



## Non-surgical methods of regulation reproductive function and contraception males of domestic animals

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### Abstract

The regulation of male reproductive function today is not limited to surgical castration; there are many other methods of controlling reproductive function. Non-surgical methods of controlling male reproductive function can be reversible and not reversible, i.e., reproductive function is preserved or completely suppressed. Castration of males or orchiectomy leads to irreversible sterility of the male, when the male completely loses the ability to reproduce. This operation can also entail some side effects: obesity, underdevelopment of the external genital organs, an increased risk of diabetes or hypothyroidism, problems with frequent urination and behavioral problems. Therefore, methods of non-surgical management of reproductive function and contraception in males are being actively developed. The article presents the latest methods of contraception and the management of the reproductive function of male domestic animals (cats, dogs): clinical application GnRH and agonists GnRH, Non-Peptide GnRH Antagonists, GnRH-Toxin Conjugate, GnRH Vaccines and other immune contraceptive vaccines, chemical sterilants for intratesticular injections, calcium chloride, zinc gluconate, chlorhexidine digluconate, hypertonic saline, sex steroids (progestines), gene silencing, kisspeptin and GnIH, targeting delivery of cytotoxins, single-dose hormonal male and female sterilianr, FSH – receptor ligand cytotoxin conjugates, Sperm Protein Reactive with Antisperm Antibodies (SPRASA) Reversible Inhibition of Sperm under Guidance(RISUG).

**Key words:** males, cats, dogs, contraception, progestin, GnRH, vaccines, intratesticular injection, cytotoxines, non-surgical methods.

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## 1. Introduction

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 (Kustritz, 2010).

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 (Cathey & Memon, 2010).

However, there are other non-surgical methods of regulating sexual function and contraception in male domestic animals that can be completely reversible or irreversible. These methods can eliminate the aggressive behavior of males, as well as prevent the development of prostate diseases.

## 2. Results and discussion

### Gonadotropin-Releasing Hormone (GnRH)

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

1. Potentially effective in males and females.
2. 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).

3. Suppression of GnRH will result in suppression of the secretion of the reproductive steroid hormones and therefore suppress sexual behavior and hormonemediated 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 (Rhodes, 2017).

### 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 (Gobello, 2007).

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 (Gobello, 2007).

Another GnRH agonist – deslorelin – was developed in an implant formulation for use in dogs and has been used in both domestic animals and wildlife. A disadvantage of the GnRH agonist approach to suppress reproductive activity is that initial administration in males typically causes the increase in LH causes an increase in testosterone that does not express itself clinically. 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 (Goericke-Pesch et al., 2013).

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. Research has shown that Suprelorin is also effective in fertility suppression in bitches and in male and female cats (Goericke-Pesch et al., 2011).

Note that in addition to contraception, GnRH agonists also cause significant shrinkage of the prostate gland, 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 (Limmanont et al., 2011).

### Deslorelin

Deslorelin in Dogs – Males. Researchers reported a mean duration of efficacy of 89 weeks, with a range of 56–132 weeks (Trigg & Yeates, 2008).

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 (Goericke-Pesch et al., 2011).

Suprelorin on male cats. 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” (Novotny et al., 2012).

### 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 & Wood, 2006).

But a significant drawback of these drugs is that they are not registered for animals and the dosage forms that are described are used only in humane medicine.

### 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. 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 (Concannon, 2006).

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).

Nowaday this compound is not under development for animals.

### 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. Then, theoretically, permanent sterility would result, and little or no “off-target” toxicity would be seen (Nett & Jarosz, 2002; Ball et al., 2006).

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 (Ball et al., 2006).

### 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 (Donovan et al., 2012).

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. 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 (Sad et al., 1993; Robbins, 2004; Wang et al., 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).

### 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 (Oliveira et al., 2013).

#### Zinc Gluconate

Zinc solutions that cause testicular degeneration and permanent sterility have been developed for direct in-

tratesticular 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 for use in male dogs 3-10 months of age for chemical (non-surgical) sterilization. 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 (Oliveira et al., 2013).

#### 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.

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. The maximum responses in both the biochemical and histological parameters related to chemo-sterilization were noted at the 15- or 20-mg doses (Leoci et al., 2019; Jana & Samanta, 2007).

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 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 & Samanta 2010).

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 (Leoci et al., 2019).

### **Chlorhexidine Digluconate**

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 (Cathey & Memon, 2010).

### **Hypertonic Saline**

A study conducted in 40 rats compared orchietomy 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 orchietomized. The study was undertaken to investigate an alternative, minimally invasive approach to castration in human patients with metastatic carcinoma (Leoci et al., 2019).

At 30 days after injection, the rat testes were slightly atrophied, and testosterone levels were similar to those for animals that had an orchietomy. 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 orchietomy and medical castration” but that further laboratory work would be required to ascertain the potential utility of this approach in dogs.

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

### **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 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 et al., 1981). These drugs work through negative feedback at the level of the brain and pituitary. 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 (Max et al., 2015).

### **Progestins**

Progestins are a class of compounds that are structurally similar to progesterone, and mimic its biological effect. Based on the principles of negative feedback exogenous progestins should suppress gonadotropin secretion in males, thereby disrupting spermatogenesis (England, 1997).

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 & Wood, 2006).

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 (Donovan et al., 2012).

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

### **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). 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 (Dissen et al., 2012).

### **Kisspeptin and Gonadotropin-Inhibitory Hormone (GnIH)**

Kisspeptins, which were identified in 2001, are expressed in neurons of the hypothalamus. These neurons synoptically 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., 2006).

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 downregulation of the hypothalamo-pituitary-testicular axis response to kisspeptin following continuous administration" (Fellman et al., 2006).

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., 2006).

### 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, 2010).

### 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 antispermato-genic 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 & Roby, 2010). 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 (Gupta et al., 2012).

### 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 (Jana & Samanta, 2010).

### 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 (Yan Cheng & Mruk, 2010).

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 (Lohiya et al., 2014).

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 & Guha, 2010).

### 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. 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.

### 3. Conclusions

Non-surgical methods of contraception and regulation of sexual function are an excellent alternative to surgical castration, when it is necessary to suppress the reproductive function of males for a certain time in order to treat diseases of the prostate gland and reduce aggressive behavior.

Also, non-surgical methods of contraception are good in the case of complete and irreversible suppression of reproductive function in males who do not want surgical intervention (contraindications to surgery, age, intolerance to the components of anesthesia), as well as for stray dogs for the purpose of humane treatment (no postoperative complications, do not require care, methods are simple and quick to use).

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