

Non-surgical methods of contraception and sterilization

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Abstract

The Humane Society of the United States estimates that each year between 8 and 10 million dogs and cats enter shelters and 4–5 million of these animals are euthanized due to lack of homes. Many veterinarians within the United States recommend surgical sterilization for population control in dogs and cats. However, there are non-surgical methods to control reproduction. Pharmacologic methods of contraception and sterilization can be safe, reliable and reversible. Hormonal treatments using progestins, androgens, or gonadotropin releasing hormone (GnRH) analogs act to either directly block reproductive hormone receptor-mediated events, or indirectly block conception via negative feedback mechanisms. Immunocontraception, via vaccination against GnRH, the luteinizing hormone receptor or zona pellucida proteins, is also possible. Intratesticular or intraepididymal injections provide a method for non-surgical sterilization of the male dog and cat. Additional methods have been employed for mechanical disruption of fertility including intravaginal and intrauterine devices and ultrasound testicular ablation. Alternative approaches to surgical sterilization will be reviewed.

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Keywords: Contraception; Non-surgical; Sterilization; Canine; Feline

1. Introduction

Over half of all households in the United States own a dog or cat [1]. Although exact figures are unknown, the Humane Society of the United States estimates that each year between 8 and 10 million dogs and cats enter shelters and 4–5 million of these animals are euthanized [2]. Unwanted dogs and cats may be reservoirs or vectors of transmissible diseases to man and to economically valuable domestic species. In Great Britain, feral cats kill approximately 100 million birds and mammals each year [3]. There also are ethical implications of euthanizing millions of animals each year. The purpose of this review article is to present a

50-year perspective of research on non-surgical methods for limiting pet reproduction.

Traditional neutering of companion animals has been accomplished through surgical methods of sterilization, namely ovariectomy (spaying) and orchidectomy (castration). However, for understandable reasons, not all owners have their pets surgically sterilized. For purpose-bred bitches and queens, the safest, most effective and least expensive method to prevent unwanted matings is indoor confinement and segregation from intact males. Even for those pets not intended for breeding, pet owners may still be reluctant to consider surgery. In a survey in Sao Paulo, Brazil, 56.5% of owners of adopted shelter dogs were against surgical sterilization, citing compassion (58.1%), unnecessary procedure (11.4%), cost (9.5%), and behavior change (4.8%) as reasons against this method of limiting pet reproduction [4]. In addition, when considering feral cat and dog populations where permanent sterilization is desired, surgical methods can

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be too time consuming and expensive to be performed on a large-scale.

Currently, several alternatives to surgical sterilization are being investigated. For the purposes of this discussion, contraception will be defined as a reversible method for blocking fertility (and will not include pregnancy termination). Pharmacologic methods of contraception and sterilization can be safe, reliable, and reversible. Hormonal treatments, including progestins, androgens, or analogs of gonadotropin releasing hormone (GnRH), act either directly to block reproductive hormone receptor-mediated events or indirectly block conception via negative feedback mechanisms. Immunocontraception via vaccination against GnRH, the luteinizing hormone (LH) receptor, or zona pellucida proteins are also possible. Intratesticular or intraepididymal injections provide a method for non-surgical sterilization of the male dog and cat. Additional methods have been employed for mechanical disruption of fertility, including intravaginal and intrauterine devices and ultrasound testicular ablation. Research investigating non-surgical approaches to contraception and sterilization will be reviewed.

2. Hormonal down-regulation

Hormonal down-regulation is an alternative for temporary suppression of fertility in breeding animals. Exogenous steroid hormones suppress fertility indirectly via inhibition of pituitary gonadotropin secretion and release (mainly LH) [5].

2.1. Progestins

2.1.1. Female dogs and cats

Megestrol acetate, a synthetic progestin, is a tasteless, odorless crystalline powder. Megestrol acetate is rapidly metabolized when given orally, with a half-life of 8 days in the dog [6]. Megestrol acetate has been used extensively for temporary estrus suppression in the bitch. When megestrol acetate was administered to bitches at a daily dose of 2.2 mg/kg body weight orally for 8 days (beginning in early proestrus), estrus was suppressed in 92% of cases [7]. Pyometra, a reported side effect of megestrol acetate treatment, developed in 0.8% of treated bitches [7]. Megestrol acetate was also effective at suppressing estrus in queens when given at a dose of 5 mg/cat orally for 5 days and then once weekly [6,8]. Reported side effects of prolonged megestrol acetate treatment in dogs included: increased appetite leading to weight gain; lethargy or restlessness [6,8]; marked mammary stimulation with hyperplastic and/or

neoplastic changes; clinical and pathologic changes typical of diabetes mellitus [9,10]. Similar side effects have also been reported in queens [6,11,12].

Medroxyprogesterone acetate (MPA) is a long-acting injectable progestin that has been used to suppress estrus in the bitch and queen but to a more limited extent than megestrol acetate, due to the high incidence of side effects. Occurrence of uterine disease was common in MPA-treated animals. The prevalence of uterine lesions on histopathology (after ovariohysterectomy) was 45% for bitches treated with MPA for estrus suppression, compared to 5% for untreated animals [13]. In addition to uterine lesions, subcutaneous administration of MPA in dogs has resulted in clinical signs consistent with adrenocortical suppression (e.g. alopecia, hair discoloration, thinning of the skin and mobilization of subcutaneous fat) [14]. It is noteworthy that MPA is not recommended for use in cats [15].

Proligestone (14 α ,17 α -propylidene-dioxy progesterone) is a unique progestin with weaker progestational activity than other synthetic progestins [16]. Proligestone is marketed in Europe (Delvosteron, Intervet) as an injectable canine contraceptive. The manufacturer claims that it is safe to use for prevention, delay or suppression of estrus when given to female dogs at an initial dose of 10–30 mg/kg SQ, with repeated administration 3 and 7 months later [17]. It can also be given to female cats (1 mL subcutaneously), causing estrus suppression for about 6.5 months [17]. In clinical trials, this regimen did not promote development of uterine disease or mammary tumors [16].

Canine and feline contraception through hormonal manipulation has been practiced for many decades, with the first report by Murray and Eden [18]. However, most of our understanding regarding the side effects of progestin administration in dogs comes from research on human contraceptives for which dogs served as animal models. In 1962, the U.S. Congress passed the Kefauver-Harris Amendment that mandated all drugs developed for use in humans must first be extensively tested in animals [19]. The current recommendations from the Food and Drug Administration are to administer new human contraceptives to dogs at doses 1, 10 and 25 times the anticipated clinical dose for humans [20]. Reported adverse effects depend upon the type of progestin administered, dose, time of treatment, treatment regimen, and age of the animal [21,22]. In beagles treated with doses of MPA 1–25 times the human contraceptive dose for 4 years, a dose-related increase in mammary dysplasia was reported [16,23]. However, it is important to note that the canine mammary gland undergoes pathologic changes following progestin administration in a way not

commonly seen in other species [24,25]. Therefore, dogs may have a unique sensitivity to the mammary tumor promoting effect of progestins via progestin-induced growth hormone induction [26]. Perhaps there are species differences in the relative affinities of progesterone receptors for contraceptive steroids [24]. Progestin administration induces progesterone receptor synthesis in the mammary glands and uterus of adult beagle bitches but not in other laboratory animals [27].

2.1.2. Male dogs

Spermatogenesis is regulated by follicle stimulating hormone (FSH) and LH. Based on the principles of negative feedback previously described for the female, exogenous progestins should suppress gonadotropin secretion in males, thereby disrupting spermatogenesis. In male dogs, daily oral treatment with megestrol acetate (2 mg/kg) for 7 days produced no change in semen quality; higher doses (4 mg/kg) produced only minor secondary sperm abnormalities. However, subcutaneous administration of MPA (20 mg/kg) produced a rapid response (within 3 days), with significant decreases in sperm motility, morphology and output [28]. Because of the rapidity of the response, the authors postulated that the effect was mediated by the direct action of progestins on epididymal spermatozoal maturation and transport. However, semen quality was not adversely affected when MPA was given at dosages of 4 mg/kg [29] or 10 mg/kg [28].

2.2. Androgens

2.2.1. Female dogs and cats

Androgens have also been used for contraception in female pets. Weekly intramuscular injections of 110 mg of testosterone propionate have been used to prevent estrus in bitches [30]. In addition, oral administration of 25–50 mg of methyltestosterone twice weekly inhibited estrus in bitches [30]. Mibolerone is a synthetic androgen that was marketed for estrus suppression in dogs and cats [31,32]. The dose for mibolerone in bitches varied with body weight and breed [6]. For bitches up to 12, 12–23, 23–45 and over 45 kg, the mibolerone dosage is 30, 60, 120 and 180 µg/day, respectively. Any German Shepherd Dog or any Alsatian-derived mixed breed should receive the maximum daily dosage (180 µg/days); the reason for the higher dosage requirement within Alsatian lineage is unknown [33]. If treatment is initiated at least 30 days prior to the onset of proestrus, estrus can be postponed for up to 2 year with continuous therapy. Following cessation of the treatment, return to estrus will occur

within 70 days on average (range, 1–7 months) [31]. Continuous treatment up to 5 year has been done, but it is generally not recommended to treat continuously for more than 24 months. The most common side effects reported in dogs are clitoral hypertrophy and vaginitis [8,32]. Other side effects include increased body odor, urinary incontinence and urine spraying, mounting behavior, cervical dermis thickening and epiphora [6,8,31]. Mibolerone is also contraindicated for use in Bedlington terriers due to an increased risk of hepatic dysfunction.

The oral dosage for mibolerone in queens is 50 µg/days [32]. Lower doses did not suppress estrus in queens. This dose is near the toxic dose; hepatic dysfunction has been observed in queens at doses of 60 µg/days, with mortality ensuing at doses of 120 µg/days [6,8]. Cervical skin thickening and clitoral hypertrophy was observed in cats and did not resolve after drug withdrawal [32].

2.2.2. Male dogs

While administration of exogenous progestins has not reliably interrupted spermatogenesis, administration of exogenous androgens has been more effective. Subcutaneous administration of 5 mg/kg of testosterone esters (testosterone propionate 0.6 mg/kg, testosterone phenylpropionate 1.2 mg/kg, testosterone isocaproate 1.2 mg/kg, testosterone decanoate 2.0 mg/kg) to male dogs resulted in a significant decline in sperm motility (within 3 weeks after treatment) that persisted for 3 months [28]. Daily oral administration of 50 mg of methyltestosterone to male dogs for 90 days decreased daily sperm output [34]. Chronic administration of danazol, a synthetic derivative of 17 α -ethinyl testosterone, to male dogs resulted in loss of the spermatogenic elements and azoospermia [35]. These effects were reversible within 60 days [35]. Other than a transient elevation in serum-amino-transferase, hepatic function during danazol treatment was unaltered [35]. Administration of anti-androgens, like flutamide or cyproterone, resulted in only a slight, transient influence on spermatogenesis [5].

Intramuscular administration of exogenous prolactin (600 µg/kg of body weight weekly for 6 months) to male dogs resulted in severe asthenozoospermia, teratozoospermia and oligospermia or azoospermia within 6 weeks of treatment [36]. Prolactin is a protein hormone secreted by the anterior pituitary gland. In men, hyperprolactinemia resulting from pituitary adenoma results in oligo- or azoospermia. At the end of 3 months of treatment in dogs, degenerative changes within the seminiferous tubules were evident on testicular biopsy. Within 3 months of drug withdrawal,

the sperm count normalized, mating produced pregnancy, and offspring exhibited no developmental abnormalities [36]. It was noteworthy that serum concentrations of testosterone, LH and FSH were not significantly affected by prolactin treatment [36]. The authors speculated that prolactin may be having a direct effect on the testes rather than functioning via hormonal down-regulation [36].

2.3. Gonadotropin releasing hormone (GnRH) agonists

It is well known that GnRH acts as the master reproductive hormone through regulation of the release of LH and FSH from the pituitary. In males, LH regulates testosterone synthesis, whereas FSH is necessary for the initiation and maintenance of spermatogenesis. Testosterone is needed for spermatogenesis and for the development of secondary sexual characteristics, including behavioral characteristics such as territorial marking (spraying), mounting and aggressiveness. In females, both LH and FSH are required to stimulate the ovarian changes leading to ovulation.

Within the past decade, GnRH analogues have been developed to suppress fertility. Sustained exposure to GnRH reduces GnRH-stimulated gonadotropin secretion through GnRH receptor down-regulation, internalization and signal uncoupling. Hormonal down-regulation was used to achieve reversible contraception.

2.3.1. Female dogs

Subcutaneous administration of nafarelin (32 µg/days) via an implanted osmotic pump or daily injections postponed puberty and estrus in female dogs [37]. Similar effects have been reported with subcutaneous administration of azagly nafarelin via an implanted osmotic pump at doses of 16 µg/days [38]. Cycling bitches returned to estrus between 2 and 18 weeks after cessation of treatment [37,38]. Prepubertal bitches displayed their first estrus 3–4 months after cessation of treatment [37,38]. Subcutaneous administration of deslorelin via a slow-releasing implant suppressed estrus for up to 27 months in female dogs [39]. Deslorelin administered subcutaneously daily to prepubertal female dogs for 23 months delayed puberty, followed by normal fertility [40]. The predominant side effect of GnRH analogues for contraception in bitches is the induction of estrus in anestrus bitches within 1–4 weeks following initiation of the treatment [37,38]. Treatment should be initiated before 4 months of age, within a period of 60 days following an ovulatory estrus, within 7 days of

parturition or following 7 days of oral exogenous progestin treatment (megestrol acetate (2 mg/kg/days)) to prevent estrus induction [37,38,41].

2.3.2. Male dogs

Several studies have examined the use of deslorelin as a male contraceptive in 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 [42]. Treatment-induced effects on fertility were completely reversible [39,43]. Testosterone and LH concentrations and semen quality returned to normal by 60 weeks after implant administration [42]. The threshold concentration of deslorelin necessary for suppressing spermatogenesis in male dogs was >0.25 mg/kg of body weight [39]. A long-acting deslorelin implant (Suprelorin[®], Peptech Animal Health) is commercially available in Australia and New Zealand. The manufacturer claims that this product will result in contraception for at least 6 months in 98% of male dogs. Serial administration of multiple implants at 6-months intervals did not result in adverse effects or diminished efficacy [44].

In addition to deslorelin, daily subcutaneous injections of nafarelin (2 µg/kg/days) decreased basal testosterone concentrations and resulted in infertility within 3 weeks after the onset of treatment [45]. Within 8 weeks of cessation of treatment, normal fertility was restored [46]. Two other GnRH agonists have been used for contraception in male dogs. Administration of a single subcutaneous injection of leuprolide acetate (1 mg/kg) to intact male dogs decreased ejaculate volume, increased the percentage of morphologically abnormal spermatozoa and significantly decreased serum testosterone and LH concentrations for 6 weeks [40]. Return to normal spermatogenesis occurred 20 weeks after treatment [40]. Subcutaneous administration of buserelin implants (6.6 mg) to intact male dogs decreased testosterone concentrations to basal values and resulted in infertility within 3 weeks of implant administration [47]. The contraceptive effect persisted for an average of 233 days [47].

2.3.3. Wild carnivores

Gonadotropin-releasing hormone agonists have provided safe and reversible contraception in wild carnivore species. Long-acting deslorelin implants at dosages of 3–15 mg, depending on body weight, induced contraception lasting >1 year in male and

female African wild dogs [48], male and female red and grey wolves [49], male and female cheetahs [48], male and female leopards [48], female lions [48], female Fennec foxes [49], and the male black-footed cat [49]. Female wild dogs and wolves responded less consistently compared to other species; deslorelin administration commonly resulted in estrus induction, with pregnancies occurring in three bitches [48,49]. A few female lions and cheetahs displayed estrus following implant administration, but did not allow mating [49]. Simultaneous administration of progestins to three female African wild dogs and one lioness did not suppress the induced estrus. In males, fertility was not interrupted for 6 weeks after the insertion of implants [48,49]. In addition, in two male bush dogs, long-acting deslorelin administration did not suppress reproductive function [49]. No side-effects or behavioral changes were noted following deslorelin implant administration [48,49] and normal estrous cycles and spermatogenesis resumed 12 months following implant administration [48,49].

2.4. GnRH antagonists

The GnRH antagonists block pituitary GnRH receptors, resulting in suppression of gonadotropin release. Subcutaneous administration of acyline (110 µg/kg) to bitches within the first 3 days of proestrus resulted in a short, anovulatory estrus with a return to proestrus within 3 weeks of treatment [50]. No side effects were observed.

3. Immunosterilization/immunocontraception/immunocastration

Over the past two decades, efforts have been made to develop a vaccine that could suppress fertility in both female and male canids and felids. Several targets of immunocontraception have been identified, including GnRH, LH and its receptor, sperm antigens, and oocyte zona pellucida (ZP). The physiologic effects of antibody titers against GnRH included suppression of reproductive behavior in both males and females, suppression of synthesis and secretion of gonadotropins and steroid hormones, arrested gametogenesis and gonadal atrophy [51,52]. Antibody production against the LH receptor suppressed estrous cycles in females and lowered serum testosterone concentrations in males [53,54]. Antibody production against sperm antigen and the ZP would prevent sperm from binding to the oocyte and therefore prevent fertilization.

3.1. Immunization against GnRH

Development of a GnRH vaccine for immunocontraception is problematic for several reasons. Native GnRH is not naturally immunogenic; GnRH is a small decapeptide hormone that is well conserved throughout all mammalian species. Therefore, under normal conditions, GnRH is recognized by the immune system as self (allogenic). Subsequently, administration of a vaccine derived from native GnRH results in no antibody production or a short-lived, weak response because the animal is tolerant to its own hormones. However, if GnRH is altered in a way that induces recognition as a foreign material, e.g. coupling it with another molecule with many antigenic determinants, an IgG response will occur [55].

To enhance antigenicity, GnRH molecules have been conjugated to various antigens to mobilize T-helper cells. Vaccination with fusion protein composed of canine GnRH and the T helper cell epitope p35 originating from canine distemper virus F protein was strongly immunogenic and resulted in loss of testicular function in dogs [52]. Vaccination of male dogs with a fusion protein of GnRH conjugated to tetanus toxoid had similar effects, which were reversible once antibody titers waned [51]. Vaccination of male cats using the same vaccine resulted in a similar antibody response, but did not result in infertility [51]. In addition, vaccination of male dogs with N-terminal modified GnRH conjugated to tetanus toxoid did not result in infertility [56]. Additional carriers that have been conjugated to GnRH for vaccination in pigs and cattle include *Mycobacterium tuberculosis* hsp 70 and ovalbumin, respectively [57,58]. A GnRH vaccine (Improvac[®], CSL Animal Health) is commercially available in Australia for use in mares; following two vaccinations (4 weeks apart), estrous activity ceased within 6 weeks, with the duration of contraception exceeding 54 weeks in most mares [59].

3.2. Luteinizing hormone and receptor immunization

Immunocontraception targeting LH and its receptor have been successful in domestic carnivores. Reproductive function in males dogs immunized against LH was severely impaired for up to 1 year [60]. Vaccination of the bitch and queen with a bovine LH receptor vaccine resulted in estrus suppression for >11 months [53,54], with a return to a normal physiologic reproductive state after antibody titers declined.

3.3. Sperm antigen immunization

Sperm antigens are an excellent target for contraceptive vaccines because these proteins are viewed as “foreign” to the immune system of both male and the female [61]. Anti-sperm antibodies affect both fertilization and fertility. However, the entire spermatozoon cannot be used for vaccine development because it shares several antigens with other somatic cells [62]. Therefore, research has focused on delineating appropriate sperm-specific epitopes that would increase immunogenicity (specifically within the reproductive tract) and efficacy. Lactate dehydrogenase (LDH-C4) and acrosin have been isolated as two of the main sperm-specific antigens [61]. To date, sperm antigen immunization has not resulted in a satisfactory control of fertility [62].

3.4. Zona pellucida immunization

Immunization against ovarian antigens was first proposed more than 30 years ago when it was discovered that rabbits exposed to hamster ovary tissue produced antibodies that blocked fertilization [63]. The zona pellucida (ZP) is a protective layer of proteinaceous, acellular, gelatinous material that covers the outer surface of the ova in mammals. In the dog and cat, secretion of the ZP originates from the oocyte, as demonstrated by transmission electron microscopy and immunogold staining [64]. Fertilization is a complex process that begins with binding of sperm to the ZP. Sperm specifically bind to zona pellucida 3 receptor (ZP3) and undergo the acrosome reaction, resulting in the release of enzymes that digest the ZP, allowing sperm to enter and fuse with the oocyte [65]. Antibodies against ZP block sperm receptor sites, rendering ova impervious to sperm. The ZP3 receptor has unique epitopes, which make it an ideal target for immunocontraception. However, immunization with ZP proteins from the same species has little effect [66]. Porcine ZP3 (pZP3) has been consistently utilized in vaccine development because it is readily available from abattoirs and has antigenic similarities to the ZP3 of dogs and cats [65].

3.4.1. Female dogs

Although vaccination against pZP3 resulted in infertility in $\geq 75\%$ of bitches [67,68], vaccination against recombinant canine zona pellucida 2 (rec-dZP2) protein failed to prevent pregnancy in bitches [67]. Following immunization with pZP3, serum progesterone concentrations did not increase during estrus,

suggesting ovulation failure as the cause of infertility rather than impairment of sperm binding sites [69]. Immunocontraception of dogs with pZP3 inhibited follicular development due to atretic changes in the ZP [67]. Ovarian cysts were the most common ovarian pathology, seen in 64% of bitches immunized with porcine ZP3 [70]. Bitches immunized with crude PZP proteins had follicular cysts lined with a thin layer of granulosa cells [70]. Bitches immunized with partially purified PZP proteins had ovarian cysts that were lined by a basement membrane with a clump of luteinized cells [70]. These histologic changes provided an explanation for estrous cycle aberrations (prolonged proestrus bleeding and estrous behavior) observed in bitches following immunization with pZP3. While the exact pathophysiology of these ovarian changes has not been elucidated, it appears to be immune-mediated [70]. In some bitches with high anti-ZP3 antibody titers following vaccination, autoimmune oophoritis against ZP T-cell epitopes occurred, resulting in permanent sterilization [68].

3.4.2. Female cats

Immunocontraception utilizing pZP3 has been successful in several species to date, including African elephants [71], feral horses and burros [72–74], rabbits [75,76], white-tailed deer [77,78], seals [79,80], and hamsters [81]. However, vaccination of the queen against ZP has not resulted in immunocontraception. A single dose of a commercially-manufactured pZP3 vaccine (SpayVac™, SpayVac™-for-wildlife, Inc.) is a successful immunocontraceptive in many wild species, but did not prevent estrous cyclicity or pregnancy in queens [82]. Although queens produce high concentrations of anti-pZP3 antibodies, these antibodies have a low affinity for feline ZP3 protein. Vaccines constructed against native soluble-isolated zona pellucida (SIZP) from the ovaries of pigs, cows, cats, ferrets, dogs, and mink were also tested in female cats [83]. Although all queens developed anti-SIZP antibodies, they all became pregnant during a breeding trial [83]. Consistent with the previous study, the SIZP from these species was immunogenic in the cat, but SIZP antibodies did not bind to feline ZP in situ, and fertility was not blocked [83].

4. Intratesticular, intraepididymal and intra vas deferens injections

Chemical castration is another non-surgical approach to male contraception. Chemical agents injected into the testis, epididymis or vas deferens cause infertility by inducing azoospermia in male

animals. The technique is not technically challenging, is inexpensive and is suitable for large-scale sterilization programs in both dogs and cats [84], although the tail of the epididymis in cats is smaller and more difficult to locate. Sedation typically is used for this procedure in dogs and general anesthesia is necessary for cats. The scrotal area is first clipped and disinfected. Then, a 22 gauge (dogs) or 27 gauge (cats) 1/2 in. needle is inserted percutaneously into the structure of interest (testis, epididymis or vas deferens).

4.1. Male dogs

Intratesticular injections have been investigated as a method of inducing aspermatogenic orchitis and male contraception for more than five decades [85]. The procedure for intratesticular injection involves inserting the needle from the caudal pole of the testis and gently pushing it towards the other pole, depositing the injection homogenously as far as possible through the tissue [86]. Injecting an adjuvant agent, such as Freund's complete adjuvant (FCA) or Bacillus Calmette Guerin (BCG), directly into the testis incites a local inflammatory response that enables lymphoid cells to gain access to testicular tissue, resulting in autoimmune response. A single intratesticular injection of FCA or BCG (10–25 units) resulted in severe oligospermia or azoospermia without granuloma formation or the development of circulating anti-sperm antibodies [61,86,87]. The few spermatozoa that may be present were immotile [87]. Infertility occurred within 6 weeks and lasted for several months [61,86,87]. Intratesticular injection of high doses (>75 units) resulted in a severe granulomatous reaction [86].

In addition to using bacterial cell wall products, reproductive toxins can be directly injected into the testis. Intratesticular administration of a 100 mg solution (0.5 mL total volume/testis) of methallibure, dexamethasone, metopiron, niridazol, α -chlorohydrin or danazol caused testicular and epididymal atrophy and azoospermia in dogs [88]. Within the past 3 year, the FDA has approved a product labeled for chemical castration via intratesticular injection in the male dog [89]. The procedure involves injecting a predetermined amount of zinc solution based on scrotal width into each testis of puppies 3–10 months of age [90]. Zinc is considered non-mutagenic, non-carcinogenic, and non-teratogenic [91]. Neutersol Injectable Solution[®] is a zinc gluconate solution neutralized to a pH of 7 by arginine [90]. Histopathologic findings within 2.5 months of injection included almost complete fibrosis of the seminiferous tubules and Leydig cells [92].

Intratesticular injection of a 70% glycerol solution did not result in azoospermia and sterility in dogs [93].

As an alternative to intratesticular injection, zinc arginine can be injected into the tail of the epididymis. Intraepididymal injection of 50 mg of zinc arginine (0.5 mL/testis) resulted in azoospermia within 90 days following injection [91]. Histologic examination of the testes revealed normal seminiferous tubules with atrophy of the rete testes, an absence of spermatozoa within the epididymis and ductus deferens and no sperm granuloma formation [91]. Intraepididymal saline injection did not induce azoospermia [84].

Injections of sclerosing agents (3.5% formalin solution in phosphate buffered saline or 1.5% chlorhexidine gluconate in 50% DMSO) into the tail of both epididymides in dogs resulted in irreversible azoospermia via chemical occlusion, with secondary testicular atrophy [84]. However, intraepididymal treatment with formalin alone induced only temporary azoospermia or oligospermia in treated dogs [84]. A single bilateral intraepididymal injection of chlorhexidine in DMSO to male dogs resulted in the development of azoospermia by 91 days after treatment [84]. Two bilateral intraepididymal injections of chlorhexidine in DMSO resulted in the development of azoospermia within 35 days after treatment. A single injection directly into the vas deferens with sclerosing chemical agents (10% silver nitrate, 3.6% formaldehyde in ethanol, 5% potassium permagnate, 100% ethanol, or 3.6% formaldehyde) resulted in irreversible infertility similar to intraepididymal injections with the same agents [94].

Studies using these models of male contraception report no or minimal signs of discomfort observed following injection, with variation depending on the route of administration and agent injected. Afferent nerve endings associated with pain sensation are located on the scrotal skin and in the capsule of the testis rather than within the testicular and epididymal parenchyma. A transient increase in testicular diameter may follow the injection within 24 h, resulting in pain secondary to swelling and this may persist for as long as 7–15 days [61]. Additional local and systemic reactions reported after intratesticular injections include scrotal ulceration and dermatitis, scrotal self-mutilation, preputial swelling, vomiting, diarrhea, anorexia, lethargy and leukocytosis [89,90]. It is important to note that depending on the treatment method, dogs may remain fertile up until 60 days post-injection due to sperm reserves present in the epididymis [90]. Also, unlike surgical castration, this kind of chemical sterilization does not eliminate gonadal sources of testosterone [90].

4.2. Male cats

Intraepididymal injections induced chemical vasectomy in tom cats. Injection of a 4.5% solution of chlorhexidine digluconate into the tail of both epididymides resulted in azoospermia or severe oligospermia [95]. Unlike in the dog, sperm granulomas and spermatocoeles were consistently observed in cats following intraepididymal injections [95]. In addition, transient scrotal swelling and pain persisted for up to 2 weeks following intraepididymal injection in cats.

5. Other non-surgical methods for contraception or sterilization

5.1. Female dogs

Intravaginal spermicides and mechanical barriers and intrauterine devices (IUD) have all been used as contraceptives in women. Contraceptive vaginal lubricants used in women have been tested with canine semen. For example, RS-37367 is a non-surfactant imidazole oxalate with high spermatostatic potency when evaluated using ejaculated dog spermatozoa [96]; exposure of canine spermatozoa to RS-37367 for 5 min resulted in progressive immobilization that was not reversible even with extensive washing of the sperm [96]. Intravaginal spermicides have not been developed for use in canine contraception. In dogs, mechanical intravaginal contraceptives have a high failure rate. The Agrophysics Breeding Control Device (Agrophysics, Inc.) was a commercially marketed intravaginal device reported to provide a non-surgical, reversible method for contraception in female dogs [97]. The intravaginal device was inserted into the vestibule and vagina of the bitch to prevent copulation. However, problems with retention and local irritation resulted in an unacceptably high failure rate. In one study, 50% of bitches fitted with the device were bred and 25% became pregnant [97].

An IUD for canine contraception, commercially marketed by Biotumer Argentina SA, was highly effective in preventing pregnancy after breeding [98]. The contraceptive activity of the IUD resulted from both mechanical disruptive effects as well as spermaticidal effects of metallic ions released by electrolytic copper [98]. The manufacturer recommends leaving the IUD in place for 2 year (this is the effective life of the electrolytic copper) [98]. Over a 2-year interval, no side effects were observed, except for one bitch that developed persistent estrus that resolved when the device was removed [98]. However, IUDs are not

practical for use in dogs due to the difficulty of transcervical cannulation.

5.2. Male dogs and cats

5.2.1. Non-invasive mechanical sterilization

Ultrasound is a form of acoustic vibration with frequencies higher than the auditory range. Ultrasound has been used to suppress spermatogenesis through a combined effect of heat and mechanics. The testes of male dogs and cats were treated with a high-intensity focused ultrasound manufactured by Whitewater Electronics [99]. Each treatment consisted of 1–2 W/cm² for 10–15 min administered one to three times at 2–7 days intervals [99]. Ultrasound treatment significantly suppressed spermatogenesis without affecting testosterone concentrations [99]. In separate studies, a burst (20–120 s) of ultrasound energy (3–19 W) was focused onto the vas deferens [100] or epididymides [101,102] of anesthetized dogs. The ultrasound induced thermal coagulative necrosis of subsurface structures resulting in luminal occlusion within 2 weeks after treatment. However, skin burns occurred in approximately 20% of cases [100,101].

5.2.2. Reproductive toxins

In addition to targeting GnRH using immunocontraception, toxins conjugated to GnRH can be used to disrupt the hypothalamic-pituitary-gonadal axis. Toxins must be carefully selected as to be safe in other tissues without GnRH receptors. The internalization of GnRH receptors following ligand binding localizes the cytotoxic effects to pituitary gonadotroph cells. Pokeweed antiviral protein has been conjugated with GnRH and administered to intact male dogs as three daily injections (100 µg/kg) [103]. Serum testosterone and LH concentrations and testicular volume decreased after treatment and the effects of male contraception persisted for 5–6 months [103]. No adverse effects were noted other than transient (<24 h) arthralgia in a few dogs.

Other reproductive toxins used for male contraception include ketoconazole, embelin, and α -chlorohydrin. Ketoconazole is an inhibitor of cellular division and has been shown to exert spermatostatic effects in several species including the dog, rabbit, monkey and man. Within 4–24 h of oral administration of ketoconazole (50–246 mg/kg) to male dogs, the motility of ejaculated sperm declined at an accelerated rate compared with control samples from the same animals [104]. The decline in spermatozoal motility was correlated with the presence of ketoconazole in the seminal plasma. In addition, serum testosterone

concentrations were profoundly suppressed following oral ketoconazole treatment. At high doses, ketoconazole was poorly tolerated by the gastrointestinal tract and can cause hepatotoxicity [105]. However, similar spermatostatic effects occurred following treatment with other orally administered 1-substituted imidazole compounds in dogs without gastrointestinal and hepatic side effects [106].

Embelin (*Embelia ribes*) is an indigenous benzoquinone plant used in Asia for the prevention of pregnancy [107]. Oral treatment of embelin (80 mg/kg every other day for 100 days) in male dogs caused a significant decrease in testicular weight and variable degrees of spermatogenic arrest mainly at the spermatocyte state (absence of post-meiotic cells) [107]. Within 8 months following embelin ingestion, normal spermatogenesis was restored. Embelin treatment did not result in any adverse effects as noted on serum biochemistry and liver histology [107]. Alpha-chlorohydrin is an alkylating agent that causes depletion of spermatogenic elements from the seminiferous tubules. A single high dose (70 mg/kg) of α -chlorohydrin or chronic administration (8 mg/kg body wt for 30 days) inhibited spermatogenesis within 33 days in dogs [108]. These effects were reversible within 100 days following treatment [108].

6. Conclusion

Within the United States, gonadectomy remains the procedure of choice for permanent sterilization of companion animals. However, no single method of reproduction control will be a panacea for the pet overpopulation problem. Commercial development of products for hormonal down-regulation with exogenous steroid hormones (Delvosteron, Intervet) or GnRH agonists (Suprelorin[®], Peptech Animal Health), immunocontraception (SpayVac[™], SpayVac[™]-for-wildlife, Inc.) and intratesticular injection (Neutersol[®], Addison Biological Laboratory, Inc.) provide non-surgical alternatives for contraception and sterilization.

Acknowledgement

We thank Pam Holthofer and Bernadette Stang for their assistance in the collection of references for this review.

References

[1] Olson PN, Moulton C. Pet (dog and cat) overpopulation in the United States. *J Reprod Fertil Suppl* 1993;47:433–8.

[2] The Crisis of Pet Overpopulation. The Humane Society of the United States. http://hsus.org/pets/issues_affecting_our_pets/pet_overpopulation_and_ownership_statistics/the_crisis_of_pet_overpopulation.html, accessed January 23, 2006.

[3] May RM. Control of feline delinquency. *Nature* 1988;332:392–3.

[4] Soto FR, Ferreira F, Pinheiro SR, et al. Adoption of shelter dogs in a Brazilian community: assessing the caretaker profile. *J Appl Anim Welfare Sci* 2005;8:105–16.

[5] Neumann F, Diallo FA, Hasan SH, et al. The influence of pharmaceutical compounds on male fertility. *Andrologia* 1976; 8:203–35.

[6] Plumb DC. *Veterinary Drug Handbook*, 4th ed., Ames: Iowa State Press; 2002.

[7] Burke TJ, Reynolds Jr HA. Megestrol acetate for estrus postponement in the bitch. *J Am Vet Med Assoc* 1975;167:285–7.

[8] Burke TJ. Pharmacologic control of estrus in the bitch and queen. *Vet Clin North Am Small Anim Pract* 1982;12:79–84.

[9] Weikel Jr JH, Nelson LW. Problems in evaluating chronic toxicity of contraceptive steroids in dogs. *J Toxicol Environ Health* 1977;3:167–77.

[10] Beck P. Effect of progestins on glucose and lipid metabolism. *Ann N Y Acad Sci* 1977;286:434–45.

[11] Hayden DW, Barnes DM, Johnson KH. Morphologic changes in the mammary gland of megestrol acetate treated and untreated cats: a retrospective study. *Vet Pathol* 1989;26: 104–13.

[12] Peterson ME. Effects of megestrol acetate on glucose tolerance and growth hormone secretion in the cat. *Res Vet Sci* 1987;42: 354–7.

[13] Von Berkly AG, Townsend WL. The relationship between the prevalence of uterine lesions and the use of medroxyprogesterone acetate for canine population control. *Aust Vet J* 1993;70: 249–50.

[14] Nelson M. After-effect of injection. *Vet Rec* 1977;100:78.

[15] Jackson EKM. Contraception in the dog and cat. *Br Vet J* 1984;140:132–7.

[16] van Os JL, van Laar PH, Oldenkamp EP, et al. Oestrus control and the incidence of mammary nodules in bitches, a clinical study with two progestogens. *Vet Q* 1981;3:46–56.

[17] Evans JM, Sutton DJ. The use of hormones, especially progestagens, to control oestrus in bitches. *J Reprod Fertil* 1989; 39:163–73.

[18] Murray GH, Eden EL. Progesterone to delay estrum in bitches. *Vet Med* 1952;47:467–8.

[19] FDA. Introduction to total drug quality. DHEW Publication #74-3006; 1973.

[20] Goldenthal ET. Current views on safety evaluation of drugs. *FDA Papers* 1968;2:13–8.

[21] Jochle W. Pet population control in Europe. *J Am Vet Med Assoc* 1991;198:1225–30.

[22] Misdorf W. Progestagens and mammary tumors in dogs and cats. *Acta Endocrinol (Copenh)* 1991;125:27–31.

[23] Frank DW, Kirton KT, Murchison TE, et al. Mammary tumors and serum hormones in the bitch treated with medroxyprogesterone acetate or progesterone for four years. *Fertil Steril* 1979;31:340–6.

[24] Owen LN, Briggs MH. Contraceptive steroid toxicology in the Beagle dog and its relevance to human carcinogenicity. *Curr Med Res Opin* 1976;4:309–29.

[25] McKenzie BE. Guidelines and requirements for the evaluation of contraceptive steroids. *Toxicol Pathol* 1989;17:377–84.

- [26] Rutteman GR. Contraceptive steroids and the mammary gland: is there a hazard?—insights from animal studies. *Breast Cancer Res Treat* 1992;23:29–41.
- [27] Briggs MH. Progestogens and mammary tumours in the beagle bitch. *Res Vet Sci* 1980;28:199–202.
- [28] England GC. Effect of progestogens and androgens upon spermatogenesis and steroidogenesis in dogs. *J Reprod Fertil Suppl* 1997;51:123–38.
- [29] Wright PJ, Stelmasiak T, Black D, et al. Medroxyprogesterone acetate and reproductive processes in male dogs. *Aust Vet J* 1979;55:437–8.
- [30] Gannon J. Clinical aspects of the oestrus cycle in the greyhound. *Racing Greyhound* 1976;1:12–22.
- [31] Johnston SD, Root Kustritz MV, Olson PNS. *Canine and feline theriogenology*. W.B. Saunders Co.: Philadelphia; 2001.
- [32] Burke TJ, Reynolds HA, Sokolowski JH. A 280-days tolerance-efficacy study with mibolerone for suppression of estrus in the cat. *Am J Vet Res* 1977;38:469–77.
- [33] Sokolowski JH. The evaluation of efficacy of mibolerone for estrus prevention in the bitch. *Proc Cheque Canine Contracep B* 1978;24–30.
- [34] Freshman JL, Olson PN, Amann RP, et al. The effects of methyltestosterone on reproductive function in male greyhounds. *Theriogenology* 1990;33:1057–73.
- [35] Dixit VP. Chemical sterilization: effects of danazol administration on the testes and epididymides of male rabbit. *Acta Biol Med Ger* 1977;36:73–8.
- [36] Shafik A. Prolactin injection, a new contraceptive method: experimental study. *Contraception* 1994;50:191–9.
- [37] McRae GI, Roberts BB, Worden AC, et al. Long-term reversible suppression of oestrus in bitches with nafarelin acetate, a potent LHRH agonist. *J Reprod Fertil* 1985;74:389–97.
- [38] Rubion S, Desmoulin PO, Riviere-Godet E, et al. Treatment with a subcutaneous GnRH agonist prevents puberty in bitches. In: *Proc 5th international symposium on canine and feline reproduction*; 2004.p. 56–8 [abstract].
- [39] Trigg TE, Wright PJ, Armour AF, et al. Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function in male and female domestic dogs. *J Reprod Fertil Suppl* 2001;57:255–61.
- [40] Lacoste D, Dube D, Trudel C, et al. Normal gonadal functions and fertility after 23 months of treatment to prepubertal male and female dogs with the GnRH agonist [D-Trp-6, des-Gly-NH2-10] GnRH ethylamide. *J Androl* 1989;10:456–65.
- [41] Wright PJ, Verstegen JP, Onclin K, et al. Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin. *J Reprod Fertil Suppl* 2001;57:263–8.
- [42] Junaidi A, Williamson PE, Cummins JM, et al. Use of a new drug delivery formulation of the gonadotrophin-releasing hormone analogue Deslorelin for reversible long-term contraception in male dogs. *Reprod Fertil Dev* 2003;15:317–22.
- [43] Dube D, Assaf A, Pelletier G, et al. Morphological study of the effects of an GnRH agonist on the canine testis after 4 months of treatment and recovery. *Acta Endocrinol (Copenh)* 1987;116:413–7.
- [44] Trigg TE, Doyle AG, Walsh JD, et al. Advances in the use of the GnRH agonist deslorelin in control of reproduction. In: *Proc 5th international symposium on canine and feline reproduction*; 2004.p. 49–51 [abstract].
- [45] Vickery BH, McRae GI, Briones WV, et al. Dose response studies on male reproductive parameters in dogs with nafarelin acetate, a potent LHRH agonist. *J Androl* 1985;6:53–60.
- [46] Goodpasture JC, Bergstrom K, Vickery BH. Potentiation of the gonadotoxicity of cytoxan in the dog by adjuvant treatment with an LHRH agonist. *Cancer Res* 1988;48:2174–8.
- [47] Riesenbeck A, Klein R, Hoffmann B. Down regulation, a new and reversible approach to eliminate testicular function in the dog. *De Praktische Tierarzt* 2002;83:512–20.
- [48] Bertschinger HJ, Trigg TE, Jochle W, et al. Induction of contraception in some African wild carnivores by downregulation of LH and FSH secretion using the GnRH analogue deslorelin. *Reprod Suppl* 2002;60:41–52.
- [49] Bertschinger HJ, Asa CS, Calle PP, et al. Control of reproduction and sex related behaviour in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations. *J Reprod Fertil Suppl* 2001;57:275–83.
- [50] Gobello C, Herno G, Rodriguez R, et al. Use of the GnRH antagonist, acycline, on estrous cycle interruption in the bitch: a preliminary report. *Theriogenology* 2005;64:13 [abstract].
- [51] Ladd A, Tsong YY, Walfield AM, et al. Development of an antifertility vaccine for pets based on active immunization against luteinizing hormone-releasing hormone. *Biol Reprod* 1994;51:1076–83.
- [52] Jung MJ, Moon YC, Cho IH, et al. Induction of castration by immunization of male dogs with recombinant gonadotropin-releasing hormone (GnRH)-canine distemper virus (CDV) T helper cell epitope p35. *J Vet Sci* 2005;6:21–4.
- [53] Saxena BB, Clavio A, Singh M, et al. Effect of immunization with bovine luteinizing hormone receptor on ovarian function in cats. *Am J Vet Res* 2003;64:292–8.
- [54] Saxena BB, Clavio A, Singh M, et al. Modulation of ovarian function in female dogs immunized with bovine luteinizing hormone receptor. *Reprod Domest Anim* 2002;37:9–17.
- [55] Mitchison WA. The carrier effect in the secondary response to hapten protein conjugate. *Eur J Immunol* 1971;1:10–8.
- [56] Ferro VA, Khan MA, McAdam D, et al. Efficacy of an antifertility vaccine based on mammalian gonadotrophin releasing hormone (GnRH-I)—a histological comparison in male animals. *Vet Immunol Immunopathol* 2004;101:73–86.
- [57] Zeng XY, Turkstra JA, Tsigos A, et al. Effects of active immunization against GnRH on serum LH, inhibin A, sexual development and growth rate in Chinese female pigs. *Theriogenology* 2002;58:1315–26.
- [58] Stevens JD, Sosa JM, deAvila DM, et al. Luteinizing hormone-releasing hormone fusion protein vaccines block estrous cycle activity in beef heifers. *J Anim Sci* 2005;83:152–9.
- [59] Imboden I, Janett F, Burger D, et al. Influence of immunization against GnRH on cycling activity and estrous behavior in the mare. In: *Proceedings for the 15th international congress on animal reproduction*; 2004.p. 152 [abstract].
- [60] Lunnen JE, Faulkner LC, Hopwood ML, et al. Immunization of dogs with bovine luteinizing hormone. *Biol Reprod* 1974;10:453–60.
- [61] Talwar GP, Naz RK. Immunological control of male fertility. *Arch Androl* 1981;7:177–85.
- [62] Naz RK, Gupta SK, Gupta JC, et al. Recent advances in contraceptive vaccine development. *Hum Reprod* 2005;20:3271–83.
- [63] Ownby CL, Shivers CA. Antigens of the hamster ovary and effects of anti-ovary serum on eggs. *Biol Reprod* 1972;6:310–8.
- [64] Barber MR, Lee SM, Steffens WL, et al. Immunolocalization of zona pellucida antigens in the ovarian follicle of dogs, cats, horses and elephants. *Theriogenology* 2001;55:1705–17.

- [65] Aitken RJ, Paterson M, van Duin M. The potential of the zona pellucida as a target for immunocontraception. *Am J Reprod Immunol* 1996;35:175–80.
- [66] Dunbar BS. Antibodies to zona pellucida antigens and their role in fertility. In: Wegmann TG, Gill TJ, editors. *Immunology of reproduction*. New York: Oxford; 1983. p. 505–34.
- [67] Srivastava N, Santhanam R, Sheela P, et al. Evaluation of the immunocontraceptive potential of *Escherichia coli*-expressed recombinant dog ZP2 and ZP3 in a homologous animal model. *Reproduction* 2002;123:847–57.
- [68] Mahi-Brown CA, Yanagimachi R, Hoffman JC, et al. Fertility control in the bitch by active immunization with porcine zonae pellucidae: use of different adjuvants and patterns of estradiol and progesterone levels in estrous cycles. *Biol Reprod* 1985;32:761–72.
- [69] Mahi-Brown CA, Huang Jr TTF, Yanagimachi R. Infertility in bitches induced by active immunization with porcine zonae pellucidae. *J Exp Zool* 1982;222:89–95.
- [70] Mahi-Brown CA, Yanagimachi R, Nelson ML, et al. Ovarian histology of bitches immunized with porcine zonae pellucidae. *Am J Reprod Immunol* 1988;18:94–103.
- [71] Fayrer-Hosken RA, Grobler D, Van Altena JJ, et al. Immunocontraception of African elephants. *Nature* 2000;407:149.
- [72] Kirkpatrick JF, Liu IM, Turner Jr JW, et al. Long-term effects of porcine zona pellucida immunocontraception on ovarian function in feral horses (*Equus caballus*). *J Reprod Fertil* 1992;94:437–44.
- [73] Kirkpatrick JF, Liu IM, Turner JW, et al. Antigen recognition in feral mares previously immunized with porcine zona pellucidae. *J Reprod Fertil Suppl* 1991;44:321–5.
- [74] Turner Jr JW, Liu IKM, Kirkpatrick JF. Remotely delivered immunocontraception in free-roaming feral burros (*Equus asinus*). *J Reprod Fertil* 1996;107:31–5.
- [75] Bhatnagar P, Gupta SK, Sehgal S. Immunogenicity of deglycosylated zona pellucida antigens and the inhibitory effects on fertility in rabbits. *Int J Fertil* 1992;37:53–63.
- [76] Sehgal S, Gupta SK, Bhatnagar P. Long-term effects of immunization with porcine zona pellucida on rabbit ovaries. *Pathology* 1989;21:105–10.
- [77] Miller LA, Johns BE, Killian GJ. Immunocontraception of white-tailed deer using native and recombinant zona pellucida vaccines. *Anim Reprod Sci* 2000;63:187–95.
- [78] Skinner SM, Killian GJ, Miller LA, et al. Characterization of antigenicity and immunogenicity patterns of native and recombinant zona pellucida proteins in the white-tailed deer (*Odocoileus virginianus*). *J Reprod Fertil* 1994;101:295–303.
- [79] Brown RG, Bowen WD, Eddington JD, et al. Temporal trends in antibody production in captive grey, harp and hooded seals to a single administration immunocontraceptive vaccine. *J Reprod Immunol* 1997;35:53–64.
- [80] Brown RG, Bowen WD, Eddington JD, et al. Evidence for a long-lasting single administration contraceptive vaccine in wild grey seals. *J Reprod Immunol* 1997;35:43–51.
- [81] Hasegawa A, Koyama K, Inoue M, et al. Antifertility effect of active immunization with ZP4 glycoprotein family of porcine zona pellucida in hamsters. *J Reprod Immunol* 1992;22:197–210.
- [82] Gorman SP, Levy JK, Hampton AL, et al. Evaluation of a porcine zona pellucida vaccine for the immunocontraception of domestic kittens (*Felis catus*). *Theriogenology* 2002;58:135–49.
- [83] Levy JK, Mansour M, Crawford PC, et al. Survey of zona pellucida antigens for immunocontraception of cats. *Theriogenology* 2005;63:1334–41.
- [84] Pineda MH, Reimers TJ, Faulkner LC, et al. Azoospermia in dogs induced by injection of sclerosing agents into the caudae of the epididymides. *Am J Vet Res* 1977;38:831–8.
- [85] Freund J, Lipton MM, Thompson GE. Aspermatogenesis in the guinea pig induced by the testicular tissue and adjuvants. *J Exp Med* 1953;97:711–26.
- [86] Naz RK, Talwar GP. Immunological sterilization of male dogs by BCG. *Int J Androl* 1981;4:111–28.
- [87] Talwar GP, Naz RK, Das C, et al. A practicable immunological approach to block spermatogenesis without loss of androgens. *Proc Natl Acad Sci USA* 1979;76:5882–5.
- [88] Dixit VP. Chemical sterilization of male dogs (*canis familiaris*) after single intratesticular administration of methalibure (ICI-33828), dexamethasone, metopirion (SU-4885, Ciba), niradazole (33644-Ba, Ciba) α -chlorohydrin (U-5897) and danazol. *Indian J Exp Biol* 1979;17:937–40.
- [89] Anon. FDA approves first injectable solution for dog sterilization. *FDA Consumer*; 2003. p. 3.
- [90] Neutersol Information Web site. Available at: www.neutersol.com/Study.htm, accessed October 17, 2005.
- [91] Fahim MS, Wang M, Sutcu MF, et al. Sterilization of dogs with intra-epididymal injection of zinc arginine. *Contraception* 1993;47:107–22.
- [92] Tepsmethanon V, Wilde H, Hemachudha T. Intratesticular injection of a balanced zinc solution for permanent sterilization of dogs. *J Med Assoc Thai* 2005;88:686–9.
- [93] Immegart HM, Threlfall WR. Evaluation of intratesticular injection of glycerol for nonsurgical sterilization of dogs. *Am J Vet Res* 2000;61:544–9.
- [94] Dixit VP, Lohiya NK, Arya M, et al. The effects of chemical occlusion of vas deferens on the testicular function of dog: a preliminary study. *Acta Eur Fertil* 1975;6:348–53.
- [95] Pineda MH, Dooley MP. Surgical and chemical vasectomy in the cat. *Am J Vet Res* 1984;45:291–300.
- [96] Vickery BH, Goodpasture JC, Bergstrom K, et al. Assessment of a new spermicidal agent against ejaculated dog and human spermatozoa in vitro. *Fertil Steril* 1983;40:231–6.
- [97] Wildt DE, Seager SWJ. Reproduction control in dogs. *Vet Clin North Am* 1977;7:775–87.
- [98] Volpe P, Izzo B, Russo M, et al. Intrauterine device for contraception in dogs. *Vet Rec* 2001;149:77–9.
- [99] Fahim MS, Fahim Z, Harman J, et al. Ultrasound as a new method of male contraception. *Fertil Steril* 1977;28:823–31.
- [100] Roberts WW, Chan DY, Fried NM, et al. High intensity focused ultrasound ablation of the vas deferens in a canine model. *J Urol* 2002;167:2613–7.
- [101] Roberts WW, Wright EJ, Fried NM, et al. High-intensity focused ultrasound ablation of the epididymis in a canine model: a potential alternative to vasectomy. *J Endourol* 2002;16:621–5.
- [102] Fried NM, Roberts WW, Sinelnikov YD, et al. Focused ultrasound ablation of the epididymis with use of thermal measurements in a canine model. *Fertil Steril* 2002;78:609–13.
- [103] Sabour K, Ball BA, Nett TM, et al. Effect of GnRH conjugated to pokeweed antiviral protein on reproductive function in adult male dogs. *Reproduction* 2003;125:801–6.

- [104] Vickery BH, Burns J, Zaneveld LJ, Goodpasture JC, Bergstrom K. Orally administered ketoconazole rapidly appears in seminal plasma and suppresses sperm motility. *Adv Contracept* 1985;1:341–53.
- [105] Heiberg JK, Svejgaard E. Toxic hepatitis during ketoconazole treatment. *Br Med J* 1981;283:825–6.
- [106] Goodpasture JC, Hiller MB, Lewis B, et al. Same day appearance of orally administered, spermicidal 1-substituted imidazoles in dog ejaculates. *J Androl* 1987;8: 230–7.
- [107] Dixit VP, Bhargava SK. Reversible contraception like activity of embelin in male dogs (*Canis indicus* Linn). *Andrologia* 1983;15:486–94.
- [108] Dixit VP, Lohiya NK, Arya M, et al. Reversible changes in the testes and epididymides of dog treated with alpha-chlorohydrin. *Acta Biol Med Ger* 1975;34:1851–6.