon (now Merck)), and acyline. Cetrorelix has activity in monkeys, dogs, rats, and humans (for a review, see Reissman et al. 2000). In humans, Cetrotide and Antagon, for example, are used for short-term treatment to suppress GnRH to prevent premature ovulation in women undergoing controlled ovarian stimulation for fertility treatments.

Work on the GnRH antagonist antide dates back to at least the 1990s. Danforth et al. (1991) characterized antide as a promising compound and reported that in primates, antide manifested prolonged (several weeks) and reversible inhibition of pituitary gonadotropin secretion after a single high-dose injection. No agonistic actions of antide were seen in vitro. Antide was reported to have no apparent noxious or toxic effect on pituitary cells in culture; the actions of antide are immediately reversible upon removal of antide from pituitary gonadotropes. Researchers concluded that the long-term inhibition of gonadotropin secretion by antide in vivo is not due to deleterious effects of this compound at the level of the pituitary gonadotrope.

Serono was developing antide (Iturelix) for endometriosis and prostate cancer in humans in the early 2000s but development was suspended in 2005. Serono become Merck KGaA Serono in 2007 and there is no reference to antide on the Merck Serono website as of the date of this update.

Merrion Pharmaceuticals, LLC has been developing MER-104, an enteric-coated oral formulation of the GnRH antagonist acyline. The injectable form of acyline has been shown to have "potential as a contraceptive regimen in man." A dose-ranging study with MER-104 demonstrated "measurable serum levels of drug at all doses and dose-related suppression of serum LH and serum testosterone. Liver function and creatinine were unaffected by treatment. The data indicate that MER-104 tablets may have potential in the treatment of prostate and breast cancer, endometriosis, prostate hyperplasia, and as part of a male contraceptive" (Amory et al. 2007).

"Newer third- and fourth-generation decapeptide antagonists have been reported to have increased potency and durations of action and are likely to further R&D at least for human applications. However, durations are reported in days rather than weeks and even micro-particle

preparations have effective durations reported in weeks rather than months … [such] short-term GnRH antagonists regimens might be useful to prevent the undesired effect of estrous induction in agonist-treated anestrous animals" (Concannon 2006).

3.2.2.2 Non-Peptide GnRH Antagonists

Non-peptide (small molecule) GnRH antagonists have the potential to be developed as oral, mucosal, and/or dermal formulations delivered via drug-release technologies that differ from typical peptide-release implants (Concannon 2006). These small molecule GnRH antagonists show species differences: They were designed to bind the human GnRH receptor and block activity. They are significantly smaller than the peptide antagonists; the larger GnRH peptide antagonists will bind the GnRH receptors in rats, humans, dogs and likely most all mammalian species, showing cross-species activity. However, it has been demonstrated that the small molecule drugs do not work in all species. Although they bind and antagonize the GnRH receptor in some species, minor differences in the receptor structure between species can result in a particular compound being effective in rats and humans, for example, but not in dogs. Therefore it cannot be assumed that small molecule GnRH compounds developed for use in human health will necessarily work in dogs and cats. Each compound will require testing in the target species (Cui, 2000).

Several biotechnology companies have worked on or are working on non-peptide GnRH antagonists. Note that these examples are not necessarily all inclusive.

For example:

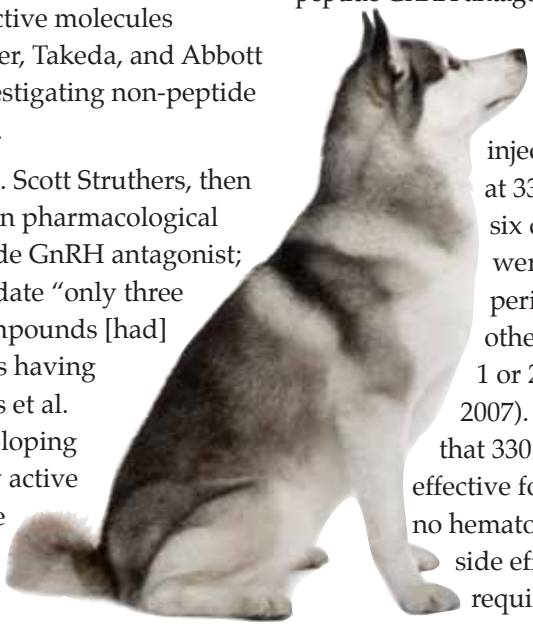
Research at Merck Laboratories advanced the development of non-peptide orally active molecules (DeVita 1999). As of 2006, Merck, Bayer, Takeda, and Abbott Laboratories were all engaged in investigating non-peptide GnRH antagonists (Concannon 2006).

In 2007 researchers, including Dr. R. Scott Struthers, then of Neurocrine Biosciences, reported on pharmacological characterization of a novel non-peptide GnRH antagonist; researchers also noted that as of that date "only three small-molecule GnRH antagonist compounds [had] been reported at scientific meetings as having been evaluated in humans" (Struthers et al. 2007). Neurocrine BioSciences is developing its lead compound, elagolix, an orally active nonpeptide GnRH antagonist. In June 2010, the company entered into an exclusive worldwide collaboration with Abbott Laboratories related to developing and commercializing elagolix and all the company's next-generation GnRH antagonists specifically for men's and women's health applications. Under the terms of the agreement, Neurocrine and Abbott are working together towards regulatory approval and commercialization of the GnRH antagonist compounds. The Phase III trial of elagolix for endometriosis began in mid-2012, and approval is expected in the US in 2016 (Neurocrine Sciences website 2012). Abbott is bearing all costs of development, marketing, and commercialization and in return will receive a percentage of the global sales of GnRH antagonist compounds (Neurocrine Science Press Release, June 16, 2010). Abbott has an animal health organization but to our knowledge, this compound is not under development for animals.

3.2.2.3 GnRH Antagonist in Dogs

As noted in the section above, one method of blocking the action of GnRH is to make a small peptide or molecule that is similar in structure to GnRH, and binds with the GnRH receptor without activating it, e.g., an antagonist. Although several peptide GnRH antagonists are approved for various uses in humans, none is approved for use in dogs or cats as of mid-2012.

A 2007 review article notes that although the effect of GnRH antagonists in female and male dogs was described in the 1980s and third-generation GnRH antagonists have been studied in dogs more recently, the data are limited. As noted previously, first-generation GnRH antagonists were characterized by limited duration of activity and had a tendency to produce allergic reactions and other systemic adverse reactions (Gobello 2007). There are no publications describing research on non-peptide GnRH antagonists in dogs.



3.2.2.3.1 Males

In a dog study, a single subcutaneous injection of the GnRH antagonist acyline at 330 μg/kg suppressed semen quality in six dogs for 2 months. Libido and erection were unaffected throughout the 2-month period in three of the dogs, while three other dogs did not display these actions for 1 or 2 weeks following treatment (Gobello 2007). Another study in dogs demonstrated that 330 μg/kg given at 2-week intervals was effective for decreasing semen quality; there were no hematological, biochemical, local, or systemic side effects noted (Valiente et al. 2007). The requirement for such frequent treatments

could be solved by availability of a sustained-release formulation.

In a 2009 study of acyline in domestic dogs (Garcia Romero et al. 2009), researchers assessed how a single dose affected serum concentrations of FSH, LH, and testosterone. Researchers took blood samples before treatment with acyline and throughout a 29-day post-treatment period. As described in the study abstract "Serum concentrations of FSH, LH, and [testosterone] varied throughout the study period … Gonadotrophins decreased below pretreatment concentrations 60 minutes after injection, whereas [testosterone] took 90 minutes to decrease below baseline … FSH, LH, and testosterone decreased until Day 9, when they reached their nadir … Both gonadotrophins and testosterone began increasing on Day 14 after treatment, although FSH and T serum concentrations still remained below baseline on that day … FSH and testosterone rebounded above baseline on Day 29, whereas LH concentrations were similar to baseline at this time … No local or systemic side effects were detected in any dog following acyline treatment. In conclusion, a single acyline treatment safely and reversibly decreased serum gonadotrophin and [testosterone] concentrations in dogs for 9 d[ays]."

In another study (Garcia Romero et al. 2012), researchers investigated the response of testosterone to GnRH challenge over a 30-day period in male dogs treated with acyline versus male dogs treated with a placebo. Dogs in both groups were serially challenged with buserelin, a GnRH agonist, and blood samples were taken before and after injection of the agonist. There were no differences in basal testosterone serum concentrations between the treated and placebo dogs prior to treatment. Post-treatment assessment indicated a "significant interaction between treatment and day" and independent analysis of each group indicated that "basal testosterone varied in the acyline but not in the placebo group." The researchers reported that "on Days 1, 3, 7, 10 and 14, the response to the agonist differed between groups, becoming similar on Days 21 and 30. It was concluded that, in dogs, a single administration of the GnRH antagonist kept the canine gonadal axis from physiologically responding to agonistic challenge during 14 days."

3.2.2.3.2 Females

Acyline has been studied for pregnancy termination in female dogs. Animals received either acyline or a placebo mid-pregnancy. Treated dogs received a single dose of either 110 μg/kg or 330 μg/kg. Pregnancy was terminated successfully in acyline-treated animals but not in placebo-treated animals. There appeared to be no difference related to the timing of pregnancy interruption. Researchers concluded that acyline prevents normal estrus and ovulation in female dogs when administered during proestrus, and that pregnancy will be terminated approximately 1 week after administration at 30 to 35 days of gestation (Valiente et al. 2009). Researchers have also investigated the use of acyline to prevent the characteristics post-treatment "flare-up" related to the use of a GnRH agonist. Hermo et al. (2006) reported that a single acyline injection "failed to prevent post GnRH agonist stimulation in anestrous bitches." See also a second Valiente et al. 2009 publication, *Effect of a GnRH antagonist on GnRH agonist-implanted anestrous bitches*, listed in the bibliography.

3.2.2.4 GnRH Antagonists in Cats

In a 2010 paper (Risso et al.), researchers reported on the use of acyline in queens relative to ovulation, development of ovarian follicles, and maintenance of pregnancy. One experiment involved seven queens representing 24 "proestrous periods." Queens "were randomly assigned to treatment with either acyline (n=17) or a placebo (n=7). All queens were mated with a fertile tomcat. In the [acyline-treated] and [placebo] groups, cessation of estrus occurred 7.0+/-1.3 d[ays] and 7.0+/-1.7 d[ays] after treatment [respectively], ovulation occurred in 2 of 17 and all seven estrous periods [respectively], and pregnancy rates were 1 of 16 and 7 of 7, respectively … intervals from treatment to the onset of the ensuing proestrus were 18.4+/-1.7 and 120+/-17.2 d[ays], [respectively]."

In a second experiment, "14 pregnant queens were randomly allocated, according to their mating date, to treatment with acyline in early pregnancy … mid pregnancy … late pregnancy … or injection of a placebo in early … mid … or late pregnancy … No pregnancies were prematurely terminated and post-treatment [serum progesterone] concentrations did not differ among treatment groups." Investigators concluded that in queens, "GnRH withdrawal by acyline prevented ovulation when given in early follicular phase (proestrus) but did not significantly affect luteal function during pregnancy."

A paper on the use of acyline (Garcia Romero et al. 2011) describes a study on the effects of the GnRH antagonist acyline on the testis of the domestic cat. As described in the study abstract "… mature cats were orchidectomised unilaterally (right testis) on Day 7 … or Day 15. On Day 0, 330 μg/kg acyline was administered subcutaneously to all the animals. Left orchidectomy was carried out on Day 15 … Day 30 … and Day 60 … Sperm were recovered from the epididymis and the testes were evaluated grossly, histologically, and immunohistochemically.

Significant differences … were found between days for epididymal sperm motility, vigor, abnormal morphology, germinal epithelium height, spermatocytes, spermatids, spermatozoa, lumen, and cellular debris. Conversely, no significant differences were found for gross testicular and tubular characteristics, spermatogonia, Sertoli and Leydig cells, and intertubular compartments. It was concluded that a single dose of acyline reversibly impaired spermiogenesis and sperm motility for 2 weeks."

There are no publications on the use of non-peptide GnRH antagonists in cats.

3.2.2.5 GnRH Antagonists: Summary of Advantages and Disadvantages

Advantages	Disadvantages
Proven to suppress fertility in males and females for short periods	Need to be given frequently to achieve effects
Suppress sexual behavior – females will not come into estrus during treatment; males will behave as castrates	No depot or long-acting formulations have been developed for use in animals
Reversible – when the drug is discontinued, reproduction should resume within a reasonable period of time (could be used in animals intended for breeding)	Reversible – when the drug is discontinued, reproduction should resume within a reasonable period of time. Note that irreversibility is preferred for many pets by their owners and for unowned and "community" dogs and cats.
May be less expensive to manufacture (non-peptide antagonists)	Some of the first-generation peptide antagonists may cause histamine release in dogs
May be given orally (non-peptide antagonists)	
Effective within a short period after treatment initiation (hours)	

3.2.3 Overview of GnRH-Toxin Conjugates

Another approach to suppressing GnRH involves ablation of the gonadotrophs, which are cells in the pituitary that have GnRH receptors and secrete LH and FSH. Coupling GnRH to a toxin or protein-synthesis inhibitor is a way of delivering the toxin or inhibitor directly to only one type of cell – those that have GnRH receptors.

The concept is that the GnRH conjugate (GnRH plus a toxin) will bind to the GnRH receptors in the target cells of the pituitary (gonadotrophs). This GnRH receptor/GnRH-toxin conjugate complex will then be internalized, and the toxin will be released from the complex only in those specific cells, causing them to die. (Nett and Jarosz 2002, Ball et al. 2002). Then, theoretically, permanent sterility would result, and little or no "off-target" toxicity would be seen.

The specificity of the toxin delivery is a potential issue related to this approach. The pituitary gland is full of other important cell types, such as cells that make growth hormone and hormones that stimulate the thyroid and adrenal glands, among others. Showing that the GnRH-toxin conjugate is safe to other pituitary cells will be important. GnRH receptors are also found in nontarget tissues (e.g., the heart and colon); therefore theoretically these receptors could also internalize toxin and be killed, thus having unintended toxicity. Also, assuring that conjugation of the toxin to the GnRH molecule is consistent and complete, both in the bottle and in the body, is important so animals don't get exposed to free toxin.

See Chapter 4, section 4.2.3 for an update on Gonex, Inc., which became Cedus, Inc., a company that began working on a GnRH-toxin conjugate in the early 2000s.

3.2.3.1 GnRH-Protein Synthesis Inhibitor Conjugate

In the 2002 *Contraception and Fertility Control in Animals* report, it was noted that an approach to control of GnRH involving the use of GnRH conjugated to a protein synthesis inhibitor – in this case pokeweed antiviral protein (PAP) – was under investigation by Dr. Terry Nett of Colorado State University. Linking GnRH to PAP was reported to allow the protein synthesis inhibitor to be delivered specifically to gonadotroph cells when the GnRH binds to the GnRH receptor in the pituitary and is internalized as part of normal cellular processes. The target gonadotroph cells in the anterior pituitary gland synthesize and secrete LH and FSH. Once destroyed, the gonadotrophs are unable to produce LH or FSH and therefore the treated animal is rendered infertile, i.e., cannot produce viable sperm or eggs.

Sabeur et al. (2003) reported on a study to evaluate the effect of a GnRH analogue conjugated to the cytotoxin PAP obtained as a plant extract, on reproductive function in 12 adult male dogs. Four received GnRH–PAP every hour for 36 hours via IV catheter; four dogs received GnRH–PAP as a bolus injection daily for three consecutive days; one dog received a single bolus; and three served as controls, receiving GnRH without the PAP. Twenty-five weeks after the initial treatment, all treated dogs received a single administration; dogs in the control group received GnRH analogue. Testosterone and LH serum concentrations were monitored and testis size was measured for 9 months after treatment. Researchers found that serum testosterone concentrations were significantly lower after treatment in the bolus and hourly groups than in the control group, and that administration once a day for 3 days "appeared to result in a greater suppression of pituitary LH release than did hourly administration for 36 hours of an equivalent dosage." Pituitary function returned approximately 5 months after the first and second administrations, as measured by increases in LH and testosterone. Researchers concluded that "administration of the conjugate GnRH–PAP at a 25-week interval resulted in a major disruption of reproductive parameters in male dogs; this effect was maintained for 11–12 weeks after a second injection of GnRH–PAP" and noted that "further studies are required to determine whether this approach may be useful to disrupt reproductive function in this species permanently and should include a more thorough dose-ranging study" (Sabeur et al. 2003).

A 2006 publication described a study in which the objective was to examine the ability of the conjugate to disrupt reproductive function in two groups of peripubertal male dogs (16-32 weeks old). The 7 dogs in Group I received a second treatment 20 weeks after the initial treatment; the 3 Group II dogs were treated in the same time frame but using a different dosage pattern. There were 5 control dogs. Group I dogs not responding to the two initial treatments were treated a third time, at 12 months following initial treatment. The initial GnRH-PAP treatment did not affect testis growth, LH release or

serum testosterone concentrations in the target animals, but administering "a higher dose of GnRH-PAP after puberty resulted in a marked and rapid decline in testis size, serum testosterone concentration and LH responsiveness in 9 of 10 dogs. Suppression of reproductive function was maintained in treated dogs for 18-50 weeks; four dogs had suppression of reproductive activity through the end of the study." Researchers concluded that although "GnRH-PAP given after puberty markedly suppressed reproductive activity, [the] variability in the response and duration of suppression after treatment, additional research would be required to determine efficacy for nonsurgical sterilization of the male dog" (Ball et al. 2006).

At the time of this update, it appears that this particular conjugate is no longer being studied.

3.2.4 Overview of GnRH Vaccines

About 40 years ago, it was hypothesized that if an animal could be treated in such a way as to stimulate an immune response to GnRH, the GnRH antibodies would interfere with the action of GnRH and this could result in infertility. But since GnRH is a small decapeptide that is normally present in all mammals, it is not recognized as "foreign" by the immune system. The challenge to immune suppression of GnRH was to develop a suitable vaccine.

Research has been conducted on GnRH vaccines for a number of years. In order for GnRH vaccines to be effective, the treated animal (or human) must develop an immune response significant enough to neutralize GnRH for a period of time. Since it is difficult to raise an effective immune response to a small self peptide, the general approach to constructing GnRH vaccines is to couple the small GnRH peptide to a large foreign protein (a hapten). A number of conjugates have been used to enable or attempt to enable the animal's immune system to recognize the coupled protein as foreign and make antibodies against the complex, some of which will bind to and inactivate GnRH. In addition to the GnRH-hapten conjugate, various adjuvants are used to further stimulate the immune response. Historically, developing such an immunity to many different experimental GnRH-hapten conjugates has been variable and it can take several months before immunity, and therefore infertility, develops fully.

In general, formulations of these GnRH vaccine preparations, when tested in laboratory animals, dogs, and other species, have required multiple injections and generated a weak, short-lived antibody response. Examples of novel approaches that have been tried include a synthetic GnRH vaccine with T-helper epitopes (Sad 1993), the use of a recombinant GnRH immunogen (Robbins 2002), or estrus shock protein fusion (Wang et al. 2010) but these approaches were considered impractical for long-term contraception because multiple injections were needed to cause the response, the response was short lived, not all animals responded, or the technology was early stage.

After approximately 40 years, why is there no commercialized vaccine available for use as a contraceptive in companion animals? The main technical hurdles have been:

- Inconsistency of the immune response
- Need for multiple injections to maintain results
- Injection site reactions due to the use of adjuvants
- Difficulties in formulating the antigen and consistent conjugation of GnRH
- Inconsistency in the duration of effect among treated animals
- Difficulty and expense of doing large-scale, multiyear studies

Nonetheless, since GnRH vaccines can be effective in both genders and most mammals, work has continued on GnRH vaccines for animals. For example:

- In the early 2000s, MetaMorphix, Inc., a Maryland-based biotechnology company, was working on a number of animal health projects, one of which was an immunocontraceptive vaccine. Work on the use of the vaccine in dogs and cats was published (e.g., Baker et al. 2004, Robbins 2002 and 2004, Robbins et al. 2004). As of 2011, MetaMorphix was disposing of its assets during bankruptcy proceedings and there was no indication that the immunocontraceptive work had progressed.
- In 2004, Pfizer Animal Health obtained a GnRH vaccine for treatment of benign prostatic hyperplasia (BPH) in dogs as part of its acquisition of the Australian company CSL and its US subsidiary Biocor. The product received a conditional license from United States Department of Agriculture (USDA) for the BPH indication but the product was never developed for contraception and is no longer available for treating BPH.
- GonaCon™, a GnRH vaccine, has been developed by the National Wildlife Research Center (NWRC) of the USDA APHIS Wildlife Services (WS). After beginning initial research in 1991, NWRC began developing the single-shot, multiyear contraceptive for white-tailed deer in 2005. The vaccine received USDA licensure for use in white-tailed deer in 2010. The GonaCon vaccine induces a long-lasting contraceptive response with a single

injection; a single shot can successfully keep female mammals infertile for 1 to 4 years without boosting, and infertility is reversible over time as antibody levels decline. GonaCon has been shown to produce high GnRH antibodies and prevent pregnancy in several species – deer, wild rats, squirrels, cats, dogs, domestic and feral pigs, rabbits, coyotes, wild horses, and bison – following a single dose. (Fagerstone 2006).

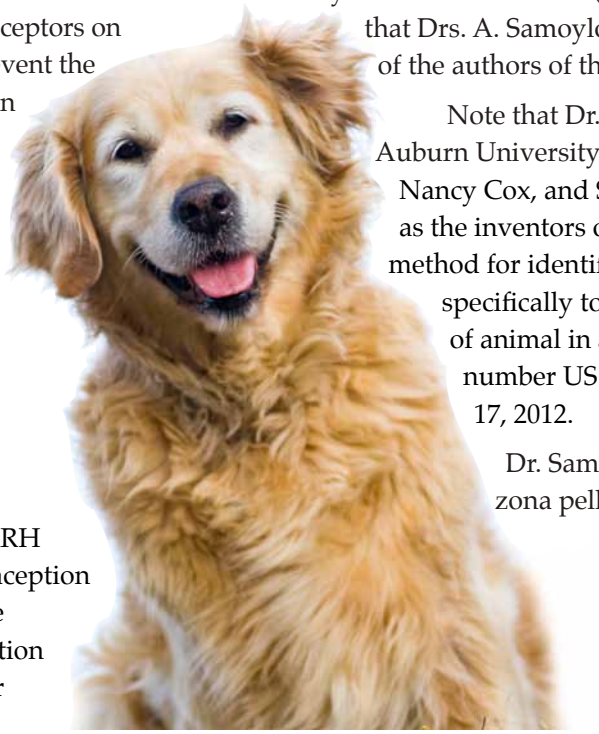
> "To make the GonaCon vaccine, multimers of a synthetic GnRH are coupled to a limpet hemocyanin. This is combined with an adjuvant developed at the NWRC (AdjuVac™) that enhances immunogenicity. The AdjuVac is a modification of the USDA-licensed Johne's disease vaccine Mycopar™ that contains small amounts of killed *Mycobacterium avium*. As noted, GnRH is a decapeptide produced in the hypothalamus and carried to the anterior pituitary gland, where it results in release of FSH and LH. In males, LH and FSH are required for testosterone production by the testes and for spermatogenesis. In females, LH and FSH are required for estrogen production, follicle formation, and ovulation. When linked to the large, foreign limpet protein, the resulting GnRH molecule "looks like" a giant new protein that the animal's immune system has never encountered. As a result, when GonaCon is injected into an animal, the animal's immune response makes antibodies to both the limpet protein and to the animal's own hypothalamic GnRH. The antibodies inhibit GnRH interaction with receptors on pituitary gonadotrope cells, and prevent the normal cascade of hormone secretion that is required for gamete production" (Fagerstone 2006).

The GonaCon vaccine has been tested in dogs and cats (see sections 3.2.4.1 and 3.2.4.2).

- Dr. Tatiana Samoylova, of Auburn University's Scott-Ritchey Research Center, received a Michelson Grant in Reproductive Biology in December 2011 (see Chapter 4, section 4.3.3.2) to fund work on a single-dose phage-GnRH construct vaccine for long-term contraception in male and female cats and dogs. The abstract submitted in a patent application naming Dr. Samoylova and two of her team members as inventors provides the following description:

> "Disclosed are recombinant bacteriophage constructs and related heterologous peptide sequences for contraception in animals. The disclosed recombinant phage constructs bind to antibodies against gonadotropin-releasing hormone (GnRH) and can be administered to an animal to generate an immune response against GnRH, including generating anti-GnRH antibodies. The disclosed recombinant phage may comprise an amino acid sequence of gonadotropin-releasing hormone (GnRH), epitopic fragments, variants, or functional mimics thereof. Also disclosed are methods for making and selecting such recombinant phage constructs and compositions that comprise such constructs (e.g., compositions for inducing an immune response against GnRH including pharmaceutical or veterinary compositions used as vaccines). Also disclosed are recombinant polynucleotides comprising genomic nucleic acid of the recombinant phage constructs disclosed herein" (faqs.org/patents/app/20120156215).

A study in mice to evaluate the formation and specificity of phage-GnRH conjugates indicated that such synthetic phage-peptide constructs "are stable and stimulate anti-GnRH immune responses." This study did not involve any fertility-related assessments (Samoylov et al. 2012). Note that Drs. A. Samoylov and T. Samoylova are two of the authors of this 2012 study abstract.



Note that Dr. Samoylova, along with Auburn University colleagues Henry J. Baker, Nancy Cox, and Stephen Ditchkoff, are listed as the inventors of a patent involving "a method for identifying a peptide that binds specifically to oocytes of a target species of animal in a species-specific manner," number US 8,158,366 B2, issued April 17, 2012.

Dr. Samoylova has also worked on zona pellucida (ZP) phage construct vaccines (see section 3.3.1.2).

3.2.4.1 GnRH Vaccines in Dogs

GnRH vaccines have been tested extensively for their contraceptive effects in dogs (early citations include Faulkner 1975 and Gonzalez 1989). Dogs have been immunized with various GnRH peptides coupled to such large proteins as ovalbumin, thyroglobulin, keyhole limpet hemocyanin, and tetanus toxoid. In addition, various adjuvants have been used, including Freund's complete adjuvant, water and oil adjuvants, aluminum hydroxide gel, and CpG (Schanbacher 1983, Singh 1985, Vickery 1989, Ladd 1994, Baker 2002).

Baker et al. (2004) reported on the effectiveness of a leukotoxin-GnRH antigen administered with the molecular immunostimulatory adjuvant CpG oligonucleotide. The formulation was tested in 8-month-old dogs, prepubescent and adult female cats, and prepubescent male kittens (see GnRH Vaccines in Cats, below). None of the dogs (n=3 females and n=5 males) developed antibodies against GnRH following primary vaccination and booster vaccination 7 months later.

As noted previously, Pfizer Animal Health obtained a conditional license from USDA for a product called Canine Gonadotropin Releasing Factor® (GnRH(F)) Immunotherapeutic, a vaccine indicated for benign prostatic hyperplasia in male dogs. The label indicated that an initial injection should be followed by a booster 1 month later and then every 6 months to maintain effect. The product was not indicated for use as a contraceptive. A study presented at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control was conducted to determine if this vaccine would also be effective for immunocastration of male dogs. Researchers hypothesized that in addition to stimulating anti-GnRH antibody formation and decreasing testosterone concentration, vaccination would decrease male reproductive behavior. Six intact postpubertal male dogs were injected with two subcutaneous injections of GnRH(F) every 4 weeks for 20 weeks. Blood samples were taken at the time of the initial vaccination and again at Weeks 4, 8, 12, 16, and 20. Researchers measured serum GnRH antibody titers and serum testosterone. Titers were detectable in all dogs by Week 8 and decreased after Week 12. There was little to no change in behavior. At Week 8 and Week 12, mean testosterone concentrations were consistent with those of castrated dogs, but as GnRH antibody titers decreased, testosterone concentrations began to increase. Researchers concluded that "this vaccination protocol resulted in a short period of immunocastration in male dogs, with inconsistent effects on sexual behavior" (Peed and Kutzler 2010). In another study a B-cell adjuvant was added to the Pfizer BPH vaccine and administered to 20 male dogs in four different treatment groups to investigate serum GnRH antibody titers, serum testosterone levels and semen qualities during a 6-week post-vaccination period. Researchers found that adding B-cell adjuvants did not result in improved short-term immune response or decreased fertility compared to the GnRH vaccine with booster protocol (Brennecke et al. 2009).

As noted above, USDA has developed a GnRH immunocontraceptive vaccine called GonaCon for use in female white-tailed deer. The vaccine has been studied in a number of species including dogs (and cats).

In a study, researchers immunized three healthy male Beagle dogs with a single, intramuscular injection of 0.6mL GnRH-KLH with AdjuVac (i.e., GonaCon), and monitored their breeding soundness and fertility biweekly for 1 year. Serum anti-GnRH antibody concentrations, serum testosterone concentrations, testicular size, prostate size, and semen analysis were assessed. Two dogs became infertile within 3-4 weeks of immunization; infertility was maintained for about 14 weeks. A second 14-week period of infertility occurred in one of the dogs 8 weeks after the animal's recovery from the initial period of infertility. All three dogs maintained "excellent libido throughout the

study period" and all three "experienced severe injection site reactions within days following administration of the vaccine" which "persisted throughout the duration of the study." Researchers concluded that "a single injection of this vaccine formulation is neither safe (due to … severe local reaction), nor effective at inducing long-term suppression of reproductive function in dogs" (Griffin et al. 2004).

In an 82-day study published in 2009 (Bender et al.), USDA and US Centers for Disease Control and Prevention (CDC) researchers investigated the feasibility of simultaneous vaccination with GonaCon and a commercially available rabies vaccine (Defensor® 3, Pfizer) in female dogs of mixed breed. The rationale for the study is based on the belief that to maximize the effectiveness of "herd immunity" resulting from rabies vaccination, "immunocontraception provided at the time of rabies vaccination should reduce fecundity and dog abundance." Three groups of animals were divided as follows: Group 1 (six animals) received a single injection of GonaCon and was intended as an internal control for the rabies vaccinations in dogs in Groups 2 and 3; Group 2 (six animals) received a single injection of rabies vaccine; and Group 3 (six animals) received a single injection of GonaCon plus a single injection of rabies vaccine. Researchers determined anti-GnRH antibody and rabies VNA on Days 0, 13, 27, 61, and 82 (study end). The study "demonstrated the potential to use this immunocontraceptive in breeding-age female dogs without affecting parenteral rabies immunization in 100% of vaccinated animals." Researchers further noted that this study may represent "the first use of the immunocontraceptive GonaCon in female dogs … future research should determine the potential effects of immunocontraception upon duration or immunity and efficacy against relevant challenge viruses."

In a 2010 Stakeholders Announcement from USDA, the agency stated that the findings described in the study "could aid in the development of new vaccination programs, as well as a combined rabies-contraceptive vaccine, for use with free-ranging and feral dog populations. USDA hopes to pursue partnerships with industry and others for future development and potential registration" (APHIS WS, April 8, 2010).

A study undertaken by the Mexican Ministry of Health and USDA APHIS WS, supported by the Rabies Program of the CDC:

> "tested an improved formulation of GonaCon™ in Hidalgo State with the participation of the State Health Services as well as the local institutions and organizations during 2011. Three groups of 6 female dogs were used in this study. The first group received rabies vaccine, the second GonaCon™ and the last group received GonaCon™ and rabies vaccine. Vaccines were delivered by IM injection. All animals were placed under observation and evaluated clinically during a 61-day period. Results of the medical and clinical evolution of the animals, as well as the blood serum results for CBC, BCP, VNA, THR and GNRH measurements and comparisons on D0, D31 and D61 will be presented. The preliminary conclusions show that adverse effects of GonaCon™ were less frequent and in lower intensity than reported in the previous dog study [Bender et al. 2009, see above]. The immune responses to the rabies and GonaCon™ vaccines were not limited by the simultaneous administration of these products. Also, observations of the macro and microscopic lesions will be presented that are consistent with findings of the previous GonaCon™ study" (Lecuona et al. 2012).

In 2009, researchers at the CDC Rabies Program reported on efforts to develop a recombinant combined vaccine for rabies prevention and immunocontraception, noting that "an effective sterilant based on rabies vaccines has the potential to create a supportive measure of public acceptability and to reduce associated clinic visit costs."

The team:

> "inserted the coding sequence of gonadotropin-releasing hormone (GnRH) into different locations within the rabies virus ERA glycoprotein (G) gene, and demonstrated that the amino terminus (N), antigenic site IIa, and the junction between the ecto- and cytoplasmic domains (C) of the G were suitable sites for GnRH insertion. The rescued recombinant rabies viruses ERA-N-GnRH and ERA-C-GnRH grew as well as the parental ERA

virus, reaching 1x10(9)ffu/ml in cell culture. Insertion and expression of the GnRH were stable in the viruses after multiple passages *in vitro*. To increase immunogenicity of the GnRH peptide, two copies of GnRH, aligned in tandem, were fused to the N terminus of the G. The recombinant rabies virus ERA-N-2GnRH was recovered and grown to high titers in cell culture. All GnRH-carrying rabies viruses induced antibodies against GnRH in immunized mice and protected 100% of the animals after rabies virus challenge. The recombinant viruses reacted strongly with the serum from a GonaCon-immunized animal" (Wu et al. 2009).

Jung et al. (2005) reported on the administration of fusion proteins produced in *E. coli* and made up of canine GnRH and T helper cell epitope p35 "originated from canine distemper virus F protein and goat rotavirus VP6 protein." In male dogs previously immunized with CDV vaccine, injection of the fusion proteins induced antibody higher than that of GnRH-rotavirus VP6 protein or GnRH alone. Spermatogenesis degeneration was present in the male dogs immunized with the fusion protein. Researchers concluded that the vaccine "acted as a strong immunogen and the antibody to GnRH specifically neutralized GnRH in the testes … and implies a potential application of GnRH-based vaccines for immunocastration of male pets."

A company called Amplicon Vaccine, LLC describes the composition of a vaccine called Repro-Bloc™ as "a series of GnRH genes [which] are cloned onto Ovalbumin carrier gene which is held in an E. coli based expression vector … the purified protein is added to an emulsifying agent, oil, dead [M]ycobacterium butyricum and a urea + phosphate buffer." The website refers to studies in mouse, swine, dogs, cats, lamb, caribou and several cattle. A 2010 PowerPoint® presentation on the company website describes several studies on heifers in the US and bulls in Brazil and notes that results of studies on other species such as cats and dogs are available under confidentiality agreements (amplicon-vaccine.com).

3.2.4.2 GnRH Vaccines in Cats

In a small study involving six male cats immunized with GnRH antigen (GnRH conjugated to tetanus toxoid) in an adjuvanted formulation, five of the six had GnRH antibodies, but there was little or no effect on serum testosterone levels, even after multiple injections (Ladd 1994).

In a study using GnRH conjugated to ovalbumin in 30 male cats (Enright 1995), cats were immunized at 0, 4, 8 and 12 weeks of the study. The study was carried out only until Week 20, so long-term effects were not observed. In the high-dose group, good suppression of serum testosterone and sperm production was seen.

A study described at the 2nd International Symposium on Non-Surgical Methods for Pet Population Control involved immunizing cats via primary vaccination and a booster 4.5 months afterward. At 2-4 week intervals researchers assessed serum testosterone and testicular size in males, serum progesterone in females, and antibody titers in all animals. Fecal estradiol was assayed every other day for females, while estrous behavior in queens was recorded each day. Data were gathered for 9-1/2 months after initial immunization (Baker et al. 2004).

- In six out of six treated adult female cats, contraception was maintained throughout the 38-week study period and no estrous behavior was recorded. Ovariohysterectomy performed on treated and untreated cats revealed that ovaries and uteri of treated cats were dramatically smaller, while the ovaries and uteri of control cats were well developed; control cats cycled normally during the study.
- Five female 3-month-old kittens were given the vaccine with CpG; one received the vaccine with no CpG; and two kittens served as controls. Failure to attain normal reproductive cycling occurred in four kittens in the vaccine plus CpG group and in the kitten receiving vaccine alone. After administration of the booster, the fifth kitten in the vaccine plus CpG group was contracepted. The GnRH antibody response rose sharply over 6 weeks and then reached a plateau. Ovariohysterectomy of all cats at the end of the study showed that as with adult females, these organs were dramatically smaller than those of controls.

- Five male 3-month old kittens were given the vaccine with CpG; four received the vaccine with no CpG; and four kittens served as controls. Control kittens progressed to normal pubescence (i.e., developed measurable serum testosterone) during the study period, while serum testosterone was undetectable in all five immunized cats at study end. The vaccinated kittens did not develop secondary sex characteristics, and "fighting behavior was noted to be absent in vaccinated cats." Control cats developed normal secondary sex characteristics, testicular size, and typical "intermale aggression." Surgical castration at study end showed severely atrophic testicles in all treated cats. One treated cat had one identifiable testicle, which was not identifiable 1 year later.

As noted previously, a GnRH vaccine was being developed by MetaMorphix, a company founded in Baltimore, Maryland. The vaccine was constructed as a recombinant protein, which would have made the eventual manufacturing process more amenable to scale-up and commercialization.

One study using the MetaMorphix GnRH Vaccine presented data in 15 male or female prepubertal cats given the GnRH antigen subcutaneously at 8 and 12 weeks of age and again at approximately 2 years of age. All immunized cats developed anti-GnRH antibodies by Day 28 post-injection. After the second immunization at Day 28, titers increased and peaked at ~Day 84. Titers were maintained for at least 606 days, with a significant booster effect seen at 2 years. No reproductive activity was seen in immunized females, testosterone was suppressed in males, and testicular and ovarian function was suppressed for the duration of the study (Robbins 2002). There was no mention of any breeding studies done.

In a 2004 study, the MetaMorphix recombinant GnRH vaccine was tested in intact domestic male (n=4) and female (n=10) cats at two different dose levels. One male cat served as a control. Animals were 8-9 weeks old at study start. The vaccine consisted of the antigen IPS-21, a commercially available adjuvant, and the immune-stimulant dimethyl dioctadecyl ammonium bromide. Doses were administered at several time points (Days 0, 28, and 643) during the study. All 15 animals experienced injection site reactions that resolved within 28 days post-vaccination in the majority of animals. The 14 treated animals all developed titers to GnRH, which peaked at Day 45; 13 of 14 cats maintained these titers for more than 20 months. Estrous behavior was not observed in any of the females, and none became pregnant. Three of the four males had serum testosterone concentration below the level of detection following the second immunization. Serum chemistry was normal. Histology performed on the testes and ovaries of two male and two female cats was consistent with LH and FSH suppression. As noted, multiple injections were required to stimulate and maintain "biologically relevant titers" for more than 20 months (Robbins et al. 2004). Unfortunately MetaMorphix is no longer in business (see Chapter 4, section 4.2.3).

In a short-term study of USDA-developed deer product GonaCon in cats, a single administration blocked testosterone production and spermatogenesis in male cats for at least 6 months. Twelve male cats were divided into groups of three. One group served as controls; the remaining groups received a single immunization of GonaCon at 50, 200, or 400 mg. Researchers monitored GnRH antibody titer, serum testosterone concentration, and scrotal size monthly; semen was collected at 6 months post-injection. In all immunized cats, GnRH antibodies were detected by 1 month post-treatment and were persistent during the course of the study. There was no dose dependency related to titers. Six of the nine treated cats "were classified as responders based on high GnRH antibody titers (greater than 32,000). By 3 months post-treatment, responder cats had undetectable testosterone concentrations and testicular atrophy. Nonresponder cats had GnRH titers of 4,000–32,000 and testosterone concentrations intermediate between responder and sham-treated cats." At 6 months post-treatment, control cats and nonresponder cats had similar sperm counts. One of the six responder cats produced non-motile sperm. Interstitial cells that were present in responder cats "were pale and shrunken" compared to those of control cats. There was marked tubular atrophy with vacuolated Sertoli cells and a lack of germ cells in responder cats. Researchers

concluded that "single-dose GnRH treatment resulted in testosterone concentrations and semen quality consistent with immunocastration in a majority of cats treated" (Levy et al. 2004).

A subsequent study of long-term fertility control in female cats with GonaCon tested the efficacy and duration of activity of a single-dose on the fertility of adult female cats in a laboratory setting. Fifteen cats received a single dose; five cats served as controls. On Day 120, a breeding trial commenced. Control (sham-treated) cats had a median time of 4.4 months to conception compared to the median time of 39.7 months in immunized cats. Fertility was suppressed for 1 year in 93% of the cats, 2 years in 73%, 3 years in 53%, and 4 years in 40% of the treated cats. At the end of the study, 5 years post-immunization, four cats were still infertile. Infertility was accompanied by a cessation in estrous cyclicity and with weight gain. Approximately 2 years after vaccination, five of the 15 vaccinated cats (27%) developed late-onset, persistent, non-painful granulomatous injection site masses. The researchers concluded that "GnRH immunocontraception is an ideal candidate for further development," particularly for feral cat control (Levy et al. 2011).

In a review article, Levy (2011) describes the use of GnRH vaccination to achieve long-term contraception in male cats. A breeding trial reported in 2004 assessed the fertility of 12 male cats vaccinated with a single administration of GonaCon. Nine responded to vaccination with "high antibody titers;" the "median onset of testosterone becoming undetectable was 2 months (range 1–12 months) and the median duration of effect was 14 months (range 5–33 months). One cat still had undetectable testosterone at the end of the observation period 34 months after treatment. Loss of detectable testosterone was generally followed in 1–2 months by azoospermia, and restoration of normal sperm counts lagged behind recovery of testosterone by 2 months." In the nine responding cats, semen characteristics prior to treatment and after the recovery of fertility were similar. The three cats that did not respond with high antibody titers experienced minimal to no suppression of testosterone. In this study, "the average time from introduction of the female cats to successful breeding was 12 months (range 3–12 months) for the responding cats, 5 months (range 5–6 months) for the poorly responding cats, and 3 months (range 0– 9 months) for the sham-treated cats. In one extreme case, GnRH antibody titer did not begin to increase until 6 months post-vaccination, testosterone was not suppressed until 12 months, and azoospermia did not occur until 14 months. In this cat, the contraceptive effect lasted 14 months, after which GnRH antibody titer waned, normal testosterone concentration and semen characteristics recovered, and the cat sired a litter." The reason for this delay in response is not known.

3.2.4.3 GnRH Vaccines: Summary of Advantages and Disadvantages

Advantages	Disadvantages
Proven to suppress fertility in both males and females	Immune response may be inconsistent in individual animals
Suppress sexual behavior – females will not come into estrus during treatment, males will behave as castrates	Depending on the formulation, might cause injection site reactions
Reversible – when the vaccination boosters are discontinued, reproduction should resume (could be used in pets intended for breeding)	Reversible – when the vaccination boosters are discontinued, reproduction should resume (irreversibility is preferred by many pet owners and for unowned and "community" dogs and cats)
Under certain circumstances, may be possible to achieve longer duration of effect desired by many pet owners and for unowned and "community" dogs and cats	Difficult to predict when reproduction would resume after cessation of treatment, as this would depend on the gradual decrease in circulating antibodies to GnRH
	May be need for multiple boosters to develop and maintain effect
	Slow onset of activity (may be more than 2 to 3 months) as animal mounts immune response

3.3 Other Vaccine Approaches

3.3.1 Zona Pellucida (ZP) and Egg and Sperm Vaccines

3.3.1.1 Overview

Creating an immune response to some component of the egg or sperm could theoretically cause infertility by interfering with the fertilization of the egg, sperm transport, and/or binding of sperm to the egg. This approach to fertility control works in either the ovary or the reproductive tract, and in all cases is effective only in females.

Since the effect is dependent on the animal mounting an immune response, the suppression of fertility will be gradual, likely over several months. Similarly, the effect can be expected to wear off slowly, resulting in a gradual resumption of fertility over several months or even years, depending on the formulation. This waning effect, or "reversibility," is likely to be variable among individual animals.

Sexual behavior should not be affected by this approach, as the whole cascade of hormones, which controls sexual behavior, will be normal in vaccinated animals, as there should be no disruption in the pituitary-gonadal axis (GnRH, LH and FSH and sex steroids). In other words, female animals should still come into estrus, and show normal mating behavior, but not become pregnant. In situations in which mating behavior is unwanted (commonly for both pet and free-roaming cats and dogs) this approach would not be desirable.

3.3.1.2 ZP Vaccines

Many years of research have been devoted to exploring the use of vaccination with components of the layer of proteins that surrounds the mammalian egg – the zona pellucida (ZP). The zona looks transparent and is made up of several glycoproteins, that is, proteins with various sugar molecules attached in specific ways. In each species, the ZP proteins are similar but not exactly the same (for a review, see Prasad 2000). In some species, the DNA that codes for the zona proteins has been isolated and sequenced, so the amino acid sequence of the proteins is known.

Much research related to veterinary applications has been done using PZP (porcine ZP), purified from pig ovaries obtained from slaughterhouses. The PZP can then be formulated in a variety of ways and used as a vaccine antigen, which can be injected into female animals to raise an immune response. Various injection schedules have been used in various species. Fertility is blocked for some period of time, and booster vaccinations are generally required to maintain the infertility. Unlike GnRH vaccines which are used in both sexes, ZP vaccines are only useful in female animals.

ZP vaccines have, in some cases, caused a side effect of inflammation of the ovary, which might be due to raising an immune response to the zona on the eggs in the ovary. If the ZP preparations were not sufficiently purified, they might have contained other components of ovarian tissue that could account for this effect, which has not been seen in all studies. Different ovarian pathology was seen in a study using a subcomponent of the ZP (Paterson et al. 2002), in which a disruption of the follicles and depletion of the primordial follicles was seen, either of which could result in irreversible sterility.

Using PZP obtained from slaughterhouse material presents some challenges: Collection is labor intensive and supplies may be limited. Using ovaries obtained at slaughter is also a disadvantage because it is difficult (and may be impossible) to manufacture this material under good manufacturing practice (GMP) guidelines, a requirement for regulatory approval by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) (but not for the wildlife products approved by the US Environmental Protection Agency (EPA)). (See Chapter 6 for an overview of regulatory considerations related to non-surgical approaches, including a discussion of the differences in the US regulatory landscape between products used in wildlife population control, regulated under the aegis of the EPA, versus products used in companion animal population control, regulated under the aegis of the FDA Center for Veterinary Medicine (CVM).) It is likely that in order to achieve regulatory approval and commercialization of a ZP product for use in owned dog and cats, a manufacturing method that does not involve isolation of PZP from slaughterhouse material may be required. Second-generation ZP vaccines

using recombinant or synthetic ZP antigens have been investigated (Dunbar 2002, Srivastava et al. 2002) and the cDNAs for ZP proteins from several species have been cloned (Harris 1994, Yonezawa 2001).

ZP vaccines that incorporate recombinant proteins might be less expensive and easier to produce than those based on slaughterhouse material. ZP is highly glycosylated, and the fact that recombinant proteins expressed in bacteria will be missing this post-translational modification can be expected to be associated with reduced antigenicity; however, yeast can be engineered to produce glycosylated recombinant proteins, though this glycosylation may not be identical to that which occurs in dogs and cats (ACC&D Think Tank, Immunocontraceptive Approaches for Sterilization in Cats in Dogs, 2009).

Vaccination with ZP does not affect sexual behavior in the females of the species in which it is useful as a contraceptive (e.g., horses). Females will still come into estrus, because there is no effect of this immunocontraception of GnRH or sex steroid levels.

Although there are many papers published on the use of ZP vaccines, there is no commercial product available for use in companion animals. The most extensive use of these vaccines is in wildlife, where they have been successfully used for many years to reduce fertility and overpopulation. First-generation (i.e., PZP-based) research in cats and dogs was deemed largely unsuccessful (Briggs, personal communication 2012). Cats did show a robust immune response to PZP vaccination, with measurable anti-PZP antibodies in their serum, but had normal post-vaccination fertility. It was concluded that the cat ZP proteins were different enough from the pig PZ proteins that the anti-pig antibodies would not bind the cat ZP, and therefore normal fertility was maintained. When cat ZP antigens were used as antigens, there was some evidence of reduced fertility in treated cats, although numbers were small (Jewgenow 2000, Eade 2009).

A porcine ZP vaccine (SpayVac™) for use in seals was developed by the Canadian company Immunovaccine, Inc. (formerly Immunovaccine Technologies) and tested in a few other species. The product is not commercially available at this juncture (Root Kustritz 2009) and the company's primary focus is not on animal health applications. However, Pfizer Animal Health licensed the platform technology for livestock vaccines in development in 2009 and in May of 2012, Immunovaccine announced a collaboration with "one of the world's leading animal health companies" for developing companion animal vaccines (imvaccine.com/releases.php?releases_id=274). The specific indications were not disclosed.

In April 2011, the US Bureau of Land Management (BLM) and US Geological Survey (USGS) announced a 5-year wild horse contraceptive study at the BLM's short term holding facility in Pauls Valley, Oklahoma. The pasture breeding study is said to be testing the effectiveness of two formulations of SpayVac to determine if treatment can reduce foaling rates in wild horse mares (BLM press release April 26, 2011).

In February of 2012, The Humane Society of the United States™ (HSUS) announced EPA approval of ZonaStat-H, the first contraceptive vaccine for use in mares for controlling populations of wild horses. ZonaStat-H has been "used to treat more than 1,600 wild, sanctuary and tribal horses annually at dozens of trial sites across the US" and is produced by the Science and Conservation Center of Billings, Montana (HSUS press release February 17, 2012). Further discussion of uses in wildlife and livestock is beyond the scope of this update. Please see Chapter 6 for a description of regulatory considerations related to approval and use of non-surgical contraceptives for animals.

3.3.1.2.1 ZP in Dogs

Dog ZP proteins are ZP1, ZP2, and ZP3, and the sequences of these proteins have been reported (Harris 1994). Using porcine ZP to immunize dogs has yielded inconsistent results. In some studies, bitches vaccinated with PZP showed marked ovarian pathology, but the mechanism was undefined. Some dogs have shown inhibition of fertility (Gwatkin 1980). In work published in 2002, although a significant anti-PZP antibody response was detected, moderate and inconsistent inhibition of pregnancy was seen in mated bitches (Liu and Ball 2002). The ovaries of bitches immunized with PZP appeared normal. It may be that when the dog generates antibody to the PZP, the antibodies have inconsistent binding to the dog ZP, and therefore do not block conception in all cases.

Others are approaching this problem using molecular biological techniques. By defining the genes that code for dog ZP glycoproteins, and then creating recombinant dog-specific ZP (dZP) proteins, researchers hypothesize that it may be possible to create a more species-specific antigen that might prove more effective. For example, researchers immunized three groups of female dogs (n=3 per group) with recombinant dZP2 conjugated to diphtheria toxoid, recombinant dZP3 conjugated to diphtheria toxoid, or diphtheria toxoid alone. (Note: diphtheria toxoid

was used as a hapten – a large protein to augment the immune response.) Dogs immunized with dZP2 and dZP3 generated antibodies against the diphtheria toxoid and the respective dZP. When dogs were mated, the dogs previously immunized with the dZP2 conjugate all became pregnant while three of four animals receiving the dZP3 conjugate did not conceive. The researchers noted that "the block in fertility was associated with anti-dSP3 antibody titers. Ovarian histopathology revealed that the block in fertility … is probably manifested by inhibition [in the] development of follicles and is due to atretic changes in the zona pellucida." The results, considered preliminary by the team, indicated that using dZP3 immunization might be useful in dog population control "providing that adequate antibody titers are achieved" (Srivastava 2002).

Wheir et al. (2005) reported on a study to evaluate the efficacy of native and recombinant ZP protein in inducing permanent sterility in female dogs aged 4-6 months at study start. Three types of ZP (porcine ZP, native rabbit ZP, and recombinant human ZP), and two types of adjuvant (modified Freund's Complete Adjuvant mFCA or CpG (C-phosphate-G)-DNA, an adjuvant that is interpreted by the immune system as a sign of bacterial invasion) were involved.

Test articles were: (Group 1) PZP with mFCA, (n=6); (Group 2) PZP, RZP (native rabbit ZP) + mFCA (n=6); (Group 3) PZP + CpG-DNA; (Group 4) fZP3 (recombinant human ZP) + mFCA; (Group 5a) mFCA control; and (Group 5b) CpG-DNA control. Researchers found that all the dogs receiving mFCA experienced "serious injection-site lesions." However the use of CpG-DNA did not result in adverse reactions; therefore, dogs immunized with vaccines containing mFCA did not receive boosters while CpG-DNA dogs did. Dogs in Groups 1, 2, and 3 showed reduced follicular development and increased atretic granulosa cell clusters. The implications of these results were that "with time, the ovaries would be depleted of developing follicles … [and that] … further studies have to be carried out for longer periods of time to evaluate fertility and time until full ovarian depletion and sterility are accomplished." Titers in Group 4 animals were quite low but since a decrease in follicles and an increase in atretic granulosa cell clusters were seen, these effects might be improved via booster immunization.

In general, the main technical hurdles to commercialization of a ZP vaccine for dogs have been:

- Inconsistent immune response and efficacy with the porcine ZP antigen
- Ovarian pathology seen in some studies but not others
- Need for multiple injections to maintain results
- Injection site reactions due to the choice of adjuvants
- Inconsistency in the duration of effect among treated dogs
- Difficulty and expense of doing large-scale, multiyear studies in dogs
- Difficulty in scaling up purification of PZP under GMP
- Difficulty developing recombinant antigens for use in dogs (although there appears to be progress in this area).

3.3.1.2.2 ZP in Cats

ZP vaccines have not been widely studied in cats. The commonly used porcine ZP antigen is not effective when used in cats. Although cats can react to PZP by producing serum anti-PZP antibodies, these antibodies do not appear

to interact with feline ZP (fZP) (Jewgenow 2000). This lack of effect of anti-porcine antibodies on feline fertility indicates that a more suitable antigen for cats may be cat ZP proteins, and some experimental evidence for this is discussed below.

A study that evaluated a PZP vaccine (SpayVac) for immunocontraception in domestic kittens indicated that although high anti-porcine ZP antibody titers were achieved, the formulations tested did not prevent estrous cycling at maturity or reduce fertility. Immunohistochemical assays indicated that the antibodies produced by treated cats "recognized porcine ZP but not feline ZP" (Gorman et al. 2002).

A PZP vaccine study designed to test safety in zoo felids (27 female felids representing 10 species) revealed

behavioral manifestations of estrus (15 animals) and the six animals assayed for antibodies against PZP showed antibody production. All the felids underwent ovariohysterectomies 3-13 months post-treatment, and no histopathologic signs of inflammatory damage to the ovaries were found. Although efficacy in terms of contraception was not assessed specifically, "two of the three felids housed with an intact male became pregnant during the study, one of which gave birth to healthy cubs" (Harrenstien et al. 2004).

Levy et al. (2005) screened a panel of native ZP antigens isolated from the ovaries of cows, cats, ferrets, dogs, and mink to ascertain immunocontraceptive activity in cats. Vaccines (using the SpayVac formulation technology) were constructed and a breeding trial commenced 20 weeks post-immunization. All cats became pregnant, producing an average 4.1+/- 0.7 viable kittens per litter. Antibodies did not bind to feline ZP *in situ*. Fertility was not impaired.

In work reported in 2004 (Ringleb et al.), the impact of feline ZP glycoprotein B-derived synthetic peptides on *in vitro* fertilization of cat oocytes was investigated. Researchers hypothesized that an immune response against fZPB (one of three feline ZP proteins) increases the possibility of permanent contraception by destruction of intra-ovarian oocytes. The research sought to identify immunologically relevant epitopes of fZP, and to test the amino-acid sequence of these epitopes for their contraceptive potential in cats. One of six immunogenic epitopes within the amino acid sequence of fZPB expressed an anti-fertility effect *in vitro* when antibodies against the synthetic peptide were added to the fertilization medium. Researchers concluded that the results were promising, and that the specific immune response and anti-fertility properties of a synthetic vaccine would have to be examined *in vivo*.

In a study at Murdoch University School of Veterinary and Biomedical Sciences (Eade 2007, Eade et al. 2009), PZP polypeptide and feline ZP A, B and C subunits expressed by plasmid vectors were evaluated as anti-fertility vaccine candidates for domestic female cats. Cats received three injections of the various ZP vaccines, and ZP-antibody response, ovarian histology and fertility after mating were compared. Vaccination with native porcine ZP polypeptide induced anti-porcine ZP antibodies but these antibodies did not cross-react with feline ZP and no effect was seen on fertility *in vivo* after mating. Vaccination with the feline ZP vectors did elicit circulating antibodies specific for feline ZP. Changes in ovarian histology were not elicited. Researchers noted that conception rates in mated females were 25% and 20%in the ZPA and ZPB+C vaccinated groups respectively, compared with 83%in the control group , but cautioned that samples sizes were small (7/8 control cats mated, 4/7 fZPA cats mated; 5/5 feline ZPB+C cats mated) and statistical significance was not achieved. Nonetheless, researchers concluded that feline ZPA and ZPBCC subunits are potential candidate antigens for immunocontraceptive vaccines in the domestic cat.

3.3.1.2.3 ZP Vaccines: Summary of Advantages and Disadvantages	
Advantages	**Disadvantages**
Proven to suppress fertility in females (in some studies and not others); efficacy shown in equids, some ruminants, pinnipeds, and elephants but not yet in carnivores	Ineffective in males
Reversible – when the vaccination boosters are discontinued, reproduction should resume (may depend on the antigen used); could be used in pets intended for breeding	Reversible – when the vaccination boosters are discontinued, reproduction should resume (irreversibility is preferred by many pet owners and for unowned and "community" dogs and cats)
	Immune response may be inconsistent in individual animals and will vary depending on the preparation of the antigen
	Depending on the formulation, can cause injection site reactions
	Difficult to predict when reproduction would resume after cessation of treatment, as this likely depends on the gradual decrease in circulating antibodies to ZP
	May require multiple boosters to maintain effect
	Will not affect sexual behavior – dogs and cats will still come into estrus and have normal cycles; in induced ovulators, this could lead to repeated pseudopregnancies with associated elevations in progesterone that could cause uterine and mammary pathology
	Difficult to purify the ZP under GMP conditions
	Slow onset of activity (may be more than 2-3 months) as animal mounts immune response
	May cause inflammation of the ovary
	Response varies by species

3.3.1.3 Vaccination against Egg and Sperm Proteins

Using proteins found exclusively in the ovum or on the sperm as vaccine antigens is another approach to immunocontraception. If antibodies can be raised to these "self" proteins, they could theoretically interact with egg or sperm to inactivate fertilization. An advantage to this approach is that the target protein can be chosen to be one that is only found in either sperm or eggs, and therefore, "off- target" effects should be minimized.

3.3.1.3.1 Vaccination against Egg Proteins

Proteins within the mammalian egg might be used as antigens in immunocontraceptive vaccines to elicit an immune response and cause infertility. It is important to identify proteins that are only expressed in the egg. If target proteins are found in other organs as well, such as liver or kidney, they could not be used for a vaccine because other tissues would be affected. If novel, egg-specific proteins could be identified, they might be used to generate an immune response and inhibit fertility. If such proteins could be isolated and the cDNA cloned, it might be possible to make a recombinant antigen to be used as a vaccine.

Why is a recombinant antigen desirable? It is unlikely that ovary extracts would be a practical source of material for the type of manufacturing that would be required to make an approvable product, which requires GMP manufacturing. With a well-defined, simple recombinant protein, manufacturing could be significantly more straightforward.

The original 2002 *Contraception and Fertility Control in Animals* report described the work of Dr. Scott Coonrod, then at the Department of Cell Biology at the University of Virginia (UVA), who constructed dog and cat ovarian cDNA libraries, and had begun the process of isolating proteins to be expressed for immunogenicity and fertility trials (Coonrod 2002). Dr. Coonrod has since joined the Baker Institute for Animal Health at Cornell University, where his work has identified an egg protein that plays an important role in reproduction. See section 3.6.3 for information on this emerging area of research.

Research on egg specific proteins for use as immunocontraceptive antigens has not yet matured to the point of identifying and testing protein targets in studies in cats and dogs.

3.3.1.3.2 Vaccination against Sperm Proteins

Researchers have been investigating proteins that are only expressed in sperm, with the goal of using these proteins as the basis of anti-fertility vaccines (for an overview see Frayne 1999, Naz 2000). As is the case with egg proteins, the idea is to find proteins that are not expressed anywhere in the body, except sperm, to decrease the potential side effects of using these vaccines to generate an immune response. Unlike the ZP or egg protein vaccines, sperm vaccines aim to generate what is known as a mucosal immune response, in which the lining of the reproductive tract in females produces anti-sperm IgA type antibodies, which could then bind the sperm and prevent conception. In studies in mice (Naz 2002), relatively long-term contraception (approximately 300 days) was achieved after vaccination.

Some studies have shown that in laboratory animals, immunizing females with unique sperm proteins can cause them to mount an immune response to sperm, which then inhibits fertility (hamsters: Gaudreault et al. 2002, monkeys: Deng et al. 2002). These antigens have been tested in males as well, but caused testicular inflammation.

Since the immune system interprets sperm antigens as "foreign," they "are a target for contraceptive vaccines" but because the spermatozoon "shares several antigens with other somatic cells … [it] cannot be used for vaccine development." Because of this, researchers have sought to find sperm-specific epitopes in order to improve vaccine immugenicity and efficacy. As of the date of the publication quoted here (Kutzler and Wood 2006), two important sperm-specific antigens were isolated: lactate dehydrogenase and acrosin, but "Although this approach could theoretically be applied to dogs and cats, the sperm antigen immunization approach has not resulted in a satisfactory control of fertility."

Work reported at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control involves landscape phage-peptide contraceptive vaccine constructs in which the peptides "mimic sperm that bind to zona pellucida (ZP) proteins at fertilization." One of the benefits of such an approach might be reduced cost since such vaccines should be reasonably stable and the components are expected to be readily available at a reasonable cost. Researchers hypothesized that "Administration of the vaccine would result in an anti-sperm antibody response that would interfere with sperm delivery or function in the male or female genital tract, leading to a contraceptive effect. Due to the natural ability of phage to stimulate B and T cell responses (without adjuvants), the vaccine may also inhibit spermatogenesis and steroidogenesis via induction of cytokine reactions in males." Four candidates were tested in year-old male dogs, who received an initial intramuscular injection followed by boosters at 3 weeks and 7 weeks. Analysis of testosterone levels and sperm collected from study dogs indicated that all tested constructs "induce[d] production of high levels of serum IgG antibodies that persisted for at least 5-6 months. Testosterone levels varied during the study … [and] testicular widths in all dogs were decreased when measured 2-3 months after the second booster immunizations." The study demonstrated that "the identified phage-peptide constructs may be useful in the design of immunocontraceptive agents for dogs (Samoylova 2010)."

3.3.1.3.3 Egg and Sperm Proteins: Summary of Advantages and Disadvantages	
Advantages	**Disadvantages**
Suppress fertility in females (in some studies and not others)	Ineffective in males
Reversible – when the vaccination boosters are discontinued, reproduction should resume (may depend on the antigen used); note that in instances in which permanent sterility is the desired outcome, this approach would be a disadvantage	Immune response may be inconsistent in individual animals, and may vary depending on the preparation of the antigen
Defined recombinant proteins easier to manufacture under GMP	Requires the development of an IgA response in the female reproductive tract, which is difficult to assess experimentally when compared to a serum IgG response
	Depending on the formulation, might cause injection site reactions
	Difficult to predict when reproduction would resume after cessation of treatment, as this would depend on the gradual decrease in circulating antibodies
	May require multiple boosters to maintain effect
	Will not affect sexual behavior – animals will still come into estrus and have normal cycles
	Slow onset of activity (may be more than 2-3 months) as animal mounts immune response

3.3.2 Luteinizing Hormone (LH) Receptor Vaccines

Very little research has been done using LH receptor protein as a vaccine adjuvant. The idea is that if an immune response can be raised against the receptor of a hormone (such as LH), antibodies could block the action of that hormone, by occupying the receptor and blocking its interaction with the hormone. As hormone receptors are seen as "self" – that is, not a foreign protein – the immune system must be "fooled" into mounting an immune response, creating challenges similar to those encountered in trying to generate an immune response to GnRH or ZP. This approach theoretically would be similar to vaccination with GnRH, in that both fertility and sexual behavior should be blocked.

When purified bovine LH receptor protein was used as a vaccine antigen in dogs, an immune response was mounted (anti-receptor antibodies were elicited), causing a reduction in fertility (Saxena et al. 2002). Bovine LH receptor vaccine administered to cats suppressed corpus luteum function in cats for approximately 1 year; this effect was reversible (Saxena et al. 2003). In this experiment, an implant containing purified LH receptors from bovine ovaries obtained from slaughterhouses was used, a difficult approach to commercialize.

In 2006, Hao and Saxena discussed the potential for use of chimeric proteins containing human lutropin receptor and chorionic gonadotropin epitopes as an immunocontraceptive vaccine (Hao and Saxena 2006).

The report from the 2009 ACC&D Scientific Think Tank on Immunocontraceptive Approaches for Sterilization of Dogs and Cats notes that "targeting LH or FSH receptors may not be practical since there are many tissues outside the reproductive system that contain receptors for these molecules and that might be affected adversely" (Golden 2009).

3.3.2.1 LH Receptor Vaccines: Summary of Advantages and Disadvantages

Advantages	**Disadvantages**
Suppresses estrus in females	Not shown to be effective in males
Suppresses sexual behavior	Difficult to manufacture under GMP without defining recombinant antigen
Reversible – when the drug is discontinued, reproduction should resume within a reasonable period of time; could therefore be used in animals ultimately intended for breeding	Depending on the formulation, might cause injection site reactions
	Immune response may be inconsistent in individual animals and may vary depending on the preparation of the antigen
	Difficult to predict when reproduction would resume after cessation of treatment, as this would depend on the gradual decrease in circulating antibodies to LH receptors

3.4 Chemical Sterilants

Current commercialized chemical sterilants for dogs and/or cats are administered via injection directly into the testis, though one approach under development is administered via subcutaneous injection or orally. One approach has been approved for use in male dogs in various markets, including the US, where it was taken off the market for business reasons, obtained by another Sponsor, and is pending reintroduction. Another formulation is approved for the Brazilian market.

3.4.1 Zinc Gluconate

Zinc solutions that cause testicular degeneration and permanent sterility have been developed for direct intra-testicular injection.

One such solution consisting of zinc gluconate and L-arginine was developed, approved by the CVM under the trade name Neutersol® in 2003, and then withdrawn from the US market in 2005, apparently due to issues between the manufacturing and marketing companies. The formulation was approved by the FDA for use in male dogs 3-10 months of age for chemical (non-surgical) sterilization.

Ark Sciences, Inc., located in New York City, acquired full rights to the original formulation in 2007 and has renamed the product Esterilsol® in markets outside the US and Zeuterin™ in the US. The company reports that Esterilsol is registered in four countries, and Zeuterin will be launched in the US in 2013. In Mexico, Panama, Bolivia, and Colombia, Esterilsol is approved for dogs 3 months and older. Ark Sciences indicates the cost to nonprofit organizations will be, on average, $15 per dog (price will vary based on dose, which depends on the size of the dog's testicles). Although, as noted, the product to be launched in the US is called Zeuterin, we will use the term Esterilsol to represent the product, since it is available in a number of markets under that name (see Chapter 4, section 4.2.4.2). Esterilsol is approved for use in cats in Columbia, but not in other markets.

A zinc gluconate/arginine/dimethyl sulfoxide (DMSO) product called Infertile® was launched by Rhobifarma Industria Farmaceutica Ltda in Brazil in March of 2009. Available information indicates that a single treatment with Infertile provides permanent sterilization for 72% of dogs. Dogs not responding within 30 days were treated a second time; the second treatment resulted in sterilization. Infertile is similar to Esterilsol but with important differences. The formulation contains a small amount of DMSO as a carrier to aid in the distribution of the drug within the testicle. In addition, Infertile's formulation contains approximately two times the concentration of zinc gluconate contained in Esterilsol. Although arginine is not listed as an ingredient on the Infertile packaging, the product's Sponsor and lead researchers confirm that it is included as a neutralizing agent. Concerns about this product include the relatively small sample sizes in the published studies and an efficacy rate less than that demonstrated in the pivotal effectiveness study conducted to support the approval of Neutersol by the CVM. With further study and possible refinement of formulation, Infertile may have potential to aid in advancing sterilization programs (ACC&D Preliminary Statement on Infertile 2009). See Chapter 4, section 4.2.4.5.

Another zinc gluconate-based product called Testoblock® has been studied in dogs in the Brazil. A 2007 publication (Oliviera et al. 2007) describes the product as "a proprietary zinc-based solution containing 0.1 M of zinc gluconate that is pH neutralized in BioRelease Technologies, LLC's (Birmingham, AL, USA) physiological vehicle (13.1 mg zinc/mL), that is designed for intratesticular injection. The vehicle is non-irritating and aids in sequestering the zinc moiety within the testicular tissue. Testoblock is similar to Neutersol in that it is a zinc gluconate formulation that is neutralized by the amino acid arginine. The solution is then mixed with a proprietary BioRelease Technologies vehicle which is formulated to deliver the zinc gluconate in a slow-release pattern … (this was not evaluated in the present experiment)."

A zinc-based product called Talsur was developed by the National Institute of Immunology (NII) of India 1988 and tested in 1990-1991. The product was a zinc tannate formulation. Experimental use in a street-dog control program revealed that 22% of treated dogs developed complications (Animal People October 1998); excessive scrotal swelling was noted (Animal People May 2007). The product was discontinued.

3.4.1.1 Zinc Gluconate in Dogs

Esterilsol is injected using a 28-gauge (or finer) needle directly into the testes, where it destroys sperm cells, followed by eventual shrinking and scarring of the epididymis and seminiferous tubules. Dogs treated before puberty never become fertile; however, dogs that are sexually mature at treatment may remain fertile for up to 6 weeks (the delay is due to the time required for passage of sperm from the epididymis and vas deferens that were produced prior to treatment. Studies described in the Neutersol Freedom of Information (FOI) summary (available on the CVM website, fda.gov/AnimalVeterinary) indicate this treatment is 99.6% effective in causing sterility in young male dogs (aged between 3 and 10 months; n=270). (Success was defined as an animal that displayed aspermia (no semen ejaculated), azoospermia (no spermatozoa in the ejaculate), necrospermia (spermatozoa in the ejaculate are dead or motionless), or oligospermia (sperm concentration less than 20 million spermatozoa per mL) 6 months post-injection.) The studies also reported testosterone levels were reduced by 41%–52%, compared with a much greater reduction caused by surgical castration. The "effects of Esterilsol on hormone-dependent diseases and behaviors have not been established … It is important to note that testosterone levels range widely among dogs" (ACC&D *Product Profile and Position Statement on Zinc Neutering* April 2012). Safety was evaluated both in controlled laboratory conditions and in a large (270 dog) pivotal field study. In the field study the majority (97.5%) of dogs treated exhibited no sign of pain when zinc gluconate was injected into the testicles using recommended procedures. Sedation and analgesics were used for some dogs to minimize stress and discomfort. Adverse reactions as reported in the FOI Summary occurred in approximately 6.3% of the dogs treated, including 1.1% that required medical treatment. Local reactions included mild testicular swelling, evidence of pain, swelling of the prepuce, dermatitis, ulceration, infection, and bruising or drying of the scrotum. Systemic adverse reactions included elevated white cell count, vomiting, anorexia, lethargy, and diarrhea. Ark Sciences emphasizes the importance of following the recommended treatment techniques to minimize adverse reactions. Current administration protocol includes light sedation and administration of a non-steroidal anti-inflammatory drug (NSAID).

In a study of pet-owner acceptance, ease of use, and short-term outcomes, 103 dogs were treated with Neutersol; treatment was "well accepted by the dogs." Dogs went home to "their free-roaming environment" post-treatment and their owners observed them. There were no post-treatment activity restrictions. This field study was conducted on Isabella Island in the Galapagos. Note that dogs "of all ages" were included in the study and dosed based on testicular size, per a dosing chart. Necrotizing injection site reactions occurred in four large dogs (3.9% of the treated population) "receiving injection volumes near the maximum label dose." These reactions required orchiectomy and extensive surgical repair in two dogs. Researchers concluded that "the use of zinc gluconate injection is particularly useful in situations involving "large-scale use in dogs, particularly in remote locations … [and that] further investigation is needed to identify risk factors in dogs for adverse reactions to zinc gluconate and to develop strategies for avoidance." Researchers emphasized the importance of proper injection technique to avoid exposing non-target tissue and noted "possible contributing causes to complications … include … improper after-treatment management, and characteristics unique to the Galapagos environment" (e.g., "the warm climate and habit of dogs to lie on the hot sand and lava rocks") (Levy et al. 2008).

See Chapter 5, section 5.4.1.3 for a discussion related to programs involving the use of zinc gluconate (Esterilsol) in field studies in frontier markets.

In a study of the use of zinc gluconate "either associated or not to dimethylsulfoxide," researchers divided 29 "sexually mature" male dogs into 5 groups: Group 1 (control; saline); Group 2 (zinc gluconate 13.1 mg); Group 3 (zinc gluconate 26.2 mg); Group 4 (zinc gluconate 13.1 mg and DMSO 0.5%); and Group 5 (zinc gluconate 26.2 mg and DMSO 0.5%). Dogs were examined at Day 15, Day 0, and every 15 days post-treatment for 6 months to assess testicular size and sperm quality and quantity. In Groups 3, 4, and 5, cell motility declined significantly at five collection points. Two control dogs and four Group 5 dogs were surgically neutered at 12 months post-treatment. Histopathology indicated "testicular degeneration, decreased number of germ cells, areas of atrophy, disruption of seminiferous tubule architecture, and loss of germ and Sertoli cells in Group 5 dogs … [indicating that] the association of DMSO (0.5%) to zinc gluconate (26.2 mg) may be indicated as a contraceptive method for male dogs" (Soto et al. 2007).

In a study of Infertile, researchers concluded that zinc gluconate formulated with DMSO was clinically safe and effective and did not produce behavioral changes or discomfort in dogs (n=11) treated with an injection of 26.2 mg/mL zinc gluconate and 0.5% DMSO in both testicles. Histology revealed lesions "compatible with permanent sterilization" (Soto et al. 2009).

Researchers studying Testoblock evaluated the effectiveness of intratesticular injection as a contraceptive in 15 mixed-breed dogs. Five dogs served as controls, five dogs assigned to Group 2 ranged in age from 8 months to 1 year, and five dogs assigned to Group 3 ranged in age from 2 to 4 years. Dogs in Groups 2 and 3 received 0.2–1.0 mL Testoblock depending on testicular width. Histopathological analysis revealed changes that "suggested irreversibility" and researchers concluded that the use of Testoblock intratesticular injection "effectively impaired spermatogenesis" (Oliveira et al. 2007).

A recently published study of Testoblock assessed "whether the efficacy of zinc gluconate (Testoblock) as a chemical contraceptive in male dogs was compromised in the presence of metamizole sodium (a nonsteroidal anti-inflammatory/analgesic agent)." Ten sexually mature mixed-breed dogs received intratesticular injection of Testoblock at 0.2–1.0 ml/testis, based on testis width. Five dogs also received metamizole sodium (aka sodium dipyrone) orally at 25 mg/kg three times a day for 2 days, starting 2–3 hours post injection. All 10 dogs had transient testicular swelling for 3 days post injection. All 10 dogs were azoospermic at Day 60 post injection; seven dogs were azoospermic at 120 and 180 days post injection and three had apparent aspermia. Researchers found no significant differences in any clinical parameters between the two groups and concluded that administering "metamizole sodium concurrent with an intratesticular injection of a zinc-based solution did not interfere with chemical sterilization and it improved animal welfare" (Oliveira et al. 2012).

The low cost, lack of need for anesthesia and relative safety involved in the proper use and administration of products containing zinc gluconate for sterilization of male dogs are characteristics that can be expected to be appealing to the shelter and related communities as well as clients and veterinarians. The effect of these treatments on behavior in male dogs has not been evaluated, and there are few data on the effects of surgical castration on male dogs.

3.4.1.2 Zinc Gluconate in Cats

In a 2010 presentation at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control summarizing potential options for non-surgical approaches in cats, Dr. Julie Levy of the University of Florida reported on unpublished data provided by Ark Sciences about the use of zinc gluconate (Esterilsol, Ark Sciences) in cats. One hundred and fifteen cats were treated at 6 months of age and monitored for 12 months. Cats were heavily sedated and treated with dosage of 0.2-0.4 mL/testes. Treatment resulted in testicular atrophy, reduced testosterone, and absence of sperm.

3.4.2 Calcium Chloride

Research on use of calcium chloride as an intratesticular injection for sterilization of dogs "and other large mammals" was reported as early as 1978. Use in cats was reported by Jana and Samanta at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control in 2010, who noted that ease of injection is the primary "practical advantage" of this approach, while "the primary disadvantage is slow onset of action (4-6 weeks) and inter-individual variability in level of discomfort during injection." The latter may be addressed by basing injection volume on testicular volume rather than body weight.

For an ACC&D review article on the use of calcium chloride for non-surgical contraception, see http://www.acc-d.org/ACCD%20docs/ACCD-RecommCalcChlor2.pdf.

3.4.2.1 Calcium Chloride in Dogs

In a study of sterilization of 24 male stray dogs with a single injection into each testicle of calcium chloride, a 5-, 10-, 15-, or 20-mg dose in dogs caused significant atrophy of testicular tissue. Epididymal sperm counts and testosterone concentrations were significantly decreased at all doses. The 15- and 20-mg doses provided a higher level of efficacy than the two lower doses. This method of chemical sterilization was found to be economical and effective, with no adverse effects noted. All animals tolerated the intratesticular injections of calcium chloride and exhibited a slight increase in firmness of testis on palpation. Most dogs, including those injected with normal saline, displayed signs of mild discomfort approximately 1 to 5 minutes after injection. Researchers attributed this to fluid pressure. Every dog had mild testicular swelling by 24 hours after injection, and swelling was most evident in treated dogs between 48 and 72 hours post-injection. The swelling decreased gradually at 3 weeks. Injection of 5 mg of calcium chloride did not induce uniform results as evaluated by histology after removal of the testes.

Significant morphological changes were associated with the 10 mg dose, and the 15 mg dose "resulted in total necrosis in seminiferous tubules and interstitial Leydig cells, with replacement by a fibrocollagenous band." Researchers were able to palpate only a "small testicular remnant" at 4 weeks after the 20 mg calcium chloride injection. Therefore, "a dose-dependent relationship [resulted] when [calcium chloride] was used to induce sterilization in the male dog. The maximum responses in both the biochemical and histological parameters related to chemo-sterilization were noted at the 15- or 20-mg doses." Researchers concluded "calcium chloride induces necrosis of the entire testicular tissue, [which is consistent] with previous studies with this chemical agent in the testis of the rat and in domestic animals" and that "an intratesticular injection of CaCl(2) at specified doses could be a suitable method of sterilization in preference to surgical castration of dogs"(Jana and Samanta 2007).

3.4.2.2 Calcium Chloride in Cats

In a study reporting on the use of calcium chloride in the cat, cats (6/group) received a single bilateral intra-testicular injection of 0.25 ml 5%, 10%, or 20% calcium chloride dehydrate containing 1% lignocaine hydrochloride per testis:

> "At Day 60 post-injection, cat testes were collected and examined, and showed complete testicular necrosis and replacement by fibrous tissue; very low sperm counts; and reduction of serum testosterone by at least 70% in [the] 20% dose. Androgenic enzyme activities and their expressions were also reduced in all the treated groups … intra-testicular testosterone concentration was also low. Increased testicular lipid peroxidation, with reduced antioxidants and mitochondrial membrane potential, were evident following calcium chloride treatments" (Jana and Samanta 2011).

A pilot efficacy and safety study of a single intratesticular injection of calcium chloride in causing sterility of male adult cats was also reported at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control (Baran et al. 2010). Four male cats were selected for this study; treated cats were injected with a 10-, 20-, or 40-mg concentration of calcium chloride at 0.2 ml/per testis, and one control cat was injected with sterile saline at the same rate.

Researchers assessed testicular size and serum testosterone level was assessed prior to injection, on Day 0 and every 20 days for a 2-month period. Semen analyses were done at these time points as well. Ability to collect semen and analysis of semen collected at 20, 40, and 60 days post-injection were defined as the "primary indicators of treatment effectiveness." Semen could not be collected from the high-dose cat after injection, possibly because "the high dose of calcium chloride injected into the testes could have caused serious damage in seminiferous tubules." Collection from the other cats was successful.

The other three semen samples (two treated cats and one control cat) contained live motile sperm; the two treated animals were found to be oligospermic, with less than 20 million spermatozoa/mL of ejaculate, and the control animal was found to have sperm greater than 20 million per mL. Testicular histology showed dose-dependent degenerative changes.

On Days 1-7 post-treatment, researchers assessed general attitude, appetite, ability to walk, scrotal pain, rectal temperature, and scrotal swelling. Although the scrotum of all the cats was swollen or the testis was sore or irritated to a minor degree post-injection, researchers did not note significant safety-related findings.

Routine surgical castration was utilized at Day 60, and histomorphological analysis was conducted on the testes. The authors concluded that "efficacy of calcium chloride [at 40 mg] in inducing sterilization was supported by the necrosis of the seminiferous tubules and interstitial cells, along with the significant fibrosis. Results indicate that intratesticular injection of calcium chloride (40 mg) is a well-tolerated and effective method for non-surgical chemical sterilization of male cats."

3.4.3 Chlorhexidine Digluconate

In the 1980s work was done with chlorhexidine digluconate with or without DMSO injected into the epididymis of dogs and cats (Pineda and Hepler 1981, Pineda and Dooley 1984, respectively).

- Injections of 3.0% chlorhexidine digluconate in 50% DMSO in eight dogs resulted in azoospermia for all but one dog (which had low sperm count, low motility, and many abnormal sperm) by Days 35 or 42. Dogs were monitored for 952 days, and all dogs remained azoospermic. Injections of 4.5% chlorhexidine digluconate to dogs resulted in azoospermia by Day 28, but dogs were not monitored for long-term effects. Some transient edema was observed in dogs in both studies, but no other adverse effects were observed.
- In eight cats, epididymal injection of 4.5% chlorhexidine digluconate reduced or eliminated sperm production for 140 days (the duration of the study) without major

adverse effects. The authors note that this method of "chemical vasectomy … appears to be safe and may be suitable for large-scale sterilization programs … "

Recent work on a single testicular injection of chlorhexidine solution as a chemical sterilant in male dogs was presented at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control (Aiudi et al. 2010). Forty-two healthy dogs were sedated and divided in two groups of 21 animals. One group was treated with 2 ml of 5% chlorhexidine solution injected percutaneously into the dorsal cranial portion of both testes, and the second group injected with 1 ml of saline solution. Researchers monitored testosterone in all dogs every week for 60 days. Chlorhexidine-treated dogs showed testicular tenderness and local swelling at 96 hours post-treatment, which regressed within 15 days. At Day 60, testicular ultrasonography revealed bilateral nodular lesions. Libido was reduced and prostatic volume and parenchyma were normal. Analysis of semen indicated azoospermia and a substantial decrease in the volume of ejaculate. Control animals showed no changes in libido, semen quality, testicular, epididymal or prostatic characteristics. Following surgical castration at Day 60, "longitudinal sections of testes revealed an area of necrosis and fibrosis beside the epididymis extended to the tubuli seminiferi recti, rete testis and ductuli efferentes; histological examination showed degeneration of the seminiferous tubules associated with a significant alteration of the germinal epithelium cells … [researchers concluded that] a single percutaneous administration of 5% chlorhexidine digluconate solution into the testicular parenchyma should be considered an effective non-surgical sterilization method without local or systemic adverse effects."

3.4.4 Vinylcyclohexene Diepoxide

The basis for the use of 4-vinylcyclohexene diepoxide (VCD) as a sterilant for female dogs has been the hypothesis that VCD treatment will accelerate elimination of primordial follicles in dog ovaries leading to ovarian failure, eliminate estrous behavior and cause permanent sterility. VCD works by up-regulating cellular and molecular processes of apoptosis, thereby accelerating the natural process of follicular atresia. In mice, it has been shown that over 2 weeks of daily injections are required to cause ovarian follicles to become atretic. The more practical preferable mode of delivery for causing sterility in female dogs would be continuous release to attain 100% sterility and a hormone environment equivalent to that of sterile individuals (Mayer 2006). SenesTech, the company developing this approach in dogs, is also working on a product to sterilize rat populations in Asian rice fields (see Chapter 4, section 4.2.4.6).

VCD is an industrial chemical that is an intermediate related to production of a number of products including synthetic rubber and flame retardants. VCD has been shown to cause loss of small, preantral ovarian follicles in rodents and, since this occurs *in vitro* and *in vivo* models, appears to directly act on those follicles. The literature contains reports of various pathological consequences of the use of VCD in accelerated ovarian failure (i.e., menopause) rodent models, but "these studies used higher concentrations, longer duration, and different routes of administration (Van Kempen et al. 2011). Note that VCD is a known dermal carcinogen, as noted in the relevant material safety data sheet (MSDS).

In a field study on a Navajo reservation in Arizona, researchers treated eight 12-week-old female puppies and eight 6-month old female dogs with 80 mg VCD, 160 mg VCD, 240 mg VCD or vehicle each day for 6 days. On Day 30 ovaries were removed for histopathology. Primordial follicles in VCD-treated dogs were reduced significantly compared to vehicle-injected control dogs. Researchers reported that blood chemistry was normal, growth in puppies was not affected, the older dogs did not lose body weight, and all animals were "healthy and active" at 2.5 years post-treatment (Mayer 2006). There does not appear to be any additional publications describing research on VCD to sterilize dogs.

3.4.5 Hypertonic Saline

A study conducted in 40 rats compared orchiectomy versus an injection of a hypertonic (20%) saline solution into the testicles of laboratory rats. Twenty rats were treated with hypertonic saline and 20 rats were orchiectomized. The study was undertaken to investigate

an alternative, minimally invasive approach to castration in human patients with metastatic carcinoma.

At 30 days after injection, the rat testes were slightly atrophied, and testosterone levels were similar to those for animals that had an orchiectomy. Histologically, the epididymis was unaffected by the saline injection. Adverse effects were not observed in treated animals. Researchers indicated that "intratesticular hypertonic saline injection seems to be an alternative method in the future to its rivals such as orchiectomy and medical castration" but that further laboratory work would be required to ascertain the potential utility of this approach in dogs (Emir et al. 2008).

Note that advantages and disadvantages may vary depending on the specific approach.

3.4.6 Chemical Sterilants: Summary of Advantages and Disadvantages

Advantages	Disadvantages
Permanent sterility	Irreversible
Low cost	Potential for improper administration and related side effects
Convenience	Side effects may necessitate surgery and require provisions for follow up
Ease of use	
Surgery and anesthesia not required (sedation strongly recommended)	
Potential for large-scale use in public health-related settings	

Note that continued testosterone production at some level, albeit reduced from normal (characteristic of use of zinc gluconate), and presence of testicles in males may be viewed either as advantages or disadvantages in a given owner, population or situation.

3.5 Sex Steroids

Hormonal down-regulation involving the administration of exogenous steroid hormones can serve as a method of suppressing fertility. These drugs act, in general, via several mechanisms, which may include suppression of GnRH through negative feedback or by direct effects on the uterus, sperm transport, or other mechanisms.

A variety of modified versions of the sex steroids have been synthesized and are used for therapeutic purposes in human and animal medicine (Okkens 1981). These drugs work through negative feedback at the level of the brain and pituitary (see Chapter 2). They reduce the level of GnRH, impair fertility and have local effects on the reproductive tract that interfere with fertility. However, they may have a number of side effects which can make them undesirable therapies for cats and dogs.

3.5.1 Progestins

Progestins are a class of compounds that are structurally similar to progesterone, and mimic its biological effect. They are typically used to manage reproduction in female dogs and cats, and progestins may also be used for dermatological and behavioral indications in animals. Use of progestins in companion animals is subject to "variability in individual animal response based on an animal's genetic and metabolic characteristics … [and is] cumbersome, in many instances unreliable, and subject to the timing at which the drug is administered. Thorough assessment via physical and reproductive examination is required prior to using progestins in companion animals, as is consistent follow-up monitoring" (Jöchle, personal communication 2012).

Side effects of the progesterone-type drugs vary depending on when treatment is given in relationship to stage of the estrous cycle and can include:

- Uterine hyperplasia and pyometra

- Diabetes
- Changes in hair coat (hair loss, discoloration)
- Increased incidence of mammary tumors
- Increased sodium and water retention
- Lethargy
- Weight gain

Use of progestins also "increases the tendency towards diabetes, mammary tumors, fibroepithelial mammary hyperplasia, [and] adrenocortical suppression" (Kutzler, personal communication 2012), and Dr. Michelle Kutzler maintains that "progestins should never be used in cats" (Kutzler, personal communication 2012). Dr. Sandra Goericke-Pesch notes that in cats "the [progestin-related] risks of uterine disease, mammary tumours, fibroadenomatosis or diabetes mellitus have to be taken into account – especially in predisposed animals. Modern, effective pharmacological alternatives are available for managing oestrous suppression and unwanted pregnancy" (Goericke-Pesch 2010).

There are species differences in response to the use of progestins and a given dosage "will have different effects on the cycle depending on the time of treatment relative to the stage of the estrous cycle" that vary from days' to months' duration of effect (Romagnoli and Concannon 2003).

Kutzler and Wood (2006) note that publications regarding the use of "hormonal manipulation" for contraception in cats and dogs date back to 1952, and much information related to side effects was generated in dog studies in support of applications in humans.

Progestins available for contraceptive use in the US include MGA, medroxyprogesterone acetate (MPA), and proligestone; these and others are available outside the US. MGA was approved by the FDA and marketed as Ovaban® by Schering Plough Animal Health (it is known as Ovarid® in the UK and other parts of the EU). It is no longer on the market, but unapproved "copies "of this compound are available via the Internet. MPA was known by the trade names Provera® and Depo-Provera and is not approved by the FDA but also is available on-line from compounding pharmacies. Proligestone's trade name is Delvosteron®. It is not approved by the FDA and does not appear to be available from compounders in the US.

3.5.1.1 Bitches and Queens

- Megestrol acetate "has been used extensively for temporary estrus suppression in the bitch," (Kutzler and Wood 2006) and is also used to alleviate false pregnancy in bitches and treat dermatological and behavioral indications in queens. Megestrol acetate has been associated with side effects such as "increased appetite leading to weight gain; lethargy or restlessness; marked mammary stimulation with hyperplastic and/or neoplastic changes; clinical and pathologic changes typical of diabetes mellitus … Similar side effects have also been reported in queens" (Kutzler and Wood 2006). The most serious side effect of megestrol acetate is endometrial hyperplasia which favors pyometra (Asa, personal communication 2012).
- Orally administered megestrol acetate for 1 week prior to and 1 week following deslorelin implant placement can prevent the deslorelin flare and resulting estrus in domestic dogs as well as wild canids and felids (AZA Wildlife Contraception Database, St. Louis Zoo).
- Plumb's Veterinary Drug Handbook, 7th Edition lists the following additional adverse effects of megestrol acetate:
- In dogs – changes in behavior or hair color, mucometra, endometritis, cystic endometrial hyperplasia, acromegaly, adrenocortical suppression and, rarely, lactation
- In cats – profound adrenocortical suppression, adrenal atrophy, polydipsia/polyuria, personality changes, possible hepatotoxicity
- An unapproved product, FeralStat® (megestrol acetate), was marketed over the Internet in the US for contraception in feral cats from 2009-2011. The product was produced by a compounding pharmacy to be added canned cat food and fed to cats for fertility suppression. The distributor characterized FeralStat as a "stop gap" measure to prevent reproduction until a colony could be trapped and sterilized. The dose of megestrol acetate that was contained in FeralStat was significantly lower than that used historically; its safety and effectiveness do not appear to have been studied (ACC&D 2010).
- MPA has been administered to bitches and queens as a long-acting injectable treatment, but this has not suppressed estrus as effectively as MGA, and in bitches was associated with a "high incidence of side effects"

including uterine disease and skin- and hair-related manifestations. MPA should not be used in cats (Kutzler and Wood 2006).

- *Plumb's Veterinary Drug Handbook, 7th Edition* notes that "if MPA is administered subcutaneously, permanent local alopecia, atrophy, and depigmentation may occur" and recommends injecting subcutaneously in the inguinal area.
- Proligestone is characterized by "weaker progestational activity than other synthetic progestins" (Kutzler and Wood 2006). (MSD/Merck Animal Health) is approved in certain ex-US markets as an injectable contraceptive for dogs. The label for the product in the UK notes "urine sugar levels [should be] observed carefully during the month after dosing" and "bitches may accept the male for some days after medication with the product in pro-oestrus." Proligestone can also be given to female cats … causing estrus suppression for about 6.5 months" (Kutzler and Wood 2006). Covinan® is a product previously marketed by the company Intervet (now Merck/MSD Animal Health). For bitches this proligestone is labeled for multi-dose treatment every 5 months. The label notes that "Return to normal oestrous [sic] activity occurred within 9 months in 75% of animals and within 12 months in 90% of animals following a single injection of Covinan." For queens the label notes "Dosage regimes similar to those given for bitches are advised except that, for temporary postponement of calling, the injections may be given in either di-oestrus or anoestrus. Because cats are seasonally poly-oestrous, the reoccurrence of calling after medication is very variable. However, the majority of queens will call 6 ½ months after injecting Covinan for the suppression or temporary postponement of calling."

3.5.1.2 Male Dogs and Cats

"Based on the principles of negative feedback … exogenous progestins should suppress gonadotropin secretion in males, thereby disrupting spermatogenesis." In male dogs, semen quality did not change or changed insignificantly when MGA was administered orally; subcutaneous administration of MPA [medroxyprogesterone acetate] at 4 mg/kg or 10 mg/kg did not affect sperm quality; "however, subcutaneous administration of MPA 20 mg/kg produced rapid response (within 3 days) with significant decreases in sperm motility, morphology and output" (Kutzler and Wood 2006). Higher doses can be expected to be accompanied by higher rates of side effects (Asa, personal communication 2012). Use of progestins in male cats increases the tendency towards diabetes, mammary tumors, fibroepithelial mammary hyperplasia, adrenocortical suppression, and other side effects seen in queens (Kutzler, personal communication 2012).

There have been no progestin drugs that have been approved by regulatory bodies for use in male dogs or cats.

3.5.2 Androgens

Androgens are natural or synthetic steroids that control and stimulate male sex characteristics. Mibolerone (Cheque® Drops, an orally administered product formerly produced by Pharmacia, now part of Pfizer Animal Health), is a synthetic androgen that was used to prevent estrus in dogs. Mibolerone (MIB) works via negative feedback to block the release of LH and has been used to lengthen the anestrous period to postpone estrus and to treat false pregnancies. It was not recommended for use in dogs before the first estrous cycle because it can stunt growth, nor was it to be used in breeding bitches. The Cheque Drops label stated that the product can be used daily as desired but should be discontinued after 24 months of use, making it unsuitable for owners wishing to have continuous contraception for their bitches. Treatment must be started at least 30 days before the next estrus, or the treatment may not be effective. MIB has been declared a Class III drug in the US and is no longer marketed for use as a commercial veterinary product, but it can be obtained from compounders and "underground" producers, who are providing this androgenic material to bodybuilders.

Side effects are numerous and may be acute; the drug is contraindicated in Bedlington terriers (Kutzler and Wood 2006) and should not be co-administered with progestins or estrogens (*Plumb's Veterinary Drug Handbook, 7th Edition*). Plumb's lists the adverse effects of MIB in female dogs as follows:

- Prepubertal female: premature epiphyseal closure, clitoral enlargement, vaginitis
- Adult bitch: mild clitoral hypertrophy, vulvovaginitis, increased body odor, abnormal behavior, urinary incontinence, voice deepening, riding behavior, enhanced

clinical signs of seborrhea, epiphora (tearing), hepatic changes (intranuclear hyaline bodies), increased kidney weight (without pathology), hepatic dysfunction (rare)

MIB is contraindicated for use in cats because the effective dose is very close to the toxic dose (i.e., narrow therapeutic index) and "cervical skin thickening and clitoral hypertrophy was observed in cats and did not resolve after drug withdrawal" (Kutzler and Wood 2006). In addition, "mibolerone has been reported to cause thyroid dysfunction in cats" (*Plumb's Veterinary Handbook, 7th Edition*).

3.5.3 Anti-Androgens, Anti-Estrogens and Aromatase Inhibitors

Drugs have been developed for human use that either interfere with the production of testosterone or estrogen, or inhibit binding of testosterone or estrogen to their receptors.

These compounds are mainly used to treat prostate or breast cancer in humans. They have not been used in animals, so efficacy is unknown. Most of the compounds for humans require daily pills or injections – in some cases several times a day – and are impractical for veterinary use. Although theoretically they could be used as contraceptives, because of practicality and expense, research on their veterinary use has not been done. They are not discussed further in this document.

3.5.4 Progestational and Androgenic Drugs: Summary of Advantages and Disadvantages

Advantages	Disadvantages
Suppresses estrus and fertility in both sexes (depending on the drug)	Unacceptable side effects
Some approved veterinary drugs have regulatory approval and are available in some markets[5]	Time of administration during the estrous cycle determines effectiveness, duration, and side effect profiles
	Consistent veterinary monitoring required due to significant and sometimes life threatening side effects seen with use
	Maximum duration of effectiveness is unpredictable
	Lack of products for use in cats
	Minimal effectiveness of progestins in male dogs
	Minimal/inconsistent availability of approved veterinary versions

3.6 Miscellaneous Research of Interest

3.6.1 Gene Silencing

One potential approach to non-surgical contraception is gene silencing, which essentially involves turning off genes that code for proteins essential for reproduction. It is believed that gene silencing would be unlikely to reach 100% efficacy, although levels of 95% to 99% are regarded as quite possible (Whitcomb 2010, S. Johnston, personal communication 2012). It is not known what level of silencing would be required for permanent sterilization.

Agents that can be used for gene silencing include small interfering RNA (siRNA) that can bind to specific messenger RNA (mRNA) molecules and increase or decrease their activity; and chemically modified oligonucleotides, such as antisense oligonucleotides, that bind to complementary sequences in DNA and RNA and disrupt their transcription or translation.

At a 2009 ACC&D Scientific Think Tank, Gene Silencing Potential for Sterilization of Cats and Dogs, participants identified some of the ways that a gene silencing agent might be delivered into a target cell, discussed the research that would need to be undertaken to better understand the molecular aspects of male and female dog and cat reproduction, and discussed the potential regulatory and other practicalities involved in developing and obtaining approval for a product whose activity is based on gene silencing (see http://www.acc-d.org/ThinkTanks for more information).

Researchers at the Oregon Health & Science University

[5] At this time, availability of approved veterinary drugs is difficult to determine in a given market and varies a great deal. In addition, a company with an approved drug may choose not to market that drug. Human generics and compounded versions are typical (Jöchle, personal communication 2012).

(OHSU) and the University of Iowa (UI) (Dissen et al. 2012) have conducted proof-of-principle work involving the use of RNA interference to silence a gene needed for fertility. One of the issues related to gene silencing is the potential for unintended silencing or repression of non-target genes.

The team's objectives were:

1. "identifying a gene within the hypothalamic-hypophyseal-gonadal axis that is required for fertility;
2. choosing a method that can selectively silence the gene of interest with minimal off-target effects;
3. devising a minimally invasive method of silencing genes of interest in a cell-specific manner;
4. utilizing a delivery vehicle that allows this silencing effect to be maintained for the live [sic] span of the animal … "

Two studies were conducted – one in rats and one in monkeys. The EAP1 (Enhanced at Puberty 1) gene was selected as the target fertility-related gene. Suppressing this gene disrupts reproductive cyclicity. A viral-based vector "that allows infection of a very broad spectrum of species and cell types" was used to contain and deliver specifically engineered small inhibitory RNA (siRNA). Researchers concluded that the studies demonstrated "targeting RNAi to a gene required for reproductive fertility and delivered to the hypothalamus is capable of suppressing fertility" and noted that the development of delivery systems to target the hypothalamus ought to enable the development of "the tools to silence genes essential for reproduction in a non-invasive, effective and sustained manner in dogs and cats."The team included Dr. Beverly Davidson of UI, who has received a Michelson Grant in Reproductive Biology to conduct additional proof-of-principle work.

3.6.2 Kisspeptin and Gonadotropin-Inhibitory Hormone (GnIH)

Two peptides have been discovered "and have … emerged as important regulators of the reproductive axis." They are kisspeptins and gonadotropin-inhibitory hormone (GnIH), and they provide "a novel approach to studying the physiological regulation of reproduction, as well as its pathology and also open new avenues for pharmacological or vaccinal control of fertility." (Fellman 2010)

Both are members of a family of peptides known as RFAmide or RFAmide-related peptides. (See Ebling and Luckman 2010 for a detailed technical discussion).

Kisspeptins, which were identified in 2001, are expressed in neurons of the hypothalamus.

"These neurons synaptically contact GnRH neurons and they express steroid hormone receptors. Their responses to gonadal steroids suggest that [depending on their location], kisspeptin neurons … are involved in the negative feedback regulation of gonadotropin secretion [or] … may contribute to generating the preovulatory gonadotropin surge in the female" (Fellman et al. 2010).

There may also be a role played by "locally produced kisspeptins" as indicated by "the ability of the LH surge to induce ovarian expression of KiSS-1 at the preovulatory period. … In the male, recent results suggested a down-regulation of the hypothalamopituitary testicular axis response to kisspeptin following continuous administration" (Fellman et al. 2010).

Researchers note that kisspeptins are also characterized by metastasis suppressor effects, "effects on motility, chemotaxis, adhesion and invasion have also been documented" and a system in which kisspeptin is involved affects certain secretory functions in the endocrine pancreas. Signaling in which kisspeptin is involved "may participate in implantation of the mammalian embryo, placenta formation, and maintenance of pregnancy" (Fellman et al. 2010).

GnIH, a hypothalamic neuropeptide that inhibits the release of gonadotropin at the level of the pituitary, is found in the pituitary, hypothalamus, and "several brain regions." It was discovered in quail in 2000. Researchers have identified its receptor and characterized its binding activity, and have suggested "that GnIH acts directly on the pituitary via GnIH receptors to inhibit gonadotropin release. GnIH may also act on the hypothalamus to inhibit GnRH release" (Fellman et al. 2010).

Fellman et al. (2010) note that these peptides have "emerged as important regulators of the reproductive axis, underscoring the importance of further investigations into the neural, cellular, and molecular mechanisms by which [they] act. Their potential for the manipulation of the gonadotropic axis and gametogenesis deserves a very particular interest."

3.6.3 Egg Proteins and Peptides

The 2002 *Contraception and Fertility Control in Animals* report described the work of researchers at the Department

of Cell Biology at the University of Virginia (UVA), who constructed dog and cat ovarian cDNA libraries and had begun the process of isolating proteins to be expressed for immunogenicity and fertility trials (Coonrod 2002). Dr. Scott Coonrod, previously at UVA, is currently working in this field at Cornell University College of Veterinary Medicine Baker Institute for Animal Health, and Dr. John Herr has continued work in this area at UVA.

Certain of these proteins are important because they are required for the oocyte-[egg]to-embryo transition, "which has the potential to make them good drug targets or targets for autoimmune responses." Two of the most relevant proteins that have been isolated are peptidyl arginine deiminase (PAD6) and maternal antigens that embryos require (MATER). Researchers discovered that in mouse models, removing the thymus gland in young females produces an autoimmune response that results in destruction of oocytes, completely depleting the germ pool and causing infertility. "The key was figuring out what the immune system is seeing in the egg, and it's MATER." Therefore, if MATER can be expressed in a recombinant form and injected into an animal, the animal's immune system could be "tricked" into destroying oocytes before they can become fertilized. It may be possible to generate a large amount of recombinant MATER that is identical to the MATER that is expressed in the oocyte, take the MATER and the gene that encodes MATER, and create a vaccine (Coonrod, personal communication 2012).

In order to use egg-specific proteins as antigens in vaccines that could result in single shot sterilant, innovations are required in vaccine construction, since vaccines used to date for immunocontraception require multiple booster injections to maintain effectiveness. One potential method of structuring a vaccine for longer-term effect involves the use of virus-like particles (VLPs). VLPs are noninfectious particles that are similar in structure to infectious viruses but are noninfectious because they don't contain viral nucleic acid. Examples of human vaccines that use VLPs include FDA-approved human hepatitis B and human papillomavirus vaccines. Their small size facilitates their uptake by dendritic cells and macrophages and "enables diffusion to lymph nodes" (Schiller 2010).

VLPs from the parvovirus capsid have a rigid conformation that makes them good candidates as immunogens or adjuvants. They have been used in dog and cat vaccine formulations to incite potent and long-lived immune responses against a range of associated infectious-disease-based antigens. Therefore, it is hypothesized that "permanent sterility can be achieved in dogs and cats by using vaccine formulations that contain hormonal and oocyte-restricted antigens that have been conjugated with these VLPs" (Coonrod 2010).

For dog and/or cat contraception, it is not known if plateau antibody levels created by this type of vaccination would be above or below those needed for contraception (Schiller 2010).

At the time of this update, Dr. Coonrod and his colleagues are testing MATER expressed within a VLP protein in mice and assessing the immune response and level of germ cell depletion, as a precursor to potential studies in cats (Coonrod, personal communication 2012).

Dr. Herr and his colleagues are screening phage libraries. A phage is similar to a bacterial virus and "displays various peptide structures on its surface." The researchers are seeking oocyte biomarkers – "peptides that bind to the surface of immature egg cells." Dr. Herr has received a Michelson Grant in Reproductive Physiology (see Chapter 4, section 4.3.3.2) to help fund the project. The team is "screening [the peptides] against different organs to see which phage only targets the egg cell … [and] … plans to use those peptides as a drug to target the surface of the eggs and deliver another peptide that will induce apoptosis and kill the eggs." This will involve what Dr. Herr calls "hunter and killer" peptides – the "hunter" will target the surface of the oocyte, and the "killer" will cause the oocyte to die. At this point, the "killer" peptide has been identified (Burnham 2011).

In ongoing work at the Herr laboratory at the UVA School of Medicine, researchers are identifying, cloning, and characterizing human testis genes and their proteins in terms of their expression in human tumors. This area of research is known as "cancer-testis antigens." Whether or not this approach will identify additional targets for immunocontraception of dogs or cats is unknown.

3.6.4 Targeted Delivery of Cytotoxins

The use of targeted delivery of cytotoxins for sterilization in dogs and cats involves applying the power of potent biological toxins to kill just the cells that are targeted, in this case specific sperm, egg, or hormone-producing cells required for reproduction.

Three factors must converge for this approach to be effective (Rhodes 2010):

1. A toxin has to be purified and attached to something that will take it to its target. This "transport molecule" could be an antibody that binds to a specific protein on a cell surface, or a hormone that binds to a specific hormone receptor.

2. The particular cell type to be destroyed has to have a specific "dock" for the deadly payload, to bind tightly to the cell and deliver the toxin to that cell alone. This "dock" could be a hormone receptor or a specific cell surface protein that an antibody can grab onto.

3. The researcher has to make sure that the "dock" is only on the cells to be killed and nowhere else, so that other "non-target" cells in other parts of the body are not harmed, causing unwanted side effects."

In addition, if the effect is to be permanent, and only require one treatment, the destroyed tissue must be unable to regenerate (Levy, personal communication 2012).

3.6.4.1 Single-Dose Non-Hormonal Male and Female Sterilant

At the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control in 2010, Drs. Joseph S. Tash and Katherine F. Roby of the Center for Reproductive Sciences at the University of Kansas Medical Center (KUMC) described KU-AS-272, an antispermatogenic targeting the testis and causing sterilization of male rats following a single high dose. Since the ovary contains the same protein KU-AS-272 protein targets and homologous granulosa cells, data have shown that a single oral administration of KU-AS-272 in female mice reduced ovarian weight and endocrine hormones. The researchers' goal is to develop KUAS-272 as a single-dose sterilant in both male and female dogs and cats (Tash and Roby 2010). Gupta et al. (2012) reported on the effects of several KU-AS-272 dose levels administered to rats and concluded that "the data collected thus far indicate that KU-AS-272 at 12 mg/kg and higher may have achieved the desired sterilizing block to spermatogenesis with total loss of spermatogenic cells." Researchers are expecting 60-day data, pending at the time of this publication, will ascertain whether sterilization was in fact attained. Mating trials in the rats and additional proof-of-concept studies in dogs and cats are planned.

3.6.4.2 FSH Receptor Ligand-Cytotoxin Conjugates

Cytotoxins that target the follicle-stimulating hormone receptor (FSHR), a protein found in specific cells of the male and female reproductive systems that are crucial for fertility, may act as potential chemosterilants. Dr. William Ja, a professor at the Scripps Research Institute in Florida, has been working on such an approach for developing cancer therapeutics, and is now applying the same principle to ablating Sertoli and granulosa cells to cause permanent sterility in animals. His work involves developing a compound by combining a ligand, that is, a molecule that binds to a receptor on a cell, with a toxic molecule. Dr. Ja has received a Michelson Grant in Reproductive Biology (see Chapter 4, section 4.3.2.1) to enable him to work on potential compounds that target the FSHR.

3.6.4.3 Reversible Inhibition of Sperm under Guidance (RISUG)

RISUG®, a chemical complex of styrene maleic anhydride and DMSO, is being developed as a sterilant for men under the trademark Vasagel™ (in the US). The product is intended for contraception and suppressing testosterone in men.

Work in the rat and the monkey indicate that once the drug is injected into the epididymis, a stable "implant" is created, which leads to azoospermia and contraception. Delivery of the drug into the testes impedes testicular blood circulation, "all of which together lead to regression

of the seminiferous tubules along with the Sertoli cells and the testicular interstitial tissue and its contained Leydig cells. Thus, the source of testosterone production is depleted."

According to the researchers "this method may potentially be a good technique for obtaining contraception and testicular tissue regression and may be quite effective in male dog sterilization" (Chauhan and Guha 2010).

3.6.5 Retinoic Acid Receptor Antagonists

BDADs (Bis-(dichloroacetyl)-diamines) are compounds believed to inhibit biosynthesis of testicular retinoic acid, resulting in reduced spermatogenesis.

The BDAD WIN 18,466 has an interesting 50-year history, summarized in a 2011 paper (Amory et al. 2011). Originally the compound was intended as an amebicide. Testing in rodents revealed that while the compound caused marked impairment of spermatogenesis, it did not affect other tissues significantly *in vivo*. Investigation in dogs and rhesus monkeys in the 1960s showed that administering WIN 18,466 orally resulted in "a complete arrest of spermatogenesis in testicular biopsies." Work by Asa et al. (1996) and Munson et al. (2004, see below) indicated that WIN 18,446 was a safe, effective, reversible oral contraceptive in male wolves and cats. Development of WIN 18,446 for contraception in men was discontinued upon the discovery that men taking WIN 18,446 experience unpleasant side effects when they drink alcohol.

As noted, Munson et al. (2004) investigated whether the particular drug metabolism of cats precludes extrapolation of the safety and effectiveness of WIN 18,446 for contraception seen in other mammals, including humans. Researchers determined that WIN 18,446 was a safe and effective contraceptive for male cats; testosterone concentrations decreased during treatment (Munson et al. 2004). Based on this particular study of five male cats, it appears that, in toms, inhibition of spermatogenesis lasts longer than approximately 2.5 months but not as long as 5 months. No follow-up work in cats was discovered in a March 2012 Internet search; however, researchers are continuing to investigate the potential to utilize BDADs for contraception in men.

In a more recent study, researchers sought to investigate how a specific BDAD (WIN 18,466) "can inhibit spermatogenesis by blocking the ability of vitamin A to drive germ cell developments." The techniques utilized in this study are expected to be helpful to researchers conducting screening for novel retinoic acid biosynthesis inhibitors for possible development as male contraceptives. (Hogarth et al. 2011).

Another publication (Chung et al. 2011) described a study that investigated the use of low doses of the pan-retinoic acid receptor (RAR) antagonist BMS-189453. "Spermatogenesis was disrupted, with a failure of spermatic alignment and sperm release and loss of germ cells into lumen, abnormalities that resembled those in vitamin A-deficient and RAR(alpha)-knockout testes. Importantly, the induced sterility was reversible." Recovery of spermatogenesis was seen at the histological level after dosing at systematically modified dosing regimens. Researchers noted that "Results suggest that testes are exquisitely sensitive to disruption of retinoid signaling and that RAR antagonists may represent new lead molecules in developing nonsteroidal male contraceptives."

3.6.6 Sperm Protein Reactive with Antisperm Antibodies (SPRASA)

In 2004, Chiu et al., University of Auckland, reported on the discovery of SPRASA, which is a sperm protein targeted by anti-sperm antibodies in some men who are infertile. Since "only [antisperm antibodies] from infertile men react with SPRASA [it is suggested] that this novel protein may be important in the processes of fertility." The Department of Obstetrics and Gynecology at the university is studying the role of SPRASA in human and animal infertility. Dr. Larry Chamley, an author of the 2004 publication, has received a Michelson Grant in Reproductive Biology to study the immunocontraceptive potential of SPRASA (see Chapter 4, section 4.3.3.1). A 2008 publication (Wagner et al.) coauthored by Dr. Chamley described SPRASA as highly conserved, demonstrated that SPRASA is expressed by oocytes as well as sperm, and suggested that "this protein has an important function in fertility." Dr. Chamley has also studied the responses of possums, considered an invasive pest in New Zealand, to immunocontraceptive vaccines. Responses were found to vary (Holland et al. 2009).

4.0 Overview of Companies, Organizations, Institutions, and Agencies Involved in Researching or Developing Approaches to Non-Surgical Contraception in Dogs and Cats

Please note that some endeavors involve more than one entity or type of entity and that there may be others in the area of cat and dog population control not contained in this report. Some may be proprietary and therefore no information is available publicly. Organizations and institutions sponsoring or conducting relevant non-proprietary research not included here are encouraged to contact Alliance for Contraception in Cats & Dogs (ACC&D) so that their projects may be included in future updates. Please see Chapter 3, section 3.6, for additional information on emerging areas of research that may provide approaches to non-surgical contraception in dogs and/or cats.

4.1 Setting the Framework: Overview of Existing Non-Surgical Products

This overview was presented by Linda Rhodes, VMD, PhD, at the 4th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control held in 2010 and organized by ACC&D, which is profiled in section 4.3.3.1. At the time Dr. Rhodes was chair of the board of ACC&D.

Several small updates have been made. ACC&D (acc-d.org) was founded in 2000 and incorporated as a nonprofit, tax-exempt organization in 2005. A key part of the ACC&D mission is to bring together people at international symposia – people with a passion for more tools for pet population control, people with knowledge of basic research and science, experts in drug and vaccine development and manufacture, and companies interested in investing resources for new products – to talk and collaborate, spark new ideas and make real progress, not just in demonstrating that a technology works, but in developing technologies and drugs to the point at which they can achieve regulatory approval and can be used around the world. Proceedings of ACC&D's four International Symposia are available at acc-d.org/ACCD%20 Symposiaacc-d.org.

Pet owners and pet population management organizations now have access to products that were unavailable just a few years ago. This overview describes those products and illustrates that success is possible. It has been updated from the 2010 International Symposium version as appropriate.

As mentioned elsewhere in this report, for many years, basic researchers and people and organizations working on dog and cat population control have been interested in developing an alternative to surgical spay and neuter for sterilization and/or contraception of animals.

Hundreds if not thousands of research studies have been published showing progress towards the goal of non-surgical contraception, but until recently no products were available for use in dogs and cats, with the exception of progesterone-related drugs that provided short-term fertility suppression, but usually at the expense of a risk of undesirable side effects.

When the 1st International Symposium was organized by ACC&D in April of 2002, there were no new[6] products available for either contraception or sterilization of dogs and cats. Some information was presented on Neutersol®, an intra-testicular injectable zinc gluconate to sterilize male dogs that would subsequently achieve Food and Drug Administration (FDA) approval in 2003 and be marketed to veterinarians in the United States (US). At that same meeting, an Australian company, Peptech Animal Health, presented information on deslorelin, a drug in a class called gonadotropin-releasing hormone (GnRH) agonists. Data showed suppression of fertility in male and female dogs for up to 12 months, but there was as yet no approved product. The deslorelin product obtained its first approval and was launched in 2004 in Australia and New Zealand as Suprelorin®, an implant labeled for use in male dogs for 6-month suppression of fertility.

At the 2nd International Symposium in 2004, the landscape didn't change. More information was available on Neutersol and Suprelorin, and although several other seemingly promising technologies were presented, they were all in the research stage – none was "ready for prime time."

[6] Megestrol acetate and Depo-Provera® were available at this time.

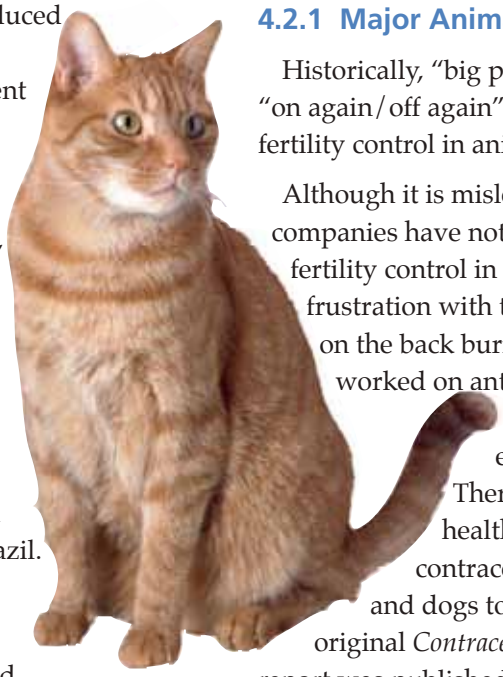
By the 3rd International Symposium in 2006, Neutersol was unfortunately no longer being marketed and was unavailable for use; in 2005, production and distribution of Neutersol were discontinued.

However, there was news from Dr. Marc Antoine Driancourt, of the company then known as Intervet (and now part of MSD/Merck Animal Health). He presented data on the use of another GnRH agonist implant, containing azagly-nafarelin, called Gonazon™ (not to be confused with GonaCon™). This product was shown to suppress fertility in female dogs for up to 12 months. Dr. Driancourt announced that it would achieve regulatory approval in Europe soon after the meeting, which it did.

During 2006, we waited to see the marketing of Gonazon for bitches in the European Union (EU), but although it was approved by regulatory agencies, it has never been introduced. In the meantime, in September 2006, Peptech Animal Health registered a 12-month version of Suprelorin in Australia for use in male dogs. Approval followed in July 2007 in Europe for the 6-month version for male dogs and in April 2010 for the 12-month version. Peptech Animal Health was acquired in 2011 by Virbac, putting a major animal health company "in the game."

As of 2010, and still at the time of this publication, there are no products with FDA approval for contraception or sterilization on the market in the US. Suprelorin 6- and/or -12-month implants are on the market in Australia, New Zealand, and Europe for fertility control in male dogs.

Esterilsol® (ex-US)/Zeuterin™ (US), a product identical to Neutersol and owned by Ark Sciences, Inc. is an intra-testicular injection for sterilizing male dogs, and is used in puppies and adults. Esterilsol was introduced in Mexico in 2008 and made available to private-practice veterinarians, government programs, and non-governmental organizations (NGOs) in that country. As of March 2012, Esterilsol was approved in Mexico, Columbia, Panama, Bolivia and the US, and is scheduled to be launched in the US in 2013. Ark Sciences has announced plans to extend distribution to other countries.



Another zinc gluconate product called Infertile®, with a different formulation than that of Esterilsol, was introduced in Brazil in 2009 and is only available in Brazil.

Progress is illustrated by another development, albeit in wildlife contraception. The group from the United States Department of Agriculture (USDA) National Wildlife Research Center (NWRC) developing GonaCon (not to be confused with Gonazon), a GnRH vaccine for use in contraception in deer, announced that in the US the Environmental Protection Agency (EPA) would be regulating contraceptives for wildlife. In 2009, the EPA announced approval of GonaCon for use in white-tailed deer. Although not approved for use in companion animals, research indicates that GonaCon may be an effective contraceptive in cats, and may have some potential in dogs with adjustments in formulation.

In summary, after many years of research on the use of GnRH agonists for fertility suppression, two companies have committed the resources to achieve regulatory approval of products, and one is being marketed in major markets. A sterilant that is effective for male dogs is approved in four Latin American countries and is expected to be available in the US in 2013; it is showing great promise in sterilizing male dogs, and could be particularly useful in dogs that might never have been able to be castrated surgically. A vaccine that is approved for use in deer may also be effective in cats and potentially dogs.

This progress, plus the exciting development of new interest in the area of research in cat and dog contraception and sterilization inspired by the Michelson Prize & Grants (see section 4.3.3.2), indicate that strides are being made in providing safe and effective alternatives to surgical spay/neuter for dog and cat population control (Rhodes 2010, updated 2012).

4.2 Companies

4.2.1 Major Animal Health Companies

Historically, "big pharma" animal health has had an "on again/off again" relationship with contraception and fertility control in animals.

Although it is misleading to say large animal health companies have not been interested in contraception and fertility control in animals, societal factors as well as frustration with technological issues put the market on the back burner while animal health companies worked on antiparasitics, antimicrobials, anti-inflammatories, and vaccines for economically important diseases. There appear to be fewer major animal health companies involved in non-surgical contraception and fertility control in cats and dogs today than there were at the time the original *Contraception and Fertility Control in Animals* report was published in 2002.

4.2.1.1 Historical Involvement

Although at the time of this update Virbac is the only major animal health company with approved, marketed non-surgical contraceptive products (although none in the US), there are a number of examples of animal health company initiatives undertaken in non-surgical contraception over the years:

- Pharmacia, which ultimately became part of Pfizer Animal Health, provided funding support for research into a chemical sterilant in the late 1970s.
- Several large animal health divisions of major human pharmaceutical companies pursued GnRH vaccines in the 1970s and 1980s. One project, in the 1970s, led to the discovery of the principle of down-regulation of GnRH receptors caused by giving continuous GnRH agonists, but the product concept was not deemed commercializable and the project was dropped. Another GnRH project in the late 1980s did not produce a product felt to be reliable enough and was discontinued.
- The Carnation Company and Upjohn started to develop a mibolerone (MIB)-based contraceptive dog food in 1975. Carnation was hopeful of quick approval from the FDA, and planned to distribute the dog food through veterinarians and, eventually, retail outlets. However, the project did not come to fruition, at least in part because of fears that a product for animals might be used to induce abortions in women or cause abortions in women who consumed pet food.
- Pfizer Animal Health announced an agreement with Peptech Animal Health (see section 4.2.3) in December of 1998 by which Pfizer agreed to fund a 12-month period of research as well of the development of methods for scaling up manufacture of a long-acting implant for reversible castration of companion animals. Pfizer did not continue this project, and the technology was eventually returned to Peptech. Note that the Peptech-sourced product eventually developed by Peptech and acquired and marketed by Virbac is a version of the implant developed following the cessation of work by Pfizer. See section 4.2.1.2 for details of Virbac's eventual acquisition of Peptech.
- Pfizer Animal Health obtained a GnRH vaccine indicated for canine benign prostatic hyperplasia (BPH) in early 2004 as a result of Pfizer's acquisition of the Australian company CSL and its US-based subsidiary Biocor. USDA granted a conditional license for use in treating BPH in 2004, but a full license was not obtained and the product is not available at this juncture. See Chapter 3, section 3.2.4.1, for information on a canine contraception study involving this product, which was not labeled for use as a contraceptive. The product was known as Canine Gonadotropin Releasing Factor® Immunotherapeutic.
- Protherics PLC formed an alliance with Janssen Animal Health for a GnRH vaccine for use in animals. Protherics PLC was acquired by BTG plc in December 2008. BTG focuses on development, manufacture, and marketing of specialized hospital products for critical care and cancer in humans, primarily in the UK, US, and Australia.
- United Biomedical, Inc. (UBI) had an alliance focused on a GnRH vaccine, with an unnamed animal health company. At this juncture UBI is not pursuing the GnRH vaccine. See the table in section 4.2.3.
- Intervet (now Merck/MSD Animal Health) developed the GnRH agonist Gonazon which was approved in Europe for injection into salmonid fish and as an implant for preventing gonadal function in bitches via long-term blockade of gonadotropin receptors. Studies have been done in cats as well. See Chapter 3 for information on the dog and cat studies. Unfortunately, as noted, the product for dogs was never launched.
- In 2006, following the removal of the zinc gluconate sterilant Neutersol from the market in 2005 (see Addison Biological Laboratory, Inc., section 4.2.3), Abbott Animal Health announced the company's intention to re-launch Neutersol; however, this did not occur. The product was subsequently acquired by Ark Sciences, which has re-launched the product as Esterilsol in several markets and is planning to re-launch the product in the US renamed Zeuterin (see section 4.2.4.2).

4.2.1.2 Virbac Acquisition of Peptech Animal Health (Suprelorin)

Peptech Animal Health launched Suprelorin (6-month implant containing 4.7 mg deslorelin) in Australia in December 2004 and in New Zealand in September 2005. Peptech Animal Health went on to register Suprelorin 12 (12-month implant containing 9.4 mg deslorelin) in Australia in 2006. In July 2007, Suprelorin (6-month implant) was approved in Europe; in May of 2008, it was launched in Europe and Virbac became the exclusive distributor there. Sales in Europe were initiated in 2008. Virbac Australia was appointed to market Suprelorin in Australia in 2009. Suprelorin (12-month implant) was approved for sale in Europe in April 2010. One year later, Peptech Animal Health was acquired by Virbac. In May 2010, Virbac presented a small animal symposium entitled Deslorelin in Practice at the 7th EVSSAR Congress. For proceedings, see zoovet.ee/product/docs/2050981722.pdf.

4.2.2 Smaller Companies

The fact that large animal health companies are not pursuing basic R&D in contraception and fertility control in animals, coupled with the fragmented nature of the market, has presented opportunities for smaller animal health and life sciences companies to collaborate with partners, or, in some cases, work on their own, to develop, commercialize, and ultimately market products. It appears that fewer companies are engaged in development and commercialization activities in the non-surgical contraception and fertility control space as of the date of this update than at the time of the original report. Note, however, that given the number of smaller life sciences and biotechnology companies working on reproduction and related areas, it is possible that there are additional, unannounced animal health projects underway.

4.2.3 Update on Entities Profiled in the 2002 Report

The entities shown in Table 4-1 below were profiled in the original 2002 *Contraception and Fertility Control in Animals* report, which covered horses, wildlife, and production animals in addition to dogs and cats.

Table 4-1: Entities Profiled in the 2002 Report

Company	Status

Addison Biological Laboratory, Fayette, MO	Addison Biological Laboratory is a private animal health company that manufactures and sells a number of veterinary products including production animal products and dental, dermatology, otology, and wound care products. Neutersol was developed by Pet Healthcare International, Inc., and introduced in the US in 2003 by Addison Laboratories after it was approved by the FDA Center for Veterinary Medicine (CVM) for permanent sterilization of male dogs from 3-10 months of age. In 2005, production and distribution were discontinued after a business divorce between Pet Healthcare and Addison Laboratories. Plans to reintroduce Neutersol by Abbott Animal Health in 2009 were cancelled for undisclosed business reasons. Ark Sciences currently owns all Neutersol rights and intellectual property and is re-commercializing the product. See section 4.2.4.2 and Chapter 3, section 3.4.1.
Aphton Corporation, Miami, FL	At the time the 2002 report was developed, Aphton had been working on a GnRH vaccine for treating human prostate cancer, and it was believed that the technology may have had animal health potential. However, in May of 2006 Aphton filed a Chapter 11 petition. The only additional reference to Aphton Corporation found in an Internet search notes that the bankruptcy case had not been settled as of June 2010.
CSL (Commonwealth Serum Laboratories) and associated entities, Australia and New Zealand	Pfizer Animal Health acquired CSL and its US operation Biocor in 2004, and obtained a conditional license from the US Department of Agriculture (USDA) for the CSL GnRH vaccine for treatment of benign prostatic hyperplasia (BPH) in dogs. As noted earlier in this chapter, no permanent license was obtained and the product is not available. The vaccine was not labeled for use as a contraceptive; an initial injection, booster after one month, and subsequent boosters at 6-month intervals were required for treatment of BPH. See Chapter 3, section 3.2.4.1, for information about a study of the potential of this vaccine for fertility control.
Gonex, Inc., Boulder, CO	Gonex was launched in 1995, initially to develop GnRH-based technology invented by company founders Drs. Terry Nett and Michael Glode. In April 2002, then Gonex COO Dr. Paul Jarosz noted that the objective at that time was to provide the market with an injectable product that can sterilize male and female companion animals via a single injection. The company's technology chemically linked GnRH to a protein synthesis inhibitor (pokeweed antiviral protein). See Chapter 3, section 3.2.3.1, for information on this technology. Gonex changed its name to Cedus, Inc. In 2009 Drs. Nett and Weber (then the COO) filed a patent application, and Application Number 20110281299 was issued in November 2011. The application notes "the present invention provides novel, modified pokeweed antiviral proteins, nucleic acids that encode the proteins, conjugates that incorporate the proteins, and methods to make and use the proteins. The present invention also provides methods to administer the conjugates to animals, for the purpose of directing toxin to particular cells." The application names Colorado State University Research Foundation (now known as CSU Ventures) and Cedus, Inc. as assignees, but there appears to have been no update to the Cedus/Gonex website since 2005. Cedus won 3rd place and an $18,000 prize in a 2006 national business plan competition sponsored by Purdue University Life Sciences. The CSU Ventures website lists Gonex as one of the startups emerging from CSU but as noted above, the associated URL is not up to date. In August of 2010, an article in the Kansas City Business Journal quoted Cedus CEO Kevin Scott and noted that Cedus had presented at the 2009 Kansas City Animal Health Investment Forum. The Morris Animal Foundation (MAF) has funded a study undertaken by Dr. Nett and called *Development of New, More Efficacious Technology to Chemically Castrate Male and Female Dogs*. The study appears to have been extended in December 2011. Dr. Nett is a professor in the CSU Department of Biomedical Sciences and a member of the university's Animal Reproduction and Biotechnology Laboratory. His current research is focused on "obtaining a better understanding of factors that regulate synthesis and secretion of hormones that control reproduction, particularly the gonadotropins. Gonadotropin-releasing hormone (GnRH) stimulates synthesis and secretion of gonadotropins by interacting with membrane receptors in the anterior pituitary gland. Highly potent analogs of GnRH are being used to deliver cytotoxic moieties specifically to gonadotrophin-producing cells in the anterior gland with the goal of developing a treatment to control reproductive rate of wild and selected domestic animal populations to treatment of hormone-dependent cancers in humans" (csuvth.colostate.edu/directory/person.aspx?m=NzMxNDE5OTI2).

Immucon, Montreal, QC, Canada	Immucon was specializing in reproductive technologies; specifically, the company developed the P34H Sperm Fertilizing Ability Test diagnostic test for infertility in human males and at the time of the 2002 report was working on oral contraceptives for men and women and reversible contraceptive vaccines for men, women, and animals. A posting dated April 21, 2011 on the Immucon website (immucon.com) notes that due to market uncertainty and technical challenges, the shareholders had halted development of products based on protein P34H.
Immunovaccine Technologies (now called Immunovaccine, Inc.), Halifax, NS, Canada	Immunovaccine Technologies (IVT) began as an animal health company that successfully developed SpayVac™, a contraceptive vaccine for use in seals, although the vaccine was never approved or marketed. The vaccine is based on a proprietary vaccine development technology called DepoVax™. Pfizer Animal Health is developing two livestock vaccines using the company's proprietary vaccine delivery technology; however IVT is now focusing on human health applications of the DepoVax technology, particularly in oncology and infectious disease. In 2012 the company announced a collaboration with an unnamed animal health company to use DepoVax in the development of companion animal vaccines. Indications have not been specified publicly. See imvaccine.com/releases.php?releases_id=274.
MetaMorphix, Inc. (Savage, MD) and Metamorphix Canada, Saskatoon, SK, Canada	MetaMorphix was a privately held animal health company with several technology platforms. At the time of the 2002 report the company was pursuing immunocontraception for animals using technology they had obtained from Biostar. In February of 2011, the company announced its intention to sell all, or substantially all, of its assets as part of reorganization under Chapter 11. The collateral subject to auction sales did not specifically list any asset related to immunocontraception for animals. This GnRH vaccine technology that had been under development was returned to the academic institution at which it originated (personal communication 2012).
Peptech Animal Health, Milperra, NSW, Australia	Peptech developed Ovuplant®, a sustained-release implant of the GnRH agonist deslorelin, for use in timing ovulation in horses, and Suprelorin, also a sustained-release GnRH agonist implant, for 6-12 month suppression of reproductive function of male dogs. Ovuplant was first approved in 1995 in Australia and New Zealand, in the US in 1998, in the UK in 2005, and several European countries in 2007. The 6-month version of Suprelorin was approved in Australia in 2004, New Zealand in 2005, and Europe in 2007. A 12-month version was approved in Australia in 2006 and Europe in 2010. Peptech Animal Health was acquired by Virbac in May 2011. See Virbac, section 4.2.1.2.
United Biomedical, Inc., Hauppauge, NY	United Biomedical (UBI) is a privately held immunotherapeutics and immunodiagnostics company that employs proprietary processes to design and manufacture synthetic peptide products for human and animal health. UBI technology is based on refining peptides to act via the immune system. The pipeline is made up of biologicals for the treatment and prevention of Alzheimer's disease, AIDS, and allergy in humans, as well as animal health vaccines. As of 2002, the company was working on a number of animal health applications, including a long-acting immunocontraceptive for male and female companion animals. Duration would be achieved via administration of annual boosters. The company indicated it was conducting trials of the vaccine in dogs in collaboration with an undisclosed animal health company. The current UBI website indicates a veterinary portfolio consisting of a vaccine for growth promotion and boar taint (swine), PCV-2 vaccine (swine), PRRS vaccine (swine), FMD vaccine (cattle), and allergy vaccine (canine); and refers to a longer longer-term plan to address health needs in companion animals. As of August 2012, the company was not working on a pet contraceptive (Wang, personal communication).

Zonagen, Woodland Hills, TX	Zonagen was founded in 1987 to commercialize work done by Dr. Bonnie Dunbar, formerly of Baylor University in Waco, Texas, on a zona pellucida (ZP) contraceptive vaccine for use in animals and possibly humans. The collaboration ended in a dispute over issues of technology transfer and intellectual property issues that can arise. In 2006, the company name was changed to Repros Therapeutics, which is reported to be working on drugs for male fertility, type 2 diabetes, uterine fibroids, and endometriosis. There is no reference to animal health applications on the Repros website. Work related to non-human species appears to have ceased.

4.2.4 Companies Currently or Recently Involved in Non-Surgical Contraception and Fertility Control for Dogs and/or Cats

4.2.4.1 Amplicon Vaccine, LLC, Pullman, WA

The Amplicon Vaccine, LLC website (ampliconvaccine.com) describes the composition of a vaccine called Repro-Bloc™ as "a series of GnRH genes [which] are cloned onto ovalbumin carrier gene which is held in an *E. coli* based expression vector … the purified protein is added to an emulsifying agent, oil, dead [M]ycobacterium butyricum and a urea + phosphate buffer." The company website refers to studies in mouse, swine, dogs, cats, lamb, caribou and cattle, and a PowerPoint® available on the site describes several studies in heifers and bulls. No information on studies in other species appears to be available to the public.

4.2.4.2 Ark Sciences, Inc., New York, NY

Ark Sciences (arksciences.com) was founded in 2007 by Joseph Tosini, who has focused his career on the field of community development. The company has resurrected the zinc gluconate male canine sterilant formerly known as Neutersol and has renamed the product Esterilsol (ex-US) and Zeuterin (US). According to Ark Sciences the company now owns all intellectual property rights. The formulation has been approved for use in the US for male dogs from 3- 10 months of age, and in Mexico, Bolivia, Panama and Colombia for dogs 3 months and older. In Colombia, it is also approved for use in cats. Ark Sciences has announced plans to expand to other countries in the future. Currently, the product can be used on a limited basis with special permission in some countries in which it does not have regulatory approval. The US launch is expected in 2013. Ark Sciences reports that the company expects the label claim to be expanded to male dogs 3 months and older by that time.

Ark Sciences' website describes the company as a social entrepreneurship venture that combines social impact and investor return. The company's strategy is to work with nonprofit animal welfare organizations involved in population control to make it possible to sterilize male dogs that would otherwise be contributing to homeless pet populations. According to a July 2011 Ark Sciences presentation, 10,000 dogs have been sterilized using its product in a field trial in Mexico to establish the safety and effectiveness of the product. The company also reports that the US Army has taken the product to earthquake-affected areas in Japan to sterilize dogs left without homes, and that Bangladesh has ordered 50,000 doses to be used in a dog population control project.

The company reports that post-treatment sterility is permanent, and although sedation is strongly

recommended prior to administration, anesthesia is not required. In an experienced administrator's hands, as many as 12 dogs can be sterilized per hour. Post-treatment clinical observation is suggested for 10- 15 minutes when dogs should be alert enough to be released. The company has developed training programs utilizing Master Trainers (veterinarians) who train other veterinarians. See Chapter 3, section 3.4.1, for more details on use of this product.

An October 2012 Veterinary Information Network article announced the impending return of the product to the US market, renamed Zeuterin, and provides comprehensive information on how the product works, how to use it properly, how Ark Sciences is training veterinarians and supporting the product, and how it has evolved since its initial foray into the US market as Neutersol. See news.vin.com/VINNews.aspx?articleId=24708.

4.2.4.3 Crinetics Pharmaceuticals, Inc., San Diego, CA

Crinetics Pharmaceuticals, Inc. (crinetics.com) specializes in applications of neuropeptide-receptor targeted therapeutics for the treatment of endocrine diseases and cancers in humans. The company is researching an ovarian cancer drug candidate that can be expected to cause sterility as a side effect. Dr. Scott Struthers, who is Crinetics' President and Chief Scientific Officer, believes that the drug may cause sterility in domestic animals as well. Crinetics has received two Michelson Prize grants (see section 4.3.3.2) to pursue the use of its technology in dogs and cats: a 2-year grant entitled *Novel Toxin Conjugates for Non-surgical Sterilization via Gonadotroph Ablation* and a

3-year grant to study *Targeted Ablation of GnRH Neurons for Non-surgical Sterilization.*

4.2.4.4 FeralStat, Old Lyme, CT

Note that FeralStat® is not a company. Rather, FeralStat was the brand name of a megestrol acetate (MGA) oral contraceptive developed by the late Dr. John Caltabiano, a

Connecticut veterinarian, who during his career helped re-home unwanted pets and in the 1990s launched a nonprofit mobile spay/neuter and vaccination clinic for cats (Tait's Every Animal Matters – TEAM). FeralStat appeared to be marketed independently of the organization; it was available via a website.

MGA has not been approved by the CVM or any European regulatory agency as a contraceptive for cats, but has been used, most commonly in Europe, for this purpose. The particular dose of MGA in FeralStat has not been studied for safety and/or effectiveness. According to the website, FeralStat was to be added to canned food weekly and positioned as a "stop gap" strategy to prevent reproduction in a feral cat colony until the colony could be trapped and permanently sterilized. The product was marketed over the Internet, prescribed over the telephone and was not distributed wholesale to veterinarians for re-sale. Although FeralStat is no longer available, numerous feral cat caregivers have reported acquiring generic MA from private veterinarians and administering it at the dose level recommended for FeralStat. FeralStat came on and went off the market during the period between the 2002 *Contraception and Fertility Control in Animals* report and this update.

While FeralStat was still available, ACC&D did not recommend use of the product in part due to the absence of safety and efficacy studies, but did note that since the dose of MGA was "significantly lower than that used historically," it may have been "possible that the lower dose is effective and not associated with significant side effects." The organization has developed a position paper on FeralStat (www.acc-d.org/ACCD%20docs/PPPP-FeralStat.pdf).

4.2.4.5 Rhobifarma Industria Farmaceutica Ltda, Brazil

In 2009, the Brazilian company Rhobifarma Industria Farmaceutica Ltda launched Infertile, a zinc gluconate product with a different formulation than that of Esterilsol/Zeuterin (section 4.2.4.2). The company distributes the product. Infertile is an injectable sterilant for male dogs administered via a single injection into each testicle. The product consists of zinc gluconate (at twice the strength of Esterilsol/Zeuterin), argenine and dimethyl sulfoxide (DMSO) as a "carrier" to aid in the distribution of the drug within the testicle.

Development of Infertile was funded by an animal welfare advocate committed to advancing animal welfare by providing safe, effective, affordable, and easy-to-administer alternatives to surgical sterilization. The product is approved by Brazil's Ministry of Agriculture, the agency that oversees all veterinary products in Brazil. The Sponsor plans to expand introduction to other countries, but as of 2012 Infertile was only available and approved in Brazil.

The instructions for use of Infertile include a recommendation to use an analgesic for post-injection pain management (see Chapter 3, section 3.4.1).

In June of 2012 a research project at Universidade de Sao Paulo was launched to "evaluate and compare the level of pain and inflammation that chemical sterilization by [Infertile]" may involve. Study dogs will be assigned to one of four groups. Three groups will receive Infertile 15 minutes after administration of dipyrone, tramadol, or meloxicam. The fourth group will receive standard

anesthesia and undergo surgical orchiectomy. Dogs will be monitored for 7 days post-surgery and various scales will be applied to assess pain in the four groups. The principal investigator is D. T. Fantoni; the study is expected to end in May of 2013 (study announcement, 2012).

See http://www.acc-d.org/About for ACC&D's Preliminary Statement on Infertile.

4.2.4.6 SenesTech, Inc., Flagstaff, AZ

SenesTech, Inc. was founded in 2002 and products under development are based on technology licensed from the University of Arizona. The technology involves the use of 4-vinylcyclohexene dipoxide (VCD) (see Chapter 3,

section 3.4.4) to provide chemical acceleration of ovarian senescence. SenesTech is currently working on two contraceptive products: ContraPest® for rice-rat control and ChemSpay™ for contraception in bitches. According to its website (senestech.com), the company is developing strategic partnerships with international industry partners and nonprofit institutions to accelerate the translation of its platform technology into a marketable approach to the rice-rat problem. SenesTech currently regards rodent management within agriculture as its primary market and chemical spay as its secondary market.

Work on ChemSpay has been based on the hypothesis that VCD treatment will accelerate elimination of primordial follicles in dog ovaries leading to ovarian failure, eliminate estrous behavior and cause permanent sterility. The preferable mode of delivery would be continuous release to attain 100% sterility and a hormone environment equivalent to that of sterile individuals (Mayer 2006).

For a brief time, SenesTech had a relationship with an organization called 600 Million Stray Dogs Need You, which was founded by People for the Ethical Treatment of Animals (PETA) co-founder Alex Pacheco, and claimed to be developing an orally available compound that could result in permanent sterilization, using VCD as the active compound. In April 2011 SenesTech notified ACC&D that they had severed ties with that organization and its founder, and that "neither 600 Million nor Mr. Pacheco [has] any right, title, license or interest in our ChemSpay product or any other [SenesTech] product" (acc-d.org/600Million).

4.2.4.7 Vaxin, Inc., Birmingham, AL

Vaxin, Inc. (vaxin.com) is a life sciences company with a proprietary technology "that consists of non-replicating adenovirus and bacterial vectors that can deliver antigens non-invasively to the nasal passages or to the skin to elicit a protective immune response." At the 4th International Symposium on Non-Surgical Contraceptive Methods for Pet Population Control, Dr. Kent R. Van Kampen explained that research indicates use of adenoviral vectors that express antigens to induce antibodies that block fertility in male and female dogs and cats is possible. Immunogenicity against antigenic epitopes of infectious agents has been demonstrated to induce both humoral and cellular immune responses. Dr. Van Kampen noted that "the [potential for] application of this scientifically sound, proven technology for companion animal contraception" may be compromised by factors that are not science related, such as regulatory requirements and funding issues (Van Kampen 2010).

In December 2011, Vaxin and the Scott-Ritchey Research Center at the Auburn University College of Veterinary Medicine were awarded a Michelson Grant (see section 4.3.3.2) to continue development of a vectored GnRH contraceptive vaccine to control dog and cat

overpopulation. According to a Vaxin press release (vaxin.com/FAFAuburnRelease.pdf), "the three-year project draws upon the science of Vaxin's vaccine technology already tested in humans for influenza and the Scott-Ritchey Research Center's commitment to develop contraceptive vaccines for companion animals." Drs. Van Kampen of Vaxin and Henry Baker and Nancy Cox of Auburn University are the lead investigators.

4.3 Research: Organizations and Foundations, Government Agencies, and Academic and Research Institutions

This section of the report deals with research sponsored or undertaken by various organizations and research that is

emerging and may offer new approaches to contraception. We are pleased to report that there appears to be significantly more research and involvement in this field by organizations, foundations, government agencies, and academic and research institutions than there was when the original report was published. In some instances work will have been mentioned in other sections of this report but is also mentioned here due to the nature of its sponsorship. See also Chapter 3, section 3.6.

4.3.1 Update on Research Described in the 2002 Report

The entities shown in Table 4-2 below were profiled in the original 2002 *Contraception and Fertility Control in Animals* report, which covered horses, wildlife, and production animals in addition to dogs and cats.

Table 4-2: Research Profiled in the 2002 Report

Group and Investigator(s) (2002 Report)	Update
AFSSA Nancy Wildlife Health and Management Unit, Malzéville, France Dr. Franck Boué et al.	Dr. Franck Boué presented work (Verdeir et al. 2002) related to developing a sperm-antigen-based immunocontraceptive vaccine for canine species. Foxes were the model used in the study, in which several auto-antigens were identified as "potentially interesting for the development of a contraceptive fox vaccine." Researchers tested three types of vaccines for delivering the auto-antigens: synthetic peptides, recombinant proteins produced *in vitro* by *E. coli* or baculovirus, and recombinant antigens inside a non-replicating *E. coli* "ghost" containing only the protein of interest. While these approaches "induce[d] immune response against the injected agent … the antibodies did not recognize the native protein and no impact on the ovary or on the spermatozoa was observed." At the 2nd International Symposium on Non-Surgical Methods for Pet Population Control in 2004, Dr. Boué described efforts to develop immunological tools to assess the humoral and cellular immune responses in sera and vaginal fluids, in support of development of a canine immunocontraceptive vaccine (Boué 2004). See section 4.3.4.4 for additional related research published in 2005. Current focus of the organization appears to be on wildlife issues. Further discussion is beyond the scope of this update. See www.anses.fr/index.htm.
Baylor College of Medicine, Houston, TX Dr. Bonnie Dunbar (currently at the University of Nairobi)	See Zonagen, section 4.2.3.
Center for Reproductive Science/Technology, University of Missouri, Columbia, MO Dr. Min Wang Dr. Mostafa Fahim (deceased 1995)	The 2002 report described work done by Dr. Min Wang and based on earlier work by the late Dr. Mostafa Fahim on the safety and efficacy of zinc gluconate neutralized by arginine for use as an intra-testicular injection for chemical castration in male dogs. This work was commercialized initially by Addison Biological Laboratory (Neutersol, see section 4.2.3) and, subsequently, by Ark Sciences (Esterilsol in ex-US markets, Zeuterin in the US). See section 4.2.4.2 for more information about the impending US recommercialization of this zinc gluconate formulation.
College of Veterinary Medicine, Colorado State University, Ft. Collins, CO Dr. Terry Nett	See Gonex in section 4.2.3; see Chapter 3, section 3.2.3.1.
College of Veterinary Medicine of the Scott-Ritchey Research Center, Auburn, AL Dr. Brenda Griffin (currently at University of Florida) Dr. Henry J. Baker	The Scott-Ritchey Center has been involved in research related to non-surgical contraception in animals for some time and, in fact, was instrumental in starting ACC&D. The Center continues to be involved in research and is working with Vaxin on a project funded by a Michelson Grant. See section 4.3.3.2.

College of Veterinary Medicine, University of California, Davis (UCD) Dr. Irwin K. Liu Dr. Linda Munson (deceased) Dr. Barry Ball (currently at the University of Kentucky)	The 2002 report described Dr. Irwin K. Liu's work on developing zona pellucida vaccines. At that time he had completed a study (Liu & Ball 2002) showing that dogs immunized with pig zona pellucida were not contracepted effectively. His group was working on developing a ZP-based immunocontraceptive treatment for horses. Dr. Liu has continued to work on issues related to equine reproduction, which is beyond the scope of this update. Dr. Linda Munson published her work on the use of GnRH agonist treatment in cats (Munson 2001) and a review article, *Contraception in Felids* (2006), in which she indicated that limited availability of non-progestin contraceptives and side effects associated with some agents in felids has limited the fertility control options in felids. Unfortunately, Dr. Munson passed away in 2010. Dr. Barry Ball was working to identify sperm proteins in dogs (Sabeur et al. 2002) and to test the GnRH protein synthesis inhibitor in male dogs (Ball 2002). Dr. Ball is now the first Albert G. Clay Endowed Chair in Equine Reproduction at the University of Kentucky; he is no longer involved in dog research.
College of Veterinary Medicine, University of Florida, Gainesville, FL Dr. Julie Levy	Dr. Julie Levy has continued to research the population dynamics of feral cats under various management situations and test potential approaches to non-surgical contraception in cats. Her work and resulting publications have spanned approaches from GnRH and ZP vaccines to retinoic acid receptor antagonists to chemical castration with zinc gluconate. See www.vetmed.ufl.edu/about-the-college/faculty-directory/julie-levy/ for a profile of Dr. Levy and a list of some of her publications. See Chapter 3, sections 3.2.4.2, 3.3.1.2.2, 3.4.1.2, and 4.3.2 of this report.
College of Veterinary Medicine, University of Georgia, Athens, GA Dr. Richard Fayrer-Hosken	Dr. Richard Fayrer-Hosken continues his work on contraception in wildlife, which is beyond the scope of this update.
Cornell University College of Veterinary Medicine Baker Institute for Animal Health Dr. Scott Coonrod	Dr. Scott Coonrod, formerly of the University of Virginia, has joined Cornell University College of Veterinary Medicine as an associate professor at the Baker Institute for Animal Health. See Chapter 3, section 3.6.3 for an update of Dr. Coonrod's work in this area. See also University of Virginia, below.
Cornell University Institute for Animal Welfare, Cornell University, Ithaca, NY Dr. Paul Curtis	The 2002 report described wildlife-related work at the Cornell Institute for Animal Welfare, which is beyond the scope of this update. Note: See Dr. Patrick Concannon listed below under New York State College of Veterinary Medicine, also affiliated with Cornell University.
Division of Veterinary and Biomedical Sciences and the Department of Microbiology, Murdoch University, Perth, Australia Investigator not specified at the time	The 2002 report noted that investigators were using naked DNA coding for zona pellucida proteins as an antigen for the immunization of the cat. At that time T-cell responses were seen in a small group of animals, but no long-term contraception trials had been reported. See Chapter 3, section 3.3.1.2.2 for information about a 2007 Murdoch University study of the contraceptive potential of porcine and feline zona pellucida A, B and C subunits in domestic cats.
Institute for Zoo and Wildlife Research, Berlin, Germany Dr. Katarina Jewgenow	Dr. Katarina Jewgenow continues to publish a range of research related to fertility and contraception in animals. For example: reduced germ cell apoptosis during spermatogenesis in the teratospermic domestic cat (2009); functional role of feline zona pellucida protein 4 trefoil domain: a sperm receptor or structural component of the domestic cat zona pellucida (2009); seasonal profiles of ovarian activity in Iberian lynx based on urinary hormone metabolite analyses (2009); cryopreservation of mammalian ovaries and oocytes (2011); the molecular detection of relaxin and its receptor RXFP1 in reproductive tissue of *Felis catus* and *Lynx pardinus* during pregnancy (2011).

Institute for Molecular Biology, National Chung Hsin University, Taichung, Taiwan Dr. Jualang Hwang	Dr. Jualang Hwang's laboratory is focusing on 1) using linear array epitope (LAE) approaches to develop therapeutic vaccines and 2) studying the post-translational modification of DNA topoisomerase. His web page notes that an anti-GnRH vaccine has been developed using LAE technology, and other molecular vaccines such as LAE vaccine against HER2 for HER2 over-expressed breast cancer therapy and LAE for Alzheimer's disease prevention are under development. There appears to be no activity related to the application of this technology to develop a contraceptive vaccine for dogs and/or cats.
Lethbridge Research Center, Lethbridge, Alberta, Canada Drs. Cook, Kastelic, and McAllister	The work described in the 2002 report involved the use of GnRH vaccines in cattle. Updating is beyond the scope of this document.
National Wildlife Research Center, US Department of Agriculture, Ft. Collins, CO Dr. Kathy Fagerstone Dr. Lowell Miller	The work described in the 2002 report involved wildlife. Updating wildlife applications is beyond the scope of this report; however the GonaCon vaccine developed by NWFC for deer has been tested in dogs and cats and has been administered with a canine rabies vaccine. See Chapter 3, section 3.2.4, and section 4.3.4.1.
New York State College of Veterinary Medicine, Cornell University, Ithaca, NY Dr. Patrick Concannon (Also see others at Cornell University above)	Dr. Patrick Concannon has worked for many years on canine reproduction and the canine estrous cycle. In 2006 he made a presentation entitled *Use of GnRH Agonists and Antagonists for Small Animal Contraception* (Concannon 2006) at the ACC&D 3rd International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control. He was also on the organizing committee of the 7th International Symposium on Canine and Feline Reproduction in July 2012, which included several reports on new research in the area of contraception.
Norwegian School of Veterinary Science, Oslo, Norway Dr. W. Farstad	The work described in the 2002 report was related to reproduction in the fox and assisted reproduction in various canid species. More recent work appears to have been undertaken in lambs. This work is beyond the scope of this update.
Rutgers University, New Brunswick NJ Dr. Larry Katz	The work described in the 2002 report related to various methods to address deer overpopulation and is beyond the scope of this document. (Dr. Katz no longer does research in reproduction.)
University of Liège, Belgium Dr. John Verstegen (now at Minitube International)	The 2002 report described work of Dr. John Verstegen on the use of GnRH agonists in the bitch. As of 2010, Dr. Verstegen became the executive vice president of international research and development at Minitube International in Wisconsin. Minitube specializes in assisted porcine, bovine, equine, and canine reproduction technologies.
University of Pretoria, Ondertepoort, Republic of South Africa Dr. Henk Bertschinger	Dr. Bertschinger's present work includes the use of porcine zona pellucida (PZP) in managing reproduction in elephants and Suprelorin (deslorelin) GnRH agonist in managing reproduction in cheetahs. Further updating is beyond the scope of this report.
University of Virginia, Charlottesville, VA Dr. Scott Coonrod (now at Cornell University College of Veterinary Medicine Baker Institute of Animal Health). See section 3.6.3 for an update of his work. Current: Dr. John Herr	The 2002 report described oocyte-related work undertaken by Dr. Scott Coonrod, then at the University of Virginia, on identifying oocyte-specific proteins in dogs and cats under Dr. John Herr. Dr. Herr is continuing work on oocyte ablation and has received a Michelson Grant (see section 4.3.3.2) to pursue work on egg ablating drugs for use in dogs and/or cats (Burnham 2011).

Vertebrate Biocontrol Cooperative Research Centre (CRC), Commonwealth Scientific and Industrial Reseach Organization (CSIRO) Wildlife and Ecology, Canberra, ACT, Australia Dr. Mark Bradley	The 2002 report described work on various methods to address wildlife control. Updating is beyond the scope of this report. See CSIRO/CRC section 4.3.4.3 for other information.
Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA Dr. Stephen M. Boyle (retired) Dr. Beverly Purswell (retired)	The 2002 report described work by Drs. Stephen M. Boyle and Beverly Purswell to develop species-specific orally administered contraceptive baits. In the interim, Drs. Boyle and Purswell have retired. Dr. Boyle continues to be involved in the development and testing of a contraceptive vaccine, in this case for feral swine, using a live, attenuated strain of *Brucellosis suis* VTRS2 developed at Virginia Tech. Drs. Lowell Miller and Steven Olsen of the National Wildlife Research Center are collaborating on this research (Boyle, personal communication 2012). Further discussion of the swine project is beyond the scope of this document.

4.3.2 Universities Working on Non-Surgical Approaches

Note that much of the work described in Chapter 3 has been done at universities so this table may not include all universities at which work on non-surgical approaches is occurring. Since this field is evolving continually, ACC&D may not be aware of every academic research project. Please notify ACC&D of any project that may be included in future updates of this report.

An asterisk (*) in Table 4-3, below, denotes a Michelson Grant in Reproductive Biology recipient (as of July 2012). See section 4.3.3.2 for information about the Michelson Prize & Grants in Reproductive Biology.

Table 4-3: Universities Involved in Researching Non-Surgical Approaches to Dog and Cat Contraception/Sterilization

University	Researchers	Area(s)
Auburn University, Auburn, AL	*Tatiana I. Samoylova, PhD in collaboration with Drs. Nancy Cox, Valery Petrenko, Bettina Schemera, Frank Bartol and Mark Carpenter	Phage-GnRH constructs (see Chapter 3, section 3.3.1.3.2 for a description of Dr. Samoylova's work on Phage-ZP constructs)
Baker Institute, Cornell University	Scott Coonrod	Egg-ablating drugs delivered via virus-like particles (see Chapter 3, section 3.6.3)
Bose Institute, Kolkata, India	Dr. Kuladip Jana	Calcium chloride as a sterilant (see Chapter 3, section 3.4.2)
Center for Reproductive Services, University of Kansas Medical Center	Joseph S. Tash and Katherine F. Roby. Note that Dr. Gunda Georg at the College of Pharmacy, University of Minnesota, is collaborating with Drs. Tash and Roby on this research.[7]	Antispermatogenic (see Chapter 3, section 3.6.5).
Interdisciplinary Center for Male Contraceptive Research and Drug Development	Joseph S. Tash and Gunda Georg	Under the auspices of the Contraceptive and Reproductive Health Branch of National Institute of Child Health & Human Development (NICHD), the Center and collaborator Dr. Gunda Georg (see above) are spearheading a multi-university effort to develop reversible non-hormonal male contraceptive agents.

[7] Researchers listed in this table may have collaborators at other institutions.

Iowa State College of Veterinary Medicine, Ames, IA	*Douglas E. Jones, MS, VMD, PhD	GnRH vaccine delivery device. Dr. Jones has been involved in developing mathematical models of immunity, which has led to research related to developing a vaccine implant that responds to the immune status of the person or animal, enabling release of the vaccine when the immune response is decreasing.
National Jewish Health, Denver, CO (hospital and medical research facility)	*Phillippa Marrack, PhD and Michael Munks, PhD	Attenuated recombinant herpesviruses. Dr. Marrack's research is related to T-cell function.
National University of La Plata, Argentina	*Cristina Gobello, DrVetMed, Dipl ECAR. Also Drs. C.Valienta, and Y. Corrada (and other publication-specific authors)	Several, including GnRH agonists and antagonists. Dr. Gobello has authored or co-authored a number of publications related to reproduction and fertility control in companion animals. (See Chapter 3, sections 3.2.1 and 3.2.2 for examples of published work for which Dr. Gobello is the lead author.)
Oregon Health & Science University	Sergio R. Ojeda DVM and Gregory A. Dissen, PhD	Interfering RNA/gene silencing (see Chapter 3, section 3.6.1)
School of Medical Science and Technology (SMST) and National Institute of Medical Science and Technology, Kharagpur, India	Dr. Sujoy K. Guha	Reversible inhibition of sperm under guidance – RISUG™ contraceptive polymer (see Chapter 3, section 3.6.4.3)
Scripps Research Institute, Jupiter, FL	*William W. Ja, PhD	Cytotoxin conjugates (see Chapter 3, section 3.6.4.2)
UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ	*Paul R. Copeland, PhD	Targeting selenoprotein P for use in male contraception. Dr. Copeland has identified and characterized important factors involved in programming ribosomes to incorporate selenocysteine, an amino acid involved in many human proteins. These proteins include glutathione peroxidase 4, which is required for male fertility.
Université de Franche-Comté, Besançon Cedex, France	D. Fellman, F. Pralong, P. Y. Risold	Kisspeptin and GnRH (see Chapter 3, section 3.6.2)
University of Aldo Moro of Bari, Valenzano, Italy	Dr. Raffaella Leoci	Calcium chloride as a sterilant (see Chapter 3, section 3.4.2). Note that Dr. Leoci has also worked on the use of ultrasound for sterilization of male dogs. Note: As of November 2012, Dr. Leoci was in the planning stages of a study to compare various visual identification methods to mark dogs as sterilized (Lissner, personal communication 2012).
University of Arizona, Tucson, AZ	*Benjamin Renquist, PhD	Interfering RNA/gene silencing (see Chapter 3, section 3.6.1)
University of Auckland, New Zealand	*Larry Chamley, PhD	Sperm protein (SPRASA, see Chapter 3, section 3.6.6)
University of Iowa Medical School, Iowa City, IA	*Beverly L. Davidson, PhD	Interfering RNA/gene silencing (see Chapter 3, section 3.6.1)

University of Newcastle, Callaghan, NSW, Australia	*R. John Aitken, ScD, FRSE	Developing a humane non-surgical sterilization method for use in domestic animals. Note that Dr. Aitken has published on a number of topics related to reproduction, including the cell biology of male germ cells, particularly spermatozoa, and certain aspects related to ovarian follicles.
University of North Carolina at Charlotte, Charlotte, NC	Nathaniel Fried, Ph.D.	Working on non-invasive laser vasectomy with funding from NICHD. According to the university's Biomedical Optics Laboratory website: "Preliminary experiments … have demonstrated that it is possible to use therapeutic focused ultrasound to noninvasively target the vas deferens for thermal coagulation, scarring, and occlusion." Dr. Fried has co-authored a number of studies related to non-invasive laser coagulation of the canine vas deferens. See maxwell.uncc.edu/nmfried/index.html and maxwell.uncc.edu/bmolab/pages/sterilization.html.
University of Pennsylvania, Philadelphia, PA	*Ralph G. Meyer, PhD	Poly(ADP-ribose) metabolism-related pharmacological strategies for treatment of male infertility and non-surgical sterilization of cats and dogs.
University of Virginia (UVA), Charlottesville, VA	*John C. Herr, PhD	Egg-ablating recombinant vaccines. Dr. Herr is focusing on work that could lead to the development of a non-surgical sterilant that targets oocytes before they become eggs (see section 3.6.3). His laboratory is also working on understanding human testis genes and how they are expressed in various types of human tumors.
University of Western Australia, Crawley, Western Australia	*Megan Lloyd, PhD	Recombinant viral vector. Note that Dr. Lloyd has published on infertility caused by immunization with recombinant murine cytomegaloviruses expressing murine zona pellucida (O'Leary et al. 2009).
West Bengal University of Animal and Fishery Sciences, Calcutta, India	Dr. P. K. Samanta (now retired)	Calcium chloride as a sterilant (see Chapter 3, section 3.4.2). Dr. Samanta is now partially retired, but still involved in a local field trial (Lissner, personal communication 2012).
Yale University. New Haven, CT	*Meenakshi Alerja, PhD	Developing a non-surgical sterilization method in mice. Note that Dr. Alerja has published on activation of the G-protein-coupled receptor GPR54 by kisspeptins during puberty (Dumaiska et al. 2008).

4.3.3 Organizations and Foundations

There has been a significant increase in the level of nonprofit – and to some extent – government support of research and general focus in the area of contraceptives for dogs and cats since the original report in 2002. At least one of these entities has pledged to also play a role in commercializing new products.

One product, sponsored by The Humane Society of the United States™ (HSUS), received regulatory approval in February 2012 from the EPA following years of use in the field on a research basis. The product is a vaccine, called ZonaStat-H, intended and approved solely for use in fertility control in female wild horses. Although ZonaStat-H is not

a product for dogs or cats, it represents perhaps the first known animal health product sponsored by a nonprofit to receive approval (see humanesociety.org/news/press_releases/2012/02/EPA_Announces_First_Fertility_Control_Vaccine_for_Wild_Horses.html). (See section Chapter 3, 3.3.1 for a discussion of porcine zona pellucida-based vaccines.)

Overviews of different organizations' and foundations' involvement and approaches are reviewed below in alphabetical order.

See Chapter 6 for an overview of regulatory considerations related to non-surgical sterilization and/or contraception products for use in dogs and cats, including a discussion of issues related to un-approved products obtained from compound pharmacies or other sources in lieu of development under the prescribed pathways for regulatory approval.

4.3.3.1 Alliance for Contraception in Cats & Dogs, Portland, OR

ACC&D was founded by contraceptive researchers Drs. Henry Baker, Stephen Boyle and Brenda Griffin in 2000 as a program of Auburn University and was then incorporated as an independent 501(c)3 nonprofit organization in 2005. The organization's mission is to expedite the successful introduction of methods to non-surgically sterilize dogs and cats and to support the distribution and promotion of these products to humanely control cat and dog populations worldwide. ACC&D represents the interests of the animal welfare community in obtaining more efficient methods of preventing unwanted litters of cats and dogs. The Board of Directors and Scientific Advisory Board include leaders and experts in veterinary medicine, reproduction, animal health, drug development, public health and animal welfare. Experts include senior staff/scientific advisors for the American Society for the Prevention of Cruelty to Animals® (ASPCA®), The HSUS, Dogs Trust, and, formerly, the World Society for Protection of Animals (WSPA).

The organization advocates science-based approaches that can be developed into effective, safe products that meet applicable regulatory standards, are commercializable, and can be dispensed affordably and conveniently in low-income and frontier market situations. Another focus of the organization is providing scientifically-sound and animalwelfare-oriented information about available products and work in this field to stakeholders in animal welfare, animal health, population management, and public health. See acc-d.org for additional information.

ACC&D has the support of its Council of Stakeholders, which provide multi-year operational financial support as well as strategic insight on stakeholder interests and networking. As of November 2012 the Council consists of seven organizations: Amber and Adam Tarshis Foundation, the ASPCA, The HSUS, Parsemus Foundation, Petco® Foundation, PetSmart Charities®, and the Regina Bauer Frankenberg Foundation. For updates, see acc-d.org.

4.3.3.1.1 ACC&D Symposia

ACC&D has organized and hosted four international symposia focusing on non-surgical contraception in companion animals. Proceedings of the 2002, 2004, 2006, and 2010 symposia are available for review and downloading at acc-d.org/ACCD%20Symposia. A fifth symposium will take place in June 2013.

4.3.3.1.2 ACC&D Think Tanks

ACC&D has held four "Think Tank" meetings since 2009 to bring together experts in a given field to discuss specific opportunities relating to developing non-surgical contraception and fertility control for use in cats and dogs. Think Tanks convened to date include:

- **Contolled Release for Depot and Implant Technologies, as it Applies to Developing Non-Surgical Alternatives to Sterilize Cats and Dogs** (April 2012, sponsored by Found Animals® Foundation and the Animal Assistance Foundation)
- **Population Dynamics Modeling and Field Studies to Improve Development of Technologies for Non-Surgical Sterilization of Cats and Dogs** (June 2011, sponsored by Leonard X. Bosack & Bette M. Kruger Foundation and PetSmart Charities; follow-up development of a computer model to evaluate both surgical and non-surgical interventions in free-roaming cat populations funded by the ASPCA in 2012)
- **Immunocontraceptive Approaches for Sterilization of Dogs and Cats** (November 2009, sponsored by Found Animals Foundation and the Animal Assistance Foundation)

- **Gene Silencing Potential for Sterilization of Cats and Dogs** (October 2009, sponsored by Found Animals Foundation and the Animal Assistance Foundation)

Think Tank summaries are available for review and downloading at acc-d.org/ThinkTanks.

4.3.3.1.3 ACC&D-Sponsored Studies

ACC&D provided support in 2006 to SenesTech for early work in efficacy and safety of its technology in dogs. According to a position on research updated in 2012 and available on ACC&D's website, "ACC&D does not directly conduct or fund the research necessary in the development

of new contraceptives and non-surgical sterilants. However, [ACC&D] may facilitate research on promising formulas by providing counsel on study design, standards of animal use, and routes for potential funding. ACC&D may conduct or fund non-terminal research involving animals in field projects designed to increase learning about existing products: those that have regulatory approval and are commercially available for dogs and/or cats, or extra-label use of other approved products."

Other grants awarded by ACC&D have supported field-based efforts to "advance knowledge about [the] effective use of Esterilsol, the non-surgical sterilant for [use in] male dogs." See Chapter 3, section 3.4.1 and section 4.2.3 for information about the history of Esterilsol (known as Zeuterin in the US) and its precursor, Neutersol.

Ongoing Project: Esterilsol Behavior Study

A current behavioral study in Puerto Natales, Chile seeks to address the behavioral effects of Esterilsol compared to those of surgical castration in male dogs. The study, an international collaboration among the Chilean National Agriculture Service, the Italian Instituto G´Caporale, the University of Pennsylvania, and the Canadian Atlantic Veterinary College and Veterinarians without Borders/Vétérinaires sans Frontières-Canada (VWB/VSF), is being carried out by VWB/VSF. A report on the study is expected in 2013. Ark Sciences, the company commercializing Esterilsol, has provided training. A February 2012 visit report by then-ACC&D Senior Director Karen Green is available at http://www.acc-d.org/ACCD%20docs/VWBChileStudyKGReport.pdf.

Previous Projects

Esterilsol Small Grants Program (ESGP)

This program was inaugurated in 2009 to help nonprofit organizations wishing to use Esterilsol in their sterilization programs and to gather data regarding the field-based use of the product. Five grants, funded by a variety of groups and individuals, were awarded to groups working in Colombia, Dominican Republic, Samoan Islands, Galapagos Islands, Chile, Sierra Leone, and Kenya. See acc-d.org/EsterilsolGrants for specific information.

Sterilization Program and Field Study in Guatemala

ACC&D sponsored this 2009 VWB/VSF field-based project in Todos Santos, Guatemala (with funding from Parsemus Foundation – see section 4.3.3.4) in which 126 male dogs were sterilized using Esterilsol. (See www.acc-d.org/ACCD%20docs/SummaryGuatemalaReportFinal.pdf for a report and photos of the project.)

4.3.3.2 Found Animals Foundation/Michelson Prize & Grants in Reproductive Biology, Los Angeles, CA

Philanthropic investor Dr. Gary Michelson formed the nonprofit Found Animals Foundation in 2005 to address the problem of cat and dog overpopulation. In October of 2008 Dr. Michelson instituted the Michelson Prize in Reproductive Biology, a $25 million prize for the first method shown to permanently sterilize cats and dogs and to have a viable pathway to regulatory approval. The prize is supported by the $50 million Michelson Grants in Reproductive Biology program. Grants are intended to fund promising research in pursuit of non-surgical sterilization products or technologies for use in dogs and cats. Found Animals Foundation seeks proposals from researchers for up to $250,000 USD per year for up to 3 years of funding.

The goal of the Michelson Prize & Grants Program is development and commercialization of a single dose, non-surgical sterilant that could be administered in the field at a reasonable cost. By offering the Michelson Prize in Reproductive Biology, Found Animals encourages researchers to take on the challenge of non-surgical sterilization for dogs and cats. Found Animals Foundation believes a low cost, non-surgical method of sterilization would allow large populations of cats and dogs to be

sterilized to reduce the number of homeless and unwanted animals that are killed each year in shelters.

Found Animals will award the $25 million Michelson Prize to the first entity to provide a product that has, at minimum, the following characteristics (michelson.foundanimals.org/michelson-prize/michelson-prize-criteria):

- *Single dose, non-surgical sterilant*
  - Administered in a single patient encounter
  - Must not require general anesthesia
  - May require sedation or local anesthesia
  - Subcutaneous implant is considered non-surgical for the purpose of the Michelson Prize
  - Must induce permanent sterility for the reproductive lifetime of the species, defined as at least 10 years after administration
  - Will generally require 3 years of data with a trend line with no expectation of recovery; may require that study extends for additional time
- *Safe and effective in male and female, cats and dogs*
  - Must be one product for both species and genders
  - Must be no more dangerous than surgical gonadectomy
  - Must be safe for treated animal, environment (including predators), and person administering the product
  - Efficacy must be greater than or equal to spay/neuter. For the purposes of experimental design, a lower standard of proof may be acceptable
  - Must not induce clinical abnormalities or pathological lesions at 1X dose in safety studies
  - Must not be carcinogenic, mutagenic, or clastogenic
  - Must not be a "bait" formulation (i.e., administered without handling of treated animals)
- *Ablates sex steroids and/or their effects*
- *Suitable for administration in a field setting*
  - May require refrigeration/cold chain but no frozen storage, water source, or other electricity than refrigeration
  - Must have shelf life of at least 2 years
  - Must not require sterile environment/surgical theater
- *Viable pathway to regulatory approval*
  - As defined by CVM as a prescription product in dogs and cats
  - Regulatory approval will be sought by Found Animals
- *Reasonable manufacturing process and cost*
  - Target is a low-cost product to deliver to shelters
  - Must be no greater than $50 cost per dose to deliver labeled, packaged product to the Foundation or other distributor, with less than $25/dose preferred when manufactured at commercial scale

- Importantly, Found Animals plans to license the prize-winning approach and then take it through the US regulatory approval process and to market, with the goal of making a product available in a timely manner and affordably to the target market.
- The Michelson Grants also provide funds for research with promise of leading to an approach that meets their priorities. As of this update, 22 grants have been approved and 18 grants have been awarded and announced publicly, totaling more than $8.7 million (S. Johnston and K. Palfrey, personal communication October 2012).
- Grant applications are (and future prize applications will be) reviewed by the organization's Scientific Advisory Board under the leadership of the Michelson Prize & Grants' Director of Scientific Research, Dr. Shirley Johnston, a veterinarian, with a specialty in animal reproduction (theriogenology) the author of a textbook on canine reproduction, and the founding dean of the College of Veterinary Medicine at Western University.

- Table 4-4, next page, provides information regarding recipients of Michelson Grants. Readers are advised to consult the Michelson Grants in Reproductive Biology at michelson.foundanimals.org/ for information on grant amounts and durations, and periodic additions to the grantee list. This table is taken directly from the Michelson Grants website (except for references to specific sections of this report which provide more detail on the work) and has been updated as of July 14, 2012.

Table 4-4: Michelson Grants in Reproductive Biology

Grantee	Project Title[8]
R. John Aitken, ScD, FRSE, University of Newcastle, Callaghan, NSW, Australia	"Development of a human non-surgical sterilization method for domestic animals."
Meenakshi Alfreja, PhD, Yale University, New Haven, CT	"Development of a non-surgical sterilization method in mice."
Larry Chamley, PhD, University of Auckland, New Zealand	"SPRASA – An Immunocontraceptive with a Difference."
Paul R. Copeland, PhD, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ	"Targeting Selenoprotein P for male contraception in mammals."
Beverly L. Davidson, PhD, University of Iowa Medical School, Iowa City, IA	"Inducing stable infertility by RNA interference – proof of principle studies."
Cristina Gobello, DrVetMed, Dipl ECAR, National University of La Plata (FVS-NULP), Argentina	"Prepubertal administration of GnRH agonists in domestic cats."
John C. Herr, PhD, University of Virginia, Charlottesville, VA	"Oolysins: egg ablating drugs."
William W. Ja, PhD, Scripps Research Institute, Jupiter, FL	"FSH receptor ligand-cytotoxin conjugates for permanent chemosterilization."
Douglas E. Jones, MS, VMD, PhD, Iowa State College of Veterinary Medicine, Ames, IA	"Development of a vaccine delivery device that will maintain life-long high titers of anti-GnRH antibodies."
Megan Lloyd, PhD, University of Western Australia, Crawley, Western Australia	"Contraception in companion animals using a recombinant viral vector."
Phillippa Marrack, PhD, National Jewish Health, Denver, CO	"Use of attenuated recombinant herpesviruses, expressing fertility antigens, to induce infertility in cats and dogs."
Ralph G. Meyer, PhD, University of Pennsylvania, Philadelphia, PA	"Targeting Poly (ADP-ribose) metabolism for development of a non-surgical sterilant."
Michael Munks, PhD, and Phillippa Marack, PhD, National Jewish Health, University of Colorado Health Science Center, Denver, CO	"Use of attenuated recombinant herpesviruses, expressing fertility antigens, to induce infertility in cats and dogs."
Benjamin Renquist, PhD, University of Arizona, Tucson, AZ	"Inducing stable infertility by RNA interference."
Tatiana I. Samoylova, PhD, Auburn University, Auburn, AL	"Phage-GnRH constructs and their mimics for immunocontraception of cats and dogs."
A.C. Schaefers-Okkens, DVM, PhD University of Utrecht, Netherlands	"Kisspeptin: the Endocrinological Gatekeeper to Reproductive Function. A Realistic Target for Non-Surgical Contraceptives in the Dog."
R. Scott Struthers, PhD, Crinetics Pharmaceuticals, San Diego, CA	"Novel toxin conjugates for non-surgical sterilization via gonadotroph ablation." See Crinetics, section 4.2.4.3.
R. Scott Struthers, PhD, Crinetics Pharmaceuticals, San Diego, CA	"Targeted ablation of GnRH neurons for non-surgical sterilization." See Crinetics, section 4.2.4.3.
Kent R. Van Kampen, DVM, PhD Vaxin, Inc., Birmingham, AL; Drs. Henry Baker and Nancy Cos, Auburn University, Auburn, AL	"A vectored GnRH contraceptive vaccine to control dog and cat overpopulation (with Scott-Ritchey Research Center, Auburn University)." See Vaxin, section 4.2.4.7.

[8] Note that in many instances, description of a given project beyond its title is not available. Relevant publicly available information can be found in the sections referenced in the table.

Two additional grants have been awarded through the Michelson Graduate Student Challenge program:

- Joseph Rosenthal (Cornell University, Ithaca NY), a student in the Department of Biomedical Engineering, received a $15,000 grant in the Materials and Science/Engineering Category
- Owen Siggs (Scripps Institute, La Jolla, CA), a student in the Department of Genetics, received a $15,000 grant in the Depot Formulation Category

4.3.3.3 Morris Animal Foundation, Denver, CO

Over the years a number of reproduction- and overpopulation-related studies have been sponsored by Morris Animal Foundation (MAF) to evaluate methods of non-surgical contraception in dogs and cats. Consult the MAF website (morrisanimalfoundation.org/our-research/studies.html) for updates and for information on specific studies. The studies below were conducted between the years 2000 and 2012; investigators received a combined total of $673,000 in grant funding.

Table 4-5: Morris Animal Foundation Dog and Cat Overpopulation Projects

Division	Primary Contact	Project Title	Organization Name	Status
Canine	Dr. Cristina Gobello, MV, DMV, DECAR	Use of the GnRH Antagonist, Acyline, for Pregnancy Termination (Pilot)	National University of La Plata	Completed 2007
Canine	Dr. Cristina Gobello, MV, DMV, DECAR	Use of the Non-Peptide GNRH Antagonist, NBI-42902 on Pregnancy and Estrous Cycle Interruption	National University of La Plata	Completed 2010
Canine	Dr. Terry M. Nett, Ph.D.	Development of New, More Efficacious Technology to Chemically Castrate Male and Female Dogs (see section 4.3.2, Gonex)	Colorado State University	Project extended as of 12/31/2011
Canine	Elisa Juarez	Use of a GnRH Antagonist on Estrous Cycle Interruption	National University of La Plata	Completed 2008
Canine	Paul S. Cooke, PhD	Use of Neonatal Progestin Treatment as a Permanent, Non-surgical Contraceptive Methodology in Dogs	University of Florida	Noted as Active but scheduled to end 8/12
Feline	Dr. Julie K. Levy, DVM, Ph.D.	Evaluation of SpayVac for Sterilizing Domestic Cats (Felis catus) (see Chapter 3, section 3.3.1.2.2)	University of Florida	Completed 2001
Feline	Dr. Julie K. Levy, DVM, Ph.D.	GnRH Immunocontraception for the Humane Control of Feral Cats (see Chapter 3, section 3.2.4.2)	University of Florida	Completed 2006
Feline	Dr. Julie K. Levy, DVM, Ph.D.	GnRH Immunocontraception for the Humane Control of Feral Cats (continuation of study on duration of effect) (see Chapter 3, section 3.2.4.2)	University of Florida	Completed 2009
Feline	Ana Cristina Carranza Martin	Effect of Exogenous Melatonin on Prevention of Breeding Season in the Domestic Cat	Catholic University of Cordoba	Completed 2008

Feline	Mr. Jorge Diaz	Effect of the GnRH Antagonist, Acyline, on Domestic Cat Pregnancy (Pilot)	National University of La Plata	Completed 2009
Feline	Mercedes Soriano	Effect of Prepubertal Administration of a GnRH Agonist in Domestic Cats	National University of La Plata	Completed 8//12
Feline	Dr. Cristina Gobello, MV, DMV, DECAR	Effect of Neonatal Administration of a GnRH Antagonist in Domestic Cats	National University of La Plata	Extension – results due 2/13

4.3.3.4 Parsemus Foundation, San Francisco, CA

The California-based Parsemus Foundation's website (parsemusfoundation.org) describes its purpose as "finding low-cost solutions neglected by the pharmaceutical industry."Although the foundation is heavily involved in human health-related projects with special interest in non-hormonal human male contraception, contraception and fertility control in dogs and cats is also one of the foundation's key areas of interest. The foundation website lists the following dog and cat projects as receiving its support:

- Use of calcium chloride testicular injection for neutering male dogs and cats (several collaborators)
- Confirmatory studies of ultrasound non-surgical sterilization (Dr. Raffaella Leoci, University of Bari)
- Research into transcervical intrauterine contraception or sterilization for medium and large female dogs (several collaborators)
- Promoting a transition from the use of ovariohysterectomy to ovariectomy to sterilize female dogs
- A demonstration video focused on ovary-sparing spay as an option in cases in which the health benefits of keeping the ovaries are deemed to outweigh the health risks (Dr. Michele Kutzler, Oregon State University)

The foundation's most extensive involvement in non-surgical spay and neuter has been in calcium chloride testicular injection for male dogs and cats, specifically, funding completion and publication of studies in India (Dr. Kuladip Jana, Bose Institute) and independent confirmatory trials in Italy (Dr. Raffaella Leoci, University of Bari). According to personal communication with Parsemus Medical Director, Elaine Lissner: "The foundation seeks to raise awareness of the calcium chloride approach and of the opinion of several regulatory experts that in certain circumstances and under certain conditions, veterinary use of compounded calcium chloride injection may be justified based on current published literature and the publication of the Italian results expected in early 2013." (See section 3.4.2 for more on calcium chloride and an ACC&D-issued statement and review of current studies on this technology (http://www.acc-d.org/ACCD%20docs/ACCD-RecommCalcChlor2.pdf), and Chapter 6 for an overview of regulatory pathways for new drugs intended for use in dogs and cats.)

4.3.3.5 600 Million Stray Dogs Need You, Pompano Beach, FL

600 Million Stray Dogs Need You (600 Million) was founded and continues to be led by PETA co-founder Alex Pacheco to address dog overpopulation, prevent deaths due to bites by dogs with rabies, provide "practical and affordable alternatives to labor intensive and expensive surgical sterilization," and "enable government animal control and rabies agencies to reduce their use of inhumane animal control methods." The group's focus is on developing an oral, single-dose sterilant for dogs (or "Super Birth Control Pill"). The website refers to "formulas which thus far are all safe when used as intended" and notes that due to expense "the work … is outside the US." Funds are actively sought on the group's website to advance the research. No information on the approach(es) being worked on is provided. In 2010, 600 Million began promoting its efforts, referencing a technology that was "in-hand." At that time, the organization had a relationship with SenesTech, an Arizona-based company working on chemical-sterilant-based contraceptive products for use in rats and dogs (see section 4.2.4.6) but that relationship has since been terminated by SenesTech. The current 600 Million website refers to a relationship with Planned Pethood International, which is "the non-profit division" of Denver-based Planned Pethood Plus, Inc. These two entities appear to be focused on companion animal welfare, including encouraging and providing spay/neuter services.

4.3.4 Government

4.3.4.1 United States Department of Agriculture National Wildlife Research Center, Fort Collins, CO

See information in Chapter 3, section 3.2.4, which is related to the GnRH vaccine GonaCon, developed by NWRC, approved for contraception of white-tailed deer in the US and tested in cats and dogs. GonaCon has also been incorporated into a simultaneous rabies/immunocontraceptive vaccine protocol by researchers at USDA, CDC in Atlanta, GA, and Navajo Nation Veterinary Program. See below and also Chapter 3, section 3.2.4.1 for information about simultaneous use of GonaCon and a commercially available rabies vaccine.

4.3.4.2 United States Centers for Disease Control and Prevention, Atlanta, GA

As described in Chapter 3, section 3.2.4.1, researchers at the CDC have been investigating simultaneous administration of a canine immunocontraceptive vaccine and rabies vaccine, as well as combining a canine immunocontraceptive vaccine and rabies vaccine that can induce appropriate dual immunological responses against both rabies virus and immunocontraceptive targets following a single administration in animals. A 2009 publication described a study in which GonaCon, USDA's GnRH immunocontraceptive vaccine for white-tailed deer, was administered simultaneously with a commercial rabies vaccine. In that study researchers concluded that "the use of … GonaCon did not affect the ability of dogs to seroconvert in response to the rabies vaccine. Thus, GonaCon provides a potential immunocontraceptive for use in combination with rabies vaccine to increase herd immunity and address dog population over abundance to better manage rabies (Bender et al. 2009)." A 2009 study (Wu et al.) described the development of a recombinant combination rabies/immunocontraceptive vaccine. See Chapter 3, section 3.2.4.1 for information on the Bender et al. study, the Wu et al. study, and a subsequent Lecuona et al. study (2012).

US patent application 13/062680, entitled *Rabies Virus-based Recombinant Immunocontraceptive Compositions and Methods of Use,* was filed on August 20, 2009 and published on July 7, 2011. Inventors are listed as Xianfu Wu and Charles E. Rupprecht of the CDC, and the assignee is listed as "The Government of the United States of America as represented by the Secretary of the Department of Health and Human Services, Centers for Disease Control and Prevention." The invention is characterized as follows:

> "Described herein are recombinant rabies viruses comprising a heterologous nucleic acid sequence encoding an immunocontraceptive protein, such as gonadotropin-releasing hormone (GnRH) or zona pellucida 3 (ZP3). The recombinant rabies viruses disclosed herein are recovered by reverse genetics, replicate efficiently, elicit rabies virus neutralizing antibodies and immunocontraceptive peptide-specific antibodies in vaccinated

animals, and protect vaccinated animals against wild-type rabies virus challenge. Further provided is a method of immunizing a non-human animal against rabies virus infection and simultaneously inhibiting fertility of the animal, comprising administering an immunogenic composition comprising one or more of the recombinant rabies viruses described herein" (freepatentsonline.com/y2011/0165189.html).

Note that the invention described above refers to recombinant rabies virus being "combined" with either GnRH or ZP.

With the help of local animal welfare advocates, the CDC is striving to raise $125,000 for a field trial in dogs, presumably related to the project described above. Few details are available (Briggs, personal communication 2012).

4.3.4.3 Cooperative Research Centres, Australia

The Cooperative Research Centres (CRC) program was inaugurated by the Australian Federal Government in 1990 to unite researchers from universities, government labs (including the Commonwealth Scientific and Industrial Research Organization (CSIRO)), private industry, and public-sector organizations in long-term collaborations to support R&D and education activities "that achieve real outcomes of national economic and social significance." At the time of the 2002 *Contraception and Fertility Control in Animals* report, there were three CRCs involved in work in this area: CRC for Vaccine Technology, CRC for the Biological Control of Pest Animals, and Marsupial CRC. None of these appears in a listing of 2010-2011 CRCs on the Encyclopedia of Australian Science website (www.eoas.info/bib/ASBS02735.htm).

CRC work on contraception and fertility control involves pest species only; the July 2012 CRC website refers to developing tools for "major pest species" which include "wild dogs" and "feral cats." Projects include but are not limited to the following studies: to develop new monitoring technologies to better target and monitor wild dog, fox and feral cat control programs; to develop an integrated, cross-tenure strategy, which manages or eradicates feral goat, deer, pig, and cat populations on Kangaroo Island using effective control measures, including new technologies PIGOUT® and HOG-GONE® [both poisons]; to test an alternative approach to managing foxes and feral cats – automated control using spray tunnel technology through targeting the propensity of both species to orally groom; to understand community attitudes to fauna conservation, control of feral and stray cats, and containment of domestic cats, to smoothly implement conservation policies (see invasiveanimals.com/wp-content/uploads/2011/11/Research-Portfolio-2010-11-Compressed.pdf for information). The CRC is also working with SenesTech, Inc. (see 4.2.4.6) on SenesTech's rat contraception product, ContraPest.

4.3.4.4 AFSSA Nancy, Wildlife Health and Management Unit, Malzéville, France

Dr. Franck Boué of Agence Française de Sécurité Sanitaire des Aliments (AFSSA) Nancy presented research related to development of a sperm-antigen-based immunocontraceptive vaccine for canine species at the 1st and 2nd International Symposium on Non-Surgical Methods of Pet Population Control (see section 4.3.1).

Later work by the AFSSA team (Verdier et al. 2005) was carried out to clone and sequence the fox antigenic proteins fSP8 and fSP13 as a precursor to developing methodology to characterize the antigens identified in the study described in 2002.

4.3.4.5 Food and Environment Research Agency, United Kingdom

The mission of the United Kingdom (UK) Food and Environment Research Agency (FERA) is to "support and develop a sustainable food chain, a healthy natural environment, and to protect the global community from biological and chemical risks." This includes dealing with the challenge of "humane, effective and sustainable dog population management." In a 2010 joint FERA-USDA publication the authors noted that an estimated 75% of the approximately 500 million dogs in the world "are free to roam and reproduce and may have a negative impact on human activities" (Massei et al. 2010).

In September 2012, FERA, in co-operation with the World Health Organization (WHO), World Organization for Animal Health (OIE), Royal Society for the Prevention of Cruelty to Animals (RSPCA), WSPA, International Fund for Animal Welfare (IFAW), and Humane Society International

(HSI), sponsored the 1st International Conference on Dog Population Management. At this conference, FERA expressed its continued interest in being involved with research on the use of GonaCon in free-roaming dog populations (see section 3.2.4.1). Conference abstracts are available for download from https://secure.fera.defra.gov.uk/dogs2012/bookOfAbstracts.cfm.

4.3.4.6 The AZA Wildlife Contraception Center, St. Louis, MO

Based at the St. Louis Zoo, the AZA Wildlife Contraception Center (WCC) provides zoos with information regarding contraception of captive wildlife, including numerous species of canids and felids. The Center collects data on use of contraceptives in zoos throughout the country and develops recommendations based on its communications with zoos and tissue pathology from its Health Surveillance Program. A recent project sought to better understand side effects related to use of deslorelin (Suprelorin) implants in female canids through a "retrospective analysis of medical records and pathology reports from more than 1000 subjects." The Center provides information about a variety of contraceptive interventions, including progestins, GnRH agonists, vaccines, surgery, and nicarbazin (for birds). Recommendations and cautions about products and their use are included (www.aza.org/wildlife-contraception-center). For more information, visit stlzoo.org/animals/scienceresearch/contraceptioncenter/.

5.0 Marketing Overview and Issues

5.1 Overview

Non-surgical dog and cat contraception, whether permanent or reversible, is regarded as a market with significant potential due to the sheer numbers of animals, and the general commitment of a large number of pet owners in many countries to control their animals' fertility. In addition, pet owners are increasingly expecting more pet healthcare options, and although several products have emerged, historically there have been few choices for non-surgical fertility control for the majority of pets. The market for non-surgical approaches also includes nonprofit organizations and government agencies that commit resources to controlling populations of unowned pets; this market segment may be expected to appreciate and embrace the benefits of being able to achieve dog and cat population control without the need for surgery.

The belief that non-surgical alternatives to pet population control represent a viable market is illustrated by the increasing extent of ongoing research and development efforts to create products that offer non-surgical approaches for pets and stray or feral animals. In fact, since the original *Contraception and Fertility Control in Animals* report was published in 2002, several products have been approved, for male dogs at least, and are available in various markets

The dog and cat "contraception and fertility control market" is, in fact, a number of markets at various stages of development. After some false starts and decades of research, several commercially viable products for specific applications in contraception and fertility control in animals have been approved in certain markets (e.g., Suprelorin® from Peptech/Virbac, Esterilsol®/Zeuterin™ from Ark Sciences – see Chapters 3 and 4). Because no single technology developed to date is suitable for cats and dogs and all segments of the market, researchers are continuing to refine long-standing approaches and pursuing relatively early stage technologies as well, and significant challenges to serving the broad market continue to exist.[9]

The unmet needs in the companion-animal contraception and fertility control market represent opportunities for animal health companies. Historically, however, "big pharma" animal health has been reluctant to develop products either for domestic dogs and cats or so-called low-profit segments, such as feral animals and wildlife. (Note that wildlife is beyond the scope of this document.)

Aside from the traditional obstacles to developing, delivering, and commercializing pharmaceutical and biological products (e.g., cost, business and competitive risks, scale-up and manufacturing issues, overall length of time it takes to develop products and obtain regulatory approvals), contraception and fertility control in dogs and cats present specific challenges.

The combination of different technologies, diversity among stakeholders, and the presence of very visible low-margin segments presents a challenge that can be expected to keep marketing managers awake at night. What kinds of things will they be thinking about?

There is no single approach, formulation, technology, or product to date for use in dogs and cats.

It is an understatement to say that the needs of dog and cat owners, shelters and welfare groups, and the veterinary professionals who serve these groups differ.

There are important end-user preferences that affect the ultimate attributes of products – short-term, long-term, or permanent sterilization; injection, implant, or oral formulation; and suppression or maintenance of sex hormone influenced behaviors.

The following assumptions have typically been made by individuals and groups interested in contraception and fertility control in dogs and cats:

- Spay/neuter clinics and organizations sponsoring programs such as Trap-Neuter-Return (TNR) would like something inexpensive, easy to administer, 100% effective, and permanent or long lasting for feral and adoptable animals. It should be possible to distinguish treated animals from untreated animals.
- Companion animal veterinarians may value a treatment that brings pet owners back to the practice on a regular basis, and seek a treatment that is dependable, safe,

[9] See Chapter 4, section 4.3.3.2, regarding the Michelson Prize & Grants for information about a major effort to discover and develop a commercially viable approach to permanent sterilization that is effective in male and female dogs and cats.

profitable, simple and fast, and provides the presumed long-term health benefits associated with surgical spaying and neutering. See Chapter 3, section 3.1.1 for a discussion of pros and cons of gonadectomy in dogs and cats. It is interesting to note that in a 2007 survey of small animal veterinarians, 21% indicated that there is a great need for a non-surgical alternative and 45% indicated there is no need; 51% of shelter veterinarians surveyed in 2008 indicated there is a great need and 16% indicated they felt there is no need (see section 5.6 for more information on these surveys).

- Some pet owners may prefer a permanent solution, and others object to the permanence of sterilization or may wish to breed their animals someday and therefore would like an approach that "wears off."
- For many pet owners, suppression of sexual behavior is important – they don't want their female dogs and cats coming into estrus, and owners of male dogs want to reduce marking, mounting and aggression. See Figure 5-2 and Tables 5-20 and 5-22 for reasons pet owners do and do not have their dogs and/or cats spayed or neutered.
- Breeders (i.e., reputable breeders) and owners of show animals need the flexibility of reliable fertility control and reduced sexual behavior when an animal is participating in a show or event, but want normal fertility when their animals are scheduled for breeding, or have retired from competition, to produce future champions

There are many potential customer segments and stakeholders – even within a given group there are different needs, and there are societal factors too.

Just as there is a range of technologies at different stages, there is a range of stakeholders, i.e., groups with an interest in contraception and fertility control in dogs and cats, including animal health companies; biotechnology and life sciences companies; nonprofit and governmental agencies that sterilize animals for adoption or offer community sterilization services; veterinary schools and research institutions; veterinarians; pet owners; show animal owners; breeders; regulatory agencies; and animal welfare and animal rights groups with a variety of philosophies – not to mention the animals themselves.

Since stakeholder characteristics, beliefs, needs, and wants vary among and within customer and key influencer groups, the need for extensive market research, targeting, prelaunch programs, and public relations will add to marketing costs and complexity.

Companies interested in pursuing opportunities for contraception or fertility control in animals will want to have a thorough understanding of market size and market characteristics. Knowledge about stakeholder interactions and factors that drive the price/value relationship for each end-user segment under consideration will also be important. Companies will need to consider which approach best fits where a given company is, where it wants to be, and how it wants to get there. Deciding which technology to embrace and how to define and approach such a complicated marketplace can be a "balancing act" – weighing company culture, strategic direction, internal resources, existing customer base, and product portfolio to determine the most commercially and strategically viable approach. In order to help optimize the potential of any opportunity, an analysis should be undertaken as a part of the long-range planning process, ideally before initiating product development or forming alliances, but certainly before final decisions regarding product attributes are made.

In summary: The attributes of a given product are a combination of the inherent characteristics of the science and technology behind that product; the needs, plans and competencies of the organization involved in marketing it; and the needs and economics of the mix of groups that influence the dynamics of the particular market segment. There's nothing unusual about that in and of itself – but in a market as diverse as contraception and fertility control in cats and dogs, each segment brings its own set of challenges, and, in addition to the typical costs related to product launches, first-in-market companies can be expected to bear the market-definition, education, and public relations costs inherent in bringing "pioneer" products to market. This is further complicated by the complex mix of politics and advocacy associated with pet population control and regulations aimed at dog and cat sterilization.

Potential customer segments have different expectations.

Technical characteristics, advantages, and disadvantages of approaches to non-surgical contraception and fertility control in dogs and cats vary from species to species (see Chapter 3) and, as noted above, the profile of an "ideal" product differs among end-user segments (i.e., customer groups). There are also considerations that don't relate strictly to product attributes.

For example, a contraceptive technology that produces permanent sterilization in male dogs will be effective for a pet dog as well as a stray dog. Some of the needs of the nonprofit or governmental agencies that sterilize animals for adoption or offer community sterilization services are different than those of a veterinarian with only a percentage of clients who want permanent sterilization for their male dogs – the shelter needs to desex animals quickly and economically before adoption or release; the veterinarian needs a certain margin to cover overhead and make a profit. Consequently, cost is an issue that could serve as a basis for realignment among stakeholders for mutual benefit.

For instance, organizations that have helped fund research by academic institutions could reallocate some of their resources to underwriting the costs of the products used in shelter and spay/neuter settings once the products they've help create are approved for commercial use in much the way donors subsidize the cost of surgical spay and neuter now. Shelter and spay/neuter groups might unite in buying groups that could bring volume business to companies that have products to sell. Joint ventures, partnerships, or businesses such as low-profit Limited Liability Companies (L3Cs) could be formed to take on different aspects of the development, commercialization, and marketing process – approaches that are already in use in the human health arena.

Of course the opportunities for realignment and alliances will depend on who brings what products to market and in what order, but these types of models will have to evolve if important but traditionally nonprofit segments, such as "unowned" and feral cats and dogs, are to be served meaningfully.

5.2 "Owned" Dogs and Cats

The most obvious market to target is pet owners caring for one or more cat or dog and willing and able to pay for treatment. This section reviews information on several world markets to provide a feel for the size of the owned pet market and trends in purchase of products and use of veterinary services.

5.2.1 Population and Spending Estimates

5.2.1.1 North America

5.2.1.1.1 United States

Sources vary slightly regarding the number of pet dogs and cats in the United States (US).

Table 5-1: Overview of US Pet Dog Population

	APPA	PFI	AVMA	Estimate or Average (if multiple)	US Human Population	Dogs/ Person
	In millions					
1990	52.7			52.7	248.7	0.2119
1996	54.6	55.7		55.15		
2000	68.0			68	281.4	0.2416
2001			61.6	61.6		
2003		61.5		61.5		
2004	73.8			73.8		
2005						
2006	74.8		72.1	73.45	298.6	0.2456
2007						
2008	77.5			77.5	304.4	0.2546
2009						
2010		65.5		65.5	308.7	0.2154
2011	78.2	65.9	69.9	71.3	310.5	0.2296

The growth in the population of dogs has roughly followed the growth in human population but as noted above, statistics suggest that the rate of pet-keeping may have peaked in 2008 and declined a bit since that time.

Table 5-2: Overview of US Pet Cat Population

	APPA	PFI	AVMA	Estimate or Average (if multiple)	US Human Population	Cats/ Person
	(In millions)					
1990	60.8			60.8	248.7	0.2444
1996	66.1	67.9		67.0		
2000	72.9			72.9	281.4	0.2591
2001			70.8	70.8		
2002	77.7			77.7		
2003		78.35		78.35		
2004	90.5			90.5		
2005						
2006	88.3		81.7	85.0	298.6	0.2847
2007						
2008	93.6			93.6	304.4	0.3075
2009						
2010		78.4		78.4	308.7	0.2540
2011	86.4	79.1	74.4	80.0	310.5	0.2576

Sources (Tables 5-1 and 5-2): Pet Food Institute (PFI) website lists recent source of data as Euromonitor International. Earlier resources were Ipsos-NPD (2001); American Pet Products Association (APPA) Executive Summary, 2009; en.wikipedia.org/wiki/Demographics_of_the_United_States; 2011 US population US Census Bureau estimate (census.gov/population/www/popclockus.html); American Veterinary Medical Association (AVMA) statistics (AVMA.org)

The growth in the population of cats has roughly followed the growth in human population. However, recent statistics suggest that the rate of pet-keeping may have peaked in 2008 and declined a bit since that time. The most recent American Veterinary Medical Association (AVMA) survey indicates a decline in the US dog and cat population between 2007 and 2011. This may have been a function of both economic factors during the recession, as well as changes in the US population demographics.

- Pet cats can be separated into two groups: cats that are strictly indoor cats (~50%) and cats that are outside at least part of the time (50%) (Rowan, personal communication 2012).
- In 2011, 62% of households had at least one cat or dog, outnumbering households without a cat or dog (APPA 2011).

The APPA provides the following additional relevant statistics for 2011:

Table 5-3: Miscellaneous Statistics

Dogs	Cats
■ 39% of US households own at least one dog	■ 33% of US households own at least one cat
■ 60% of owners own one dog	■ 52% of owners own more than one cat
■ 28% of owners own two dogs	■ On average, owners have two cats (2.2)
■ 12% of owners own three or more dogs	■ More female than male cats are owned (80% vs. 65%)
■ 21% of owned dogs were adopted from an animal shelter	■ 21% of owned cats were adopted from an animal shelter
■ On average, dogs owners spend $248 on veterinary visits per year	■ On average, cat owners spend $219 on veterinary visits per year
■ 78% of owned dogs are spayed or neutered	■ 88% of owned cats are spayed or neutered

Historically the trend in dog and cat population tends to follow the increase in the total number of households. Household life stage is an important determinant of pet "ownership." Almost 80% of families with children aged 5-17 have pet dogs or cats while only about 40% of single person households have dogs and cats (Rowan 2008).

According to data from the AVMA *US Pet Ownership and Demographics Sourcebook* (2012) (veterinarynews.dvm360.com/dvm/article/articleDetail.jsp?id=784088):

> "The percentage of pet-owning households declined 2.4 percent over the last five years, according to a study of 50,000 pet owners conducted by the American Veterinary Medical Association (AVMA). The percentage of households owning dogs decreased 1.9%, while households owning cats declined 6.2%, although cats still outnumber dogs as pets.
>
> "Changes in the total number of dogs and cats owned as pets are even more dramatic. There were 69.9 million pet dogs at the end of 2011, a decrease of 3% from 72.1 million in 2006, and 74.4 million pet cats in 2011, a decrease of 9.4% from 81.7 million in 2006. The decline in the total number of pet dogs and cats in the US from 2006 to 2011 is 6.4%."

These figures differ from the 2011 APPA figures presented in Table 5-4, which may be due to survey population demographics, methodology, and/or timing.

An analysis of data from several surveys provided the following information regarding sources of pet dogs and cats several years ago (Rowan 2008):

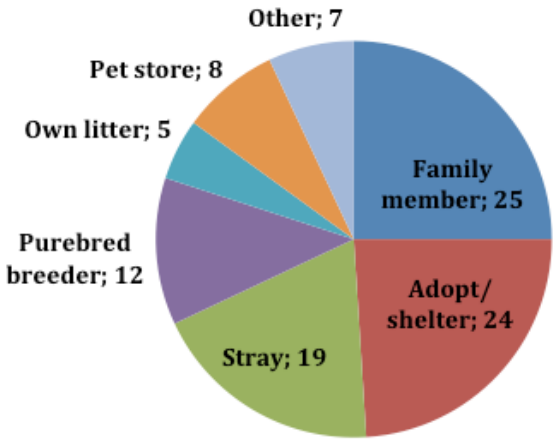
Table 5-4: Sources of Pet Dogs and Cats

Source	Dogs (%)	Cats (%)
Family/friend/neighbor, etc.	35%	28%
Stray	8%	28%
Offspring of own animal	9%	15%
Shelter/rescue/adoption	12%	12%
Pet store/newspaper	9%	3%
Breeder	17%	1.5%
Internet	0.5%	0%
Other (e.g., gift, veterinarian)	9.5%	12.5%

This source (Rowan 2008) indicated data such as these should be used as a guide only. Generally it is reported that somewhere around 15+ percent of animals are adopted from shelters or rescue centers, that a large number of cats "adopt" their owners spontaneously (e.g., they wander in off the street and stay), and that breeders and pet stores are a more important source for dogs than for cats.

A survey commissioned by PetSmart Charities® (2009) found that owners acquired their dogs and cats from the following sources:

Figure 5-1: Sources of Pet Dogs and Cats in the US by Percentage



Source: PetSmart Charities 2009

Although figures tend to vary somewhat from source to source, *Pet Product News* reports that in 2011, US pet owners spent an estimated $51 billion on their animals, broken down as shown below (petproductnews.com):

Table 5-5: Pet Owner Expenditures

Category	2010 Actual	2011 Actual	% Growth, 2010-2011	2012 Estimate	% Estimated Growth, 2011-2012
	($ billions)	*($ billions)*		*($ billions)*	
Food	18.76	19.85	5.8	20.46	3.1
Supplies/OTC medications	10.94	11.77	7.6	12.56	6.7
Veterinary care	13.01	13.41	2.9	13.59	1.3
Live animal purchases	2.13	2.14	0.5	2.15	0.5
Other services	3.51	3.79	7.9	4.11	8.4
Total	48.35	50.96	5.3	52.87	3.8

Source: americanpetproducts.org/press_industrytrends.asp

5.2.1.1.2 Canada

At the 2006 Banff Summit for Urban Animal Strategies (BSUAS), delegates indicated that a lack of reliable Canadian statistics was hampering municipal leaders and legislators in their effort to develop urban animal strategies (Perrin 2009). As a result, a national survey on urban animals was conducted in late 2008. The survey provided the following information:

Table 5-6: Overview of Dog and Cat Ownership in Canada

Cats	Dogs
35.5% of Canadian households included a cat • 4,820,085 households • 1.76 cats/household • 8,510,021 cats in Canada	**32.3% of Canadian households included a dog** • 4,384,978 households • 1.38 dogs/household • 6,070,783 dogs in Canada

Table 5-7: Age Breakdown of Canadian Dogs

	Dogs
Average age	**5.9 years**
< 1 year	7%
1-3 years	29%
4-7 years	30%
8-10 years	11%
10+ years	22%

A total of 50% of cats and 22% of dogs had not been to a veterinarian in the past 12 months. Of those that did receive veterinary care, 34% of all cats and dogs went only once during the 12-month period; 13% of cats and 34% of dogs were seen 2-3 times. Seventy-nine percent of cats and 69% of dogs were spayed or neutered. A total of 66% of respondents acknowledged that spaying or neutering was recommended by their veterinarian, was good value for the money spent, and was done with the best interest of the pet in mind. Sixteen percent of owners of "mostly indoor pets" believed that sterilization was not necessary for their animals.

Table 5-8: Annual Estimated Cost of Care (CA$)

Category	Cats	Dogs
Veterinary care	$287-294	$360-451
Kitty litter	$91-100	-
Food	$306-372	$510-514
Collars, leashes, toys, miscellaneous supplies	$36-53	$36-53
Pet insurance	$270-360	$408-455
Licensing	-	$25-39

5.2.1.2 Outside of North America

5.2.1.2.1 Europe

There are 84.7 million cats and 73.6 million dogs living in European households; 64.4 million cats and 60.2 million dogs are living in the European Union (EU) countries (FEDIAF Facts & Figures 2010).

- Estimated number of European households owning at least one pet:
  - 70 million (excluding Russia)
- Estimated percentage of European households owning at least one cat or one dog:
  - EU
    - Cats: 24%
    - Dogs: 27%
  - Europe
    - Cats: 25%
    - Dogs: 26%
- In the United Kingdom (UK), the pet population in 2009 was 8 million dogs and 8 million cats. Approximately 6 million households owned at least one dog and 5.2 million households owned one or more cats.

Table 5-9: Top 10 European Countries: Dog and Cat Populations 2010

Country *(alphabetical order)*	Dog Population	% of Households Owning at Least One Dog	Cat Population	% of Households Owning at Least One Cat
Czech Republic	3,152,000	43%	1,750,000	22%
France	7,595,000	23%	10,965,000	26%
Germany	5,300,000	13%	8,200,000	16%
Italy	7,000,000	21%	7,400,000	19%
Netherlands	1,493,000	19%	2,877,000	26%
Poland	7,311.000	38%	5.550,000	30%
Romania	4,166,000	43%	3,891,000	42%
Russia	12,520,000	20%	18,000,000	33%
Spain	4,720,000	27%	3,385,000	21%
United Kingdom	8,000,000	22%	8,000,000	18%

Source: FEDIAF Facts & Figures 2010

5.2.1.2.1.1 United Kingdom

Dog ownership has been declining, while cat ownership is increasing. Surveys conducted between 2008 and 2010 indicate that 46% of households in the UK own either a dog or cat or both (pfma.org.uk/statistics). Among dog-owning households, 78.5% have one dog; the rest have two or more. Among cat-owning households, 62.2% have a single cat and the rest have two or more. More people between ages 35 and 44 are cat owners while more people between 45 and 54 are dog owners. The bulk of owned dogs are medium (26%) and large (43.6%) sized. Small dogs (20.8%), toy dogs (5.7%), and giant dogs (3.8%) round out the population. Dog ownership is higher in urban locations. Approximately 59% of pet dogs are purebreds.

A paper summarizing the results of a random sample of 2,980 UK households indicated that in 2006, 26% and 31% of households owned cats and dogs, respectively. The actual populations extrapolated from the data collected showed a virtually equal number of owned cats (9.34 million) and dogs (9.62 million) in the survey timeframe. Notably these earlier data are higher than the 2010 estimates of 8 million each of dogs and cats in the UK. Households with outdoor space were more likely to own cats and dogs than households without outdoor space (i.e., "garden"). Households that were classified as more educated were more likely to own cats and less likely to own dogs than other households. Cats were more likely to be owned by semi-urban and rural households and by female respondents. Dog ownership significantly decreased the likelihood of cat ownership, and respondents aged 65 years or more were less likely to report that their household owned a cat than younger respondents. Households with one or more dogs and children aged 11-15 years were more likely to own a cat than other households. The likelihood of dog ownership increased as household size increased. Dogs were more likely to be owned by rural households, and less likely to be owned by households with cats or children aged 10 years or younger. Female respondents and those aged 55 or less were more likely to report dog ownership than other respondents (Murray et al. 2010).

5.2.1.2.1.2 France

There is at least one pet cat or dog in more than 50% of households. Forty-one percent of dogs and 37% of cats live in rural households; 30% of dogs and 28% of cats live in cities and towns with fewer than 100,000 residents; and 29% of dogs and 35% of cats live in cities of 100,000+ people. About 53% of families that own companion animals have one dog or cat; 45% have a dog and a cat. The majority of people who own a dog and/or cat say they acquired the animal(s) because they love animals, like the company, and/or want the pets for their children (FEDIAF Facts & Figures 2010, Reichler 2008).

5.2.1.2.1.3 Germany

The number of dogs is declining while the number of cats is increasing. There is at least one dog in approximately 13% of German households, and 16% of German households have one or more cats (FEDIAF Facts and Figures 2010, Reichler 2008). Dog and cat owners in

Germany spent $2.7 billion on pet food in 2009 (FEDIAF Facts and Figures 2010). FEDIA Facts and Figures 2010 is available at: www.fediaf.org/fileadmin/user_upload/facts_and_figures_2010.pdf.

5.2.1.3 Cat and Dog Populations vis-a-vis Human Populations: Europe and US

One way to look at the value of pets in a given society is to look at pet spending per the total human population, i.e., per capita. For example, spending on pets is about $100/person/year in the US; $20/person/year in Brazil, which is rapidly growing into a pet culture; and about $1/person/year in China. So although the total spending on pets in the US was reported to be just over $50 billion in 2011, and $1 billion-to-$2 billion in China, the US is a far more pet-oriented culture, as evidenced by the per capita pet-related spending (Rowan, personal communication 2012).

It is also interesting to understand what those numbers mean in terms of the human population. Given that some people have more than one cat or dog, these numbers are indicators, not precise measures of animals/person or people/animal (FEDIAF Facts and Figures 2010); however they do indicate the place a pet dog and cat holds in a given culture (Rowan, personal communication 2012).

Table 5-10: Dogs/Person and People/Dog and Cats/Person and People/Cat in the Top 10 European Countries and the US

Country	Humans	Dogs	Dogs/Person	People/Dog
Russia	142,905,208	12,520,000	0.0876	11.4
United Kingdom	62,041,708	8,000,000	0.1289	7.8
France	63,460,000	7,595,000	0.1197	8.4
Poland	38,192,000	7,311,000	0.1914	5.2
Italy	60,418,711	7,000,000	0.1159	8.6
Germany	81,757,600	5,300,000	0.0648	15.4
Spain	47,150,800	4,720,000	0.1001	10.0
Romania	19,042,936	4,166,000	0.2188	4.6
Czech Republic	10,535,811	3,152,000	0.2992	3.3
Hungary	9,979,000	2,856,000	0.2862	3.5

	Humans	Cats	Cats/Person	People/Cat
Russia	142,905,208	18,000,000	0.1260	7.9
France	63,460,000	10,965,000	0.1728	5.8
Germany	81,757,600	8,200,000	0.1003	10.0
United Kingdom	62,041,708	8,000,000	0.1289	7.8
Italy	60,418,711	7,400,000	0.1225	8.2
Poland	38,192,000	5,550,000	0.1453	6.9
Romania	19,042,936	3,891,000	0.2043	4.9
Spain	47,150,800	3,385,000	0.0718	13.9
Netherlands	16,696,700	2,877,000	0.1723	5.8
Hungary	9,979,000	2,240,000	0.2245	4.5

United States	Humans	Dogs	Dogs/Person	People/Dog
	308,745,538	78,200,000	0.253283	3.9
United States	Humans	Cats	Cats/Person	People/Cat
	308,745,538	86,400,000	0.279842	3.6

Source: FEDIAF Facts and Figures 2010, APPA 2011

5.2.1.4 Other Areas

5.2.1.4.1 Australia

According to 2009 figures, there are 3.4 million pet dogs and 2.35 million pet cats in Australia. Pet population numbers in Australia fluctuated during the 15 years prior to the 2009 assessment, with a moderate decline in dogs numbers noted since 1998, when the dog population was estimated at 4 million. Cat numbers rose in 2009 following a 23% decline in cat numbers from 1994 to 2007. In 2009, consumers spent AU$6.02 billion on pets, pet care products, and services. Spending on dogs accounted for almost 60% ($3.6 billion); cats accounted for 24% ($1.4 billion). Veterinary services represented the largest segment at $2.22 billion. Pet food was the second largest section, with consumer expenditures totaling $1.83 billion. The market appears to be transitioning to premium pet foods, which is believed to be the result of pet owners prioritizing the health and well-being of their pets. There are pet dogs in 6 million households (23%) and pet cats in 5.2 million households (20%) (Australian Companion Animal Pet Population Trends, 2009 at acac.org.au/pdf/ACAC%20Report%200810_sm.pdf).

In 2009, Australian pet owners contributed AU$3.6 billion to the country's economy. Australians spent a yearly average of AU$135 per pet dog and AU$33 per pet cat (acac.org.au). Overall, consumer expenditures on pets in Australia in 2009 can be broken down as follows:

Table 5-11. Overview of Consumer Expenditure on Pets in Australia in 2009

Category	AU$ million	% of total
Veterinary services	$2,219	36.9%
Pet food	$1,826	30.3%
Pet care services	$1,041	17.3%
Pet purchases	$616	10.2%
Pet care products	$319	5.3%
Total	**$6,021**	

Source: acac.org.au

5.2.1.4.2 Asia (Japan and China)

5.2.1.4.2.1 Japan

The Japanese pet market has become a trillion-yen (US$12.7 billion at September 2012 conversion rate) industry; in fact, there are now more pet dogs and cats in Japan than children under the age of 15 (Japan Pet Food Association). Pets are becoming valuable members of the family, a phenomenon unheard of 30 or 40 years ago.

Today, there are about 13.1 million pet dogs in Japan, compared to 6.6 million in 2006 and 3.7 million in 1989. In Tokyo, 410,000 dogs were registered in 2005, 1.6 times higher than in 1995. *Shukan Economist* traces the origin of the pet boom to the 1980s when the Japanese economy began to grow and Golden Retrievers become status symbols. A subsequent economic downturn caused the pet boom to subside. A 2000 TV commercial featuring a Chihuahua is believed to have reignited the pet boom, which continues, particularly for small dogs. There are pet spas, gyms, nursing care, hotels, insurance, funerals, and clothing and accessories. Dog parks apparently serve as places for pet owners to meet one another, and *koen debut* is a term used to describe the first visit for a dog owner and pet to a new dog park – dog owners even have business cards printed up with pictures of their dogs (factsanddetails.com/japan.php?itemid=795&catid=21...145).

In a survey conducted by a pet food company in 2004, Japanese dog owners provided the following reasons for having pet dogs (multiple responses permitted):

Table 5-12: Reasons Japanese Dog Owners Have Dogs (2004)

Reason	% of Owners Citing
They like dogs	66.3%
Dogs are fun to be around	65.6%
Dogs are affordable	50.4%
Dogs enrich daily life	50.4%
Dogs contribute to family life ("are indispensable for family communication")	42.1%
Dogs guard the home	28.7%

Source: factsanddetails.com/japan.php?itemid=795&catid=21...145

Information for the year 2009 indicates that the total value of the "pet market" was 1.2 trillion yen, which includes companion animals, fish, birds, and reptiles. One-third of that amount is spent on pet food – food for dogs comprises 53% of the market, cats 40%, and other pets 7%. Pet-related expenses per household averaged 18,323 yen (US$233 at September 2012 conversion rate). In single-person households, women spent more than men on their pets, with women 35-39 spending the most money on their pets. Small-breed dogs are the most popular, with nearly 70% weighing less than 10kg (22 lbs); 73% of these dogs are indoor dogs. The residential housing market appears to be keeping up with the trend – in 1999 only 3% of condominiums allowed pets, a figure that had increased to 75% in 2005. The rental housing market, however, is still not a pet-friendly market and this may account at least in part for the popularity of "cat cafes," where people can go to visit with cats for a fee of about 100 yen/hour. These establishments typically serve drinks or coffee, and offer Internet access and other amenities (factsanddetails.com/japan.php?itemid=795&catid=21&subcatid=145).

5.2.1.4.2.2 China

In 2008, the *New York Times* reported "Keeping pets has become all the rage among the affluent in China, even though some Chinese still consume dog and cat meat." Euromonitor International, a market intelligence firm, forecast spending on pet food and pet care in China at an estimated $870 million in 2008, up about 15% from the $757 million spent in 2007. Note that a large percentage of that figure is related to pet birds, fish, and reptiles (Cheney 2008).

Estimates of the number of pet dogs and cats in China can vary greatly, so greatly, in fact that it is difficult to even settle on a range for this report. The *New York Times* article cited above noted that China estimates it has 150 million pet dogs (2008). Statistics are scant because many pets are unregistered. Euromonitor puts the figure at 26.8 million, and says China has 10.7 million pet cats.

In Beijing, where about 900,000 dogs were reported to be registered as of 2010, dog owners are not allowed to have dogs taller than 36 centimeters (just over 14 inches) out of concern that large dogs might frighten people; according to one source, this has led to an overpopulation of Pekinese, Pomeranians, and Chihuahuas. In Shanghai, home to an estimated 60,000 pet dogs in the early part of the 2000s, officials estimate the city was home to 740,000 pet dogs in 2011. Officials have proposed a one-dog policy and mandatory registration of dogs. Owners would also be required to sterilize their dogs. Stores catering to dog owners have sprung up in cities, and there is at least one dog park in Shanghai (factsanddetails.com/china.php?itemid=266&catid=12&subcatid=81).

In recent years the image of dogs has improved as the Chinese middle class grows. In 2011, the Beijing-based magazine *Dog Fans* estimated that the pet dog population was growing at about 30% per year; French Poodles and Rottweilers are among the popular breeds. In an October 24, 2010 *New York Times* article, writer Michael Wines notes that "people used to be focused on improving their own lives, and they weren't really acquainted with raising dogs. But with the improvement in the economy, people's outlooks have changed. There's a lot of stress in people's lives and having a dog is a way to relieve it." Another factor may also be at work in the increasing popularity of pet dogs: "Many owners also say China's one-child policy has fanned enthusiasm for dog ownership as a way to provide companionship to only children in young households and to fill empty nests in homes whose children have grown up. As the younger generation waits longer to marry and put careers ahead of having children, parents of only children are increasingly lavishing attention on furry companions as a stand-in for the grandchildren they do not have" (nytimes.com/2010/10/25/world/asia/25dogs.html?pagewanted=all).

5.2.1.4.3 Latin America

The Brazilian market is leading the development of the pet food and pet care products markets in Latin America. It appears that, as in other geographic areas, the expansion of the middle class is one of the major drivers. During the period 2005-2010, sales of pet food and pet care products rose from $4.8 billion to $8.3 billion (Euromonitor). This represents a compound annual growth rate (CAGR) of 11.9%. As a result, the Latin American market proportion of global pet care sales rose from 7.6% in 2005 to 10.2% in 2010. Brazil is by far the largest market in the region, with pet care sales valued at $5.2 billion in 2010, followed by Mexico ($1 billion) and Argentina ($645 million). In 2010

there were 155 million pets in Argentina, Brazil, Chile, Colombia, Mexico, and Venezuela combined, compared with 116 million in 1999 (Euromonitor). Chilean households were expected to overtake pet-owning households in Argentina and Brazil in terms of spending on dog and cat food. It is estimated that there are 30 million dogs and 12.5 million pet cats in Chile. There is speculation that the dog figure includes unowned dogs.

5.2.2 Dynamics of the Spay/Neuter Decision

One of the first questions that comes to mind when spay/neuter versus non-surgical sterilization is considered is what is known about the actual impact of removing an animal's sources of reproductive hormones. We have a fair idea of what they provide relative to reproduction, with several yet unidentified shared functions of hormones for an animal's well-being (Root Kustritz, personal communication).

There is an ever-growing body of literature regarding the benefits and risks of spay/neuter (see summary below) and the optimal age for gonadectomy, but the overall conclusion appears to be that there is no single answer. As non-surgical alternatives are available it is important to be able to compare them objectively to surgical methods of sterilization.

5.2.2.1 Summary of Pros and Cons of Spay or Castration in Dogs and Cats

	Pros	Cons
Female dog	Completely effective sterilant Decreased incidence of mammary neoplasia (depending on timing of gonadectomy) Decreased incidence of reproductive tract (ovarian/uterine) disease Decreased incidence of reproductive behaviors Eliminates the risk of difficult birth (dystocia)	Surgical complications Increased incidence of urinary incontinence Increased incidence of hematologic, bone, and bladder tumors Increased disposition to knee injury Obesity Possible breed-related decreased lifespan
Male dog	Completely effective sterilant Decreased incidence of reproductive tract (testicular and prostatic) disease (except prostate tumors) Decreased incidence of reproductive behaviors Possible increased lifespan	Surgical complications Increased incidence of hematologic, bone, and prostate tumors Increased predisposition to knee injury Obesity
Female cat	Completely effective sterilant Decreased incidence of mammary neoplasia (depending on timing of gonadectomy) Decreased incidence of reproductive tract (ovarian/uterine) disease Decreased incidence of reproductive behaviors Eliminates the risk of difficult birth (dystocia)	Surgical complications Obesity Possible increase in diabetes mellitus

Source: Derived from Root Kustriz 2007 and personal communication 2012, Reichler 2009, and Rhodes, personal communication 2012). Note that this table also appears in Chapter 3, along with supporting documentation.

Marketers will need to realize that perceptions of pet owners may not reflect what is actually known about non-reproductive effects of spay/neuter surgery.

For example, pet owners may have expectations that spaying and neutering will change the behavior of their pets. In some cases, that change would be desirable; in others, there is concern that the animal's personality will change from the personality the pet owner knows and loves.

Historically, the animal welfare and veterinary communities have promoted positive behavioral changes as a benefit of spaying or neutering dogs and cats; however, there is a fairly small body of research literature addressing this assumption.

In reality, the only behavioral change for which there are data following surgical castration of dogs indicate that castration is associated with decreases in indoor urine marking, roaming, sexual mounting, and dog-to-dog aggression around females in estrus; however, it does not always decrease these behaviors. Studies report inconsistent findings on how castration status may correlate to aggressive behavior towards humans.

A study of more than 6,000 dogs suggested "that spayed female dogs tend to be more aggressive towards their owners and to strangers than intact females, but that the effects of spaying on behavior appear to be highly breed-specific." Results of the study indicated that, contrary to popular belief, there is little evidence that castration is an effective treatment for aggressive behavior in male dogs, and [castration] actually may exacerbate other behavioral problems"(Hsu and Serpell 2003, cited by Duffy 2006).

Spayed female dogs and cats will not exhibit estrous behavior or vaginal discharge and bleeding, and it seems to be widely accepted that male cats will be less likely to roam, urine spray, vocalize, and fight when they are sterilized. One study (Finkler and Terkel 2010) concluded that the 36 neutered females living in a managed Trap-Neuter-Release (TNR) and feeding program had lower levels of aggression and cortisol compared to a control group of 15 intact females. Researchers note that this study makes it "possible to suggest for the first time a possible relationship between cortisol levels and aggression in free-roaming female domestic cats" and that therefore it may be "possible that TNR has an added beneficial role in cat welfare in addition to that of control of population size."

In summary, credible studies indicate that neutering reduces urine spraying and roaming in search of mates by male cats, and spaying eliminates estrous-associated behaviors in female cats, including aggression, vocalization and perhaps efforts to escape outdoors in order to mate. The impact of sterilization on canine behavior is less clear, and may be breed dependent. Sterilized males will be less likely to fight other male dogs when estrous females are present, and will likely roam less in search of females in estrus. No clear relationship has been established between aggression towards people and canine neuter status.

5.2.2.2 What Drives Owners' Decisions and What Are Some Statistics?

Information regarding what factors encourage and discourage pet owners from having their pets spayed or neutered is useful to entities developing or considering developing non-surgical approaches – which factors might be ameliorated by non-surgical alternatives and which might not. Information about spay/neuter rates, costs, and programs are likely to affect development, commercialization, and marketing decisions.

5.2.2.2.1 In the United States

A 2011 survey of pet owners in the United States (US) by American Pet Products (americanpetproducts.org/) estimates that 22% of owned dogs and 12% of owned cats have not been spayed or neutered.

Studies show that many are unclear on the appropriate age for sterilization. In one survey, 17% of the people contacted regarding the appropriate age to spay or neuter did not know. In that survey, 29% of the people contacted believed it was not appropriate to spay a female before the first estrus and 8% felt it was necessary to wait until the animal had produced at least one litter (PetSmart Charities, 2009).

Using the percentages of intact pet dogs and cats (22% and 12% respectively) and the overall pet dog and cat population figures (78.2 million and 86.4 million respectively) from the 2011-2012 APPA National Pet Owners Survey (americanpetproducts.org/press_industrytrends.asp), we can calculate the number of intact pet dogs at 17.2 million and the number of intact pet cats at 10.4 million – significant numbers in the eyes of any business development or marketing manager.

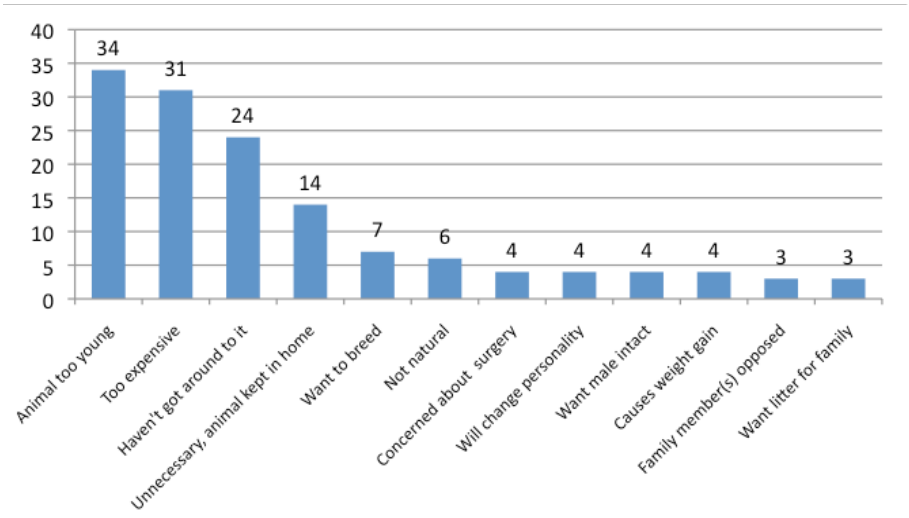
While there is certainly room to convert pet owners currently choosing surgery to non-surgical methods, these same managers will wonder why these animals haven't been spayed or neutered, and what the chances are that alternatives to surgery would encourage their owners to embrace contraception and fertility control for their pets.

Two surveys help provide insight into why dog and cat owners in the US have not spayed or neutered their

animals: a 2009 study commissioned by PetSmart Charities, as noted above; and a 2000 study commissioned by Ralston Purina®.

The 2009 PetSmart Charities study indicated the following reasons pet owners haven't spayed or neutered their dogs and cats. Responses were not broken out on a per species basis. Figures represent the percentages of respondents choosing a given reason; multiple answers from a given respondent are included.

Figure 5-2: Summary of Reasons Pet Owners Have Not Spayed or Neutered Their Dogs and Cats in the US (%)



Source: PetSmart Charities 2009

In a 2000 *State of the American Pet* study sponsored by Ralston Purina, dog and cat owners who had not had their pets spayed or neutered were asked why. Although respondents were allowed to provide more than one reason, figures represent what percentage of the time a given answer was provided compared to all answers.

Table 5-13: Summary of Reasons Pet Owners Have Not Spayed or Neutered Their Dogs and Cats in the US (2000 Ralston Purina Study)

Justification for Inaction	Dog Owners	Cat Owners	Dog/Cat Owners as a Single Group
Just haven't had it done yet	30%	27%	29%
Want to breed the animal(s) someday	21%	5%	16%
Animal(s) too young	13%	20%	15%
Procedure is cruel	5%	5%	5%
Can't afford	5%	18%	9%
Not "natural"	3%	7%	4%
Other	16%	12%	15%
Not sure	6%	9%	7%
Totals	99%	103%	100%

The table below provides the authors' subjective input regarding whether or not owners falling into a given group might or might not embrace non-surgical approaches as an alternative to spay or neuter surgery.

Table 5-14: Potential Acceptance of a Non-Surgical Alternative to Spay/Neuter

Justification for Inaction	Would They Accept a Non-Surgical Alternative?
Just haven't had it done yet	Depends on the real reason(s). These owners might: ■ view spaying or neutering as too inconvenient and time consuming ■ dislike the idea of major surgery even though they don't consider it cruel ■ believe that their animals should produce one litter before they're sterilized The availability of effective, safe alternatives to surgical spaying and neutering can be expected to appeal to pet owners who are uncomfortable with surgery or believe it's too inconvenient or cruel, but such products will not counter the "one litter" myth. Once products are available, companies, veterinarians, and advocacy groups that want to target the "one litter" consumer group will collaborate to educate owners who are holding off until their pets have a litter – according to one source, such litters are the source of an estimated 400,000 relinquished puppies in the US each year, which account for 10% of the dogs that enter the shelter system (naiaonline.org). This is likely an even bigger issue with cats.
Want to breed the animal(s) someday	Yes. Dogs and cats whose owners wish to breed them eventually are good candidates for non-permanent (aka "reversible") contraceptive products that eliminate undesirable mating-related behavior but permit a return to fertility and normal mating behavior at some time in the future. Such an approach can be expected to appeal to owners of show dogs, who understandably do not want to have to handle sexually related behaviors during competitions. To be commercially viable, such products will have to cause no harmful effects in either males or females but particularly on the treated female and on subsequent litters. Issues related to use of a given product on a pregnant animal will have to be addressed, including: ■ how the pregnancy would be affected ■ how to manage the pregnancy ■ how to address this issue in conjunction with pregnancy testing and/or ultrasound.
Animal(s) too young	Yes. However, for companies that will be marketing products shown to be safe for use in young dogs and cats, the marketing challenge will be fourfold: ■ teaching owners that animals younger than 6 months of age can be treated without ill effects ■ educating owners about the benefits of non-surgical approaches ■ demonstrating that the long-term health benefits of surgical sterilization also apply to non-surgical approaches (if they do) ■ gaining the confidence of veterinarians who have not begun to alter dogs and cats younger than ~6 months of age.
Procedure is cruel	Maybe, if the pet owner views the surgical procedure, rather than the outcome, negatively.

Can't afford	Maybe. This will depend in part upon the pricing of non-surgical alternatives relative to surgery. Maybe, especially if the reluctance reflects a combination of affordability and concerns about surgery, rather than a reluctance to spend money on the pet. Some pet owners, for whom the charge for a spay or neuter in a veterinarian's office would be too high, may be concerned about the quality of procedures done in clinics or shelters. In fact, there is a wide range of spay/neuter options and pricing available for pet owners from free or low-cost clinics and shelters to private veterinarians. When the cost of surgery is divided by the average life span of a pet dog or cat, the annualized expense is negligible. Pet owners who don't spay or neuter their animals due to expense may not have thought about the potential costs of that decision – for example, the costs of: repairing property damage caused when a male dog digs his way out of the yard to pursue a female in estrus; injuries to or from a competing male dog, necessitating veterinary care; getting picked up by the local "dog catcher" and impounded; female animals damaging furniture or carpeting when in estrus (spcala.com/). While companies marketing contraception and fertility control products should be aware of the price/value concerns of pet owners, it may be difficult to capture those pet owners who have not even taken advantage of free or low-cost surgical spaying or neutering programs that, in some cases, even include other important health services.
Not "natural"	Depends on what "not natural" means to individual owners. Is it the cessation of sexual function and/or sexual behaviors, the changed appearance in males, or a presumption that animals should be left "as nature intended?" ■ sexual function: If it is important that the pet has the potential to reproduce, the owner could consider products that are nonpermanent ■ sexual behaviors: If it is important that the pet behave "naturally," the owner could consider an approach that prevents reproduction but does not alter behavior ■ appearance of male dogs: There is a product called Neuticles®, testicular implants that can be used to preserve the appearance of male dogs and cats that have been surgically castrated. Intratesticular injections that sterilize without requiring removal of the testicles may help address this concern. ■ nature no matter what: Responsible pet ownership requires management of reproduction and sexually related behaviors.

The 2009 PetSmart Charities survey also examined reasons people *do* have their dogs and/or cats spayed or neutered:

Table 5-15: Reasons Pet Owners Have Had Their Dogs or Cats Spayed or Neutered in the US

Rationale	Male (dogs and cats)	Female (dogs and cats)
It's the right thing to do	70%	67%
To prevent unwanted pets	63%	73%
To keep my pet from reproducing	64%	71%
To reduce pet overpopulation	62%	62%
For the health benefits	35%	43%
To eliminate certain behavioral issues	50%	21%
To stop my female pet from going into estrus	7%	69% (mainly cats)
Recommended by my veterinarian	22%	21%
It helped my pet roam less	24%	17%
It helped my pet become less aggressive	27%	10%
Recommended by the place I acquired my pet	11%	12%

License fee is lower for sterilized pets	7%	8%
Recommended by my friend/family	6%	5%
Mandatory spay/neuter law in my area	3%	4%
Landlord required it	2%	2%

Source: PetSmart Charities 2009

In the US, at least two significant surveys have indicated that pet owners consider pet overpopulation to be an important issue to them. Positioning non-surgical sterilization options as tools to better combat pet overpopulation could be meaningful to consumers, given the importance of this issue to them.

The Ralston Purina *State of the American Pet* (2000) study indicates that new tools to address pet overpopulation will address the issue of most importance to pet owners. Purina asked pet owners a range of questions, including, "Thinking about our country's pet population, which of the following issues is most important to you?"

Pet owners ranked the following statements in terms of importance:

- Reducing the pet overpopulation problem 60%
- Promoting a more pet-friendly society 15%
- Increasing preventive healthcare for pets 11%
- Increasing funding for research to improve pet health 9%
- Not sure 5%

In the 2009 PetSmart Charities study, 29% of pet owners said they were familiar with the issue of overpopulation of dogs and cats and 39% had seen campaigns providing information about it or asking for support; 52% of respondents cite dogs/cats not being altered leading to unwanted litters as the main source of overpopulation and homelessness, by far the most frequent answer given.

New non-surgical sterilization options may be effectively marketed as tools to better combat pet overpopulation and reduce euthanasia, given the importance of this issue to consumers.

5.2.2.2.1.1 Legislation

For decades, many communities have offered a discounted license rate for pets that have been sterilized. This strategy, called "differential licensing," aims to raise more funds from an audience whose animals, or their animals' offspring, are more likely to contribute to animal-control costs.

In the US, mandatory spay/neuter laws in many states require at least re-homed animals from shelters to be sterilized. According to a 2010 summary on www.animallaw.info, "Releasing agencies (animal shelters, control agencies, etc.) are required in approximately 32 states … to provide for the sterilization of all dogs or cats they transfer or adopt out. Generally, releasing agencies are required to have a sexually mature dog or cat (usually six months of age or older) sterilized by a licensed veterinarian prior to releasing it to a new owner."

Looking ahead to a time when alternatives to surgery exist to sterilize cats and dogs, or to when contraception may be an alternative, "It will be important to develop interpretation of these regulations that accept non-surgical forms of sterilization" (Zawistowski, personal communication 2012).

Estimated size of the United States market for sterilization of dogs and cats

(includes "owned" and "unowned" animals; see section 5.4 for information related to "unowned" dogs and cats)

One analysis estimated the annual size of the US market for surgical sterilization of cats and dogs at 12.5 million surgeries (Briggs 2006). Of these surgeries, approximately 11 million were performed by private veterinarians, and 1.5 million were performed on a subsidized basis by "shelters" for re-homed pets and through community outreach. An estimated 200,000 subsidized surgeries were estimated to be associated with feral cat Trap-Neuter-Release (TNR) programs for maintained colonies.

The analysis was based on American Pet Products Association (APPA) population data as of 2004. Note that some sources indicate a somewhat higher dog and cat population at that time than the population estimates as of 2011, but this may be balanced by a rising rate of sterilization since that time. Nonetheless, the analysis described here did not involve an assumption related to overall pet population growth.

This estimate includes replacing the population of dogs and cats that are forecast to die annually, in part with unaltered puppies and kittens and in part with re-homed pets, a percentage of which are already altered. An analysis of shelter intake and adoption rates was used to determine number of surgeries performed by that sector, estimating the number of re-homed pets altered. Specifics of the analysis are available at (acc-d.org/2006%20Symposium%20Docs/4Briggs.pdf).

Briggs reports that at the time, an independent analysis done of this market for a completely separate project arrived at a similar figure.

Average pricing for spay/neuter surgeries in the US nationally is not generally available. Typically the nonprofit sector prices surgery at cost or below and subsidizes delivery with charitable dollars. (Note that "price" referred to the price paid for the surgery; "cost" refers to the cost to the provider of the services.)

To get a rough estimate of potential market value, Briggs assumed an average price of $200 for private practice and $50 for nonprofit clinics. Note that these averages were derived from a fairly wide range of prices for dog/cat and male/female surgeries. Under these price assumptions, the market as described above may be as large as $2.95 billion ($2.2 billion in private practices, and $751.5 million in the nonprofit sector). Charges related to current surgical methods are largely for labor, anesthesia and surgical overhead.

Progressive communities have already revised the language of laws to specifically state that dogs and cats must be surgically sterilized, spayed or neutered. In conjunction with the initial launch of Neutersol, Texas was the first state to preemptively revise the Texas Sterilization Act (Nordyke P, see specifics at acc-d.org/2006%20Symposium%20Docs/Posters.pdf). Arizona followed suit with revisions to its state language in 2011 to open doors to future non-surgical methods.

5.2.2.2.1.2 Economic/Price Sensitivity

In the 2000 study sponsored by Ralston Purina and described above, 9% of US dog and cat owners surveyed reported they had not had their dog or cat sterilized surgically due to cost. In the 2009 study sponsored by PetSmart Charities, also described above, the combined figure was 31%.

Affordable programs continue to be available, and in fact the Alliance for Contraception in Cats & Dogs (ACC&D) estimates that there are more affordable programs in 2012 than there were in 2002. It is unclear whether differences in study design affected the answers or if there was actual significant growth in the number of people seeing expense as a major challenge. This could relate to differences in the sample or changes in the economy (Briggs, personal communication 2012).

Respondents in the PetSmart Charities survey provided the following information when asked how much they would spend on a spay/neuter surgery for their pets. Note that gender and species do not appear to have been specified:

- Average for a cat: $109
- Average for a dog: $144
- 33% would spend $100 or more
- 37% would spend $51-$100
- 29% would spend $1-$50

Respondent age and region had an influence on the amount a given respondent would be willing to spend to spay/neuter a dog or cat:

- Respondents aged 18-24: $133
- Respondents aged 35-54: $128
- Respondents aged 55+: $101
- Northeast: $164
- Midwest: $121
- South: $103
- West: $145

5.2.2.2.2 Outside the United States

There appears to be no single, reliable source delineating spay/neuter rates in European countries.

This section provides input on spay/neuter practices from a variety of sources. Information regarding a given country may therefore be separated.

In a report of the results of a 2006-2007 survey of stray animal control practices in 32 countries/regions in Europe, the World Society for the Protection of Animals (WSPA) and RSPCA International (Tasker 2008) selected four locations – Slovenia, Sweden, Switzerland, and the UK – as "success stories" in terms of dealing with stray dog populations. The report describes the landscape in regards to neutering, which is typically defined as including males and females.

In Slovenia:

> "Bitches are more likely to be neutered than male dogs. Although estimates of the percentage of dogs that are neutered are not available, the member society reports that a high percentage of sexually mature females are neutered and the number of dogs castrated is increasing year over year. It should be noted that the cost of neutering is relatively high, e.g., it costs approximately 200 EUR (~US$285 on October 14, 2012) to spay a large female dog (e.g., German Shepherd) through a private veterinary clinic.
>
> "Although there is no nationally operating, reduced-cost neutering scheme [i.e., program], a large number of municipalities run twice yearly schemes that they subsidise, and owners can have their pets neutered at greatly reduced cost. All dogs in animal shelters are neutered prior to re-homing, with the exception of very young animals whose adopters are issued with a neutering voucher permitting them to return the dog to the shelter at a later date for neutering at no extra charge.
>
> "Veterinary practitioners working in rural regions run mobile clinics at certain times of the year; they actively publicise the need for annual rabies vaccination and promote the routine neutering of pets during their clinics. This activity is supported by the veterinary administration of the Ministry of Agriculture."

In Sweden:

> "There is an enormous commitment by the Swedish people and authorities for strict dog control and an impressive degree of social responsibility where dog ownership is concerned. Owners readily comply with the law. Furthermore the high investment and status of dogs within Swedish household means that they are not readily disposed of or abandoned … The routine neutering of dogs of either sex is uncommon in Sweden; less than 7% of bitches and 4% of male dogs are neutered. Consequently there are no subsidized neutering schemes in Sweden … Responsible ownership and enforced leash laws mean that animals that aren't neutered do not breed uncontrollably."

In Switzerland:

> "… 33% of males and 50% of female dogs are neutered … Restrictive dog ownership and enforced leash laws control against accidental matings. Moreover, owners are required by law to avoid uncontrolled reproduction of their pets … Subsidised neutering schemes, run by animal welfare charities operate across Switzerland for owners who are in receipt of social benefit."

In the UK:

> "There are no published estimates of the proportion of dogs that have been neutered … Most sexually mature animals being re-homed from animal shelters are neutered prior to being placed in their new home. In addition, sexually immature animals leave shelters with a "neutering" voucher for low-cost neutering at a later date. The majority of animal welfare organizations operate subsidized neutering schemes [i.e., programs] for owners who are in receipt of … state benefits or are low-income. Local authorities run subsidized neutering schemes that operate year-round for owners on … benefit."

In the Scandinavian countries and in Germany, where pets are typically left intact and the emphasis is on responsible pet ownership and managing the reproductive behaviors of pets, spaying and neutering are somewhat rare (Jöchle, personal communication 2012). In Germany, castration of male dogs is the first choice for treatment of prostate hyperplasia, but in France, it is regarded as the last option (Reichler 2008). In some European countries, surgical contraception is seen as a form of mutilation. In Norway, it is considered unethical to neuter animals to make them easier to handle. In spite of a relatively common disposition among pet owners against surgical sterilization of their pets, younger veterinarians in Europe are performing spays and neutering and can be expected to do so as long as the procedures are profitable (Jöchle, personal communication 2012).

In a 2012 publication, Palmer et al. note that the American notion that spaying and neutering pets is a component of responsible ownership of cats and dogs "is not a view shared by veterinarians all over the world. In large parts of Europe … veterinarians are traditionally much more reluctant to neuter, particularly to neuter dogs." The authors continue:

> "In Sweden, for example, it was illegal to castrate a male dog until 1988, unless there was a specific medical reason for doing so. And the official view of Swedish veterinarians is still much more restrictive than that of their American counterparts. The section of the Swedish Veterinary Association (SVF) dealing with companion animals issued a statement (last revised in 2011) in which routine surgical neutering of dogs is rejected as sound policy (SVS 2011). This statement maintains that culturally based differences between countries concerning how dogs are kept affect the extent to which unwanted puppies are a problem. It's claimed that in Sweden, despite that only about 7% of bitches and an even smaller percentage of male dogs are neutered, any problem with unwanted stray dogs is insignificant."

The burgeoning pet insurance industry in Sweden is adding a new dimension to the discussion about spaying bitches because records on health issues for pets are available. The database of a well-established pet health insurance company in Sweden indicates that "on average 23-24% of the bitches in the database will have experienced pyometra by 10 years of age" (Egenvall et al.2001) .

As a result of the access to this specific information, "there has been growing interest in that country in spaying bitches as a means of preventing pyometra" (Zawistowski, personal communication 2012).

In a presentation at the 7th International Symposium on Canine and Feline Reproduction in 2012, Jitpean et al. noted that because only a small percentage of the dog population in Sweden is spayed "a large number of dogs are susceptible [to] diseases of the genital tract or that are associated with production of reproductive hormones." In a study to determine the occurrence of the two most significant canine diseases that can be prevented by spaying, researchers utilized the database of Agria, a Swedish pet insurance company. The records of 20,423 bitches diagnosed with pyometra and 11,758 diagnosed with mammary tumors were reviewed, and "the combined risk of contracting either of the two diseases was studied." In this particular group of bitches, 30% had developed either pyometra or mammary tumors at 10 years of age. Occurrence of the two conditions among the 110 breeds represented in the database varied with breed, and researchers noted that the occurrence was ">50% for over 20 breeds in the studied dog population. The results regarding age and breed differences in the incidence of both diseases were, on the whole, in accordance with previous reports." The researchers concluded that "these data may be valuable when deciding whether or not to perform elective spaying for individual dog[s] of different breeds" (Jitpean et al. 2012).

> attitude. Through shared international media—such as television channels focused on animal issues—the idea is spreading that routine neutering is the normal thing to do. Some small animal clinics in Denmark, which likely have a vested interest in the matter, have started to advertise and advise accordingly."

According to Kirpensteij (2008), "Although most countries will allow elective neutering of dogs and cats, regional differences exist. In Nordic countries (Sweden) elective neutering practices have been strongly discouraged, while in Holland, for instance, public opinion of castrating a male dog seems to be more problematic that spaying a bitch. Many factors associated with the client's background and beliefs and the type of animal play a role. For instance a cat spay or castration is commonly more acceptable than and dog spay or castration" (Kirpensteij, International Congress of the Italian Association of Companion Animal Veterinarians, 2008).

In the UK, 80%-85% of cats are sterilized (Rowan, personal communication 2012), but "there are no published estimates of the proportion of dogs that have been neutered [includes spay] in the UK" although there has "been a shift in the attitudes of veterinarians to the routine neutering of pets since … 1969" (Tasker).

Table 5-16: Dog Populations in European Countries and Rates of Spay/Neuter

	France	Spain	Germany	Switzerland
Dog population (000's)	8,508	4,510	6,473	480
Spayed females	25%	18%	28%	62%
Neutered males	12%	6%	25%	32%

Source: Reichler 2008

Palmer et al. (2012) also note:

> "In other parts of Europe the position is somewhere between that expressed by the American and the Swedish veterinary associations. In Britain, a position paper developed by the Ethics and Welfare Group of the British Veterinary Association (BVA 2011), and policy statements issued by the British Small Animal Veterinary Association (BSAVA 2006a, 2006b and 2006c) unanimously recommend neutering of male and female cats and of female dogs, but argue that decisions about castration of male dogs should be taken on a case-by-case basis. In Denmark … common practice has traditionally been much like that in Sweden. However, the Danish Veterinary Association has no official policy on the issue, and some of its members seem to be increasingly influenced by the American

It has been observed that in Latin America, surgical sterilization, particularly of male dogs, is generally not accepted culturally (Veterinarios sin Fronteras); however, this is not always the case. For example, experience in a Costa Rican program that sterilizes 10,000-15,000 dogs annually indicated "a slight reluctance, but by and large the people are more than willing to have their pets sterilized" (Rowan, personal communication 2012).

More than 53% of Australian households own a dog and/or a cat (Australian Companion Animal Council, 2009). In 2007, the dog population was estimated to be approximately 3.7 million, and cats numbered approximately 2.2 million. In both 1994 and 2006, over 80% of dog and cat owners believed that pets should be spayed or neutered unless specifically intended for breeding

(petnet.com.au/sites/default/files/National_People_and_Pets_2006.pdf). Attitudes were similar from 1994 to 2006; however, owner behavior appears to have undergone a change. In 1994 only 61% of dogs actually had been spayed or neutered. By 2006 this had risen to 78%. The number of spayed or neutered cats had also risen slightly, from 91% in 1994 to 93% in 2006.

In Belgium, the government announced the Multi-annual Cat Plan 2011-2016 in September 2010. The plan is intended to sterilize all but a select few of the country's estimated 1.7 million cats (the human population is 11 million). At that time it was assumed it would be passed into law, but there has been no follow-up information on the Internet. Culling had become common, with 13,000 cats killed in animal refuges in 2009, amounting to about a third of the country's strays. The initial phase of the plan called for sterilization of all cats in shelters, followed by neutering of cats sourced from breeders and other sellers. In the final phase, all cat owners will be obligated to have their cats sterilized and registered, at a cost of about 130 Euros (~US$172 as of December 18, 2012) for a female cat and 50 Euros ($66 as of December 18, 2012) for a tom. Officials from several rescue groups have commented that "they'll never be able to sterilize all the cats" and "pet owners will refuse to do it"; however, the animal welfare lobby appeared to be supportive of the plan (www.guardian.co.uk).

5.2.2.3 A Sample of Spay/Neuter Costs

Please note that much of this information comes from organization websites and that some of it is undated. If these figures are to be used for planning purposes they should be verified via additional sources. Also please note that cost basis, and therefore prices, vary by country and by source of surgery (e.g., subsidized program versus spay/neuter clinic versus private veterinary practice).

Table 5-17: Examples of Spay/Neuter Costs (Non-Subsidized)

Species	Procedure	Country	Cost*
Cat	Spay	France	$250
Dog	Spay	UK	$350-$400
Dog	Spay	Japan	$385-$650
Dog	Spay	USA	$150-$300
Dog	Neuter	USA	$200
Cat	Spay	USA	$150
Cat	Neuter	USA	$75

*Costs for countries other than the US are converted to US$ based on January 2012 conversion rates.

Table 5-18: Examples of Subsidized or Low-Cost Spay/Neuter Services in the US

Area	Program
DFW Humane Society, Dallas, TX	The Dallas-Fort Worth Humane Society sells certificates that enable dog and cat owners to go to one of several area veterinary clinics where they can redeem them for spay/neuter services. ■ Female cats: $40 ■ Male cats: $30 ■ Dogs under 20 lbs: female $55; male $50 ■ Dogs 21-35 lbs: female $60; male $55 ■ Dogs 36-50 lbs: female $65; male $60 ■ Dogs 51+ lbs: female $70; male $65
Broward, Miami-Dade, and Palm Beach counties, FL	The Pet Aid League has agreements with 20 veterinarians who will perform spay/neuter surgery. ■ Female cats: $35 (+ $5-$15extra if the cat is pregnant) ■ Male cats: $25 ■ Female dogs (based on weight): $39-$89 +$5 if the dog is in estrus or pregnant ■ Male dogs (based on weight): $39-$64)
Humane Society of Northeast Florida Putnam County Spay/Neuter Assistance Program, Hollister, FL	■ Cat neuter: $35; cat spay: $45 ■ Dog neuter: $45; dog spay: $65 (dogs under 85 lbs) ■ Additional cost for dogs over 85 lbs
Sacramento ASPCA, Sacramento, CA	■ Female cat: $45 (+$10-$30 if pregnant) ■ Male cat: $30 (+$10-$74 if cryptorchid) ■ Female dog: $50-$100 depending on weight; + $10 for female in estrus; + $ $20-$60 if pregnant) ■ Male dog: $40-$110 depending on weight; + $10-$75 if cryptorchid)
Oregon Spay/Neuter Fund Portland, OR greater metro area	The fund provides coupons redeemable at participating veterinary clinics. ■ Female cat: $49 (no extra charges) ■ Male cat: $33 ■ Female dog: $60 (+ $1 for every pound >60 lbs at some participating clinics) ■ Male dog: $49 (+ $1 for every pound >49 lbs at some participating clinics)
Animal Shelter Alliance of Portland Portland, OR greater metro area	■ $10 for all cats owned by people on a form of government assistance ■ $20 for all cats owned by people meeting low-income requirements (but not on public assistance) ■ By donation for feral cats that must be trapped to be sterilized

<table>
<tr>
<td>Chicago, IL</td>
<td>
Friends of Animals Certificates

$51: Male cat
$64: Male dog
$65: Female cat
$90: Female dog

Anti-Cruelty Society Low-Cost Clinic

$15: Cat (feral cats are free)
$90: Female dog (pit bull/pit bull mix dogs are always free)
$70: Male dog (pit bull/pit bull mix dogs are always free)

Tree House Humane Society Low-Cost Spay/Neuter Surgery

Pet or stray female cat: $75*
Pet or stray male cat: $50*
Pet or stray female dog: $135* (under 50 lbs only)
Pet or stray male dog: $95* (under 50 lbs only)
TNR Feral Cat Package: $30 (includes vaccinations, parasite treatment, ear-cleaning and more)

* Low-income individuals on public assistance receive reduced rates - call for full details

Animal Welfare League
<table>
<tr><th>Cats</th><th>Male</th><th>Female</th></tr>
<tr><td>• Any weight</td><td>$50.50</td><td>$75.25</td></tr>
<tr><td colspan="3"></td></tr>
<tr><th>Dogs</th><th>Male</th><th>Female</th></tr>
<tr><td>• <51 lbs</td><td>$95.75</td><td>$137.50</td></tr>
<tr><td>• 51-70 lbs</td><td>$140.75</td><td>$153.00</td></tr>
<tr><td>• 71-90 lbs</td><td>$155.75</td><td>$175.75</td></tr>
<tr><td>• > 90 lbs</td><td>$170.75</td><td>$189.75</td></tr>
</table>
</td>
</tr>
<tr>
<td>The Humane Alliance,
Ashville, NC</td>
<td>

$65: Neuter male dog
$65: Spay female dog
$50: Spay female cat
$35: Neuter male cat

These costs appear to be unchanged since they were cited in the 2002 Contraception and Fertility Control in Animals report.

Note that the Humane Alliance model is structured to be “breakeven” and thereby sustainable, given surgery revenue and expenses to run the clinic. ASPCA® and PetSmart Charities have helped the organization expand this model. As of November 2012, this includes a network of 110 mentored clinics using the Humane Alliance’s approach in the US (humanealliance.org).
</td>
</tr>
</table>

WSPA and RSPCA International (Tasker 2008) report notes that 58% of the European/Eurasian countries surveyed “had some form of subsidized neutering scheme … offered to people on low incomes or people with a large number of animals. However, the majority of schemes were available to owners who were resident at specific locations and were not, therefore, in operation nationwide. In most instances it was animal welfare organizations that provided this service to owners.” WSPA/RSPCA International Report can be found at: www.fao.org/fileadmin/user_upload/animalwelfare/WSPA_RSPCA%20International%20stray%20control%20practices%20in%20Europe%202006_2007.pdf.

Dr. Andrew Rowan, CEO of Humane Society International™ (HSI), notes that in Costa Rica, between 10,000 and 15,000 dog sterilizations are being performed annually at a cost (not price) of US$8-$12 per procedure. In India, a humane organization is spaying dogs for US$15-$20 dollars per procedure (Rowan, personal communication 2012). In 2009, WSPA supported 10 projects that provided working models of humane dog and cat population management in different regions of the world; together these projects have sterilized nearly 20,000 dogs and cats, vaccinated more that 35,000 and provided veterinary treatment for more than 15,000. As of 2010, the cost of surgical sterilization in the projects WSPA supported was, on average, US$7.50 ($3-$15) for medicine costs and US$30 ($10-$52) for the full costs, including staff and clinic operations costs for each dog or cat. At that time, the majority of WSPA's funds were spent on delivering surgical sterilization. Consideration of emerging and future approaches will hinge on approvability by regulatory authorities, safety, permanence, ability to generate sterility in male and female animals in a single treatment, and economic feasibility (Hiby 2010).

The organization Animal People compiled the following examples of other non-US costs (ACC&D, 2008):

Table 5-19: Spay/Neuter Costs in Non-US Markets (Expressed in US$)

Location	Organization	Cost Range Spay		Cost Range Neuter		Avg Cost Sterilization	Avg Cost Excluding Boarding/Food
		Dog	Cat	Dog	Cat		
Philippines	Palawan Animal Welfare Ass'n.						$11.02
Thailand (Bangkok)	SCAD					$23.25	$17.96
China (Beijing)	ARB	$72.81-$203.89	$13.11-$47.33	$43.69-$131.07	$7.280-$40.05		
India	Blue Cross of India					$14.11	$10.36

Other sources provide the following examples:

- The Philippine Veterinary Medical Association (PVMA) holds a free, annual spay/neuter event funded by the local Camiguin government. The 2012 event targeted 200 animals; 157 animals were sterilized in the 2011 event. If surgical sterilization is performed in a private clinic, the average price to owners is estimated at P 5,000 (US$120) (piacamiguin.wordpress.com/2011/09/30/camiguin%E2%80%99s-free-spay-neuter-for-pets-benefits-157-pet-owners-2/).
- The Philippine Animal Welfare Society (PAWS) provides spay/neuter services for dogs and cats at its PAWS Animal Rehabilitation Center (PARC), which also serves as a shelter, in Quezon City. Costs are shown in the table below (paws.org.ph):

Table 5-20: Spay/Neuter Costs in the Philippines

Animal	Cost	Additional Information
Female cat	P 1,000 (US$24)	Extra charge for purebreds and cats with pyometra or who are pregnant
Female dog	P 1,500 (US$36)	Extra charge for purebreds and cats with pyometra or who are pregnant. Additional P500 for every 10 kg in excess of 15 kg body weight.
Male cat	P 700 (US$18)	Additional charge for purebreds
Male dog	P 1,000 (US$24)	Additional P500 for every 10 kg in excess of 15 kg body weight

- The Soi Dog Foundation (Thailand) carries out sterilization and vaccination programs for stray and owned dogs and cats. Costs, covered by a "sound financial plan," are approximately US$30 to spay or neuter one animal. The cost includes drugs/surgical supplies (40%), veterinary fees (15%), clinical overhead (5%), nurse fees (5%), dog catcher fees (10%), vehicles and fuel (5%), and darts and anesthetics (12.5%). The remainder of the cost is not broken out (soidog.org/en/about-soi-dog/).

Readers wishing to learn about programs and costs in their areas are encouraged to consult local sources for up-to-date information.

Table 5-21: Spay/Neuter Costs in Thailand

Location	Animal	Cost
Pets Are Wonderful Veterinary Clinic	Male cat	600 yuan (US$96)
	Female cat	750 yuan (US$120)
	Male dog	800 yuan (US$128)
	Female dog	1,000+ yuan (US$160)
St. Anthony Animal Recovery Hospital (SAARH)	Male cat	400 yuan (US$64)
	Female cat	800 yuan (US$128)
	Male dog	> 10kg 1,000 yuan (US$160) <10kg 800 yuan (US$128)
	Female dog	>10kg 1,200 yuan (US$192) <10kg 1,000 yuan (US$160)

5.3 Show Animal Owners and Dog and Cat Breeders

Responsible commercial breeders, owner/breeders, and owners of show dogs and cats who wish to control when their animals come into estrus are likely to prefer a fertility control method that has a predictable duration and is not permanent. In addition, any contraception or fertility control product for female cats and dogs whose owners want to breed them at some time in the future will have to be shown to be free of negative effects on subsequent fertility, litter size, and quality, or on the long-term health and fertility of the offspring. Furthermore, products that do not also suppress sexual behaviors would generally appear inappropriate for this market, although some show animal owners may prefer a treatment that does not affect sex-hormone-influenced morphology (e.g., toms are expected to have "jowls").

Currently some owners of show dogs use progesterone-type products to suppress the estrous cycles of animals that are likely to come into season at inconvenient times. Using progesterone type drugs in dogs successfully requires close monitoring of the estrous cycle and is labor intensive. (See Chapter 4, section 4.1.5 for a discussion of progesterone-type drug use in dogs. Progesterone-type drugs as injections are also available for cats. See Chapter 4, section 4.2.3 for a discussion of progesterone-type drugs used in cats.) Six- and 12-month gonadotropin-releasing hormone (GnRH) agonist implants, (Suprelorin, Peptech/Virbac), are available for use in male dogs in Australia, New Zealand, and Europe; however, the period after which a given dog regains fertility after treatment is variable (see Chapter 4).

5.4 "Unowned" Dogs and Cats

The groups that deal with "unowned" animals are diverse, and their needs are diverse. Some serve owner-relinquished animals only, but many are involved in dealing with issues presented by the presence of abandoned, stray, free-roaming, and feral animals. There is long-standing global debate about how many dogs and cats make up these populations, how many "adoptable" and "unadoptable" animals are euthanized each year, what can and should be done about it, and the degree to which contraception and fertility control can influence the overall dynamics of what is typically called "overpopulation."

There is general agreement, however, that the number of unowned animals is large – and that many adoptable animals are euthanized. Furthermore, many of these animals are sexually intact. Dr. Andrew Rowan[10] estimates that there are 400 million "pet" dogs and 300 million "street" dogs worldwide. While in some parts of the world,

[10] Dr. Rowan is President and Chief Executive Officer of Humane Society International (HSI) as well as Chief International Officer and Chief Scientific Officer for Humane Society of the United States (HSUS).

poisoning, and/or shooting stray and feral cats and dogs are accepted practices (albeit becoming less accepted), groups in many countries attempt to deal with issues of population control, abandonment, and relinquishment of cats and dogs in ways that are regarded as more humane.

WSPA and RSPCA International explain that:

> "Definitions of stray dogs are inherently problematic and judgments regarding when a dog is considered to be a stray varies from country to country and may be subject to local and national regulations … any dog found unaccompanied by a responsible person in a public place may, in some countries, be considered as stray and collected accordingly. Conversely, at the other end of the scale, unwanted dogs – dogs, whose owners have revoked all caregiving responsibilities – may, if they survive for long enough, be able to reproduce and rear young. Though this generation of dogs may be considered to be genuinely ownerless and in some instances feral, their survival rates are invariably low and their reproductive success is likely to be poor. They are therefore not considered to be the main source of overpopulation. Somewhere between the two examples, dogs may be cared for by one or more members of a community, allowed to roam, and permitted to reproduce. Nevertheless, they are genuinely dependent upon human caregivers, as humans provide access to the resources essential for their survival. The reproduction rates of these dogs and their rearing success has the potential to be high because care given by humans offers the necessary protection for puppy survival … The relationship between cats and their caretakers is intrinsically different to dogs, although the same set of associations may apply but to varying degrees" (Tasker 2008).

The World Health Organization (WHO) developed four categories to characterize dog populations (cited on jeevashram.org):

- Restricted/supervised: pets; dogs that are dependent on owners who keep them under supervision
- Family dogs: dependent on owners, who restrict them only partially
- Neighborhood or community dogs: partially dependent on people; movement unrestricted
- Feral dogs: independent or dependent on human-generated waste and garbage; movement unrestricted

The Humane Society of the United States™ (HSUS) includes the following cats in its "free-roaming" category:

- Owned cats that are allowed to roam
- Owned cats that have become lost
- Previously owned cats that have been abandoned [as well as their offspring]
- Quasi-owned cats that roam freely and are fed by residents
- So-called working cats that serve as mousers

WSPA and RSPCA International have classified dogs and cats by the nature of their dependence on humans (Tasker 2008):

Table 5-22: Classification of Dogs and Cats by Their Dependence on Humans

Classification	Dogs	Cats
Stray: feral	■ No owners or caretakers ■ Generally derived from dog populations under some degree of human care "gone wild" ■ Found on the outskirt of urban and rural areas ■ Poorly socialized to human handling ■ Survive by scavenging ■ Poor survival rates ■ Low reproductive capacity	■ Unowned, independent of human control ■ Poorly socialized to human handling ■ Sub-population of free-roaming cats (may be offspring from owned or abandoned cats) ■ Survive through scavenging and hunting
Stray: abandoned/ unwanted by owners	■ Were once dependent on an owner for care ■ Owner is no longer willing to provide resources ■ May or may not be fed by other members of the community (food may be delivered intermittently) ■ Survive by scavenging or hunting ■ Poor survival prospects once there is no longer a caretaker to provide food or shelter	■ Were once dependent on an owner for care ■ Owner is no longer willing to provide resources ■ May or may not be fed by other members of the community (food may be delivered intermittently) ■ Survive by scavenging or hunting ■ May or may not be socialized to human handling
Stray: owned, not controlled	■ Free-roaming dogs ■ "Latch-key" dogs ■ Community or neighborhood dogs ■ Either entirely free to roam or may be semi-restricted at particular times of the day ■ Dependent upon humans for resources ■ May or may not be sterilized ■ Potential for high reproductive capacity and rearing rates	■ Free-roaming cats ■ "Kept" outdoors ■ Either entirely free to roam or may be semi-restricted at particular times of the day ■ Dependent upon humans for resources ■ May or may not be sterilized ■ Potential for high reproductive capacity and rearing rates
Owned, controlled	■ Totally dependent upon an owner for care and resources ■ Generally under close physical control of the owner ■ Confined to the owner's property or under control when in public places ■ Reproduction usually controlled through sterilization, chemical means, or confinement	■ Totally dependent upon an owner for care and resources ■ May vary from totally indoor to indoor/outdoor, outdoor but confined to pen or garden ■ Generally reproduction may be controlled through sterilization or confinement

HSUS notes that feral cats are unsocialized animals "who may be one or more generations removed from a home environment and who may subsist in a colony of similar cats," and that it can be difficult to differentiate between free-roaming and feral cats (hsus.org).

The popular and professional literature is rife with statistics and although there is some – and even great – semantic and numerical disparity among them, it is agreed that shelter and "street" or "feral" populations consist of owned animals

at large, previously owned animals, free-roaming animals that are fed by humans, "unsocialized" animals that receive some support (e.g., food and even medical care) from humans, and "unsocialized" animals that haven't had homes or support from people. While it is difficult to find reliable information regarding abandoned, stray, or feral animal populations worldwide, a few estimates are given below. Note that a detailed examination of the issues related to feral animals is beyond the scope of this report, but some information is included at the end of this section on lessons learned as a result of the use of chemical castration to help manage unowned or "community-owned" dog populations.

In the US:

- Estimates of the number of abandoned, stray, or feral cats in the US vary. Feralcat.com estimates that there are 60-100 million; Alley Cat Allies (alleycat.org and www.alleycat.org/page.aspx?pid=667) estimates the number at between 30 and 60 million; and Centonze and Levy estimate that stray and feral cats account for 35% to 45% of the entire known cat population (Centonze and Levy 2002). According to the American Society for the Prevention of Cruelty to Animals® (ASPCA®), it is impossible to determine how many stray dogs and cats live in the US; estimates for cats alone range up to 70 million (ASPCA 2012).
- An estimate presented in 2010 indicates that approximately 50 million feral cats produce approximately 145 million kittens in the US. This compares to 26 million kittens produced by approximately 88 million owned cats in the US. The sterilization rate among the feral cats is estimated at 1.2%; it is 85% among owned cats. (Levy 2010).
- In 2002, it was estimated that 5% of pet dogs in the US are acquired by their owners as strays; 24% of pet cats are taken in as strays (purina.com). A PetSmart Charities survey indicated that 19% of dogs and cats are taken is as strays but did not separate data by species (PetSmart Charities 2009).
- A survey showed that "sexually intact" dogs and cats were 2-3 times more likely to be relinquished by owners in the US than dogs and cats that had been spayed or neutered (New 2002).
- The National Council on Pet Population Study and Policy (NCPPSP) *Shelter Statistics Survey 1994-97* found that approximately half of the pets (42.8% of dogs, 50.8% of cats) surrendered were not neutered (www.petpopulation.org/statsurvey.html). Another source indicates that 55% of surrendered dogs and 47% of surrendered cats are intact (ohlonehumanesociety.org). Although these statistics focus on the US, it is likely that owner failure to neuter pets that are unsupervised at least part of the time is an important contributing factor to shelter populations in general. Therefore shelters are interested in sterilization to address a demographic of animals more likely to contribute to shelter intake to well as to prevent litters.

Outside the US:

- An estimate of 2 million "strays" on UK streets is considered low (celiahammond.org).
- Cats are not native to Australia. They were introduced probably around the time of first European settlement. During the 19th century, thousands of cats were released in the gold fields to control mice. Cats were also popular with settlers to keep down the number of rabbits and native rats. Many were released – or escaped – into the bush, where they were able to fend for themselves. These cats did not depend on humans for food or shelter and in effect became wild, or "feral." They spread rapidly across most of the continent. It is estimated that there could be 400,000 feral cats in New South Wales and around 12 million across Australia. Cats in NSW are categorized as domestic, stray, or feral. Feral cats have been declared a pest species and are subject to abatement programs (www.environment.nsw.gov.au 2011).

- There are an estimated 3,000,000 street dogs in Bulgaria (bulgarianstreetdogs.com) and a March 2012 census is expected to provide an estimate of 11,000 stray dogs in the capital city Sofia (animalmedicalcarefoundation.com/bulgaria.html).
- In Japan, according to the Japanese animal health company Nippon Zenyaku Kogyo (Zenoaq)

(www.zenoaq.jp/english/aij/1201.html, www.zenoaq.jp/english/aij/1102.html):

- 112,690 "unwanted" dogs and 228,373 "unwanted" cats were euthanized by Japanese animal control agencies in 2008, which constitutes a dramatic decrease from the 1997 level of between 600,000 and 700,000 for dogs and cats combined. The decrease in euthanasia of unwanted dogs is greater than the decrease in cats.
- 81% of abandoned dogs are adults; 71% of abandoned cats are kittens.
- 80% of cats euthanized by animal control agencies are kittens.
- Less than 5% of owners surveyed in 2008 adopted their dogs from a public or private shelter.
- The reason most given for relinquishing a dog is owner death or illness; this justification accounts for about one-third of canine relinquishments; the second most cited reason is that the owner is moving. These two reasons taken together account for one-half of the dog relinquishments.
- Puppy mills are a source of animals brought to shelters; although puppy mills must register with local authorities, "the standards for registration are still minimal and inspection infrequent due to a lack of manpower." More than 88% of abandoned dogs (~2,700 dogs) at the rural Tokushima Animal Welfare Center were euthanized in 2008 (Hoon and Fabre 2010).
- Most stray dogs are abandoned rather than relinquished to shelters and are the sources of "dogs that have gone wild" (Hoon and Fabre 2010).
- Some hunters abandon hunting dogs after the hunting season and purchase new dogs the following season (Hoon and Fabre 2010).

5.4.1 Dealing with "Free-Roaming" Animals

A thorough review of this topic is beyond the scope of this document; however, comprehensive sources, not limited to the US, can be found at: www.vetmed.ucdavis.edu/strays.htm. For a summary document, developed by WSPA and RSPCA International and describing stray dog control practices in Europe Stray Animal Control Practices (Europe), see: fao.org/fileadmin/user_upload/animalwelfare/WSPA_RSPCA%20International%20stray%20control%20practices%20in%20Europe%202006_2007.pdf. There are numerous examples of TNR programs to be found on the Internet.

Availability of current (e.g., zinc gluconate [Esterilsol/Zeuterin – see Chapters 3 and 4]) non-surgical sterilization methods and those expected to emerge will likely play a role in population control and general health programs for free-roaming dogs and cats. One can envision their use, for example, in rabies programs that involve dog population control. See section 5.4.1.3 for information about the use of Esterilsol in population control programs for male dogs and "lessons learned" by groups involved in non-surgical projects.

5.4.1.1 A Look at Methods of Dealing with Free-Roaming Dogs

The following information and reported methods of stray dog control were noted in the Europe Stray Animal Control Practices (Europe) document cited above. Information was derived from questionnaires distributed to 34 animal welfare groups operating in 30 countries located in Europe and Eurasia during 2006-2007:

- Statutory holding periods varied from 3-60 days in the countries capturing stray dogs.
- 32% of countries capturing stray dogs euthanized animals that were not returned to owners or placed in a new home.
- 6% of countries capturing stray dogs euthanized all animals upon capture, without waiting for the holding period to end.
- 3% of countries capturing stray dogs legally forbid euthanasia of healthy stray dogs and mandate life-long care for those healthy stray dogs for which homes cannot be found.
- There appears to be a general lack of numerical data collected by "authorities" in responding countries
- No country reported centralized monitoring of dog populations, demographics, or ownership trends.
- Methods of controlling stray dogs and cats vary "greatly" among surveyed countries
- Stray cats are "more likely to be culled than stray dogs."
- 22 countries (70% of those surveyed) have laws requiring licensing or registration of dogs. In 15 of those countries, this was considered ineffective in helping to reduce stray numbers … "because the law was neither enforced nor adhered to by owners."

Discussion of country-specific information generated by the survey is beyond the scope of this document; however, the full report contains a great deal of interesting input and can be accessed

at: fao.org/fileadmin/user_upload/animalwelfare/WSPA_RSPCA%20International%20stray%20control%20practices%20in%20Europe%202006_2007.pdf.

In a review of Capture, Neuter and Return/Release (CNR) programs in developing areas, Jackman and Rowan (2005) point out that the "free-roaming dog populations have emerged as both animal welfare and public health problems in developing countries. Free-roaming dogs face high mortality, malnutrition, starvation, disease, and abuse; account for 99% of cases of rabies transmission worldwide (WHO 2004); and are associated with more than 60 other diseases (Beck 2000, Reece 2005). Additional social problems with free-roaming dogs include road accidents, fighting, noise, fecal contamination, spread of rubbish, and uncontrolled breeding." Dog attacks on livestock are also considered a serious issue in many communities.

Human public health preventive measures should be paralleled by programs for dog rabies control (WHO 2004, cited in Jackman and Rowan 2005). While sterilization of free-roaming dogs appears to be a desirable added tool to reduce the dogs needed to treat in rabies vaccination programs, capture-and-kill methods used historically have been condemned as "ineffective and cruel," and in some instances those practices began to decrease in the late 20th century. CNR programs are modeled on Trap-Neuter-Release (TNR) programs for feral cats. Sterilization via CNR, along with "vaccination, habitat control, and responsible pet ownership … are now replacing … capture-and-kill" (Jackman and Rowan 2005).

The benefits of CNR programs for dogs include (Jackman and Rowan 2005):

- Controlled population and reduced mortality "discourage migration and compensatory breeding … to fill ecological niches left by dog losses." New dogs are less attracted to a given territory.
- Puppy populations are reduced.
- Less post-sterilization-procedure stress occurs because dogs are returned to their own territory.
- There is significant public support in areas in which there is opposition to catch-and-kill programs.

5.4.1.2 A Look at Methods of Dealing with Free-Roaming Cats

In the US, the sterilization rate in the feral/free-roaming cat population is very low – about 2%. A large percentage of cats are pregnant – 15% on average – and there is a seasonal pattern; therefore, effects of any non-surgical approach on pregnant queens and their fetuses would have to be known prior to use in the field or in veterinary practices (Briggs, personal communication 2012).

Although less humane methods are also employed, many communities and organizations deal with free-roaming cats in one of four ways (definitions from Slater 2002):

- Trap, remove, euthanize – This is regarded as a short-term approach unless the sources of food and shelter that attracted the cats in the first place are removed. Otherwise, any cats that avoid the process remain in the area and continue to reproduce and the trap-remove-euthanize cycle can continue.
- Trap, remove, and relocate to another colony or a sanctuary – This is a difficult process because suitable locations must be identified and obtained, and the stress of relocation can compromise the health of the cats. In some cases, cats can be socialized and adopted, but the socialization process is time consuming.
- Trap, neuter, and return/release (TNR) – This alternative requires the assistance of veterinarians, who surgically sterilize the cats and notch or tip the ear for identification purposes, as well as caretakers, who feed and monitor the colony on an ongoing basis. Monitoring is necessary to identify any new cats moving into the colony. At its most comprehensive, this strategy is extended to TTVARM, which stands for Trap, Test, Vaccinate, Alter, Release, Maintain, and is geared towards stabilizing and managing feral/unowned cat colonies to help reduce unwanted reproduction and control disease (Cat Fanciers Association, cfainc.org). Research for this update appears to indicate that TNR is emerging as the preferred method of managing feral colonies. It is important to note that there is an excellent opportunity for these cats to receive additional veterinary care while they are being sterilized, which is also an opportunity for animal health companies interested in developing non-surgical approaches to market other feline products. As an example, in addition to being spayed or neutered, each cat receiving treatment from the Feral Cat Coalition of Oregon receives "FVRCP (distemper) and rabies vaccines, flea treatment, ear cleaning and ear mite treatment if necessary, fluids if dehydrated, treatment for minor medical conditions if present, his or her right ear tipped for future identification. Cats that appear to be suffering, as determined by a veterinarian, are tested for feline leukemia (FeLV) and feline immunodeficiency virus (FIV). Any cat testing positive is euthanized" (www.feralcats.com/FAQ.html). For a summary of studies on use of TNR to

manage feral cat populations, see www.alleycat.org/page.aspx?pid=667.

- Wait and see – This strategy, which typically occurs by default, makes no effort to manage populations and can result in the growth of colonies to crisis level, when any type of intervention can be more costly and a greater animal welfare challenge.

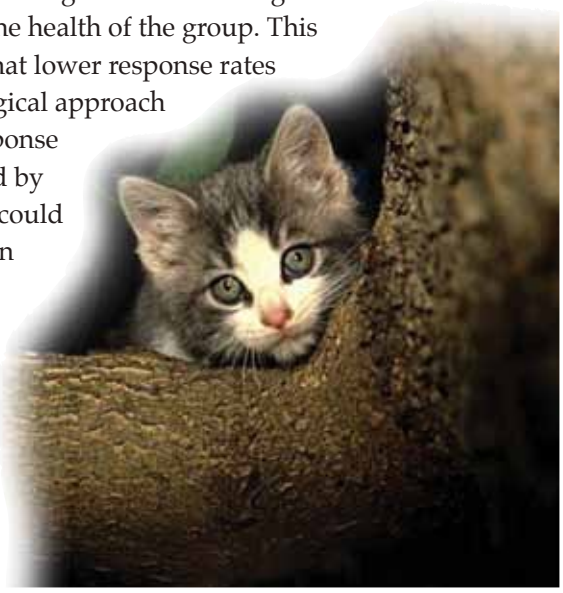
One of the issues discussed among stakeholders advocating the development of non-surgical methods of population control in cats relates to whether or not a given approach must provide permanent sterilization. Indeed, the Michelson Prize & Grants program of the Found Animals® Foundation (see section 5.9.1) is focusing on discovery and development of a single approach to permanent, non-surgical sterilization that will work in male and female cats and dogs. For some time, the working assumption made by many stakeholder groups concerned with unowned populations of cats and dogs was that since animals such as feral cats may only be able to be treated once, sterilization was required to have significant impact on population control.

Given that several of the non-surgical contraceptives that have been developed may allow long-term fertility suppression, ACC&D was interested in understanding the impact of a treatment that could cause a long-term suppression of fertility on a feral population when compared to surgery, which offers a permanent solution. Because there was some information available that a deslorelin implant might result in up to 3 years of fertility suppression in cats, preliminary modeling work was done to evaluate population impact of a 3-year contraceptive treatment in feral cats. This work resulted in a study that was published in 2009 that modeled the impact of 3-year contraception vs. surgical sterilization on the population of feral cats. Results indicated that both were similarly effective and far more effective in population control than no intervention (Budke and Slater 2009):

> "This study constructed matrix population models to explore feral cat population growth for a hypothetical population (a) in the absence of intervention; (b) with a traditional surgical sterilization-based trap, neuter, and return program; and (c) with a single-treatment 3-year non-surgical contraception program. Model outcomes indicated that cessation of population growth would require surgical sterilization for greater than 51% of adult and 51% of juvenile (<1 year) intact female cats annually, assuming an approximate 3-year mean life span. After the population stabilizes, this would equate to sterilizing approximately 14% of the total female population per year or having approximately 71% of the total female and 81% of the adult female population sterilized at all times. In the absence of juvenile sterilization, 91% of adult intact females would need to be sterilized annually to halt population growth. In comparison, with a 3-year non-surgical contraception program, an annual contraception rate of 60% of female juvenile and adult intact cats would be required to halt population growth, assuming that treated cats were retrapped at the same rate after 3 years."

Animals returned to feral colonies should be vaccinated against rabies and, if possible, identified in some way to prevent their recapture and re-treatment. Some programs use ear tipping for this purpose; however, identification of animals sterilized non-surgically is typically regarded as a barrier to acceptance of non-surgical approaches. In fact, effects of accidental re-treatment would have to be understood and addressed to the satisfaction of regulatory agencies before a product could be labeled for use on feral animals and before organizations using TNR and similar strategies would actually use it. A panel discussion entitled "A Focus on Feral and Free-roaming Felines," held during the 4th International Symposium on Non-Surgical Methods of Pet Population Control in 2010, pointed out the challenges related to how the unique characteristics of feral/free-roaming cat populations affect the structure and implementation of management strategies. The issues include (Green 2010):

- Feral/free-roaming cats can be thought of as a "herd" in terms of the health of the group. This may mean that lower response rates to a non-surgical approach than the response rate expected by a pet owner could be effective in managing a colony.
- There may be potential liability involved in capturing and



sterilizing cats that are owned but are free-roaming.

- There is a wide range of surgical spay/neuter services from a variety of sources. A subsidized, high-volume, high-quality spay/neuter program may be available in some situations while in others, the only surgical option available (e.g., private practice veterinarian) may be cost prohibitive for cats in a colony.
- Effectiveness of approaches may vary depending on variability in fertility, fecundity, and mortality in a given colony – "a shorter acting product could be very effective in areas [in which feral/free-roaming cats have] low life expectancy, but ineffective in areas [in which feral/free-roaming cats have] high life expectancy."
- Programs currently depend on ear tipping to identify treated cats; ear tipping requires anesthesia, while non-surgical sterilization may not require anesthesia.
- Financing programs ("there is no owner to 'foot' the bill").
- There is a wide range of population sizes, depending on colony location.
- Cats are not easy to count, observe, and track.
- Stress involved to cats and handlers in the capture, handling, and surgical recovery process.
- Potential opposition to permitting cats to remain in colonies.

5.4.1.3 Advocacy and Population Management Projects Utilizing Non-Surgical Methodology: Lessons Learned

Since most TNR programs utilize surgical spay/neuter, a full review of TNR programs is beyond the scope of this document on non-surgical approaches. Readers wishing to learn more will be able to find information about these programs on the Internet. As permanent non-surgical options such as Esterilsol (zinc gluconate, see Chapter 3) emerge, they can be expected to play increasing roles in community-based population control projects that now rely primarily on surgery.

Experience with non-surgical sterilization is largely limited to the use of zinc gluconate in male dogs. In 2009 and 2010, ACC&D sponsored several programs involving the use Esterilsol (Zeuterin in the US) (acc-d.org/EsterilsolGrants).

For example, a three-phased, community-based approach to canine population management conducted by Veterinarians without Borders (VWB) and Vétérinaires Sans Frontières Canada (VSF) used chemical castration in males and surgical sterilization in females and has contributed to an overall reduction in population density of dogs in 12 communities of Todos Santos, Guatemala, a community in a remote mountain region. This particular project was in part supported by ACC&D, underwritten by Parsemus Foundation and The Pegasus Foundation.

In Phase 1 of the program, VWB/VSF assessed the situation in the area and met with public health, veterinary professionals, and public stakeholders to develop the relationships necessary to move from an unsuccessful approach that involved using strychnine poisoning and killing puppies to a more humane and successful approach in the future. In Phase 2, owned dogs were counted and the number of stray dogs was estimated using mark-recapture methodology. Results of a household survey indicated a slight preference for chemical sterilization over surgical castration.

Phase 3 focused on sterilization and education, and occurred during January of 2009. In preparation, two VWB/VSF veterinarians traveled to Mexico, where they were trained in Esterilsol administration by Dr. Carlos Esquivel of Ark Sciences, Inc., the company currently marketing the product. These veterinarians in turn trained veterinarians in Guatemala, including one of the veterinarians closest to Todos Santos. A total of 216 male dogs were brought to the clinic for examination and vaccination; 126 male dogs were neutered with Esterilsol. Reasons dogs were identified as ineligible or not treated included too young or too old, and cryptorchid or scrotal pathology. The owners of 17 dogs did not want the procedure done. The team encountered challenges with handling unsocialized and aggressive stray dogs; VWB/VSF plans include pursuing new methods for handling these dogs so that they can eventually be sterilized.

Minimizing the incidence of injection site reactions that have occurred in up to 4% of dogs in other campaigns was a critical objective. Using precise injection techniques and ensuring that dogs remain still during the procedure reduce the incidence of these reactions. Sedation was used during the procedure and owners received specific home-care instructions. Only two dogs experienced adverse effects – one dog's scrotum was more dry/scaly than normal and treatment resolved the condition; the other dog developed a draining tract requiring scrotal ablation. Although male dogs were the target population for this

phase of the project, 12 female dogs were surgically spayed. This allowed the team to understand the conditions under which this type of surgery is carried out in this particular setting.

During a November 2011 visit to Todos Santos, VWB/VSF evaluated the current situation with respect to free-roaming dog status in the town, cases of rabies in dogs and humans, and to obtain community recommendations for next steps. Feedback obtained by the community was very positive and suggested that inhabitants were less fearful of the free-roaming dogs. Data from the municipality showed that there had been no cases of rabies in the previous two years; however, in the outlying agricultural area in which 23,000 people reside, cases continue.

As noted above, information on ACC&D's Esterilsol Small Grants Program undertaken in 2009 and 2010, is available at acc-d.org/EsterilsolGrants.

ACC&D has summarized "key learnings" related to introduction and implementation of non-surgical methods based on the organization's Esterilsol Small Grants Program experience. Although the "learnings" are related to the use of zinc gluconate to sterilize male dogs, the information is expected to be of value as additional non-surgical methods emerge. A distillation is presented below.

Levy et al. (2008) compared the use of intratesticular injection of zinc gluconate versus surgical castration to

Table 5-23: ACC&D Summary of "Key Learnings"

Lesson	Description
Public health concerns may facilitate use	In areas in which rabies or other zoonotic disease is a significant public health issue, "opportunities to attract government officials and pet owners with rabies control/vaccination activities can provide opportunities to engage these organizations and individuals in conversations about humane dog population control on a community or individual-animal level."
Local buy-in is a must	Organizations involved in local sterilization programs "must be able to communicate effectively with the local population. This includes proficiency in the local language as well as familiarity with cultural norms." The local population must also understand and be able to comply with post-administration care requirements, which can be challenging in areas in which "veterinarians are in short supply; dogs are often 'loosely owned' and lack supervision and access to clean, dry, and temperature-controlled housing, as well as good nutrition and fresh water; dog owners often lack telephones and vehicles, making it difficult to contact veterinarians with questions or concerns." In instances in which teams that are not locally based leave too soon after providing services, follow-up care may not be adequate, which can compromise effectiveness and lead to adverse events.
Logistics are important	An understanding of specific requirements is important. Areas of interest include, but are not limited to, regulatory requirements for approved or unapproved products in a given country, and import and customs permission regulations, policies, practices, and timelines.
Record-keeping and accuracy of records are critical; planning is important	Many dog and cat sterilization programs are conducted under field conditions and involve the use of volunteers. Because tracking the effectiveness of these programs is critical to funding, maintaining, and improving them, collecting and organizing treatment records should be emphasized and related parameters predefined. Experience has revealed that in field studies, "even for those organizations which clearly understand the importance of record-keeping, it is not uncommon for some paperwork to be lost or incomplete. Expectations for the amount and accuracy of data collected must be reasonable."
Preparation can prevent problems	Experience with Esterilsol indicates that the project preparation phase can take longer than estimated. While some types of delays cannot be foreseen, delays that can be minimized have related to obtaining the permissions noted above; delivery of products and related supplies; seasonal weather patterns, and "bottlenecks in local, regional, or national bureaucratic systems." Training of veterinarians and other project personnel should occur within a reasonable time before the start of a project. In the case of Esterilsol, Ark Sciences recommends that no more than 2 months elapse between the veterinarian's training and the use of the product. "It is critical that administration protocol (including but not limited to injection technique) be followed precisely, and specific instructions may be forgotten if there are long delays between training and use."
Proper handling and administration are key to optimizing effectiveness and minimizing adverse events	Lessons learned during the Esterilsol Small Grants Program include ensuring that product integrity is maintained (e.g., following label instructions regarding storage, product expiry, product life after opening); and using proper administration technique. In the case of Esterilsol, improper injection technique is believed to account for a wide range of project-specific adverse reaction rates. "For example, a 2004 project in the Galapagos Island of Isabella had four adverse reactions out of 103 dogs (3.9%). (Administration protocols were further refined after this project.) A 2009 project in Todos Santos, Guatemala had only one major and one minor reaction in 126 treated dogs (1.6%). And a 2010 project in Peru had three reactions in 249 dogs (1.2%)."

sterilize male dogs in a project in Isabel Island in the Galapagos archipelago. The project was a cooperative effort between Animal Balance, the Galapagos National Park Service, the Galapagos Quarantine and Inspection System, and the municipal Control and Management of Introduced Species Committee. The 4-week project provided not only neutering but also an education program "developed to promote responsible pet ownership and encourage residents to restrict their dogs from roaming in environmentally sensitive areas."

Researchers reviewed medical records of 161 male dogs that were sterilized during the program. Fifty-eight of the dogs were castrated surgically and 103 were sterilized using injectable zinc gluconate:

> "Dogs were returned to their owners for observation following castration. Wound dehiscence occurred in two skin incisions, representing 3.4% of the 58 dogs that underwent bilateral orchiectomy. Necrotizing zinc-gluconate injection-site reactions occurred in four dogs receiving injection volumes near the maximum label dose (0.8 to 1.0 mL), representing 3.9% of the zinc-gluconate procedures. Surgical wound complications were treated by superficial wound debridement and resuturing. In contrast … [the 4 dogs with necrotizing injection-site reactions] required orchiectomy and extensive surgical debridement, including scrotal ablation in two dogs … Low cost, ease of use, and cultural acceptance of a castration technique that does not require removal of the testes make zinc gluconate a valuable option for large-scale use in dogs, particularly in remote locations lacking sophisticated clinical facilities or skilled surgeons and staff."

Researchers recommended that "further investigation is needed to identify risk factors in dogs for adverse reactions to zinc gluconate and to develop strategies for avoidance."

5.5 "Shelters"

A thorough review of the dynamics and services of the shelter community, defined as animal shelters and nonprofit or governmental agencies that sterilize animals for adoption or offer community sterilization services, is beyond the scope of this document; however, the emergence of non-surgical approaches to sterilization of cats and dogs is clearly relevant to this market segment.

The market served by the shelter community is complex for a number of reasons. Any company, large or small, established or new, that contemplates marketing products for use in shelter-type situations will want to be aware of some of the forces that influence how nonprofit organizations such as shelters view contraception and fertility control for dogs and cats.

A 2004 study attempted to determine the number of "animal organizations" in the US (Rowan 2008). The list contained approximately 9,500 independent entities.

- 3,352 characterized themselves as shelters, (defined as organizations with a building at their official address that housed animals).
  - Of these, 1,554 (46%) identified themselves as being municipal.
  - 1,809 (54%) identified themselves as being private 501(c)3 (i.e., nonprofit) organizations. Though these may have a wide variety of names, many are of the type the public thinks of as "humane societies" or SPCAs. These shelters may or may not have animal control contracts or agreements to house animals for municipal agencies.
- About 75% or more of the animal protection organizations concentrate on companion animal rescue, housing, and disposal (e.g., adopting out, returning to owner, and/or euthanasia).

Although sterilization as a condition for adopting dogs and cats is not universal, surgical spaying is encouraged in countries such as the US and UK. A number of US shelters "and virtually all rescue groups sterilize dogs before making them available … and many shelters that do not do the surgery before the animals leave do require that the new owner do so. Some advocacy groups … demand laws that require spay and neuter of all dogs and cats unless people buy permission to keep their animals intact. Others seek to require shelters to spay and neuter all animals that leave their premises to avoid unwanted litters in the future" (dogsonly.org/spayfaq.html).

Non-surgical sterilization may be acceptable in some countries and to animal welfare groups that oppose

surgical procedures on the grounds that the procedures are "mutilation." In European countries, pharmacological approaches to population control have focused on the use of oral and injectable progesterone-type drugs in female dogs and cats because "surgical intervention … is often regarded by owners as inhumane and degrading to a companion animal" (Jöchle 1994). Currently used progesterone-type drugs have significant drawbacks for shelter situations because they require careful monitoring over time of the estrous cycle of the bitch or queen to be effective, and are not effective in males.

For animal shelters and nonprofit or governmental agencies that sterilize animals for adoption or offer community sterilization services, there are various issues and considerations affecting their decisions and practices. These may in turn affect their embrace of alternatives to surgical sterilization. These include, but are not limited to:

- Ascertaining which reproduction control technologies best fit the needs of shelter and feral animals.
- Availability of funding: Agencies are typically trying to stretch resources to help the most animals. Economical means of safely sterilizing animals will be popular if the agencies' criteria for performance are met.
- Type of organization and staffing decisions: Some organizations sterilize the animals they are placing for adoption only; others have subsidized programs for pet owners, often requiring proof of low income or need. Increasingly, agencies have one or multiple veterinarians on staff and involved in medical protocol decisions.
- The field of shelter medicine has grown dramatically in the past decade. The Association of Shelter Veterinarians (ASV) has grown from a grassroots group of shelter vets founded in 2002 to a formal organization with 750 members, 22 student chapters around the globe, a range of published position statements and expanded continuing education. *Shelter Medicine for Veterinarians and Staff*, edited by Miller and Zawistowski of the ASPCA, the first real text in the field, was first published in 2004 with a second edition to be published in January 2013. The second edition has a chapter on non-surgical sterilization.
- Emphasis on birth control vs. other factors: the percentage of time, funds and focus agencies give sterilization vs. their other roles including adoption and preventing relinquishment.
- Better scientific guidance and changes in practice/funding related to how to intervene in rabies and population control, especially of free-roaming cats and community dogs:
  - Rabies and population control programs are often viewed hand in hand, but not necessarily combined. Non–surgical sterilants or contraceptives that can be delivered simultaneously with a rabies vaccine can be attractive. If not permanent, then a duration of similar time to a rabies vaccine could have potential.
  - Better tools to guide interventions: Several initiatives are underway to develop simulation models to better predict and guide interventions in populations of free-roaming cats and dogs. Initially developed around spaying and neutering, these can be used also to predict the impact of deploying non-surgical tools when available.
- Location (e.g., rural, suburban, urban; local economics).
- Differentiating among intact animals and stray, abandoned, and feral animals that have already been sterilized. It can already be difficult to identify a previously spayed female cat or dog visually, and even by examination. Additional tools for variable levels of birth control, delivered without the need for anesthesia, bring up the need for means of readily identifying animals as having been treated, especially those without an owner to oversee their care.
- Degree of community, governmental, and local veterinary participation and support, which can vary widely.
- Potential to collaborate with other organizations to provide a wider range of services.

5.6 How Do Practicing and Shelter Veterinarians Feel about Pet Sterilization and Potential Non-Surgical Approaches?

The 2002 Contraception and Fertility Control in Animals report noted that veterinarians are key to the commercial success of new animal health products – they have to embrace a product and use it in their practices, or in shelters where they work, and they are often on the front line. As of the 2002 publication, there was no research available on veterinarians' attitudes towards non-surgical alternatives to spay/neuter in cats and dogs. A variety of assumptions had been made concerning veterinarians' beliefs regarding non-surgical contraception and fertility control in dogs and cats. These assumptions have ranged from "spay/neuter works and is a profit center so why would I give it up?" to "it would be great to give clients choices" as representative attitudes among private practice veterinarians. Concerns among shelter veterinarians have been believed to relate to whether cost, permanence, and ease-of-use characteristics could compete with surgical sterilization.

In 2007, ACC&D commissioned a study of dog and cat contraception and fertility control attitudes in private practice veterinarians in the US, and in 2008 the organization collaborated with ASV on a similar study of shelter veterinarians in the US. Note that some ASV veterinarians are in private practices but were included in the shelter veterinarians surveys.

Summaries of key findings are provided below. To review the complete reports, see www.acc-d.org/ACCD%20docs/VetResearchWebReport.pdf and www.acc-d.org/ACCD%20docs/ASVResearchwebreport.pdf.

5.6.1 Private Practice Veterinarians

According to the 2007 survey of private practice veterinarians, there is a fairly low level of perceived need among veterinarians for an alternative to surgical spay and neutering for use in the larger community and in their own practices. Surgery was described as sufficient. Low awareness and unknowns about potential non-surgical sterilant alternatives created some skepticism. Among those who did see a great need, comments including "more affordable," "less risk with no anesthesia," and "good for use in pet population campaigns" were mentioned.

The majority of veterinarians included in this study firmly believe that surgical spay/neuter provides clear benefits beyond non-surgical sterilization, helping to prevent health problems and behavior problems in cats and dogs of both sexes. (While new products target comparable benefits, this was not stated in the survey and the respondents' assumption may or may not be accurate.) In addition, the majority of veterinarians place at least some importance on the contribution spay/neuter procedures make to revenues, though only a small percentage views these procedures as "very profitable" relative to other procedures. Importance was also placed on the role of spay/neuter in attracting new clients to the practice.

Due to the low level of perceived need for an alternative and the value placed on a range of benefits associated with spay/neuter surgeries, research concluded that private practice veterinarians – also referred to as "general practice" veterinarians – will need to be convinced to consider an alternative. However, the data also suggest there is perceived value in some of the benefits that a non-surgical sterilant may be able to provide their clients. For instance, the majority felt it could be a better option for shelters to use and may increase the number of sterilizations performed in the community overall. For their own practice, respondents recognized the value in offering a non-surgical alternative to clients who are averse to surgery or who have pets that are not good candidates for surgery. It also is valued as a lower-cost option for pet owners with barriers to the price of surgery.

There does appear to be some interest among these veterinarians in the concept of a single-treatment female cat contraceptive with a duration of 3 years, with 52% overall "somewhat or very likely to recommend" and 75% in the northeastern US the most "likely to recommend." The main attraction of this product is the lower cost and the ability to offer another option to clients who are averse to surgery; however, veterinarians are concerned about the need to repeat the treatment, expressing doubt that pet owners would remember to bring their cat in for another treatment every 3 years. It is fairly clear that, in order for any sterilant or contraceptive product to be broadly considered by veterinarians as a viable alternative to surgical sterilization (in more than just special circumstances), it will need to deliver many of the same benefits attributed to surgical procedures, preventing health and behavior problems, providing permanent sterilization and requiring only one treatment.

5.6.2 Shelter Veterinarians

The results of the 2008 shelter veterinarian survey were assessed in comparison to the 2007 survey of private practice veterinarians, summarized in the section above.

While veterinarians in both surveys agreed that unplanned litters are the major cause of unwanted pets, there was a significant discrepancy in how shelter vets versus private practice vets felt about the potential for non-surgical sterilants as a solution.

Nearly all veterinarians surveyed (96% shelter vets, 94% private practice vets) agreed that "unplanned litters contribute significantly to the number of unwanted pets in our community."

Six in 10 veterinarians working in animal shelters indicated that there is a need for non-surgical sterilization alternatives to control the pet population; however, 6 in 10 private practice veterinarians felt just the opposite, indicating that there was little or no need for non-surgical alternatives because surgical sterilizations are adequate and provide additional behavior and health benefits to pets.

Shelter veterinarians were almost twice as likely as private practice veterinarians to recognize possible benefits of non-surgical sterilizations.

In a side-by-side comparison of input from shelter veterinarians and general practice veterinarians, respectively, shelter veterinarians were more likely to find the following possible benefits of non-surgical sterilizations highly valuable (i.e., a rating of 6 or 7 on a 7-point scale):

- Could increase the number of sterilizations performed in the community (68% vs. 50%)
- Could increase the number of sterilizations performed in the shelter/clinic (64% vs. 34%)
- Reduces the time and resources required by the veterinarian and shelter (62% vs. 32%)
- Provides a lower-cost alternative to offer clients (53% vs. 30%)
- Is a safer alternative for the animal due to fewer risks and side effects than surgical spay/neuter (45% vs. 35%)

More shelter veterinarians than private practice veterinarians indicated that it was very important that a non-surgical product requires only one treatment, provides permanent sterilization, reduces unwanted behaviors of an intact pet, and protects against hormone-related diseases.

Shelter veterinarians were more likely to categorize the following attributes as very important components of a non-surgical sterilant or contraceptive product than private practice veterinarians:

- Requires only one treatment (86% vs. 70%)
- Provides permanent sterilization (93% vs. 78%)
- Reduces unwanted behaviors of sexually intact pets (83% vs. 72%)
- Protects against some reproductive tract and hormone-related diseases (78% vs. 65%)

While shelter veterinarians indicated a greater need for non-surgical sterilants to control the feral cat population in particular than private practitioners, the shelter veterinarians were much less likely to recommend a temporary (i.e., three year) contraceptive out of concern that the cats would not receive a repeat treatment. More than 7 in 10 shelter vets were concerned that their clients would forget to repeat the treatment.

- Fewer than a third (30%) of shelter veterinarians surveyed, compared to just over half (52%) of private practice veterinarians, said they would recommend this product to their clients.
- More than 7 in 10 shelter veterinarians said they were concerned that pet owners would forget to have their cats treated again in 3 years (82% versus 72%).
- All veterinarians saw the greatest need for sterilants to be available for female cats compared to male cats and female and male dogs; however, shelter veterinarians rated the need much greater, with 62% ranking it a 6 or 7 on a 7-point scale compared to general practice veterinarians, of whom 22% gave such a sterilant a rating of 6 or 7.

5.6.3 Key Messages from the Surveys

In order for any pet sterilant or contraceptive product to be broadly considered by veterinarians in the US as a viable alternative to surgical sterilization under more than just "special" conditions, the surveys appear to indicate that the product(s) will have to deliver many of the same benefits attributed to surgical procedures, i.e, preventing both health and behavior problems, providing permanent sterilization and requiring only one treatment.

Research, development, and successful commercialization of non-surgical sterilants will require educating veterinarians regarding the potential use of these products as a means of reducing

the pet population and as an alternative or supplement to surgical sterilization.

5.6.4 Other Issues Veterinarians Can Be Expected to Consider

What are the key characteristics that any product intended for use by companion animal veterinarians will require to be successful?

- **Profitability** – Veterinary practices are businesses and therefore must be profitable. Operating a veterinary practice entails carrying a variety of fixed costs, and although those costs vary depending on the location and nature of a given practice, surgical sterilization consumes time and resources that could be employed more effectively. A product that enables veterinarians to make a good margin and frees time to see additional patients could turn a relatively expensive procedure for the veterinarian into a solution that benefits owner, pet, and veterinarian alike. It would appear likely that given the inherent costs and time requirements of performing sterilization surgery, non-surgical alternatives offer the potential for greater margin.
- **Safety to humans** – Veterinary professionals administering contraceptives must be assured that these products can be used safely. Companies contemplating developing and/or marketing such products may also wish to consider including a delivery device that would limit the possibility of accidental self-injection or other exposure as part of the product concept.
- **Animal safety** – Veterinarians are charged with protecting the welfare of the animals they treat, and products must be proven to be safe to their satisfaction.
- **Minimal liability** – Liability is clearly a concern of veterinarians in instances in which an animal does not respond to a product as expected. In addition, products with durations that vary according to the responses of individual animals (e.g., contraceptive vaccines) should be labeled so that they can be re-administered at the minimum effective time frame safely in all target animals.
- **Documented effects on behavior and health that compare adequately to spaying and neutering** – There are positive non-reproductive effects of surgical sterilization which are documented and other "effects" which are essentially widely held beliefs. These effects range from decreased incidence of disease to behavior often felt to be more likely to preserve the pet-owner bond. Veterinarians value these effects and will need to understand how a contraceptive product compares. An unfavorable comparison will create barriers to acceptance.

While the labeling required by regulatory authorities will help veterinarians understand the expected effectiveness of new contraceptive and fertility control products, in-office testing that can assess continued effectiveness (e.g., for serum anti-GnRH antibodies in the case of a GnRH vaccine) to monitor how well products are working may help vets gain confidence and minimize concerns about liability.

Companies developing and marketing such products can also help build confidence by ensuring that veterinarians have client education tools that set appropriate expectations for the performance of a given product.

5.7 Owner Willingness to Pay for a Non-Surgical Alternative

Will clients be willing to pay for an innovative non-surgical contraceptive or fertility control product?

In the US, there are relatively recent, reliable data that show that dog and cat owners are willing to take their animals to the veterinarian and pay reasonable costs for treatment.

- Annual expenses in 2010-2011 for surgical visits for dogs in the US averaged $407 and routine veterinary visits averaged $248 .americanpetproducts.org/press_industrytrends.asp)
- Annual expenses in 2010-2011 for surgical visits for cats in the US averaged $425 and routine veterinary visits averaged $219 (americanpetproducts.org/press_industrytrends.asp)

An AVMA survey (AVMA Center for Information Management 2012) indicates that in 2011:

- "The mean veterinary expenditure per household [for dogs] was $378 in 2011 … the mean expenditure per visit was $146 … the mean expenditure per dog was $227
- "The mean veterinary expenditure per household [for cats] was $191 in 2011 … the mean expenditure per visit was $122 … the mean expenditure per cat was $90
- 81.3% of dog-owning households visited a veterinarian at least once in 2011, compared with 82.7% in 2006
- 55.1% of cat-owning households visited a veterinarian at least once in 2011, compared with 63.7% 2006

- 26.9% of dog-owning households reported spending between $200 and $499 on veterinary services in 2011 compared to 29.4% in 2006; 17.9% reported spending between $100 and $199, compared to 15.8% in 2006; 20.1% of dog owning households reported no veterinary expenditures in 2011, compared to 20.9% in 2006
- 15.4% of cat-owning households reported spending between $200 and $499 on veterinary services in 2011 compared to 19.1% in 2006; 13.3% reported spending between $100 and $199, compared to 15.3% in 2006; 46.1% of cat-owning households reported no veterinary expenditures in 2011, compared to 39.2% in 2006
- Total expenditures on veterinary services for dogs and cats in the US in 2011 were ~$26.43 billion
    - Total expenditures on veterinary services for dogs were ~$19.07 billion
    - Total expenditures on veterinary services for cats were ~$7.36 billion

In addition,

- 37% of caretakers of feral animals are willing to pay for veterinary services, including sterilization (Centonze and Levy 2002).

The 2011 AVMA survey indicated that 9.7% of the most recent veterinary visits by US dog owners and their dogs were to have the dog spayed or neutered, up from 7.3% in the 2006. Among cat owners, 13.1% of the most recent visits to the veterinarian were to get the cat spayed or neutered, compared to 14% in 2006.

Veterinarians can enhance the level of services they provide by playing a role in educating new pet owners about what to expect from sterilization or contraception in terms of dog and cat behaviors and strengthening the human-animal bond, thereby contributing to a decrease in relinquishment and abandonment rates. It is estimated that in the US, preserving the human-animal bond could increase veterinary income by at least $2 billion a year.

"Simply put, if animals don't remain in their homes, they can't be cared for by veterinarians as [the animals] age. They also can't use or consume products sold by pharmaceutical or pet industries" (Olson 2002).

5.8 The "Ideal" Product

Companies that will market new non-surgical products will be courting customers who may switch the method by which they control reproduction in their pets as well as customers who would not choose surgical sterilization. In the past, researchers and animal welfare advocates tried to define the "ideal" non-surgical contraceptive, with a long list of attributes such as "cheap" and "completely safe" and "100% effective." Paradoxically, creating such a high bar for a new product discouraged both research and investment into dog and cat contraception, as various stakeholders realized the difficulty of creating such an ideal product. ACC&D has encouraged stakeholders to consider a "tool box" approach – i.e., that there may be many different approaches to companion animal contraception, and each may have its uses, limitations, and strengths in different situations.

That said, it is useful to consider what types of issues could affect the "ideal" product characteristics for various potential customers and stakeholders. Below are some examples of these considerations.

Permanent versus nonpermanent ("reversible") versus long-term

- Permanent: pet owners who do not wish to breed their animals
- Nonpermanent: pet owners who plan to breed their animals, show animal owners, responsible breeders
- Long-term: pet owners who prefer a non-surgical approach and do not wish to have their pets sterilized; TNR programs (see section 5.4) serving populations for which modeling studies have demonstrated effectiveness equivalent to surgical sterilization

Contraception/fertility control with or without sexual behaviors

- For many pet owners, one of the reasons for using a contraception or fertility control product for their pets is to eliminate unwanted sexual behaviors
- Some pet owners may wish to avoid unwanted litters, yet feel that it is inappropriate to interfere with an animal's natural behaviors

Method of administration: implant versus injection versus oral

- Injection: Injections can cause transient pain to the animal, though it can be mitigated by the body location of the "shot." Reports of vaccine-associated fibrosarcoma have made some cat owners more aware of the potential for problems at the injection site. Ideally, marketed products will be based on

formulation technology that will minimize injection pain and injection site reactions.

- Implant: Implants have the same potential for transient pain as injections. In addition, owners may find it unacceptable to feel an implant beneath the skin when they pet their dog or cat, so a rigid or large implant may be unacceptable for some owners. However, should "soft" implants be developed and approved, they may be more difficult to feel, and therefore, more acceptable. (This is not likely to be an issue for population control in feral animals.) Should an owner wish a soft implant removed, for instance, to breed the animal, the implant may be located via ultrasonography.
- Oral: Although oral contraceptives for bitches and queens have been developed, they have offered no more than 6 months of fertility control and require precise, repeated administration. The side effects of these progesterone-based products include increased risk of conditions such as pyometra and diabetes; thus their acceptance is very poor in the US and variable internationally. In order to be approvable by regulatory authorities, an oral formulation will have to be demonstrated as safe for animals, humans, and the environment. This is particularly true of bait formulations.

Dogs versus cats

- Variation in cultural attitudes towards dogs and cats may influence the ultimate product mix. Some cultures "value" one species more than the other, and expenditures not only by owners but also by animal health companies may reflect that. Owners may demonstrate more resistance to contraceptive technologies for male dogs than for the tomcat, as is the pattern in some parts of the world with regards to surgical castration.
- Physiological differences between dogs and cats may affect the relative viability of a given technology. For instance, cats appear to be more sensitive to adjuvants present in certain formulations.
- Less research on these technologies has been undertaken in cats than in dogs, so it is unclear whether the technologies that appear most promising in dogs will be applicable to cats.

Despite the difficulty posed by the variability in pet owner preferences, the fact that more than one approach to contraception and fertility control in dogs and cats is being developed may mean that ultimately, overall rates of contraception in pets will increase, animal health in general will improve, and, hopefully, there will be fewer unwanted, stray, relinquished, and abandoned animals.

(See section 5.10 for a summary of technologies and marketing issues that are likely to occur as products are developed for the pet owner market.)

5.8.1 An Initiative to Develop a Single, Permanent, Non-Surgical Sterilant for Male and Female Dogs and Cats

In the past several years there has been a renewed interest in developing a single sterilant that would be effective in male and female dogs and cats. This objective has been made more attractive to researchers and organizations that may not have targeted this opportunity previously by the establishment of the Michelson Prize & Grants in Reproductive Biology. The program was launched in October 2008 and first grants awarded in 2009.

The $25 million Michelson Prize in Reproductive Biology is offered to incent researchers to develop an ideal non-surgical sterilant for dogs and cats. The competition is open to any qualifying entity from any country. The program has been established to develop a low-cost, non-surgical method of cat and dog sterilization that will enable nonprofit organizations, animal care centers, and non-governmental agencies (NGOs) to sterilize large populations of cats and dogs and reduce the number of homeless and unwanted animals that are killed each year in shelters.

As stated on its website (michelson.foundanimals.org/about-michelson). The foundation recognizes that research required to develop and test pharmaceuticals takes time and money, and many interested parties may not have access to the resources needed to initiate and maintain this research. For that reason, Found Animals also offers the companion Michelson Grants in Reproductive Biology, research funding for promising proposals in pursuit of non-surgical sterilization technology. See Chapter 4, section 4.3 for information on projects that have received grant funding as of the publication of this document, and michelson.foundanimals.org/grant-winner-bios for updates.

The $25 million Michelson Prize will be awarded to the first entity to provide the foundation with a technology proven to have defined characteristics. Specific parameters can be found in Chapter 4, section 4.3.3.1.

The Found Animals Foundation (foundanimals.org) is supporting and managing the process (michelson.foundanimals.org/about-michelson). Importantly, they also plan to fund and be involved in the commercialization and introduction of the technology of any winning application. Pursuing product development as a private foundation for charitable purposes may well result in different business plan and requirements for return on investment than those of a for-profit company.

Note that technologies for which Michelson Grants have been received that do not ultimately meet all Michelson Prize criteria, but are effective for a given population or a given length of time, may be developed or licensed for development by their inventors. A brief perspective for animal health companies looking to advance this category of products follows.

5.9 Commercializing, Manufacturing, and Marketing Products Profitably

Companies that advance non-surgical technologies will need to make sufficient profits to amortize years of R&D and regulatory approval, as well as current operating and product costs.

Companies may target nonprofit or governmental agencies and their veterinarians – especially those who are treating a large number of animals annually – as most receptive to non-surgical alternatives; however, the assumption that low price is a priority may be a barrier, and may not always be correct, as the cost will need to be compared with costs of surgical alternatives.

Animal health companies' primary focus is on pet owners and the veterinary community that treats their cats and dogs. Companies might see opportunities to partner with private practitioners with both a higher wholesale price and ultimate retail than would be seen at the shelter, where little mark-up would likely be taken. Animal health companies may fear a tiered-pricing structure that veterinarians could feel puts them at a disadvantage with "shelters."

However, alliances among animal-related funding sources, the shelter community, and animal health companies could help create a structure in which the cost of contraception and fertility control could be subsidized – in fact, many existing "low-cost" spay and neuter programs are actually subsidized, which means the difference in what the shelter, pet owner, or prospective pet owner pays and the actual cost is made up in some way (Slater 2002).

In addition, once several non-surgical approaches are commercialized and approved for use, competition may bring pricing down. This would enable the shelter community to take advantage of affordable products that will then have a history of safe and effective use by the veterinary community behind them.

For some time, the non-surgical dog and cat contraception community has operated under the premise that a "silver bullet" single approach is not feasible due to differences in canine and feline physiology as well as gender differences. Does the new Michelson Prize & Grants initiative to find and advance a single, permanent, approvable, and commercializable non-surgical approach for male and female cats and dogs lessen the opportunity to develop other approaches? To the contrary; in fact, the quest for a single, permanent approach can be expected to encourage research in this area and it has. As noted previously in this chapter, grantees whose approaches do not ultimately meet the criteria for winning the prize will have developed intellectual property that could result in value to licensees or acquirers of those technologies as candidates for further development (Jöchle and Rhodes, personal communication 2012).

While a single, permanent solution may fit the needs of some dog and cat population management stakeholders, there will be veterinarians and owners who prefer reliable non-permanent approaches, as indicated by the commercial success in Europe, Australia, and New Zealand of the 6- and 12-month Suprelorin (deslorelin) GnRH agonist implants marketed by Virbac (see Chapters 3 and 4).

5.10 Marketing Issues in Cats and Dogs: the Bottom Line

As discussed earlier in this report, the history of research in contraception and fertility control for cats and dogs is a long one. Researchers have continued to explore non-surgical alternatives, knowing that such products could offer significant advantages in population control for dogs and cats. This research has been of interest not only to organizations that have long been involved in the welfare of animals and creating awareness of population control

issues, but also to the new organizations that continue to be founded worldwide. Research continues, the quest for funding of low-profit or nonprofit segments continues, and debate about population figures and the relative value of population control strategies continues.

Non-surgical contraception of dogs and cats is regarded as a market with significant potential due to the sheer numbers of animals, the percentage of animals that are not spayed or neutered, the fact that pet owners spend an increasing amount of money on their animals, and focus of governments on broadscale population control programs of both dogs and cats to address zoonotic disease and public health. There are some common factors that marketers may want to consider when assessing what would be required to serve pet owners and the "shelter" community.

These include:

- Dealing with an unusually fragmented market in terms of customer groups, cultures, societal factors, and desired product profiles
- Understanding clearly the profile, including the limits of a given technology in terms of the marketing opportunities it presents – and doesn't present
- Implementing public relations and education activities that target each customer group and its key influencers
  - Being open to mutually beneficial collaborations, partnerships and unique business models, especially to serve the large market for unowned pets

5.10.1 Issues that Affect Marketing of Contraception and Fertility Control for Dogs and Cats

The following table is intended as a guide and is not to be considered exhaustive.

Table 5-24: Issues Affecting Marketing of Contraception and Fertility Control for Dogs and Cats

Customer Group	What's Important to Them
Owners and all other stakeholder groups	■ Human and animal safety ■ Reassurance that there are no harmful effects on treated animals, including very young animals and animals that are pregnant when treated ■ Reassurance that new technologies and products are effective ■ Clarity on the non-reproductive effects on behavior and health and believing they compare adequately to surgical sterilization ■ Eliminating "objectionable" behaviors (e.g., yowling, spraying, roaming, fighting) that contribute to pet abandonment ■ Ease of use ■ Predictable duration of effect ■ Predictable onset of effect
Nonprofit organizations and government agencies	■ Single treatment ■ Effectiveness (likely permanent sterilization) for adoptable animals and animals treated in Trap-Neuter-Release (TNR) programs ■ Trained technician can perform the procedure ■ Low cost
Reputable breeders and show animal owners	■ Flexibility (showing schedule vs. breeding schedule) ■ Full understanding of effects, if any, on treated animals, subsequent litters
Veterinarians	■ Client choice (permanent sterilization or predictable length of control) ■ Protects against some reproductive and hormone-related diseases ■ Trained technician can perform the procedure as a cost savings ■ Is profitable ■ Has regulatory approval and is backed by clinical safety and effectiveness data

6.0 Regulatory Issues: Some Considerations for Evaluating Contraceptives for Dogs and Cats

Regulatory requirements for approval of contraceptives for dogs and cats differ depending on the mechanism of action of the contraceptive approach, as well as the country in which approval is sought. The purpose of this chapter is not to serve as a detailed guide for regulatory approval of specific products, but to give an overview of the regulatory processes and issues.

Regulatory requirements generally fall into three major categories for companion animal products:

- Effectiveness
- Safety
- Manufacturing (also called chemistry, manufacturing and controls or CMC)

Note that there are additional requirements for products intended for animals used for food, (e.g., meat and milk). Discussion of these requirements is beyond the scope of this document.

The company that is developing the product is called the Sponsor, and it is responsible for submitting all information required for review by regulatory authorities prior to approval. In addition, most regulatory bodies that review data submissions to support regulatory approval require payment of significant fees (hundreds of thousands of dollars) as part of their review requirements.

6.1 United States Regulatory Agencies

The regulatory landscape for dog and cat contraceptives in the United States (US) is complicated.

Contraceptives can be divided into two broad categories: drugs and vaccines. Drugs to be used for contraception are regulated by the Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM). Although vaccines for animals are usually approved by the United States Department of Agriculture (USDA) Center for Veterinary Biologics (CVB), in the past the FDA has indicated that the CVM will regulate vaccines used for immunocontraception for dogs and cats.

For situations in which regulatory authority may be unclear, the Sponsor may contact the agency of its choice and which it believes is most appropriate, and that agency will then confer with the other agencies to make a decision as to which one will regulate. The convention is that the Sponsor will then be sent a Memorandum of Understanding (MOU) stating the decision.

The following is an example of a murky regulatory area – the regulation of immunocontraceptive vaccines for fertility suppression in wildlife. In the past, the CVM has indicated that it will regulate all immunocontraceptive products for all species. A regulatory decision was made in 2006 to clarify the role of the FDA, USDA and the Environmental Protection Agency (EPA) in regulating contraceptives for use in wildlife and free-roaming animals. A draft MOU between the FDA and the EPA was developed in which the EPA agreed to register contraceptives and immunocontraceptive vaccines for wildlife and feral animals (e.g., white-tailed deer, wild horses). In particular the EPA agreed to regulate GonaCon™, the gonadotropin-releasing hormone (GnRH) vaccine labeled as an "immunocontraceptive vaccine for use in white-tailed deer," and the label, as approved by the EPA, further defines the product as a "restricted use pesticide."

In 2006, at the Alliance for Contraception in Cats & Dogs (ACC&D) 3rd International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control, representatives of the National Wildlife Research Center (NWRC), a governmental agency within USDA Wildlife Services (WS) that developed GonaCon, announced that, in addition to agreeing to review the application for GonaCon for white-tailed deer, the EPA would register fertility control products targeted at feral cats based on an EPA-FDA MOU (acc-d.org).

The FDA agreed to retain authority over drug-based contraceptive and immunocontraceptive vaccines for use in captive and pet animals, including livestock, companion animals (dogs and cats) and zoo animals (Fagerstone, personal communication 2012).

NWRC recently indicated that it is interested in seeking EPA registration of GonaCon for use in feral and loosely owned dogs on Native American tribal lands in the US. Additionally, approval by the EPA is considered a step towards a new fertility control tool for free-roaming dogs internationally, where the dog rabies problem is far greater than in the US, because often international regulatory agencies will accept approval by US regulatory agencies as a basis for approval in their countries. NWRC provided a letter from the EPA, dated December 28, 2012, that states: "We have reviewed your request for a determination of whether EPA would have regulatory oversight for a new proposed use of Gonacon™ for use in wild and feral free-roaming dogs. This proposed use of Gonacon™ will be targeted

for wild and feral free-roaming dogs on tribal lands. We discussed this proposed use internally and with the Food and Drug Administration. It was decided that this is a pesticidal use that EPA will have regulatory jurisdiction over." NWRC has also requested an Experimental Use Permit from the EPA to conduct a large-scale efficacy study on US Indian reservations to collect the necessary field effectiveness data for a registration" (Fagerstone, personal communication 2012).

There are two interesting issues here. First, how will it work if the EPA regulates immunocontraceptives for feral cats and dogs, and the FDA regulates the same product used in pets? Second, how would "feral" be defined? In the case of dogs, it appears that the EPA regulatory authority will only apply to feral dogs on tribal lands – a significant restriction of the target population.

Advocates are leery of feral cats being defined as "pests" as a condition for a feral cat contraceptive to be approved by the EPA. The worry is that defining feral cats as pests would then allow eradication programs and might affect the Trap-Neuter-Return/Release (TNR) programs that are currently used. This concern was raised in considering EPA registration for the wild horse contraceptive ZonaStat-H, and ultimately it was concluded that EPA registration offered benefits that outweighed the concerns (Hazard[11], personal communication 2012).

If the EPA is the regulatory review body for a new product and classifies it as a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), data requirements might be abbreviated based upon proposed labeling and the method of application – for example, an injection or implant. Data requirements for pesticides are determined by use and likelihood of exposure to humans, domestic animals, and the environment. Appropriate waivers from the requirements for individual studies may be accepted by the EPA based on the lack of exposure potential, e.g., when use of an implant is involved.

A strategy for conducting studies for any pesticide and requests for waivers should be based on a thorough understanding of how the product would be used. For example, for the subject product to sterilize feral cats, consideration should be given to the following:

- How the product will be packaged
- Draft labeling
- Where the product will be used (e.g., states, locations, rural/urban areas)
- Opportunities that may exist for human exposure and potential effects; and whether restricted use would be appropriate to minimize likelihood of such exposure and effects
- How long the contraceptive/sterilant effect lasts in target animals
- Opportunities that may exist for environmental exposure
- Effective measures of package disposal; indicate return to manufacturer if appropriate
- Identify and analyze potential effects in humans should exposure occur; develop method of treatment for any human exposure; include a telephone emergency response number

An example of a contraceptive drug that EPA regulates is OvoControl® for control of wild pigeons, geese and ducks. This product is delivered as bait and distributed in the environment, which reduces the hatchability of the eggs. The EPA took regulatory jurisdiction of this product because it is distributed in the environment, and also because it is used for wildlife management.

The way to confirm the regulatory assignment of future products is for a Sponsor to undertake the process, navigating through the complexities and politics as they unfold.

6.2 European Regulatory Agency

Europe in particular is an attractive market for non-surgical contraceptive products for pets due to what is characterized as a historical and general reluctance to surgically alter dogs and cats (see Chapter 5 for further discussion). The European Medicines Agency (EMA, previously known as the EMEA) regulates companion animal drugs and vaccines for the European Union (EU). There is a centralized procedure for the approval of innovative new drugs, so that a Sponsor can submit one set of required documents (dossier) to achieve approval in all the member EU countries. Note that Suprelorin® (deslorelin) 6- and 12-month implants have been approved in the EU for fertility control in male dogs; the product is marketed by the animal health company Virbac (see Chapter 4).

[11] Holly Hazard is Senior Vice President, Programs and Innovations, at Humane Society of the United States (HSUS).

6.3 Rest of World

Review of all international regulatory considerations and procedures is beyond the scope of this document. In general, each country has its own procedures for meeting requirements, and it cannot be assumed that registration (i.e., regulatory approval) is assured in other markets once CVM or EPA requirements are met. For example, Canadian and Japanese registration often has requirements that differ from those in the US.

6.4 General Considerations and Harmonization Efforts

For each country, a full regulatory submission has to be prepared to achieve approval for marketing. Typically, agencies require that additional clinical trials be conducted in their own countries, and additional safety testing may be needed, even though regulatory approval may have been attained in other countries. Considerable effort is required to reformat regulatory submissions and meet all requirements. In general, studies are designed to prove safety, efficacy and environmental safety, and achieve the best label claim for these products. There is an effort to harmonize registration requirements in the major markets – the US, EU, Japan and other countries are working together to draft guidelines (Veterinary International Committee on Harmonization).

6.5 Time Frame for Regulatory Approval

How quickly regulatory approvals can follow after submission of all required documentation varies widely depending on the country, the product, and the quality of the submission. Working closely with a given regulatory body during the development of a drug or immunocontraceptive can speed up the process in some cases, but it would not be unusual for the entire approval process, including required studies and regulatory review, to take up to 6 to 10 years, and the process could be longer for long-acting products. Nonetheless, products have been approved, as shown in Table 6-1.

Products listed below received regulatory approval between 2003 and 2012. The list does not include older products based on progesterone or related compounds.

Table 6-1: Contraceptive Products Approved by Regulatory Authorities in the US and EU

	EPA	FDA	USDA	EMA
Dogs	-	Neutersol® (2003), also called Zeuterin™ (males) Intra-testicular sterilant	-	Suprelorin® (males) Gonazon™ (females) (Both products are GnRH agonists)
Cats	-	-	-	-
White-tail Deer	Gonacon™ (females) GnRH vaccine	-	-	-
Canadian Geese, Wild Pigeons and Ducks	OvoControl® Nicarbazin			
Wild Horses	ZonaStat-H (females) Porcine zona pellucida vaccine			

Note that the following sections pertain specifically to the US regulatory landscape but can be expected to be similar to the general requirements of any regulatory agency with responsibility for products for dogs and cats.

6.6 Effectiveness

What claims can the label of a contraceptive product contain? The claims are based on the effectiveness of the product and are backed up by clinical data in the relevant species. Design of the efficacy claims and clinical trials to prove them must be coordinated with a clear strategy so that at the end of the development process, the market is defined and communication with customer groups can be effective.

For contraceptives and fertility control agents, claim structure must include:

- The species in which the product will be used
- Definition of the population that the product is useful for (e.g., for male and female dogs greater than 6 months of age)
- How quickly the product will show its effect (e.g., for vaccines, how long is it from the initial injection and any follow-up boosters to full contraceptive effect)
- The length of time for which the product is proved to work (duration of effect)
- The potential reversibility of the treatment (e.g., will animals regain their ability to breed when treatment is discontinued, and if so, in how long a time)
- How the product is used (injections, oral dosing, implants)
- The schedule of use (e.g., once every 6 months)
- Dose (if applicable)

For both dogs and cats, it is important for a developer of a contraceptive product to make sure that the clinical trials are conducted in the widest possible population in order to achieve the broadest claim. For example, dogs of various ages and breeds should be used for the clinical work, as some veterinarians might want to use a contraceptive in adult animals, while others will be interested in treating very young animals, should owners not want their bitches to exhibit even one estrus.

Duration and potential reversibility of effect will need to be measured in clinical trials.

If the label claim is intended to be "effective contraception for a year," breeding studies of at least a year's length will be necessary. How will efficacy be proven if the label claim is permanent sterilization? Multi-year trials over the lifetime of a pet are not practical, and so it is unlikely that a label claim such as this would be realistic unless the product showed actual tissue destruction of the testicles or ovaries. One strategy that companies developing these types of products might want to take is to initiate launch of the product with a label defining duration as 6-12 months, and then continue the studies, filing label extensions to increase the duration claim, if possible, or to demonstrate that repeat treatment extends the duration of effect.

What about products that may have a variable onset of and decline in efficacy, such as a GnRH vaccine or ZP vaccine that may provide 6 months of contraception in one animal and 2 years in another? Even in the best of cases, individual animals in a clinical trial will probably have to be followed for at least a year, making clinical efficacy trials long, labor intensive, and expensive. If claims for continued effects based on booster immunizations are desired, multi-year trials may be needed.

For vaccines, co-development of a serum antibody test may be helpful, if serum antibodies can be shown to directly correlate to suppression of fertility. This type of correlation would have to be demonstrated adequately in large clinical trials. The veterinarian could then periodically test an animal for anti-zona pellucida (ZP) or anti-

GnRH serum antibodies to predict the need for booster vaccinations. For GnRH agonist implants, efficacy should be dose-related and easier to predict than that of immunocontraceptive agents, which rely on an animal's innate immune response to treatment.

One of the major reasons pet owners spay or castrate their pets is because the animals are exhibiting unwanted sexual behaviors such as estrous behavior in females, and mounting and territory marking in males. To include label claims on their contraceptive products such as "use of this product will reduce sexual behavior," sponsoring companies may have to conduct well-controlled, blinded behavioral evaluations. Even if it can be demonstrated that the proposed contraceptive suppresses serum sex steroids, such as testosterone in males, it is unlikely that regulatory authorities will allow using this surrogate endpoint to make behavior claims. Suppression of estrous behavior may be easier to document than reduction in aggression. If behavioral claims are desired, clinical trials to evaluate behavior will have to be designed carefully. It is interesting to note, however, that the package insert for Neutersol, which was approved by the FDA without behavior studies being required, includes the following statement: "As with surgical sterilization, secondary male characteristics (roaming, marking, aggression, or mounting) may be displayed."

6.7 Target Animal Safety

Contraceptive products must be safe for the target animals, but what exactly does "safe" mean? Safety is generally proved by doing a study in the "target" animal – i.e., the animal species in which the product will be used. Ideally the resulting study shows that the normal dose and higher doses, sometimes given multiple times, cause no adverse effects. The study must include a reasonable number of animals of the appropriate ages. For example, in the Veterinary International Committee on Harmonization Guidelines for Target Animal Safety, eight animals per group are suggested – four males and four females, and guidelines suggest inclusion of 0, 1, 3 and 5X groups[12]; total animals used may be limited to no more than 32. Animals are observed for any behavioral changes, and injection or implant sites are monitored for any signs of irritation, pain or inflammation. During the study, blood tests are usually performed to measure any drug effects on serum chemistry and hematology parameters.

At the end of the target animal safety study, animals are euthanized, and full necropsies are performed – gross pathology and histopathology are required, along with serum hematology and chemistry, and other, more specialized measurements depending on the product. All procedures must be done under good laboratory practice (GLP) guidelines, which increases the costs. Designing the protocol for the study, having the study reviewed and agreed to by the CVM in the case of drug approvals, completing the study (including the histopathology), and writing up a final report can take a year and up to $500,000 or more, depending on the species and duration of the experiment.

Many agencies also require "field safety" to be evaluated in a wider population of breeds and ages, in a "real-world" situation. To satisfy this requirement, safety information (side effects, also known as adverse events) are required to be collected during field trials and reported to regulatory authorities.

Finally, after a product is approved, the FDA and other regulatory bodies worldwide require post-approval monitoring for safety, called "pharmacovigilance." This means that the Sponsor is required to put in place a way to collect reports of problems or side effects seen in animals treated with the product, and these adverse events must be reported regularly to regulatory agencies to monitor safety in the actual population of dogs and cats being treated with the product.

6.8 Human Safety

In all cases, the safety of the person handling products is a concern. If a vaccine or other injectable product has a long-lasting or permanent effect, the people administering the product will be at risk for self-injection and compromise of their own fertility, and the labeling will have to reflect these issues. It may be that some types of products could be restricted to use by veterinarians only. For Gonacon, regulated by the EPA, the label states: "Restricted use pesticide: due to non-target injection hazard. For the use by USDA-APHIS wildlife services or state wildlife

[12] 1, 3, and 5X refer to multiples of the expected dosage.

management agency personnel or persons working under their authority." These types of requirements would have to be worked out with regulatory agencies.

Exposure to toxic substances such as chemotherapeutic agents, and worries about HIV and other infectious agents in human blood, have prompted a number of companies to develop injection technology that protects the person giving the treatment. It should be possible for some type of device to be developed for veterinary contraceptive injections that would similarly protect the veterinarian or technician giving the injections to animals.

This could become an issue even in the development of immunocontraceptives that may be entering clinical (field) trials. Veterinary clinics could be reluctant to participate in a clinical trial in which their staff members may be exposed to an experimental contraceptive. Certainly it would decrease the risk of participation if the experimental immunocontraceptive were to be delivered via a device that minimized the possibility of human exposure. In fact, the company that launches its contraceptive vaccine or injectable product along with a safe mode of administration should have a definite commercial advantage.

Concerns over liability will need to be addressed both in the clinical development of the product and in its commercial use, and this may be one reason companies have been reluctant to develop these types of products.

6.9 Environmental Assessment

Particularly for the EPA, but also for other regulatory agencies, the environmental impact of contraceptives must be evaluated, and the scope of this assessment will depend on the specific product. Oral, implanted or injected drugs that are given to individual animals that therefore have limited effects on the environment generally will receive a waiver from conducting an extensive environmental assessment.

Over the years, various research approaches to contraception for wildlife and feral animals have included attempts at designing baits to place in the area where feral animals live. Baited contraceptives are very unlikely to be approved, since the probability of "off-target" animals or humans – including children – being exposed is high. Development efforts are underway to achieve a species-specific result, in which only the target species will respond to or be able to access the bait, but species specificity is a significant obstacle.

6.10 Manufacturing

In the case of drugs, manufacture of the active pharmaceutical ingredient and the formulated product must be conducted under good manufacturing practice (GMP). For vaccines, similar quality standards apply. Below are some brief observations on some of the key regulatory issues in meeting the manufacturing requirements.

Probably the least appreciated but most important step in bringing a product through commercial development successfully is developing a formulation – that is, the active ingredient in combination with excipients that help keep it stable, in solution, buffered appropriately, etc. Formulations that are used for "proof-of-concept" research or early stage efficacy studies rarely are suitable for full-scale development. The final formulation needs to be stable – i.e., the active drug or antigen has to remain intact over a reasonable shelf life. It must be determined if the product needs special storage requirements (such as refrigeration), which may impact practical use in the field. If a proposed injectable is to be delivered in a multi-dose vial, the stability of the remaining product after the first dose is removed must be determined.

The formulation must be non-irritating to tissue when injected or implanted, especially if multiple applications are required (e.g., boosters or repeat implants). The formulation must be inexpensive enough to enable a market appropriate price and deliver a reasonable profit margin (which may vary depending on the Sponsor's objectives and situation). Sterilization methods must be developed that are effective and do not degrade the active antigen or drug. Analytical methods must be developed under GLP conditions to measure the antigen or drug. If an implant is being developed, release rates of the active need to be demonstrated under a variety of conditions, and if the product is to be an injectable, syringeability must be good.

Once a few possible formulations have been defined that meet these criteria, efficacy must be confirmed in a reasonable number of animals. For a contraceptive product, these efficacy tests can take 6 months to a year, assuming the product claim is for that length of time. Depending on the fragility of the product (antigen or GnRH agonist), the process of reaching a final formulation can take several years, and cost several million dollars.

Separate formulations may be needed for various species,

especially for products requiring adjuvants in order to be effective. Even if the active drug or antigen is the same, if the formulation is different, all requirements must be met for each formulation. During formulation development, it is necessary to begin to think about how the product will be manufactured and who will actually do the manufacturing. Some larger animal health companies have manufacturing facilities and have experience in putting together the data that the regulatory bodies requires, but smaller companies may have to locate toll manufacturers. For vaccines, Sponsors often use contract manufacturing sites that have the capability of meeting and experience with USDA requirements. For the two wildlife vaccine products approved by the EPA, each is made by the research organizations that originally developed the products.

Many requirements need to be fulfilled, and the details will not be reviewed here. In general, packaging, sterility, reproducibility from lot-to-lot, stability under a variety of handling conditions, cleaning requirements, and labeling need to be worked out. Data may need to be generated on the safety of the product and its raw materials for people exposed in the manufacturing process. The impact of manufacturing on the environment must be defined. Enough product must be made under GMP conditions to conduct the pivotal safety and field studies. Manufacturing must be scaled up to meet demand once the product is launched.

Each formulation of a product needs its own manufacturing process and documentation, so it is desirable to decrease development time and costs by developing one formulation that can be used in multiple species. Sterilization of the final product is also important for injectable or implantable products, and methods need to be established and proven not to degrade the active ingredients.

For GnRH agonists and antagonists, much of this information has been developed and approved for products used in humans, so GMP-manufactured bulk drug should be readily available, and development could be restricted to identifying a final formulation and manufacturing process. For immunocontraceptives, manufacturing may be more of an issue, particularly since there may be some antigen preparations that are extremely difficult to purify in bulk under GMP conditions. For example, using pig ovaries from slaughterhouse material as the source for porcine ZP proteins is an example of a process that may be useful for research purposes, but is hard to scale up for commercial use under GMP.

Selecting the right manufacturing process or toll manufacturer can make or break a product, its ability to meet regulatory requirements, and its profitability. Fail to fulfill any of the requirements, and no matter how well the product works, it will not be approved for sale.

The costs and timing of meeting the requirements and getting successfully through the approval process with the CVM and other regulatory agencies worldwide vary depending on the complexity of the product. Experienced companies know that assembling the necessary documentation for manufacturing can take a minimum of 2-3 years, and carries a multi-million dollar price tag. In some cases, a factory needs to be built, requiring large capital investment.

6.11 Conclusions

There is a long road from demonstrating that a certain contraceptive approach can suppress fertility in a dog or a cat, and achieving regulatory approval for a product that can be marketed. Although some approaches can be shown to be safe and effective, the time and technical expertise required for developing a manufacturing process that can be scaled up and result in a stable, reproducible product is often the main obstacle to regulatory approval.

In the last 10 years, we have seen only three products (Suprelorin, Gonazon, Neutersol/Zeuterin) achieve regulatory approval for contraception in cats and dogs. Gonazon was only approved for use in female dogs in the EU (and not commercialized). Suprelorin is approved for use in Australia, New Zealand and the EU and is reported to be a commercial success. The product Neutersol has also been approved in several Latin American countries under the name Esterilsol, and is being re-launched as Zeuterin in the US in the near future.

For each potential product, the regulatory path is unique. The information presented here should be used only as historical background and general guidance. In order to understand the path for a new product, there is no substitute for expert regulatory advice by an experienced consultant. This scarcity of cat and dog contraceptives approved for use reflects how difficult the regulatory hurdles can be, but one can hope that these products and Sponsors have pioneered the way for future products.

Glossary

Given the easy Internet availability of detailed descriptions of the terms used in this report, this glossary is the glossary of basic terminology related to fertility control and reproduction that was included in the 2002 *Contraception and Fertility Control in Animals* report. It does not attempt to include all terms used in this document.

ablation – removal of a part (usually by cutting)

antibody – an immunoglobulin that is made by white blood cells in response to exposure to an antigen

antigen – a substance that is administered to an animal to elicit an immune response (usually refers to a protein substance)

bulling – when cows in estrus mount each other as if to breed; this can cause excessive activity and bruising of the hind quarters (bulls and steers may also show this behavior)

castration – technically, removal of either the ovaries of females or the testicles of males, but the term is commonly used to apply to males and is considered synonymous with "neuter"

cDNA – complementary DNA, which defines the DNA coding sequence of a gene and can be used to define the amino acid sequence of a protein

cell-mediated immune response – specific acquired immunity in which the role of small lymphocytes (white blood cells) of thymic origin (T-lymphocytes) is predominant

depot injection – an injection of a drug that is absorbed slowly over a period of time; this allows a drug to have its effect over days rather than hours

down regulation – in the context of reproduction, used to mean that sensitivity to a hormone is decreased, usually due to a reduction in the number of receptors but sometimes due to the unavailability of receptors to respond to the hormone

estrus – also known as "heat"; the time during which an female animal is ovulating and receptive to breeding by a male; it is characterized by specific hormonal and behavior changes

follicle-stimulating hormone (FSH) – one of the hormones of the anterior pituitary that stimulates the growth of ovarian follicles in females and spermatogenesis in males

gonadotropin-releasing hormone (GnRH) – the hormone that is produced in the brain, released in a pulsatile manner, and stimulates the pituitary gland to release luteinizing hormone and follicle-stimulating hormone. It is a small, 10-amino-acid peptide

gonadotroph – specific cells in the pituitary gland that have receptors that bind the peptide GnRH; these cells produce follicle-stimulating hormone and luteinizing hormone

good laboratory practice (GLP) – a framework for non-clinical studies conducted for the assessment of the safety or efficacy of chemicals (including pharmaceuticals) to man, animals and the environment

good manufacturing practice (GMP) – the list of practices to be followed in manufacturing pharmaceutical products to meet worldwide regulatory requirements

heat – also known as estrus (see estrus above)

histopathology – a study of the microscopic appearance of tissues to look for any pathological changes caused by an illness, toxin, drug treatment, etc.

IgA – immunoglobulin type A; made by immune cells in response to exposure to an antigen; this type of immunoglobulin is usually secreted onto mucous membranes of the reproductive tract, nasal passages, etc.

IgG – immunoglobulin type G; made by white blood cells in response to exposure to an antigen; this type of immunoglobulin is circulated in the blood

immunocontraception – causing an animal to become infertile by injecting it with an antigen that causes an immune response to some component of the reproductive system, such as eggs, zona pellucida, sperm or GnRH

glycosylate – to add various types of sugar molecules to a protein; many proteins are glycosylated.

luteinizing hormone (LH) – one of the hormones of the anterior pituitary that acts to cause ovulation of mature follicles

and the secretion of estrogen in females, and stimulates the testes to produce testosterone in males

luteinizing hormone-releasing hormone (LHRH) – another name for gonadotropin-releasing hormone (GnRH)

necropsy – dissection of an animal's body after death to examine organs and tissues; usually done to determine the cause of death or to study the effect of treatments (called autopsy when referring to humans)

neuter – common term usually used to mean removal of the testicles of male animals, but can also mean spay in females

oligonucleotide – a relatively small fragment of DNA (usually about 2-20 bases)

ovariectomy – surgical removal of the ovaries; generally called a "spay" when referring to female cattle

ovariohysterectomy – surgical removal of the ovaries and uterus; generally referred to as spay when referring to female companion animals

spay – when used in reference to dogs and cats, spay means ovariohysterectomy, or surgical removal of the uterus and ovaries; when used in reference to cattle, spay means surgical removal of the ovaries

zona pellucida – a transparent, non-cellular layer or envelope of uniform thickness surrounding an ooctye (egg); made up of glycosylated protein

Guide to Acronyms

ACC&D – Alliance for Contraception in Cats & Dogs
AFSSA – Agence Française de Sécurité Sanitaire des Aliments
APHIS – Animal and Plant Health Information Service (US Dept. of Agriculture)
APPA – American Pet Products Association
ASPCA – The American Society for the Prevention of Cruelty to Animals
ASV – Association of Shelter Veterinarians
AVMA – American Veterinary Medical Association
AZA – Association of Zoos and Aquariums (at St. Louis Zoo)
BLM – Bureau of Land Management (US Dept. of Interior)
CAP – chlormadinone acetate
CDA – Centers for Disease Control and Prevention (US Dept. of Health and Human Services)
cDNA – complementary DNA
CNR – Capture, Neuter and Return/Release
CRC – Cooperative Research Centres (Australia)
CSIRO –Commonwealth Scientific and Industrial Research Organization (Australia)
CVB – Center for Veterinary Biologics (US Dept. of Agriculture)
CVM – Center for Veterinary Medicine (US Food and Drug Administration)
DMA – delmadinone acetate
DMSO – dimethyl sulfoxide
dZP – dog-specific zona pellucida
EMA – European Medicines Agency
EPA – Environmental Protection Agency (US)
EU – European Union
FDA – Food and Drug Administration (US Dept. of Health and Human Services)
FERA – Food and Environment Research Agency (UK)
FIFRA – Federal Insecticide, Fungicide and Rodenticide Act
FSH – follicle-stimulating hormone
FSHR – follicle-stimulating hormone receptor
fZP – feline zona pellucid
GLM – good laboratory practice
GMP – good manufacturing practice
GnIH – gonadotropin-inhibitory hormone
GnRH – gonadotropin-releasing hormone
GnRH(F) – Canine Gonadotropin Releasing Factor®
HSHVSN – High Quality High Volume Spay Neuter
HSI – Humane Society International
HSUS – The Humane Society of the United States
IFAW – International Fund for Animal Welfare
LH – luteinizing hormone

MAF – Morris Animal Foundation
MATER – maternal antigens that embryos require
MGA – megestrol acetate
MIB – mibolerone
MPA – medroxyprogesterone acetate
NCPPSP – National Council on Pet Population and Policy
NICHD – National Institute of Child Health & Human Development
NII – National Institute of Immunology (India)
NSAID – non-steroidal anti-inflammatory drug
NWRC –National Wildlife Research Center (US Dept of Agriculture)
OIE – World Organization for Animal Health
PAD6 – peptidyl arginine deiminase
PAP – pokeweed antiviral protein
PFA – Pet Food Association
PRO – proligestone
PZP – porcine zona pellucida
RSPCA –Royal Society for Prevention of Cruelty to Animals (UK)
SPRASA – sperm protein targeted by anti-sperm antibodies
TCC – transitional cell carcinoma
TNR – Trap-Neuter-Return; also Trap-Neuter-Release
TTVARM – Trap, Test, Vaccinate, Alter, Release, Maintain
UBI – United Biomedical, Inc.
UCD – University of California, Davis
UI – University of Iowa
UK – United Kingdom
US – United States
USDA – United States Department of Agriculture
USGS – United States Geological Service
UVA – University of Virginia
VCD – vinylcyclohexene diepoxide
VLP – virus-like particles
VSF – Vétérinaires sans Frontières-Canada
VWB – Veterinarians without Borders
WCC – Wildlife Contraception Center (St. Louis Zoo)
WHO – World Health Organization
WS – Wildlife Services (USDA APHIS)
WSPA – World Society for the Protection of Animals
ZP – zona pellucida

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