

**Reproduction of Dogs in the Tropics
with Special Reference to the Population Structures,
Reproductive Patterns and Pathologies, and a Non-
surgical Castration Alternative**

Antonio Ortega-Pacheco

Faculty of Veterinary Medicine and Animal Science

Department of Clinical Sciences

Division of Comparative Reproduction, Obstetrics and Udder Health

Uppsala

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Abstract

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Dog overpopulation is considered a serious problem in developing countries and a threat to human health. To be successfully implemented, official dog control measures have to be in accordance with regional beliefs and practices of an area. For these reasons local municipal authorities, veterinarians and dog owners need to have information about the structure, size, and dynamics of growth of the canine population, as well as the prevalence of reproductive diseases. They also need to be well informed about methods for the control of reproduction in this species. The aims of this thesis were to provide this information, and to test a non-surgical method of castration of male dogs.

It was found that dogs are popular pets in urban and rural Yucatan with 72.8% of households in Merida owning a dog and 63.3-71.1% in rural communities, respectively. Households of medium socio-economical status, with large families and adequate fences were more likely to own dogs. In the city owners provided better husbandry, health care and feeding of their dogs. This was reflected in a lower mortality rate and lower rate of unwanted matings and litters.

Free roaming dogs in urban or rural communities may reproduce to their maximal capacity. This is more evident in rural communities due to less supervision, and to that only 1.8% of the dogs are neutered or spayed, compared to 3.1% in the city. The veterinary practitioners did little to promote the control of dog breeding or to reduce the relinquishing of unwanted dogs in the city. Better client education and promoting the sterilization of pets at low cost would improve the situation.

Female dogs under tropical conditions may cycle throughout the year but a marked increase was found in December. Females in estrus attract males and the testosterone concentration of the males is increased during those periods. Healthy male dogs in tropical conditions constantly produce viable sperm capable of impregnating a receptive female. Pregnant bitches, therefore, can be found all through the year. During periods of high environmental temperature, however, the percentage of pregnant females was lower. Underweight animals are less likely to become pregnant. Ovulation may also be influenced by the climatic conditions of the tropics, with a higher number of oocytes being ovulated during the summer time. Although the environmental temperature can modify seminal traits and sperm morphology in male dogs, those changes are of low grade and males remain fertile throughout the year. Day length and temperature probably induced the circannual changes in prolactin secretion found in the male dogs, but the physiological role of this hormone is incompletely known. A high environmental temperature was found to induce seasonal subfertility in bitches, probably due to an increase in the rate of embryo resorption.

Epidemiological data revealed a high prevalence of genital pathologies in both sexes. The pathologies most frequently found in the bitches, however, did not adversely affect the capacity to come into estrus or to become pregnant. In males, most of the reproductive pathologies seem to affect the spermatogenesis, and depending on the amount of testicular interstitial tissue damaged the production of

testosterone may also be affected. Aged dogs, as expected, are at higher risk to suffer testicular degeneration or to develop testicular tumors.

A new non-surgical method for neutering dogs was evaluated as an alternative method for surgical mass sterilization. Crushing of the spermatic cord leads to vascular damage and cellular hypoxia of the testis and, consequently, to infarction. The method proved to be quick, efficient and of low cost, with minimal damage to the patient. However, long-term studies are needed to prove it has no adverse reactions.

Key words: Dog, tropics, husbandry, reproductive traits, reproductive pathologies, Burdizzo.

Author's address: Antonio-Ortega Pacheco: Department of Clinical Sciences, Division of Comparative Reproduction, Obstetrics and Udder Health, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences (SLU), PO Box 7054, SE-750 07 Uppsala, Sweden (On leave from the Department of Medicine and Surgery, Faculty of Veterinary Medicine and Animal Science, Autonomous University of Yucatan (UADY), AP 4-116 Merida Yucatan Mexico). Email: opacheco@uady.mx

KAKASBAL AND THE DOG

Once upon a time there was a man who was so poor he was always in a bad mood, and never passed up a chance to mistreat his old dog. Kakasbal [spirit of evil], who is everywhere, saw that he could benefit from by playing on the dog's anger. And with this in mind he approached the dog.

—Oh you poor thing; why so sad? Tell me what is the matter.

—How can I not be sad when my master beats me all the time.

—I know that he is an ornery man; why don't you leave him?

—He is my master and I am loyal; I wouldn't leave him for anything.

—But your master is incapable of appreciating your loyalty.

—That does not matter, he is my master; I will remain loyal.

But Kakasbal wouldn't let up; he harassed the dog until, just to get rid of him the dog finally said:

—OK. You have convinced me. Tell me what I should do.

—Give me your soul.

—And what will you give me in return?

—Anything you ask me for.

—I want a bone for every hair on my body.

—I agree.

With that, Kakasbal began to count the animal's hair. But just as he was finishing, having reached the tail, the dog thought of the loyalty due his master and gave a start, causing Kakasbal to lose his place.

—Why did you move? I lost my count.

—It's these darn fleas that plague me night and day. Just start again.

A hundred times Kakasbal began his count and a hundred times he lost it when the dog jumped.

—I give up. I will count no more. You have deceived me, but in so doing taught me an important lesson. It is harder to buy the soul of a dog than it is to buy the soul of a man.

Mayan legend

*Con todo mi corazón y cariño para
Matilde, colega, amiga y amada esposa
y Carlos Fabian mi maravilloso hijo.*

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Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by the Roman numerals:

- I. Ortega-Pacheco, A., Rodriguez-Buenfil, J.C., Bolio-Gonzalez, M.E., Sauri-Arceo, C.H., Jiménez-Coello, M. & Linde Forsberg C. A survey investigation about dogs in three rural and an urban area of Yucatan, Mexico. *Anthrozoös (Accepted)*.
- II. Ortega-Pacheco, A., Segura-Correa J.C., Jiménez-Coello, M. & Linde Forsberg, C. Reproductive patterns and genital pathologies of stray bitches in the tropics. *Theriogenology (Accepted)*.
- III. Ortega-Pacheco, A., Segura-Correa J.C., Bolio-Gonzalez, M.E., Jiménez-Coello, M. & Linde Forsberg, C. Reproductive patterns of stray male dogs in the tropics. *Theriogenology (In press)*.
- IV. Ortega-Pacheco, A., Rodriguez-Buenfil, J.C., Segura Correa J.C., Bolio-Gonzalez, M.E., Jiménez-Coello, M. & Linde Forsberg, C. 2006. Pathological conditions of the reproductive organs of male stray dogs in the tropics: Prevalence, risk factors, morphological findings, and testosterone concentrations. *Reproduction in Domestic Animals (In press)*.
- V. Ortega-Pacheco, A., Bolio-Gonzalez, M.E., Colin-Flores, R.F., Sauri-Arceo, C.H., Gutierrez-Blanco E., Jiménez-Coello, M. & Linde Forsberg, C. Evaluation of a Burdizzo castrator for neutering of dogs. *Reproduction in Domestic Animals 2006*, 41, 227-232.

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Introduction

The information provided here constitutes a portion of the scientific data required to comprehend and manage the current problem of canine overpopulation in the studied area.

Background

Domestic dogs are successful breeders in almost all kinds of environments, but are always directly or indirectly dependent on man. Since their domestication more than 14 thousand years ago a strong link between dogs and humans has been established. Nowadays that link persists even stronger, since the affection for and loving care given to this species has generated a tremendous industry devoted to caring for its health, nutrition, training, etc. However, as a consequence of uncontrolled reproduction, overpopulation of this species (probably the most abundant carnivore species on earth) has become a big concern in many countries where stray and feral dogs are causing zoonotic diseases, nuisance, pollution of parks and recreation areas, damage to livestock and destruction of ecosystems. In addition, the relinquishing of dogs to animal shelters incurs great costs for the maintenance or euthanasia of dogs because not enough households are available for their re-homing.

To be effective, official planning and implementation of dog population management schemes have to be in accordance with beliefs and cultural practices of the area, and with the different epidemiological situations. The authorities must be aware of the structure and size of the local canine populations, the dynamics of population growth, reproductive diseases affecting the male and female fertility, as well as be well informed of new methods for control of the populations of this species.

Dog populations

Populations of dogs vary between different habitats, different cultures and different social strata of the human populations. In developing countries, a large number of free roaming dogs have owners and are classified according to the WHO (1990) based on the level of restriction or supervision as family dogs (fully dependent; semi-restricted) which can have access to the streets, and neighbourhood dogs (semi-dependent, semi-restricted or unrestricted). The presence and abundance of stray dogs depends on the attitudes towards dogs of the humans in these areas. Stray dogs have a great impact on human public health and the ecosystems. Due to the close contact with humans, dogs are responsible for the spread of several zoonotic diseases. Dog rabies is endemic in many developing countries and is responsible for most human deaths from the disease (WHO, 1992). In addition, at least 65 other zoonoses including ancylostomiasis, echinococcosis, leptospirosis and salmonellosis, may be transmitted to man by direct contact or contact with secretions and excretions of pets (Faulkner, 1975). Additionally, domestic dogs are potentially effective predators of the native fauna and can have competitive interactions with endemic wild carnivores (Butler *et al.*, 2004). Preying of small

mammals and birds and transmission of diseases can have disastrous consequences for ecosystems in which feral dogs become established.

After an initial exponential growth in a dog population, the birth rate begins to decrease and the rate of death increases reaching equilibrium depending on the capacity of the environment to keep this balance (carrying capacity of the environment). The carrying capacity of the environment varies with habitat and it depends on the availability, distribution, and quality of the resources (shelter, food, water). The density of a population of dogs is almost always near the carrying capacity of the environment (WHO, 1990).

Strategies to control the overpopulation of free-roaming dogs include enforcement of law, education of owners and sterilization of pets. In many developing countries, mass euthanasia of dogs is systematically used in an effort to reduce the density of free-roaming dogs and prevent the transmission of zoonotic diseases. However, this strategy cannot be effective in the long term without the enforcement of laws and education of people. Free-ranging domestic dogs are non-cooperative populations, i.e. they are not dependant on other animals of the same species to survive. Any reduction in the population density through additional mortality is rapidly compensated by better reproduction and survival. In figure 1, a hypothetical model adding mortality (a) at different magnitude and frequency to two different kinds of populations, non-cooperators (b) and cooperators (c) shows that non-cooperator breeding populations can recover quickly even from important or frequent perturbations (Courchamp *et al.*, 1999). In this model a non-cooperator species could be represented by the domestic dog, and demonstrates the inefficiency of mass euthanasia as the sole strategy for the control of canine overpopulation.

To achieve long-term reduction in dog populations, the strategies selected must include controlling the reproduction of owned dogs and controlling the environment of unsupervised dogs (WHO, 1990). Knowledge of the size and trends of dog populations in the community is essential for planning and decision-making. In addition, for an efficient control of such populations, a better understanding of the rate of reproduction and the prevalence of genital pathologies affecting the potential breeding capacity is needed.

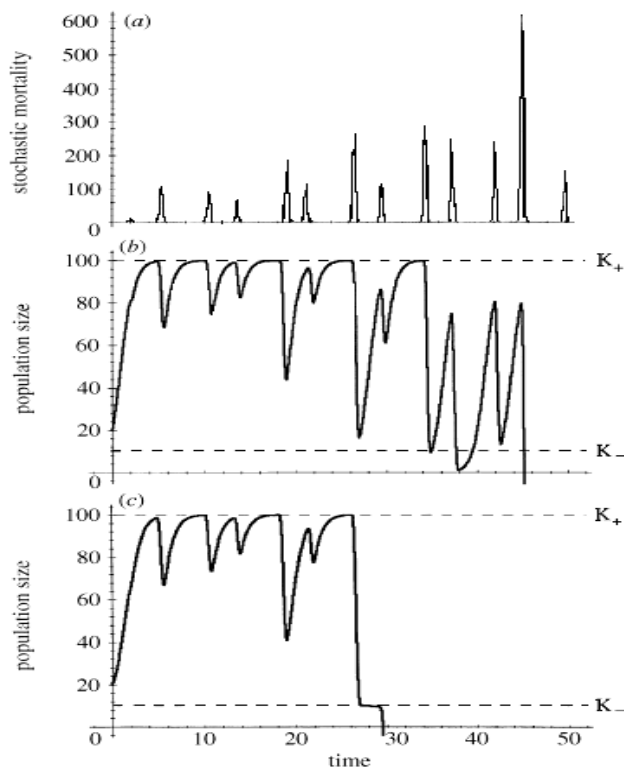


Figure 1. (a) Stochastic model accounting for mortality due to random catastrophic events. The model is set so that mortality peaks occur at random frequency and with random amplitude. (b) Population growth with time of non-cooperators when stochastic mortality is taken into account. The population becomes extinct only in extreme catastrophic events and it recovers even from a decrease down to below the minimum population size of cooperators (lower dashed line). (c) Population growth with time of obligate cooperators when stochastic mortality is taken into account. Cooperators may not recover from large catastrophic events or from frequent ones, since either would drive them below the minimum population size (From Courchamp *et al.*, 1999, with permission).

Reproductive patterns

Several types of mammals show seasonal variation in their reproduction as a strategy to synchronize the breeding and/or births to the most favourable time of the year. Whether a mammal reproduces seasonally or continuously depends mostly of the environment, e.g. food availability, rain, light, temperature. One of the first species to be domesticated was the dog, but there appears to be little information on the changes in reproduction that have occurred in this species. Domestication has brought about differences between breeds of dogs in for

instance rate of sexual maturity, number of cycles per year, and litter size (Rowlands, 1950).

Domestic dogs (*Canis familiaris*) are considered non-seasonal breeders with the exception of the Basenji which shows an annual seasonal period of estrus during the fall (Fuller, 1956). However, reproductive patterns observed in dog ancestors such as the wolf (*Canis lupus*) and other canid species e.g. the coyote (*Canis latrans*) or African wild dog (*Lycaon pictus*) may still remain and be expressed under certain conditions. Evidence of this is when domestic dogs return to the wild when they can reverse the effects of domestication so that for instance only one female in a pack gives birth during the year, even when other adult females may be present in the pack, or pup rearing may be shared by several members of the pack (Gipson, 1983). Other reproductive changes that have occurred in the dog due to domestication are a change from monogamy to a promiscuous mating system, and a pack organization. On the other hand, when wild dogs and wolves are kept in captivity, they may become more like domestic dogs in having two estrus periods in a year (Setchell, 1992). Domestication has led to improvement in reproductive efficiency, in some instances by advancing puberty, in others by increasing litter size and in others again by reducing seasonality of breeding. Not all changes are seen in all species, and more directly comparable quantitative data are needed before the effects of domestication on reproduction in the dog can be fully defined (Setchell, 1992).

Factors such as nutrition, temperature and environment can alter the estrous activity in the bitch (Sokolowski *et al.*, 1973) and increase the frequency of estrus periods during specific periods of the year. In Peru, a significant relationship between photoperiod, temperature, and month with the number of German Shepherd bitches in heat was found (Choy & Echevarria, 2005). In temperate regions, estrus periods can be observed during all months of the year. However, in the USA, a greater incidence of estrus periods is seen during late winter or early spring and again in the late summer or early autumn (Concannon, 1986). In other temperate regions, such as for instance Quebec, more bitches are observed to be in estrus during the winter and summer (Bouchard *et al.*, 1991); whereas in Britain more bitches are observed in estrus during February and March (Christie & Bell, 1971; Prole, 1973). In Sweden, like in the USA, more bitches are in estrus during winter and spring (Wikström & Linde Forsberg, 2006). In tropical regions such as Peru, south of the Equator, most German Shepherd bitches are in estrus from May to July (Choy & Echevarria, 2005), whereas in Jaipur India, estrus in stray dogs is more commonly seen during late autumn and winter (Chawla & Reece, 2002).

Domestic male dogs may remain reproductively competent year-round and be ready to mate in the presence of females in estrus (Asa, 1998). However, seasonal variations have been described in some reproductive traits such as semen quality (Takeishi *et al.*, 1975), sperm output and concentrations of glycylphosphorylcholine in seminal plasma (Taha *et al.*, 1981), and serum testosterone (Falvo *et al.*, 1980; Taha & Noakes, 1982).

Scarce and limited information about reproductive patterns of dogs under tropical conditions is available, particularly in developing countries where

knowledge of reproductive patterns in free-roaming and owned dogs is important in order to prevent overpopulations of this species.

Reproductive pathologies

The establishment of control measures of stray dog populations requires knowledge of their ecology in both rural and urban scenarios, knowledge of their behaviour in such ecosystems, and knowledge about the pathologies that may alter their reproductive capacity. Once their ecology is understood, urban dogs may serve as indicators of stress, pollution, and environmental deterioration and as models for behavioural adaptations to urban (Beck, 1973) and rural life.

Pathologies present in the dog's reproductive tract are varied and can be congenital, endocrine, autoimmune, infectious, traumatic and neoplastic. The frequencies with which they occur may also serve as epidemiological indicators with respect to the risk to develop diseases in human populations (Veeramachaneni, 2000) or in populations of owned dogs, as they share the same environment in close cohabitation. However, research about the epidemiology of reproductive pathologies in populations of domestic dogs is greatly lacking.

Genital disorders such as anatomic abnormalities, hormonal disturbances, or disorders of infectious aetiology can reduce fertility or prevent bitches from breeding or to conceive. Fertility problems in a mature bitch can occur any time during the estrous cycle as result of failure to mate, missed conception or premature termination of pregnancy (Okkens *et al.*, 1992). Similarly as for male dogs, there is scarce information available concerning these problems in the canine female population. A study of stray dogs in Mexico City indicates that the incidence of genital cystic and neoplastic alterations in bitches increase with age (Mariño *et al.*, 2003). A previous epidemiological study on reproductive pathologies in stray bitches showed that total or partial fetal resorption can occur in up to 40% of pregnant bitches with a consequent decrease in litter size (Ortega-Pacheco *et al.*, 2006).

Castration of male dogs

Castration (orchidectomy) has for many years been the method of choice to neuter male dogs. This proven procedure not only induces permanent sterilization but also reduces the testosterone levels and, consequently, reduces both the risk of developing prostatic disease and undesirable male behaviours such as roaming, aggression, mating and urination marking (Neilson *et al.*, 1997). Surgical castration, however, requires anaesthesia, surgical facilities and skilled veterinary surgeons which limit the applicability of this contraceptive procedure for extensive population control programs because of cost and diversion of veterinarians from other important professional activities (Pineda, 1986); besides, pet owners appear to be more willing to spay their bitches than to neuter their male dogs. Cost of neutering, fear of surgery (Pineda, 1986), anthropomorphic attachment to the pet (Blackshaw & Day, 1994), irreversibility of the procedure, and the associated behavioural changes (Maarschalkerweerd *et al.*, 1997) are all factors that contribute to make neutering of the male dog a less acceptable procedure to the pet owner.

Several methods of inhibiting fertility in male dogs have been under extensive research. They include chemical sterilization using intraepididymal injection of alpha-chlorohydrin (Dixit *et al.*, 1975), clorhexidine digluconate (Pineda *et al.*, 1977; Barnett, 1985; Pineda & Hepler, 1981), zinc arginine (Fahim *et al.*, 1993), or methylcyanoacrylate (Galván *et al.*, 1994); intratesticular injection of alpha-chlorohydrin (Dixit *et al.*, 1975), calcium chloride (Koger, 1978), zinc tannate (Fahim *et al.*, 1982), lactic acid (Nishimura *et al.*, 1992), or glycerol (Immegart & Threlfall, 2000). Down regulation of pituitary function has also been used for castration of male dogs. Alternatives for these approaches include 1. Immunisation against GnRH (Thompson, 2000; Mi-Jeong *et al.*, 2005); 2. Application of GnRH analogues (Páramo *et al.*, 1993, Hoffmann & Engel, 2004); 3. The use of GnRH agonists such as LHRH agonists (Sundaram, 1984), deslorelin (Trigg *et al.*, 2001), nafarelin acetate (Vickery *et al.*, 1985) and luprolide acetate (Inaba *et al.*, 1996), and 4. Competitive inhibition of 5 α -reductase (Iguer-Ouada & Verstegen, 1997; Sirinarumitr *et al.*, 2001). Medical suppression of spermatogenesis in dogs has also been achieved by using Megestrol acetate (Van den Broek & O'Farrell, 1994), Medroxyprogesterone acetate (Wright *et al.*, 1979, Páramo *et al.*, 1993; England, 1997), androgens (Freshman *et al.*, 1990), and several oral products like plant benzoquinone (embelin) (Dixit & Bhargava, 1983) and gossypol (Mushtaq *et al.*, 1996). These treatments, however, do not consistently result in sterility, require repeated applications or have not been approved for use in dogs.

An intratesticular injectable product as an alternative for surgical castration in dogs is now available (i.e. Neutersol®, Addison Biological Laboratory, Fayette, MI, USA) and has proved to be very efficient to induce sterility (Wang, 2002); however, its cost makes it prohibitive to be used in massive sterilization campaigns. Besides, it is only approved for use in pups.

The use of non-surgical, inexpensive and easy sterilisation methods of a large number of male dogs would effectively contribute to curb the growth of the pet population. Non-surgical ablation of the testicular artery using the Burdizzo clamp is routinely used in lambs and goat kids with minimal complications (Molony *et al.*, 1993). This procedure is fast, cheap and very efficient, and has also been used for vasectomy (Zufall, 1958) or neutering of humans (Herzog & Santucci, 2002) with minimal side effects. Its efficiency to neuter dogs has not yet been reported.

Aims of the Study

The overall aims of the present work have been to understand peoples' opinions about dogs and how they care for them in order to identify factors promoting overpopulation, and to determine reproductive patterns and the prevalence of genital pathologies among stray dog populations that may enhance or decrease their rate of reproduction in the studied region. A further aim was to determine the efficiency of a new non-surgical procedure to neuter dogs for large scale sterilization programs.

The specific aims of the study were:

- To investigate the structures of and peoples' opinions about dog populations in rural and urban areas of Yucatan, Mexico.
- To determine the reproductive patterns of male and female stray dogs under tropical conditions.
- To determine the prevalence of genital pathologies of male and female stray dogs in a tropical environment.
- To evaluate the efficiency of the Burdizzo clamp for non-surgical castration of dogs.

Materials and Methods

General descriptions of the materials and methods used in the studies presented in this thesis are described here. For further details, see Papers I to V.

Survey procedure (Paper I)

In Paper I, a stratified multistage sampling procedure was used to randomly select from the telephone book 400 households from the four socio-economical zones of Merida city. A questionnaire was developed and interviews made by telephone with any person older than 15 years and considered knowledgeable of the information required. In a second survey (rural survey) three rural communities with ≤ 2500 inhabitants were selected. Individual households were visited and the same questionnaire as in the city survey was used for personal interviews.

Animals

Animals used during the 12 month-studies (Papers II to IV) and for the non-surgical castration procedure (Paper V) were obtained from the Canine and Feline Control Centre, belonging to the Municipality of Merida, capital city of Yucatan, Mexico ($19^{\circ}30'$ and $21^{\circ}35'$ N latitude, and $87^{\circ}30'$ and $90^{\circ}24'$ W longitude). Euthanasia was performed if animals were not claimed within 3 days, according to federal Mexican regulations (NOM-033-200-1995) (Papers II to IV). Most of the animals were mongrels. The minimum age of the dogs that were included in the studies reported in Papers II to IV was one year. Determination of the age of the animals was based on dentition and tartar deposition on their teeth and they were classified as young (1–2 years), adult (3–5 years) or old (≥ 6 years). For each animal the size was determined by measuring the distance from the floor to the top of the withers and they were classified as small (≤ 40 cm), medium (41–50 cm) or large (≥ 51 cm). The body condition score (BCS) was determined using the method of Laflame (1977) and the dogs were classified as underweight, ideal or overweight. In Paper III, four male dogs were kept outdoors during 12 months, were fed a commercial dog food and had water *ad libitum*, and they were given away for adoption at the end of the studies.

In Paper V thirty mongrel dogs, 3 to 5 years old and weighing 13–15 kg, were maintained during the month of the study. They were fed a commercial dog food and had free access to water; at the end of the study, the animals were castrated, vaccinated, dewormed and given away for adoption.

Collection and processing of samples

Gross observation of the female and male genital tracts

In Paper II, the entire genital tracts were recovered from bitches and macroscopically evaluated for gross abnormalities. Ovaries were sliced to count the number of corpora lutea. Non-pregnant uteri and ovaries were weighed.

In male dogs, the penis, prepuce and scrotum were examined macroscopically for pathologies (Papers III and IV). Only clinically healthy dogs were included in Paper III, eliminating those with testicular or epididymal pathologies. The testicles were dissected free from the scrotum, tunica and epididymis and the testicles and epididymides were measured and weighed to the nearest 0.1g. Testicular volume was calculated as $L \times W \times H \times 0.71$ where L= length, W= width and H= height (Paltiel *et al.*, 2002) (Papers III and IV).

Microscopical evaluation of tissue samples

Vaginal smears were made from each bitch to determine the stage of their estrus cycle (Paper II).

Tissue samples for histological evaluation from the testes (Paper III, IV and V), caudae epididymidum, vasa deferentia and spermatic cords (Paper V), uterus and ovaries (Paper II) were collected. Samples were immersed in 10% neutral buffered formalin. Samples were also sliced at 2 mm thickness and embedded in paraffin. Five-micrometer sections were cut and stained with hematoxylin and eosin (HE) according to standard histology techniques.

Semen samples

In Paper III, semen samples (1st and 2nd fractions) were collected by digital manipulation once a month during 12 months. Males were stimulated for one minute before attempts to collect semen by allowing them to sniff swabs impregnated with proestrus/estrus bitch vaginal discharge. Semen volume, sperm concentration and motility were evaluated according to Oettlè (1993). Abnormalities of the acrosomes, midpieces, and sperm tails, and the presence of detached heads were evaluated by counting 200 formal-saline-fixed spermatozoa under a phase-contrast microscope at x 1 000 magnification. Abnormalities of the sperm heads were evaluated by counting 500 spermatozoa on a carbol-fuchsine-stained smear under a light microscope at x 1 000 magnification (Axnér *et al.*, 1997). In Papers IV and V, semen smears were made from the contents of the two caudae epididymidum after slicing them with a scalpel. The sperm smears were dried, fixed and evaluated as in Paper III. A sample from each cauda was also fixed in formal-saline and 200 spermatozoa in each sample evaluated in search for other abnormalities under a phase-contrast microscope at 1000x magnification (Axnér *et al.*, 1998).

Blood sampling, hormone assays and B. canis test

Blood samples were taken from the cephalic vein early in the morning every 2 weeks during 12 months (Paper III, study 1), before euthanasia (Papers II, III

study 2 and Paper IV) or at 30 min interval during 2 hours after GnRH analogue injection (Paper V). Samples were centrifuged at 400 x g for 15 minutes, and serum separated and stored at -20°C until assay.

In Paper III study 1, serum prolactin concentration was measured by enzyme immunoassay (Milenia Biotec®, Bad Nauheim, Germany). In papers III, IV and V, serum testosterone concentration was determined by radioimmunoassay (COAT-A-COUNT® Total testosterone, DPC Los Angeles CA, USA).

In Paper II, the Rapid Slide Agglutination Test (RSAT), (D-Tec CB; Symbiotics Corp., Kansas City, Missouri, USA.) was used to test the presence of *Brucella canis* antibodies in pregnant animals with embryo resorption.

Burdizzo procedure (Paper V)

In Paper V a Burdizzo clamp (Supervet™, CHIFA, Nowy Tomysl, Poland), was used. Previous to the application of the Burdizzo clamp animals received an epidural injection of ketamine (5.0 mg/kg). The Burdizzo jaws were placed over each spermatic cord and were held closed for 60 seconds. In five dogs the Burdizzo was applied over 2 areas of each spermatic cord, one close to the testis above the epididymal head and the other in the middle part of the spermatic cord. In the other five dogs, the Burdizzo was applied in the same manner but only in the middle part of each spermatic cord.

Statistical analysis

Descriptive statistics was used to show qualitative (Paper I) and quantitative variables (Papers I, II, III and IV). The association of some factors with dog ownership (type of residence, number of inhabitants in each household, presence of a fence, and socio-economical status) (Paper I) and BCS, age and size on testicular degeneration and testicular tumors (Paper IV), were tested using the EpiInfo program (Dean *et al.*, 1994). Odds ratio (OR) and 95% confidence interval (CI) at the $P < 0.05$ level were calculated.

Analysis of variance (ANOVA) was applied to determine the effect of season of the year, age, BCS and body size of dogs on the number of ovulations (Paper II), and effect of season of the year on scrotal width and seminal characteristics (Paper III), and to evaluate the effects of testicular pathologies on testicular volume and testicular and epididymal weights, and testosterone concentrations of normal dogs and dogs with testicular pathologies (Paper IV). For serum testosterone and prolactin concentrations during different seasons of the year a repeated measure analysis over time using mixed model procedures was used.

A logistic regression was used to determine the effect of season of the year, age and BCS of the bitch on the proportion of pregnant females and distribution of bitches in estrus (Paper II). A general linear model (GLM) was used to evaluate the effect of age and body weight of the dogs, and season of the year on testicular volume and testicular and epididymal weights (Paper III). All analyses were made

using the SAS program (SAS, 1989). The level of significance was set at $P < 0.05$. In Paper V, differences between GnRH-A pre-treatment and response values of testosterone were tested using a paired t-test.

Results

A survey on dog populations (Paper I)

Population structure

Differences in dog populations were found between the city and the rural areas. The number of dogs per Km² in the city was 1,163 whereas in rural areas it ranged between 75 and 390. Mean number of dogs per dog owning household in the city was 1.6 whereas in rural areas it was from 1.6 to 2.9. The ratio of people/dog in the city was 3.4 whereas it in rural areas ranged between 1.7 and 4.6. Of the households in the city 72.8% owned at least one dog whereas in rural areas this figure ranged between 63.6 and 71.1%. The probability to own a dog was 1.35 times higher ($P<0.05$) in the city than in the rural areas. Households with 5 or more inhabitants were more likely to own a dog ($P<0.05$) than households with 1-2 inhabitants. Households with adequate fences to restrain dogs were 1.56 times more likely ($P<0.05$) to own a dog than those without fences. Households of medium socio-economical status were almost 4 times more likely to own a dog ($P<0.05$) than households of low or high socio-economical status

Dog acquisition and husbandry

Most dogs in the city (57.2%) and rural villages (86.4%) were acquired at no cost donated from other owners. In the city dogs were supervised in most of the households and only 7.2% were allowed to have free access to the streets, compared to 53.7% in the rural areas. In 33.9% of the city households the dog was allowed to come into the house, spending there up to 16.5 hours per day. More than half of the dogs (56.4%) were never allowed to come inside the house and 9.7% were chained up. In rural areas, 34.4% of the households allowed dogs to come into the house and they spent up to 15 hours of the day indoors. About 53.7% of the dogs were kept outside the house all the time, and 11.9% were tied up during most of the day.

Health aspects

Vaccination against rabies covered 90.1% of the dogs in the city. Of the village dogs only 62.3% were vaccinated against rabies. The yearly mortality rate among the dogs in urban areas was 10.6% whereas in rural dogs it was high (53.0%). The most frequent reason for mortality in both cases was disease (13.3% and 51.5% for urban and rural areas, respectively).

Reproduction

In the city, 37.5% of the bitches were bred intentionally (69.2%) or by accident (30.8%) each year. The birth rate was 55.7%. Breeding had occurred in 57.3% of the rural bitches during the last year, in most of the cases by accident (91.4%) but some intentionally (8.6%). A birth rate of 85.3% was calculated. The neutering/spaying rate in bitches in the city was 3.1%, (1.2% and 1.9% for males and females, respectively), and 1.8% in rural areas, encompassing 0.5% of the males and 1.3% of the female dogs. Of the interviewed households in the city, 52.8% would accept to spay/neuter their dogs in order to control the unwanted

population, whereas most of the rural households (64.5%) were not prepared to spay/neuter their dogs.

Veterinarian opinion of dog owners

From the city veterinarians' point of view the cost of surgery was the main reason why owners were reluctant to sterilize their dogs; behavioural changes of dogs when they become adult and the costs of pet maintenance were the two most important reasons for owners abandoning them.

Reproductive patterns of stray dogs (Papers II and III)

Estrus was diagnosed in 15.7% of examined bitches and was evenly distributed over the year except for a markedly higher incidence in late autumn and winter (Paper II) (Figure 1). In the male dogs a similar pattern as that of the estrus periods was found in mean serum testosterone concentration in the free roaming male dogs, but not in the restrained dogs which had no contact with females (Paper III) (Figure 1). Significant seasonal variations were observed in reproductive traits of both female and male dogs (Table 1). In the females, although the frequency of estrus periods was not significantly different between the established seasons of the year, a significantly higher number of corpora lutea and pregnancies were observed during the warm-humid season (July to October) (Paper II) (Table 1).

Distribution of estrus periods according to season of the year, BCS, age and size of the bitches was not statistically different. The age of the bitches was not found to have any effect on pregnancy rate, but underweight animals had a significantly lower pregnancy rate (9.5%) than bitches of ideal BCS (27.5%).

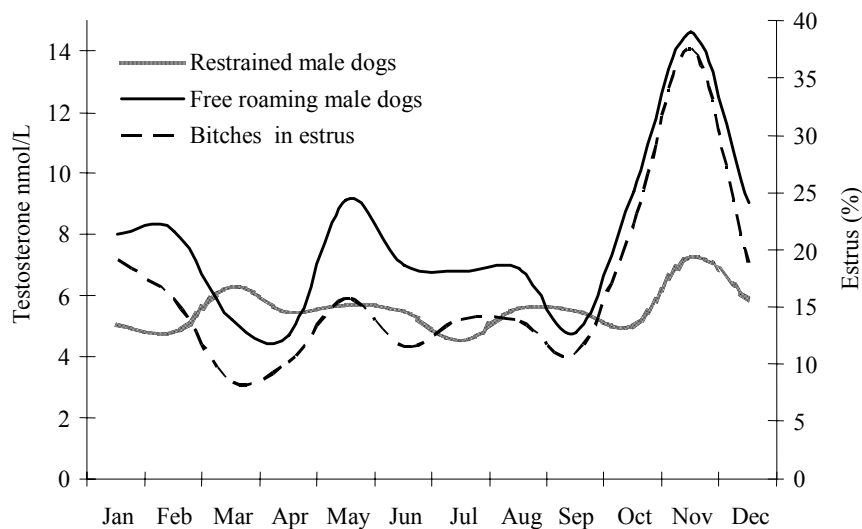


Figure 1. Annual distribution of estrus in bitches (Paper II) and mean serum testosterone concentration in male dogs (Paper III) in the tropics.

Bitches of medium and large size had significantly more ovulations (8.5 ± 0.4 and 9.6 ± 1.0 , respectively) than those of small size (7.5 ± 0.4) (means \pm SD). No differences in numbers of ovulations were observed due to BCS or age of the bitches.

In males, significant seasonal changes included variations in scrotal width, total sperm count, and sperm motility (Table 1). A circannual pattern of PRL was observed with an abrupt rise starting from March (beginning of the warm-dry season) and declining to basal levels in August (Paper III).

Values of testicular weight and volume and epididymal weight were lower during the warm-humid season (Table 1). Age had no influence on testicular volume or weight, but epididymal weight was significantly higher in old animals (2.8 ± 0.1) than in young (2.5 ± 1.0) or adult ones (2.5 ± 1.0). Body weight significantly affected testicular volume and weight, and epididymal weight, with increases of 0.7 ± 0.1 cc, 0.6 ± 0.0 g and 0.2 ± 0.0 g respectively, for every kg of body weight.

Table 1. *Seasonal variation in some reproductive traits observed in female (Paper II) and male (Paper III) free-roaming dogs in the tropics.*

	Warm-dry (Mar-June)	Warm-humid (Jul-Oct)	Fresh-humid (Nov-Feb)
Estrus periods (%)	31.9 ^a	27.7 ^a	40.4 ^a
Corpora lutea (No.)	8.0 ± 0.5^a	9.2 ± 0.6^b	8.4 ± 0.5^a
Pregnancy (%)	11.34 ^a	23.71 ^b	14.9 ^a
Scrotal width (cm)	4.9 ± 0.1^a	4.6 ± 0.1^b	4.9 ± 0.1^a
Total sperm ($\times 10^6$)	498.1 ± 102.5^{ab}	456.1 ± 112.1^b	647.5 ± 132.2^a
Sperm motility (%)	83.8 ± 2.3^a	78.8 ± 3.5^b	84.3 ± 3.2^a
Testicular weight	10.5 ± 0.3^{ab}	9.5 ± 0.3^a	10.6 ± 0.3^b
Testicular volume	11.6 ± 0.3^a	11.2 ± 0.3^a	12.3 ± 0.3^b
Epididymal weight	2.7 ± 0.1^a	2.5 ± 0.1^b	2.6 ± 0.1^b

^{a,b,c} Different superscripts within a row indicates significant differences ($P < 0.05$)

Changes in sperm morphology (Papers III, IV and V)

Seasonal changes in sperm morphology were found in both restrained and free-roaming dogs (Paper III) (Table 2). Midpiece abnormalities showed significant seasonal differences in both groups of dogs, with a higher proportion during the warm-humid and fresh-humid seasons. Distal droplets were more frequently observed in smears made from the cauda epididymis of free-roaming dogs ($49.0 \pm 15.5\%$), compared with in the ejaculates from the group of restrained dogs ($5.1 \pm 2.8\%$).

In cases of testicular pathologies (Paper IV), azoospermia was a common finding in epididymal smears especially from testicles with advanced degeneration (atrophy). Azoospermia was also present in retained testes, hypoplastic testes and in testicles with tumours. However, in some cases oligozoospermia was found with a high percentage of abnormal sperm cells (primary defects). Three of the

retained testes were oligozoospermic. Dogs with unilateral testicular degeneration, cryptorchidism or testicular hypoplasia had normal sperm morphology in samples from the unaffected testis.

Spermatogenesis was found to be still present in non-damaged areas of testicles sixteen days after application of the Burdizzo clamp (Paper V). However, several cases of azoospermia and teratozoospermia (coiled tails, double bent tails, midpiece abnormalities) were seen. Three of the five group 3a dogs (receiving two crunches in each spermatic cord) were azoospermic and the remaining 2 had spermograms with up to 95% double bent tails. The group 3b dogs (receiving one crunch in one area of each spermatic cord) showed spermograms with high percentages of midpiece abnormalities ($40.2 \pm 12.3\%$) and coiled tails ($27.5 \pm 17.3\%$).

Prevalence of reproductive pathologies (Papers II and IV)

Genital pathologies were found in 131 (43.7%) of the 300 examined bitches (Paper II) and in 135 of the 318 examined dogs (42.5%) (Paper IV). Frequencies of the different pathologies according to their probable origin are shown in Tables 3 and 4.

In females, cases of TVT (15.3%), epoöphron cysts (6.7%) and delayed puberty (6.0%) were the most common findings. The diagnosis of delayed puberty was made considering the bitch's estimated age, body weight and anatomical structure of ovaries and genital tract (Table 3). Embryo resorption was found in 26 of the pregnant bitches (45.6%) and was more frequent during the warm-dry season (March to June) (66.7%), during which time the percentage of pregnancies was low (Figure 2) Transuterine migration was observed in 20 pregnant bitches (39.2%), including those cases where embryo resorption occurred.

Of the 26 tested pregnant bitches 7.7% were seropositive to *Brucella canis* antibodies (Paper II). Neoplastic pathologies were more common in females (17.9%) than in males (10.7%), whereas infectious conditions were more commonly found in the males (17.6%) than in the females (1.2%).

Table 2. Sperm morphology and testosterone concentrations from restrained and free-roaming dogs during different seasons of the year (data from Paper III).

	Warm-dry (Mar-Jun)	Warm-humid (Jul-Oct)	Fresh- humid (Nov-Feb)
Head abnormalities (%)			
Study 1	12.1 ± 3.4 ^a	10.5 ± 2.1 ^a	9.3 ± 2.3 ^a
Study 2	11.2 ± 1.6 ^a	7.7 ± 1.1 ^b	7.9 ± 0.9 ^b
Proximal droplets (%)			
Study 1	6.0 ± 1.3 ^a	5.2 ± 1.6 ^{ab}	3.6 ± 1.9 ^b
Study 2	6.6 ± 1.7 ^a	5.8 ± 1.3 ^a	1.9 ± 0.6 ^b
Distal droplets (%)			
Study 1	5.3 ± 3.0 ^a	4.9 ± 2.9 ^a	5.1 ± 2.5 ^a
Study 2	58.9 ± 3.5 ^a	44.3 ± 3.1 ^b	44.4 ± 3.0 ^b
Detached heads (%)			
Study 1	5.4 ± 1.4 ^a	4.1 ± 1.8 ^a	3.9 ± 1.3 ^a
Study 2	6.1 ± 1.3 ^a	5.2 ± 0.9 ^{ab}	3.1 ± 0.4 ^b
Acrosomal defects (%)			
Study 1	8.4 ± 2.3 ^a	6.8 ± 1.9 ^a	8.9 ± 2.6 ^a
Study 2	8.3 ± 1.3 ^a	8.1 ± 1.3 ^a	10.0 ± 1.8 ^a
Nuclear pouches (%)			
Study 1	2.0 ± 0.9 ^a	2.6 ± 1.5 ^a	0.4 ± 0.3 ^b
Study 2	2.3 ± 0.1	1.7 ± 0.7	0.4 ± 0.2
Midpiece abnormalities (%)			
Study 1	6.4 ± 0.9 ^a	19.4 ± 6.7 ^b	20.8 ± 3.4 ^b
Study 2	5.1 ± 0.7 ^a	19.8 ± 1.9 ^b	20.1 ± 1.9 ^b
Single bent tail (%)			
Study 1	7.0 ± 2.0 ^a	3.9 ± 1.6 ^b	9.1 ± 1.9 ^a
Study 2	7.0 ± 1.1 ^{ab}	5.5 ± 1.0 ^b	10.7 ± 1.7 ^a
Coiled tail (%)			
Study 1	1.1 ± 0.7 ^a	1.1 ± 0.7 ^a	0.4 ± 0.3 ^a
Study 2	1.4 ± 0.6 ^a	0.4 ± 0.1 ^{ab}	0.2 ± 0.1 ^b
Double bent tail (%)			
Study 1	1.5 ± 0.9 ^a	1.9 ± 1.2 ^a	3.3 ± 2.3 ^a
Study 2	1.7 ± 0.3 ^a	4.1 ± 1.1 ^{ab}	4.9 ± 1.2 ^b
Normal morphology (%)			
Study 1	76.0 ± 3.5 ^a	72.90 ± 6.5 ^a	70.3 ± 3.4 ^a
Study 2	-	-	-
Testosterone (nmol/L)			
Study 1	5.9 ± 3.1 ^a	5.2 ± 4.2 ^a	5.6 ± 3.9 ^a
Study 2	5.5 ± 5.8 ^a	7.1 ± 7.6 ^a	10.0 ± 9.9 ^a

^{a,b,c} Different superscripts within a row indicate significant differences ($P < 0.05$).

Table 3. Frequency of reproductive pathologies of stray female dogs in the tropics according to their origin.

Origin	Pathology	n	%
Congenital	Epoöphron cysts	20	6.7
	Ovarian atrophy	2	0.7
	Unilateral aplasia	1	0.3
	Total	23	7.7
Neoplastic	TVT	46	15.3
	Ovarian cystadenoma	2	0.7
	Fibroleiomyoma	3	1.0
	Granulosa cell tumor	1	0.3
	Uterine granuloma	1	0.3
	Uterine carcinoma	1	0.3
Total	54	17.9	
Infectious	Oöphoritis	1	0.3
	Endometrial cyst	1	0.3
	Oviductal cyst	1	0.3
	Lymphocytic hyperplasia	1	0.3
Total	4	1.2	
Traumatic	Stump pyometra	1	0.3
	Vaginal septum	1	0.3
	Total	2	0.6
Other	Delayed puberty	18	6.0
	Serosal inclusion cysts	15	5.0
	Follicular cyst	8	2.7
	Cystic endometrial hyperplasia	5	1.7
	Vaginal polyps	2	0.7
Total	48	16.8	

In males (Paper IV), testicular degeneration was the most frequently found pathology (15.1%) followed by cryptorchidism (6.6%), testicular hypoplasia (6.6%), and TVT (5.4%). Testicular tumours were also frequent (5.3%) and these included Seminoma, Leydig cell tumour, Sertoli cell tumour and a mixed tumour (Table 4).

Table 4. Frequency of reproductive pathologies of stray male dogs in the tropics according to their origin.

Origin	Pathology	n	%
Congenital	Cryptorchidism	21	6.6
	Test. hypoplasia	21	6.6
	Infantile penis	1	0.3
	Total	43	13.5
Neoplastic	TVT	14	5.4
	Seminoma	9	2.8
	Leydig Cell Tumour	4	1.3
	Sertoli Cell Tumour	3	0.9
	Mixed tumour	1	0.3
	Total	31	10.7
Infectious	Test. degeneration*	48	15.1
	Epididymitis	4	1.3
	Epididymal abscess	2	0.6
	Testicular cyst	1	0.3
	Epididymal cyst	1	0.3
	Balanitis	1	0.3
	Total	56	17.6
Traumatic	Priapism	1	0.3
	Total	1	0.3

* Other possible origins include temperature, nutritional factors, toxins, hormonal deficiencies and excesses, vascular occlusions, obstruction to the flow of sperm, autoimmunity and age.

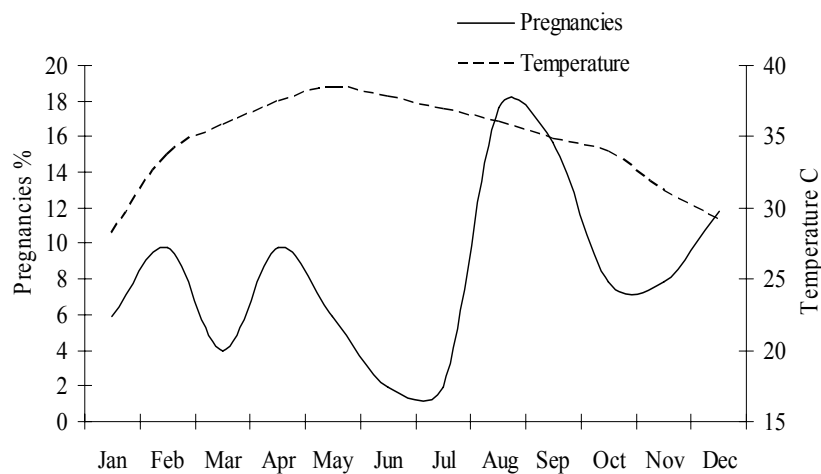


Figure 2. Annual pattern of pregnancy frequency in bitches, and of environmental temperature (Paper II).

A significant decrease in testicular volume, weight and epididymal weight was observed in testes with advanced testicular degeneration, testicular hypoplasia, and

retained or abnormal scrotal testes associated with cryptorchidism (Paper IV). In cases of advanced testicular degeneration, hypoplastic testis and retained testis, testicular volume was significantly reduced (2.6 ± 1.6 to 6.5 ± 0.5 cm³) compared to the normal contralateral testis (11.8 ± 0.7 to 12.1 ± 0.8 cm³) or to in dogs with normal testes (12.4 cm³). Similarly, testicular weight was smaller in affected testes with advanced testicular degeneration, hypoplasia and retained testis (2.8 to 5.4g) compared to the normal contralateral testis (9.7 to 10.4g) or to in dogs with normal testes (10.2g). Epididymal weight was also affected by the presence of testicular pathologies with a weight range of 1.4 ± 0.5 g to 1.9 ± 0.1 g compared to 2.6 ± 0.5 g to 5.9 ± 0.6 g for the contralateral normal testis or 2.6 ± 0.0 g for the epididymis of dogs without any pathology (Paper IV).

Risk factors for reproductive pathologies (Paper IV)

Body condition was a significant factor associated with testicular degeneration. Underweight dogs seemed to have a greater risk of developing testicular degeneration than dogs of ideal weight, but not compared to overweight dogs.

Testicular degeneration and testicular tumours were significantly more common in older dogs. Testicular degeneration was found in 18.3% of young and adult dogs from the studied population and in 33.3% of the old dogs. Older dogs showed a higher frequency (33.3%) of testicular degeneration compared to the 18.3 % found in young and adult dogs, and had an almost 7 times higher risk of developing testicular degeneration than young dogs, and 4 times higher than adult dogs. Of the total number of dogs with testicular degeneration 54.2% were of old age. Older dogs had 21.5 times higher risk to develop testicular tumours than young dogs and 4.2 times higher than adult dogs. The risk of developing testicular tumours was 14.3 times higher among cryptorchid dogs. Dog size, BCS and age appeared not to have any effect on the frequencies of cryptorchidism or testicular hypoplasia.

Observations on changes in serum testosterone concentrations (Papers III, IV and V)

No seasonal variation in serum testosterone concentration was found in the dogs (Table 2). Testosterone concentrations from the four restrained dogs did not vary throughout the year, whereas in the free roaming dogs, testosterone showed a non-significant increase (Paper III) concomitant with the increase in the frequency of bitches in estrus in December (Figure 1).

Mean serum testosterone concentration was significantly lower in dogs with pathological conditions in both testicles. Dogs with advanced degeneration of both testicles (DD) had testosterone concentrations of 0.7 ± 0.7 nmol/L, whereas concentrations of 1.2 ± 0.8 nmol/L and 0.8 ± 0.9 nmol/L were found in dogs with one cryptorchid and one abnormal testis (AC), or with bilateral cryptorchidism, and in dogs with both testes being hypoplastic (HH), respectively. Dogs having at least one normal testicle (ND, NC, NH) or two normal testicles (NN) had

significantly higher testosterone concentrations, ranging from 6.3 ± 4.4 to 8.7 ± 3.6 nmol/L (Figure 3) (Paper IV). In dogs with testicular tumours the testosterone concentration showed a large individual variation.

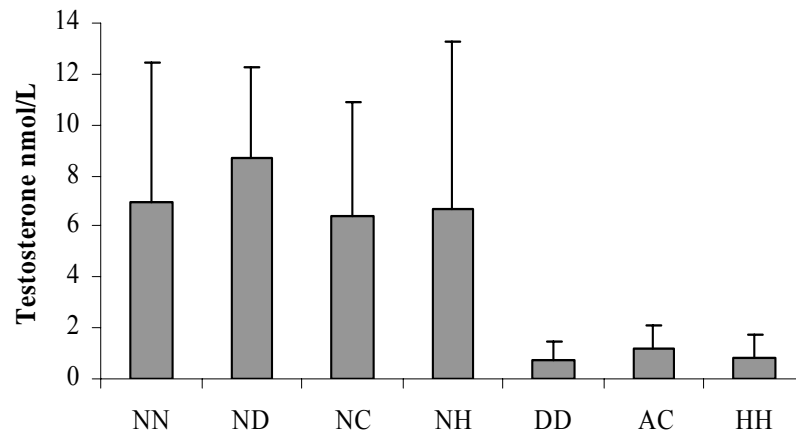


Figure 3. Mean values \pm SD of testosterone from dogs with two normal testes (NN); one normal and one degenerated testis (ND); one normal and one retained testis (NC); one normal and one hypoplastic testis (NH); advanced testicular degeneration (DD); one abnormal and one retained testis (AC) and one hypoplastic testis (HH).

The serum testosterone concentrations after gonadorelin stimulation showed a significant increase in dogs of the control group, from 4.3 ± 3.4 nmol/L to 10.2 ± 3.7 nmol/L. Castrated dogs from group 2 showed a significant decrease of testosterone and no response to gonadorelin stimulation. In the Burdizzo groups, testosterone concentrations post-treatment showed a slight, non-significant, increase after gonadorelin challenge (Paper V).

The use of a Burdizzo castrator in dogs (Paper V)

Macroscopical lesions were evident in the plexus pampiniformis and vas deferens of group 3 dogs 16 days after applying the Burdizzo. Histological examination of the plexus pampiniformis revealed cases of an acute fibrosis with inflammatory reaction and reduction of the arterial luminal area. Testicles had infarction and a degenerated parenchyma, whereas seminiferous tubules had disappeared or showed several degrees of degeneration. Interstitial tissues revealed vacuolated Leydig cells, lymphocytic infiltration, and diffuse fibrosis or total absence of Leydig cells. The deferent ducts from all dogs from group 3 showed vasitis and sperm granulomas of the vas deferens at the level where the vas had been crushed.

General discussion

Dog populations in developing countries

Populations of domestic dogs in developing countries may share a range of similar characteristics. However, different circumstances such as people's socio-economical status, education level and believes of the different regions may modify these proportions (Paper I). The proportion of dog owning households in our study was higher than the 37 to 69% reported in other developing countries (Brooks, 1990; Odendaal, 1994; Margawani & Robertson, 1995; Robinson *et al.*, 1996; Nunes *et al.*, 1997; Butler & Bingham, 2000; Fielding & Mather, 2001). This shows that dogs are popular pets in Mexico. The probability to find a dog owning household in Merida compared to in the rural villages was higher, probably as a result of the better socio-economical status and, consequently, better resources for pet maintenance. In addition, in the city people have a better acceptance of dogs especially in households of medium socio-economical status (Paper I); thus educational and sterilization campaigns should be targeting this part of the society which is responsible for a great proportion of the surplus dogs in the cities.

The high density of dogs per km² is a direct reflection of the high percentage of dog owning households in the city, in which also the suburbs are densely inhabited (Paper I). However, the density of dogs is probably underestimated since only owned dogs were taken into consideration. Although some dog owners in the city allowed their dogs to roam free in the streets, this was more common among rural dog owners (Paper I). Estimation of stray dog populations, especially in urban locations, may be achieved by using a modification of wildlife techniques such as photographic recapturings of free roaming dogs (Beck, 1973). Due to the high reproductive capacity of dogs in tropical conditions (Papers II and III) and ready availability of food and shelter (Paper I), the density of stray dogs in urban locations may be high. In rural sub-locations the households occupy large areas of land where backyard agriculture and keeping of farm animals is practiced, thus resulting in a low density of dogs (Paper I). This is also seen in rural locations in other tropical countries (Brooks, 1990; Childs *et al.*, 1998; Matter *et al.*, 2000).

The ratio of people/dog was similar or even higher in rural than in urban areas (Paper I), suggesting a similar overpopulation problem in both areas, as found in other places of Mexico (Fishbein *et al.*, 1992; Flores & Valenzuela, 2004) and other developing countries (Robinson *et al.*, 1996; Nunes *et al.*, 1997; Butler & Bingham, 2000). The ratio of people/dog in urban and rural areas of Mexico (Paper I) are below the 10:1 ratio observed in North America or found in European countries considered as acceptable by the WHO (1988).

Dog ownership in developing countries

Legal obligations pertaining to dog ownership and to the well being of dogs are regulated by sets of laws established in most countries. However, the compliance

with these laws in developing countries such as Mexico is poorly enforced.

Attitudes towards dogs may be influenced by the way they were acquired. In rural areas where uncontrolled breeding occurs, births can be expected at any time during the year (Paper II) producing surplus dogs. Most dogs in turn are donated to other owners (Paper I). These low or no cost pups can be responsible for a high number of the abandoned dogs (Hsu *et al.*, 2003). Low cost of acquisition and maintenance are probably the major reasons for the high rate of dog ownership especially in the city, where the probability of finding a household owning a dog is higher than in rural areas (Paper I). In rural villages, as reported by Orihuela & Solano (1995), the socio-economical situation is not a limitation to owning a dog since the cost of acquisition is nil or low and food is readily available from human handouts and garbage disposal. Free-roaming dogs are in most cases owned by a family or by a neighbourhood. Inadequate fencing to restrain dogs from gaining access to the street is common in rural areas of Yucatan (Paper I). Consequently, owned dogs are roaming free and are tolerated by the neighbours. In rural areas free roaming dogs searching for food can even break into houses. In some cases, due to the lack of fences, dogs in rural Yucatan like in other countries such as Sri Lanka and Ecuador are found tied up (WHO, 1988) to prevent them from having access to the streets.

People's easy access to free rabies vaccination and a better level of supervision of dogs may explain the high level of immunization observed in urban areas (Paper I). Although people in the city are positive towards rabies vaccination, immunization against other common diseases in the area is not widely used. This situation is more critical in rural areas where a high mortality rate due to infectious diseases is seen. Low economical capacity, lack of care and, probably ignorance are the major reasons for this problem (Paper I).

One of the critical factors leading to a fast growing of the canine population is the lack of control of birth rates as a result of failure to spay and castrate and to supervise the pets. Free-roaming dogs, as a consequence, have indiscriminate promiscuity and a high birth rate achieving their maximal breeding efficiency. Even when stray dogs may develop genital pathologies affecting their reproductive capacity, the percentage of pathologies in tropical conditions is not so high that it affects the population (Papers II and III). Free roaming non-gonadectomized female dogs, especially in rural areas are probably breeding at, or close to, their potential of two litters per year, resulting in a surplus of dogs which, in turn, are given away as presents (Paper I). Many pet owners do not wish to have their dog altered for emotional reasons, because of concerns about the procedures and effects of de-sexing dogs, even when the cost of surgery was not considered the main reason for not spaying or neutering dogs (Paper I). A fast and easier to perform blood-less technique such as the Burdizzo procedure (Paper V) may be proposed to owners as an alternative instead of a surgical procedure. In many cases dogs have attained the status of human beings and the owners consider that their pets have the same needs, drives and desires as they do (Levinson, 1974), and so appear more reluctant to have their dog neutered as shown by several reports (Blackshaw & Day, 1994; Fielding *et al.*, 2002). However, results from urban areas may not reflect the attitudes of people from rural areas.

Veterinarians' role to prevent dog surplus

Behavioural changes, cost of pet maintenance and dog disease were responsible for more than 50% of the abandoning of dogs (Paper I). Unacceptable behaviour is a major reason why a high number of dogs are relinquished (Beaver, 1991); most of these behaviours can be prevented or treated (Mackay, 1993; Scarlett *et al.*, 2002). Prospective pet owners must be encouraged to commit to pet ownership for the life of the animal, which includes pet maintenance. Gonadectomy is the most common and reliable method to prevent pet overpopulation. In a tropical region such as Mexico, dogs are normally sterilized at 6 to 9 months of age, or after puberty (Paper I). Veterinarians may encourage clients to have female dogs not intended for breeding neutered before the first estrus to reduce the risk of mammary neoplasia, uterine infection or prostatic disorders (Preston, 1993). In the opinion of veterinarians, high cost of surgery is the main reason for the low number of gonadectomies. Veterinarians must inform clients about the spaying/neutering procedures and in addition offer low cost neutering.

Prepuberal gonadectomy can be performed from 7 weeks of age in dogs (Howe & Olson, 2000). Apparently, a lack of information about early neutering procedures (i.e. technique, anaesthesia) minimises the number of dogs sterilized before puberty (Paper I). Scientific information points to that this practice is safe with apparently minimal side effects when compared with neutering at 7 months of age (Salmeri *et al.*, 1991). However, veterinarians must be aware of possible side effects such as changes in growth rate, obesity, perivulvar dermatitis, vaginitis, behavioral changes, urinary incontinence, increased morbidity/mortality during surgery and anesthesia, and impaired immunocompetence (Salmeri *et al.*, 1991). Other concerns regarding the early neutering include acetabulum problems, dandruff, hot spots, looser skin and poor coat quality. Veterinarians must be aware of new technologies for spaying and neutering dogs and well informed about new contraceptives that are being introduced to control reproduction of dogs.

Seasonal changes of reproductive traits

Estrus distribution. Domestic bitches are considered monoestrous, with estrus occurring any time during the year independently of season (Paper II). However, reproductive patterns including occurrence of ovulations and pregnancies may be modified by factors such as nutrition, temperature and environment (Sokolowski *et al.*, 1973). A significant relationship between photoperiod, temperature, and month with the number of bitches in estrus has been reported (Choy & Echevarria, 2005). In temperate regions estrus appear more common during winter and early spring (Christie & Bell, 1971; Prole, 1973; Bouchard *et al.*, 1991; Linde Forsberg & Wallén, 1992). In the tropics more bitches are seen in estrus during late autumn and winter, independently of their age, BCS and size (Paper II). Probably some reproductive patterns observed in the dog's ancestors may still remain and be expressed under certain conditions of the year. In West Bengal, India mating of free-ranging dogs occurs between August and October, at the end of the unfavourable monsoon season (Pal, 2005). A similar patter of estrus periods is also found in Jaipur India (Chawla & Reece, 2002). However, observations in

these regions indicate that bitches breed only once a year (Pal *et al.*, 1998) similarly to as observed in wolves (Seal *et al.*, 1979). In tropical Mexico, free-roaming dogs are commonly seen (Paper I); under these conditions dogs breed naturally and may be more commonly in estrus during less adverse environmental conditions of the year like observed in India.

Ovulations. Ovulation in the bitch occurs spontaneously 2 to 3 days after the LH surge (Olson *et al.*, 1984). In the bitch, follicles begin to luteinise during the mid-proestrus and then undergo a more rapid, extensive luteinisation during the preovulatory LH surge (Concannon *et al.*, 1989). After ovulation, the presence of well defined corpora lutea indicates the number of oocytes that have been released. The number of eggs released by the ovaries at each estrus (ovulation rate) is dependent on the number of ovulating follicles, which is genetically controlled and regulated by neuroendocrine mechanisms. However environmental factors such as photoperiod (Lincoln, 1992), nutrition and stress (Clarke & Tilbrook, 1992) can modify the number of ovulations. In the tropics, free-roaming dogs may increase their number of ovulations during summer but mechanisms involved are not clear (Paper II). Although BCS can affect the number of ovulations in other domestic species (Findlay & Cumming, 1976), in underweight dogs this is not the case since the number of ovulations was similar to that observed in dogs with ideal BCS (Paper II). Factors such as breed and body size produce differences in the ovulation rate.

Pregnancy distribution. Distribution of pregnancies in dogs throughout the year can vary. Several factors may alter it and, consequently, a distinct, repetitive seasonal distribution of births can be observed (Paper II). For example, in the USA, peaks of natal patterns are observed in the spring and early summer and a nadir during February (Tedor & Reif, 1978). In the UK a distinct seasonal pattern of canine births can be observed during the summer time (Evans, 1993) and from February to May in Sweden (Wikström & Linde Forsberg, 2006). In a tropical region like Kenya, more pregnancies are observed during October (Mutembi *et al.*, 2000), whereas in Jaipur India more pregnancies occurred during August to December (Chawla & Reece, 2002). As in other domestic species, seasonal infertility may occur during periods of high environmental temperature, probably due to increasing embryo mortality (Paper II). In farm species, the pregnancy rate may show a great variation throughout the year due to impaired fertilisation or poor embryo survival (Clarke & Tilbrook, 1992; Peltoniemi *et al.*, 2000). Nutrition can also affect pregnancy rate and litter size (Clarke & Tilbrook, 1992) similarly to as observed in underweight bitches (Paper II).

Sperm count and sperm morphology. Although significant seasonal variations in the spermogram are reported in domestic dogs (Takeishi *et al.*, 1975; Taha *et al.*, 1981), there are differences in the precise timing of the variations observed possibly because they are related to different environmental and climatic conditions. Evidence of seasonal variation in ejaculate volume, total sperm count and motility is present in stray dogs in the tropics (Paper III) but these changes are of low magnitude. Therefore, healthy dogs may produce viable sperm throughout the year. These subtle changes may be related to environmental temperature increasing the number of abnormal spermatozoa during the summer (Taha *et al.*,

1981), or increasing the number of midpiece abnormalities during the fresh-humid season of the year (Paper III). However, more precise mechanisms involved in these processes still have to be elucidated. Although environmental temperature under tropical conditions can induce deterioration in the seminal characteristics in dogs (Paper III), the semen can be of similar quality as in dogs in temperate regions (England & Allen, 1989), probably as a result of a well-adapted thermoregulation.

Testicular characteristics. Adult males of various seasonal breeding mammals can show circannual cycles of testicular involution and recrudescence, from totally arrested to highly activated spermatogenesis. This strategy minimizes the energy cost of reproductive efforts and promotes an optimal timing for the generation of viable spermatozoa in a precise period of time (Lincoln, 1981). Body weight of dogs is strongly correlated with testicular weight (Paper III). Additionally, scrotal width is strongly correlated with testicular weight and thus provides a convenient indication of testicular size and potential sperm production (Woodall & Johnstone, 1988a). A significant increase in testicular weight in a tropical region is observed in dogs during the coldest season of the year (Paper II). Although wild canids are classified as monogamous species with relatively small testis size compared to species of promiscuous mating systems (Kenagy & Trombulak, 1986), free-ranging dogs (*Canis familiaris*) through domestication have developed promiscuous mating systems with sperm competition in the genital tract of mated females. This increased sperm competition in domestic dogs produce an increase in their sperm stores with larger increases than expected for increases in body or testicular mass (Woodall & Johnstone, 1988b). This suggests a persistent spermatogenesis along the year and capability to breed a receptive female. However, some other factors such as the presence of cycling females and breeding competition probably exercise a physiological stimulus on free-ranging dogs to maintain or increase the sperm store over the year or to promote a degree of recrudescence waiting for a more adequate period of time similar to as seen in seasonal breeding mammals (Paper III).

Testosterone and prolactin secretion. Testosterone is a steroidal hormone of importance for male reproduction, including maintaining the libido and for spermatogenesis. The concentration of testosterone in dogs can fluctuate considerably due to its episodic and circadian pattern of secretion (Knol *et al.*, 1989). Its secretion is continuous throughout the year and no seasonal rhythmicity is expected (Paper III). However, some studies reveal a significant rise in the level of testosterone during August and early September (Falvo *et al.*, 1980) and during the summer (Taha & Noakes, 1982). In a tropical country such as Brazil, a significant decrease in testosterone levels was observed during the summer when temperature and humidity were high (Mello, 2005). Increases in testosterone concentrations during certain months of the year may have been associated with the presence of bitches in estrus (Paper II). Attraction in male dogs to estrous females can increase their levels of testosterone (Beach *et al.*, 1983); therefore, a physiological significance of the rise in the plasma concentrations of testosterone could be expected. In dominant wolves serum testosterone and urine-marking rates are increased during the breeding season (Asa *et al.*, 1990). In Beagles, behavioral responses to urine and vaginal secretion odors can serve as reliable predictors of

their reproductive and social behaviour and serve to communicate the reproductive status between males and females (Anisko, 1976). In free-ranging dogs, urine marking increases significantly with a peak in the period of mating, suggesting a high relationship between marking and the reproductive status of the dog (Pal, 2003). Some of the ancestral seasonal breeding patterns observed in wild canids may remain in the domestic male dog (*Canis familiaris*) and may represent some evolutionary behavioral components with geographical variations regulated by the different environments.

Prolactin secretion is highly seasonal in a wide range of mammals adapted to all kinds of climates. This pattern of secretion in dogs is circannual with an increase during the summer and a decrease in winter (Paper III), as observed in other mammals (Lincoln, 1989). Prolactin has many biological effects, most of them unrelated to reproduction; in males it may act as a signal for summer, coordinating many physiological events to this season as molting and inducing paternal behavior (Lincoln, 1989). The function of this pattern of secretion in the dogs is unknown; it may be genetically inherited from wolves, but functionally altered through domestication or other processes (Kreeger & Seal, 1992). In wolves, PRL levels varied annually in response to photoperiod, with significantly higher concentrations during the summer and a significant increase in serum LH and testosterone occurred when PRL levels were decreased (Peterson, 1984).

Reproductive pathologies

The prevalence of reproductive pathologies in the male and female dog is poorly documented in the literature, in particular those which may lead to sterility or reduced fertility. Monitoring the prevalence of reproductive diseases in stray dogs would be of great epidemiological interest to obtain more accurate data on the abnormalities of the canine reproductive tract and to assess the risk of spread of congenital or acquired diseases among dog populations in a specific area or region.

Pathologies in the female. Congenital reproductive pathologies in the bitch are rare, but the most commonly found were not important causes of infertility compared to the reproductive congenital pathologies found in the male dogs (Paper II). Transmissible venereal tumor is the most common neoplasia from the reproductive tract of female dogs (Paper II) with a high prevalence in developing countries where there is an abundance of free roaming dogs (Ortega *et al.*, 2003); a prevalence of TVT of up to 37% can be found when the density of stray dogs is high (Batamuzi *et al.*, 1992). Other genital tumors are less prevalent in the bitch (Paper II).

There are few infectious, traumatic or other conditions affecting the reproductive tract of the bitch. They are occasional findings during elective ovariectomy or when a medical problem results from these pathologies. However, bitches are more predisposed to hormonal disturbances such as follicular cysts and cystic endometrial hyperplasia (Paper II). Age of puberty in dogs can vary from 6 to 24 months (Sokolowsky, 1971) or from 9-24 months in

mongrel bitches (Hancock & Rowlands, 1949). Delayed puberty may indicate the presence of several factors that decelerate the onset of the sexual activity in a population of stray dogs (Paper II). Environmental and genetic factors can modify the onset of estrus in bitches such as the plane of nutrition and the degree of inbreeding (Phemister, 1980). Embryo resorption is a common event in dogs (Paper II) with a high frequency in stray dogs under tropical conditions especially during the summer or warm-humid season (Ortega-Pacheco *et al.*, 2006), probably due to adverse environmental conditions (i.e. high temperature and humidity).

Pathologies in the male. Congenital pathologies of the reproductive organs in stray male dogs are common (Paper IV) but seldom reported, particularly cases of testicular hypoplasia, a frequent congenital condition seen in other domestic species (Blanchard *et al.*, 1990). Environmental conditions, breed, cytogenetic abnormalities, intersex development and cryptorchidism are factors that have been associated to the development of testicular hypoplasia. Cryptorchidism in dogs is also fairly common (Paper IV) (Dunn *et al.*, 1968; Pendergrass & Hayes 1975; Kawakami *et al.*, 1984; Hayes *et al.*, 1985).

In male dogs, testicular tumors are found in higher frequency in cases of retained testes or advanced age (Paper IV). As found in the bitch (Paper II), TVT is a common neoplasia in male dogs in the studied area.

Testicular degeneration is one of the most prevalent reproductive pathologies found in male dogs (Paper IV). An infectious origin of testicular degeneration is *B. canis*, which may affect susceptible dogs (Wanke, 2004). Canine brucellosis has been diagnosed in Mexico with a high rate (11.8%) (Flores-Castro *et al.*, 1977) and its presence has been demonstrated in stray dogs in Yucatan (Paper II). However, other possible causes are high temperature, nutritional factors, toxins, hormonal deficiencies and excesses, vascular occlusions, obstruction to the flow of sperm, autoimmunity and age.

In male dogs aging is an important factor for the development of testicular degeneration and testicular tumors (Paper IV). Senile testicular degeneration and atrophy have been noticed in dogs from 7 to 10 years of age (Bloom, 1953; James & Heywood, 1979). Additionally, underweight male dogs are also at risk to develop testicular degeneration (Paper IV) with a degree of lesion probably in proportion to the daily amount of food consumption (Nduka *et al.*, 1983), duration of food restriction (Oke *et al.*, 2003) and stage of life cycle during which the restriction occurred (Larsson *et al.*, 1974).

Despite the high frequency of reproductive pathologies that potentially can affect the rate of reproduction of male dogs (Paper IV), reproduction in the population of free-roaming dogs is successful resulting in a dog surplus (Paper I).

Blood-less castration in dogs

Castration is the most reliable method to permanently sterilize male dogs. Castration of dogs reduces the testosterone levels (Paper V); consequently, it both reduces the risk of developing prostatic disease and undesirable male behaviours such as roaming, aggression, mating and urination marking (Neilson *et al.*, 1997). However, castrations are in most cases surgical and require anaesthesia, surgical facilities and skilled veterinary surgeons, which limits the applicability of this contraceptive procedure for extensive population control programs. The ideal contraceptive in the male should be effective, safe, readily available and acceptable to owners and have few side effects (Pineda, 1986). The Burdizzo clamp is widely used to castrate small ruminants with minimal postoperative complications (Molony *et al.*, 1993). It is also a safe and simple method to sterilize dogs (Paper V). This technique may be recommended for large-scale sterilisation programs in rural or urban areas of developing countries where at present few sterilizations of dogs are performed due to the high cost and people's concerns about the surgery procedure (Paper I).

The occlusion of the testicular artery and venous drainage of the testicular parenchyma produces an ischemic necrosis (infarctation) (Paper V) as observed in rams after experimental ischemia of the testicular artery (Markey *et al.*, 1994, Markey *et al.*, 1995), or in dogs with naturally occurring testicular torsion (Pearson & Kelly, 1975; Hecht *et al.*, 2004). The reduction in the blood flow through the damaged spermatic vessels can cause a lack of response of the interstitial tissue of testicles to GnRH stimulation (Markey *et al.*, 1994), and depending of the degree of occlusion the response to stimulation can be low or none (Paper V). After experimental testicular torsion in rats, there is a significant reduction in the testicular vascular perfusion leading to loss of spermatogenesis and reduction in testicular androgen production (Turner *et al.*, 2005). Despite the return of blood flow, testicular damage occurs probably due to mechanisms such as ischemia or upon reperfusion injury (Turner & Brown, 1993). Permanent damage to the vascularity of the testicular cord after induced injury (Paper V) may follow a similar mechanism of reperfusion.

Future prospects

In this study, it has been shown that people's attitudes towards dogs can have a great influence on the dog's health and rate of reproduction. However, more detailed data about factors leading to the relinquishing of dogs are needed. Dogs are rejected from households and left on the streets, to animal shelters or dog pounds; information generated from these last two sources can provide useful information about the reasons for relinquishing which may help in the decision to take specific actions. Monitoring changes in people's attitudes and changes in dog populations in both kinds of areas, urban and rural, after implementing educational programs, would provide important information regarding the success or failure of such programs. In the city, where most of the stray dogs have no owners, quantification can be achieved by using wildlife techniques for the estimation of such populations (WHO, 1990), particularly the Beck's method of photographic recaptures (Beck, 1973).

Veterinarians have an important role to play for the proportion of dogs and cats being relinquished and euthanized (Scarlett *et al.*, 2002). However, their impact in the present study seems to be low. Veterinarians must embrace new technologies not only for early gonadectomy procedures but also stay informed about the new contraceptives that are being introduced. In addition, special training in dog behaviour and early neutering may attract new clients. Monitoring changes in the number of dogs that are being gonadectomised, and reasons for relinquishing of dogs from the veterinarians' point of view after implementing courses, would be of value to assess the impact on dog populations.

This study shows that the reproductive pattern of stray dogs under tropical conditions may undergo seasonal changes but of low grade, allowing dogs to reproduce constantly throughout the year. Increased number of females in estrus in tropical conditions (Chawla & Reece, 2002), increased percentage of pregnancies and fetal resorptions, increased number of ovulations, and a deterioration in seminal components and testicular size have been reported during specific times of the year. However, factors leading to these changes should be further investigated to clarify the mechanisms involved.

The number of genital pathologies affecting the population of stray male and female dogs under the conditions of this study is high. Their aetiology should be an important area of study. Environmental conditions such as availability and competition for food, presence of infectious diseases, promiscuous mating and inbreeding in a population of stray dogs can be reflected in the frequency and type of genital disorders. Infectious agents such as *Brucella canis*, are involved in pathologies such as testicular degeneration, epididymitis and embryonic death or abortions (Wanke, 2004) and is also associated with human disease (zoonotic); for these reasons it deserves special attention for investigation. Other pathologies affecting the reproductive health of dogs should be monitored, including in owned dogs, to determine their distribution and detect possible aetiologies.

The Burdizzo clamp has proved to be efficient to induce irreversible lesions in the testicle, leading to infertility. However, to prove these points, possible side effects affecting the health of the individual, although not expected, should be elucidated in long-term studies. Vasal occlusion or vasectomy is used in dogs to induce azoospermia (Schiff *et al.*, 2003); however, this procedure is also invasive. In the light of the findings from this study, selective pressure of the vas deferens to induce sperm granulomas and azoospermia should be further investigated as a new alternative to induce infertility in male dogs.

Conclusions

- Dogs are popular and readily available pets in rural and urban regions of Yucatan Mexico. Structures of dog populations are similar in both locations but with well marked differences in the care and husbandry provided to the dogs. Socio-economical status has a great influence on the supervision of dogs. Surplus dogs are common in urban and rural areas due to a high rate of whelping particularly in rural communities where owned dogs are non-gonadectomized and are free-roaming. Veterinarians in the city have little impact on the control of dog breeding and on reducing dog surplus in the city.
- Stray male and female dogs under tropical conditions are not seasonal breeders, but environmental factors exist that can modify their reproductive pattern. Although bitches experience ovarian cycles throughout the year, the percentage of ovulations can be significantly increased during the warm-humid season (July to October), whereas percentage of pregnancies can be reduced during June and July. Healthy males are capable of producing viable sperm throughout the year with minor seasonal changes. Stimulus such as day length and presence of females in estrus can induce fluctuations in reproductive patterns in male dogs. Intrinsic factors like age, body size and BCS are also involved in the reproductive patterns of dogs.
- The prevalence of genital pathologies in the studied population of male and female stray dogs is high. In the female, however, the majority of pathologies were likely to have been of relative minor significance for fertility and few would have caused sterility. In males, congenital pathologies (cryptorchidism and testicular hypoplasia) are common, as well as neoplastic conditions such as TVT and testicular tumors. Cases of testicular degeneration were quite frequent suggesting the presence of adverse environmental factors. However, older dogs and dogs with low BCS are highly associated with this pathology. The production of testosterone and its serum concentration is reduced in cases of testicular pathological conditions depending on the amount of interstitial tissue that is damaged. However, findings in stray dogs are not necessarily representative of the real prevalence in populations of owned dogs, since the former are exposed to a wide variety of conditions including poor nutrition, poor environment and a multitude of diseases.
- Using a blood-less Burdizzo clamp induced testicular lesions leading to irreversible damage. An acute inflammatory reaction and fibrosis in the plexus pampiniformis where the pressure was applied produced a reduction in blood supply and infarction. No significant increase in testosterone concentration was obtained after gonadorelin stimulation due to the reduction of testicular blood flow and loss of testicular interstitial tissue. The Burdizzo clamp method can be used as a non-surgical, safe,

rapid and easy to perform alternative for surgical mass sterilization of dogs, or in individual cases for removal of gonadal function.

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