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Infertility in male dogs: recent advances

Infertilidade do macho canino: atualidades

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Abstract

Very little is known about male infertility in the dog. The causes of infertility are divided into two main groups, congenital infertility and acquired infertility. Congenital infertility is caused by genetic (chromosomal) abnormalities and is present at birth. Acquired infertility develops during the dog's lifetime. Infertility in male dogs is diagnosed by a good medical history, including breeding management, and a physical examination.

Keywords: dog, infertility, prostate, sperm.

Resumo

Pouco se sabe sobre as causas da infertilidade do macho canino. As causas de infertilidade são divididas em dois grupos principais, quais sejam alterações congênitas ou adquiridas. As alterações congênitas são causadas por anormalidades genéticas (cromossomais) e estão presentes ao nascimento. As alterações adquiridas, por sua vez, acontecem no decorrer da vida do animal. A infertilidade do macho canino é diagnosticada por meio de um bom histórico, incluindo manejo de cobertura e exame físico.

***Palavras-chave**: cão, infertilidade, próstata, sêmen.*

Introduction

Veterinarians are sometimes asked to diagnose and solve fertility problems in the male dog. In the case of some dog breeders, mating a stud dog with pure breed bitches proves quite lucrative and brings fame. For others, reproductive expectations are high for a dog which has won several exhibitions or competition shows or has been genetically selected after several generations. These persons are prepared to conduct several clinical investigations to know the cause and the cure of the decrease in fertility of their dog.

Very little is known about male infertility in the dog. Regarding human male infertility, the cause remains unknown in 70% to 74 % of cases (Johnston et al., 2001). In the area of human medicine, when the semen is of poor quality, most of the time assisted reproduction techniques are utilized such as *in vitro* Fertilization (IVF) or Intra-Cytoplasmic Sperm Injection (ICSI). These techniques are not routinely available to the dog, but may be used in the future (Fulton et al., 1998; England et al., 2001); therefore, the prognosis of infertility often remains very poor. However, some infertility causes can be partially or totally cured if the veterinarian is consulted early enough. A complete clinical survey of the dog is very often important as some diseases may begin by causing infertility and thereafter creating a more general health problem. But in only 10% of dogs presented with infertility may fertility be restored after diagnosis and appropriate treatment (Johnston et al., 2001).

Infertility in the male dog: the different causes

Anatomical abnormalities

Hermaphroditism or pseudohermaphroditism (for example, a dog with male external genitalia and female gonads) induces sterility. Congenital defects may include testicular hypoplasia, epididymal segmental aplasia, agenesia of the vas deferens, congenital bending of the penis bone, hypospadias or epispadias, which can cause azoospermia or incapacity to mate. If the size of the penis is too small, it may not be retained inside the vagina during copulation, which may cause a lack of volume of sperm deposited into the female genital tract. Bilateral cryptorchidism causes azoospermia, while usually unilateral monorchidism does not create any fertility problem (Rhoades and Foley, 1977). In large breeds, dimorphism may lead to mating problems when the male is so heavy that the female cannot support its weight during male overlapping (Fontbonne, 1999). Aquired anatomical abnormalities may also cause male infertility. Spermatocele or sperm granulomas, stenosis or obstruction of the genital ducts - after an infection for example - or inguinal or scrotal hernia, may lead to azoospermia or aspermia. Other acquired abnormalities are strictures after surgery or injuries and penis bone fractures. Orthopedic problems (like rachidian or hip diseases) may prevent the male from mounting the female.

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Low quality semen

Prostatic problems

A prostatic problem often plays a direct role in decreasing fertility, and, according to our data, prostatitis is one of the major cause of infertility in male dogs.

Prostatic diseases may act in many ways:

- by decreasing the volume of the ejaculate, after a hormonal anti-androgenic treatment for example;
- by altering sperm motility (ciliostasis). In the case of prostatitis, the pH of prostatic fluid is often changed. It may be acidic or alkaline (Meyers-Wallen, 1991) and stops the capacity of spermatozoa to move freely; infectious agents may also act directly on sperm cells;
- by killing sperm in situ, or making it unable to progress in the female genital tract (i.e. in the case when sperm is full of pus (pyospermia) or blood (hematospermia)).

Epididymal or testicular problems

Spermatozoa are synthesized in the testicles. They acquire motility and fertilizing ability during epididymal transit. Any disorder affecting these organs may therefore lead to infertility (Meyers-Wallen, 1991).

Urinary problems

Cystitis or uretritis may interact with sperm motility when, as already stated, they modify the acidity of the uretra.

Food-induced alcalinization of urine may have the same wrong effect.

Retrograde ejaculation

Retrograde ejaculation consists of a retrograde backflow of semen into the bladder when ejaculation occurs. This leads to either aspermia or oligospermia.

During a normal ejaculation, the hypogastric nerve is responsible for the closure of the bladder. But, a small amount of sperm always flows back into the bladder (Dooley et al., 1990). The fertility may be affected when this amount becomes too high.

In dogs, retrograde ejaculation is not well documented. This problem does not seem to be permanent. It may be influenced by the state of repletion of the bladder, as retrograde ejaculation occurs more easily if the bladder is empty. The identified causes of this problem in the dog are: urethral calculus, cystitis (due to a weak inflammation of the sphincter of the bladder), local injuries and post-surgery strictures. An increased retrograde flow of semen was demonstrated in 12 to 15 dogs sedated with xylazine.

Hormonal problems

Any hormonal trouble may interact with the hypothalamus-pituitary axe and therefore have an influence on spermatogenesis and fertility. The problem can be transient, but it is sometimes more severe. The quality of semen will often drop in the period of a few weeks to a few months. The dog will first suffer from OAT, and many bitches mated with him will not produce any pup. If no action is taken, the decrease in the semen may continue until a complete azoospermia is observed at which stage, infertility most often proves irreversible.

Central hormonal causes are not well documented. Hypopituitarism may lead to azoospermia (Johnston et al., 2001). Hypothalamic or pituitary tumors may be involved. Prolactine adenomas may play a negative role towards fertility. Idiopathic insufficiency with lack of production of gonadotropins FSH or LH may alter spermatogenesis (Johnston et al., 2001).

Testicular tumors which are responsible for excessive hormonal secretion (Sertoli cells tumors, Leydig cells tumors) may cause a decrease of spermatogenesis, even when these tumors are located only in one testicle and still quite small in size. There negative impact on fertility is due to direct destruction of testicular tissue, induction of inflammation, elevation of intrascrotal temperature, and production of estrogen or androgens that may exert negative feedback on the hypothalamus and pituitary (Johnston et al., 2001). Often, the infertility of the dog starts well before the appearance of any other clinical signs, for example, scrotal enlargement. Therefore, veterinarians may overlook the presence of a tumor if it cannot be palpated directly inside the testicles.

In our clinical practice, we have experienced several times infertile dogs presenting a hormonal profile characterized by a marked increase in the blood level of testosterone. These dogs did not suffer from any Leydig cells tumor.

Hypothyroidism is often stated as a potential cause of infertility, especially in large breeds. From our point of view, thyroxin and TSH blood level should be systematically checked when investigating the hormonal

status of an infertile dog. The link between hypothyroidism and low quality semen is still unclear. Adrenal dysfunction may also affect fertility. Usually, other clinical signs can be observed before the occurrence of infertility.

Infectious diseases

Infectious diseases probably represent a major cause of infertility in male dogs living in breeding kennels. Germs can cause prostatitis, which may alter the biochemical composition of prostatic fluids and may induce a permanent or temporary blockage of the ducts as they enter the prostate. But infections often lead to an orchitis/epididymitis, with a subsequent alteration of the quality of the semen. The hypothesized explanation could be a hematogenous spread of the organism to the contralateral testicle or induction of a systemic immune reaction after exposure of testicular autoantigens to the animal's immune system (Johnston et al., 2001).

There is no evidence that viral diseases may act directly on male infertility (Meyers-Wallen, 1991), although they can be present in seminal fluid and infect the bitch after mating, leading to infertility.

Canine brucellosis is often responsible for a rapid decrease in the quality of the semen by causing an acute and more often a chronic orchi-epididymitis. In 2 to 5 weeks, a high number of abnormal spermatozoa will appear (30% to 80%). Testicular atrophy can even appear over a longer period of time due to secondary fibrosis. After 10 to 15 weeks, the sperm analysis shows severe OAT and may reveal agglutination of spermatozoa.

Other bacterial infections have been suspected to cause male infertility. Specific bacteria like Coxiella burnetii have been identified as potential causes of orchitis in the dog. Actually there is a normal genital flora in the dog, with the same kind of usual bacteria as in the bitch. Among aerobic flora, *Streptococcus sp., E. coli, Pasteurella multocida, Staphylococcus sp., Proteus sp., Corynebacterium sp,* are often identified when a prepucial swabbing is cultured. More rarely, germs like *Pseudomonas aeruginosa* have been cultured. These germs can penetrate organs like the prostate or the testicles when local injuries occur (bites) or by ascendant progression in the genital ducts. Clinical problems may occur if bacterial concentration exceeds 1000 per milliliter (Johnston et al., 2001). *Mycoplasma* and *Ureaplasma* have often been isolated from the prepuce and urethra of infertile dogs (Meyers-Wallen, 1991).

Many authors believe that the potential role of this usual genital flora on infertility may be underestimated because most of the time orchitis or prostatitis develop a chronic form which is hardly noticed by the owner of the dog. In one study, 5 out of 9 infertile dogs suffered from prostatitis (Johnston et al., 2001). Therefore, it could be very useful to systematically collect the prostatic fraction of the ejaculate when evaluating the semen of an infertile male dog.

Fungi infections have been suspected to cause genital problems in males. *Blastomyces dermatidis* was identified in one case of orchitis and in several cases of balanoposthitis.

Drugs

Steroid hormones (such as corticosteroids, androgenic or anti-androgenic compounds, estrogens) may inhibit the central regulation of spermatogenesis or epididymal maturation and therefore accelerate a decrease in fertility. In the case of dogs involved in shows, racing or hunting, it seems quite frequent because these dogs are given different kinds of stimulants before competition.

Antifungic medicines (such as griseofulvine and ketoconazole) may act as steroid-like compounds, inhibiting the secretion of pituitary gonadotropins and playing a role in steroid hormones synthesis in coordination with p450 cytochroms.

Several other drugs may act on male fertility, including antineoplastic agents, the antiacid cimetidine, the tricyclic antidepressant amitriptyline (Johnston et al., 2001), the nonsteroidal anti-inflammatory drug naproxen (Johnston et al., 2001) and the one sulfamid: sulfasalazine.

Genetical problems

Chromosomal abnormalities that may occur in some phenotypically normal male dogs may lead to infertility with azoospermia. Sometimes the libido is normal. Such troubles include:

- 79 XXY syndrome, in which dogs often have hypoplastic testes and underdeveloped but not ambiguous external genitalia;
- XX sex reversal (78 XX males), in which dogs have normal male external genitalia and testicular or ovarian gonadal tissue. This has been described in Cocker Spaniels, Pointers, Kerry Blue Terriers, Weimaraners, Pugs, and Beagles. We recently observed one case in a Basset Fauve de Bretagne.

An immotile cilia syndrome has rarely been described (primary ciliary dyskinesia) (Johnston et al., 2001). This syndrome is due to the absence of dynein arms in the microtubules of the sperm tail. The Kartagener's syndrom is characterized in the dog by respiratory tract disease, male sterility, situs inversu and hydrocephalus (Feldman and Nelson, 1996; Keenan, 1998). It has an autosomal recessive mode of inheritance

(Feldman and Nelson, 1996).

Abnormal sexual behavior

In front of a male lacking of libido, it is always difficult to know if the underlying cause is organic or psychological. One must be aware that the same disorders causing infertility with normal libido (bad quality semen) may also in some cases affect Leydig cells and create a loss of libido (Feldman and Nelson, 1996).

Some dogs are unable to mate for many various reasons. For unknown reasons, a lack of libido may occur at any age in dogs which were previously capable of mating. Often it has been noticed that hierarchy plays an important role in this regard and that subordinate males do not even attempt to copulate with dominant females even at the peak fertilization period of their heats. Some breeds are well known for their lack of libido, like Basset-Hounds or Golden Retrievers. Some males that may have experienced a painful mating (with an aggressive or a restless bitch for example) may hesitate to try again. Young dogs may also lack experience and old dogs may lack libido.

The socialization in pups plays an important role towards the ability to mate. Pups which have been separated at a very young age (before 4 weeks) from their mother and siblings may experience problems in their future reproductive behavior. Stress just before a mating is not recommended (Feldman and Nelson, 1996). This may explain that most of the time, the bitch is brought to the male's home for mating and not the contrary.

Miscellaneous causes

Failure to achieve erection is quite rare, according to our own experience. Such failure may be due to psychological problems, pain or androgen insufficiency. Sometimes, intact male dogs have been trained not to mount bitches in heat when they are out of a special room. They will refuse to mate outside of usual conditions.

Young dogs presented with infertility with poor quality semen may have just reached puberty and should be reevaluated at an older age. Aged dogs may fail to achieve normal copulation due to lack of libido or a decrease in blood testosterone level. The quality of semen is often reduced in older males, which decreases the fertility rate. Molossoids dogs seem to have a decline in quality semen that starts earlier than in other breeds.

Some authors have noticed that males that mate too often (more than 2 or 3 times daily) may show a decline in libido (Johnston et al., 2001). On the contrary, prolonged sexual abstinence may cause a decrease in semen quality, especially in giant breeds (Fontbonne et al., 1998; Fontbonne, 1999). In humans, it sometimes creates hematospermia. The first ejaculate from a dog following a prolonged period of sexual rest contains a greater percentage of old and dead sperm that have been stored in the epididymis (Feldman and Nelson, 1996).

There could be a seasonal effect upon fertility, as fertility rates are often lower in summer, and the percentage of normal morphological spermatozoa may decrease.

Neuropathies like spinal cord injuries may enhance a decrease in libido.

Scrotal dermatidis may cause a transient decline in semen quality, due to an increase of the intrascrotal temperature. Obesity, due to the formation of periscrotal fat, may play the same role (Johnston et al., 2001). Fever may also decrease spermatogenesis. In humans, high fever causes a decline in semen quality but does not cause complete azoospermia.

Improper handling of semen may be a cause of infertility when the semen is artificially inseminated (8). Mixing with water, urine or excessive lubricant must be avoided.

Autoimmune orchitis may be associated with azoospermia. It may be connected to autoimmune thyroiditis, with incresased serum antibodies.

Little trauma, dog bites, lacerations, kicks or blows (Keenan, 1998) of testes may destroy the barrier between the blood flow and the seminiferous tubes and generate an autoimmune spermatogenic arrest due to antisperm antibodies. This disruption of the immunological barrier occurs also in the case of Brucellosis, resulting in sperm agglutination (Meyers-Wallen, 1991). Agregation of sperm and altered motility have been described in humans in the presence of E. coli (Meyers-Wallen, 1991).

Germinal cell aplasia ("Sertoli cell only") has been reported in 1% to 10% of dogs with azoospermia (Johnston et al., 2001). Fucisidosis, a lysosomal storage congenital disease affecting the function of epididymal epithelial cells and causing the retention of cytoplasmic droplets, has been described in the dog (Johnston et al., 2001).

At last, idiopathic testicular degeneration is a common cause of infertility, especially due to azoospermia, in the dog (Feldman and Nelson, 1996).

Sometimes, infertility is observed in males with normal libido and good semen quality. In humans, functional tests like *in vitro* fertilization or zona-free hamster egg penetration may be used. These tests are not routinely available to the dog. If a male dog is mated always with the same bitch, it could be useful to test mate the dog with another bitch to rule out genetic incompatibility. On the opposite, some dogs remain fertile despite morphological defects if good breeding management practices are followed (Feldman and Nelson, 1996).

Infertility in the male dog in practice

Veterinarians willing to solve a fertility problem in the male dog must first conduct a very detailed history of the general health and reproductive history of the dog. The anterior fertility will be checked carefully. The veterinarian will inquire about the results of any prior sperm analysis and the number of bitches that were successfully mated, with the litter size each time. A complete general and genital clinical examination must be conducted. Complementary examinations may, depending on the case, include sperm collection and analysis, hormonal assays, urinanalysis, ultrasonography, radiography with or without contrast compounds, serological or bacteriological tests, testicular biopsy, PCR… Of course, complementary evaluation should be designed to progress from relatively easy and inexpensive to more difficult and time consuming diagnostic tests (Feldman and Nelson, 1996).

Aspermia or oligospermia (lack of volume)

The veterinarian may suspect a problem related to:

- an incomplete collection of semen (lack of libido or interest of the dog). Normally, a male should begin to ejaculate within 20 to 30 seconds after manually induced erection;
- lack of sexual maturity;
- the existence of pain during ejaculation;
- a prostatic problem;
- a retrograde ejaculation;
- other causes like neuropathy, spinal cord injury or diabetes mellitus.

We first recommend trying to collect the dog a second time, maybe under better conditions (for example, using a teaser bitch in heat). Some experienced stud dogs will not ejaculate during manual semen collection unless presented with an estrous teaser bitch. Sometimes, they even refuse to give semen if they are not allowed to mount the bitch. Ejaculation may be effected in these dogs by treatment with GnRH (1 to 2 microg/kg SC 2 to 3 hours prior to semen collection) or hCG in order to increase testosterone level.

If the result is still a lack of volume of semen, a general clinical examination of the dog must be done in addition to a urine collection by cystocentesis after semen collection (to verify the presence of many spermatozoa in the bladder) and ultrasonography of the prostate gland. Seminal Alkaline Phosphatase should be assayed in the liquid collected (see further down paragraph 3 on “azoospermia”)

If temporary aspermia is suspected, veterinarians can perform a vaginal smear in the few minutes following a natural mating with a bitch to see if any spermatozoa can be viewed.

In the case of a reduced volume of the prostatic fraction which renders the volume of the whole ejaculate very small, treatment may easily be tried by diluting the sperm-rich fraction of the ejaculate with a synthetic diluent (used for preparing chilled semen, for example).

Treatment of retrograde ejaculation may be attempted by collecting semen when the bladder is full (which makes the sphincter close more firmly). Sympathomimetic drugs like phenylpropanolamine (3 mg/kg per os twice daily) or pseudoephedrine (4 to 5 mg/kg per os three times daily or 1 to 3 hours before semen collection or attempted breeding) may be tried (Johnston et al., 2001). In humans, spermatozoa ejaculated into the urinary bladder have been retrieved for artificial insemination.

Asthenozoospermia

Normally, dog semen samples should have more than 70% of the spermatozoa exhibiting vigorous forward motility (Feldman and Nelson, 1996). In infertile men, asthenozoospermia occurs when less than 25% of spermatozoa have normal motility (Meyers-Wallen, 1991). In dog, this terminology may be used when less than 50% of spermatozoa have a normal forward motility (Feldman and Nelson, 1996).

- This problem could be due to the following:
- the collection material that has not been correctly rinsed and may harbor spermicidal substances like detergents or toxics: recollect the dog a few hours later after having checked everything;
- an inflammation of the urinary or genital organs, such as prostatitis, cystitis, uretritis. It is useful to control the pH of semen and to perform a urinanalysis, ultrasonography of the genital tract including the prostate gland, examination of the cytology of prostatic fluid, bacteriologic examination of seminal fluid, etc
- the start of another genital problem, including testicular tumors.

Do not forget the genetically influenced immotile cilia syndroms. Diagnosis is made using electron microscopy.

Azoospermia or severe oligozoospermia

Veterinarians should be aware that in many cases of true azoospermia the size of testes remain unchanged.

The first problem is to determine if the case represents a true azoospermia or if the collection of semen was incomplete, providing only the pre-sperm fraction without spermatozoa. The semen always should be collected several times as artifactual azoospermia may occur in dogs that are apprehensive at the time of collection and may ejaculate only the first (pre-sperm) fraction (Feldman and Nelson, 1996). For similar reasons, the existence of a prior vasectomy must be ruled out.

It is recommended to assay the alkaline phosphatase concentration in seminal fluid. This concentration is much higher in the epididymis than in the testes or prostate. A normal intact dog with an incomplete ejaculation usually shows a concentration of this enzyme in seminal fluid at less than 5000 units/L, because little epididymal fluid has been ejaculated. Male dogs with true azoospermia, due to causes other than bilateral blockage of the outflow tract usually have concentrations over this level. In males with bilateral obstructive azoospermia, the concentration may be very low (sometimes as less than 10 units/L). Aspiration of the cauda epididymis may allow verification of spermatogenesis, but it may cause the formation of sperm granulomas and antisperm antibodies.

In the case of true azoospermia, ultrasonography of testes may show tumors, or heterogeneous testicular tissue, which makes the veterinarian often believe that spermatogenesis is reduced and that the seminiferous tubes are empty (England, 1991; Fontbonne et al., 1998; Fontbonne, 1999).

In azoospermic dogs with a high concentration of Alkaline Phosphatase in the seminal fluid, a complete hormonal assessment of the dog should be made (including thyroid functionality). Serum concentration of LH may be assayed, as it is normal to slightly elevated in male animals with gonadal failure. FSH could also be assayed. The decline of spermatogenesis causes Sertoli cells produce less inhibin. Subsequently, the level of FSH will increase. It is recommended to take three blood samples at 20 minute intervals (as FSH and LH are released episodically) or to stimulate the secretion of these hormones with GnRH (250 ng/kg IV, blood sample 10 minutes later).

Testicular biopsy should be considered as among the last attempts to know the exact state of decline of the spermatogenesis. If some sperm cells (for example, spermatids) remain present inside the seminiferous tubes, the prognosis may not be desperate. In some cases, karyotype could also be evaluated. But, veterinarians must be aware that in most cases, an azoospermic dog remains azoospermic (Feldman and Nelson, 1996). One case of recovery after total azoospermia has been published (Evans and Renton, 1973).

Oligo-astheno-teratozoospermia (OAT)

The diagnosis procedure of an infertile dog suffering from OAT will be the same as in the previous case: good clinical examination, ultrasonography, hormonal assessment, bacteriological culture of seminal fluid, etc. Of course, Brucella canis serological testing should always be performed, especially if sperm agglutination is observed. Everything must be done quite quickly, because most of the time OAT is a step towards complete azoospermia (Feldman and Nelson, 1996), and it may be reversible only during a short time period.

Treatment of OAT in dogs depends upon etiology. With any treatment, no improvement will occur for at least 62 to 70 days after the start of treatment, that is, the duration of a normal spermatogenic cycle. Some authors recommend to do nothing in the first run: they let the dog rest sexually and re-evaluate after 2 months, hoping that the OAT will have been transient (Feldman and Nelson, 1996).

Unilateral castration of dogs with unilateral testicular tumors may allow spermatogenesis to improve in the contra-lateral testicle.

In the case of inflammation, the return to normal fertility is unlikely to happen if fibrosis or degenerative changes have occurred.

Medical therapy for idiopathic OAT may be tried using GnRH given in regular pulses (Meyers-Wallen, 1991), GnRH agonists, gonadotropins (hCG (Keenan, 1998), FSH or PMSG) or androgens, like synthetic androgen mesterolone, which has been shown to improve seminal characteristics in three healthy dog and one dog with oligozoospermia (England and Allen, 1991). Testolactone (an aromatase inhibitor that prevents the conversion of testosterone to estradiol has been used to treat idiopathic oligozoospermia in men with variable results (Feldman and Nelson, 1996). A recent trial in dogs using an aromatase inhibitor was successful in significantly increasing the number of sperm in 4 oligozoospermic beagle dogs (Kawakami et al., 2004).

Based upon our own experience, repeated intrauterine inseminations of bitches could be successful when the quality of semen is not too bad and at least 20% to 30 % of spermatozoa show normal progressive motility. Some authors place half of the ejaculate at the tip of each uterine horn (Keenan, 1998). It has been described in the case of immune-mediated sperm agglutination (Keenan, 1998; Johnston et al., 2001). In this case, immunosuppressive dosages of glucocorticoids are not recommended as they affect spermatogenesis and may decrease fertility even more (Feldman and Nelson, 1996).

In case of genital or urinary infection, specific antibacterial compounds, depending upon the antibiogram, may be used for a long period (at least 3 weeks to one month). Although fluoroquinolones are somewhat expensive, results of their use have often been good (Meyers-Wallen, 1991). Dogs with brucellosis should not be used for breeding, even after treatment and even if they become seronegative.

Finally a dog with frequent sexual usage and low quality semen may regain fertility when used sparingly to allow spermatozoa to accumulate in the epididymis (Feldman and Nelson, 1996).

Pyospermia

Normal dogs should normally have less than 2000 white blood cells per microliter in the first and second fraction (Keenan, 1998; Johnston et al., 2001).). But the correlation between the number of leukocytes and infection is not clear.

Still, if many leukocytes are present in the sperm after centrifugation, bacterial culture of the ejaculated seminal fluid may be done. Do not forget to ask for mycoplasma and ureaplasma identification which usually necessitate specific transport and culture media (Meyers-Wallen, 1991). Definitive diagnosis of infection of the urinary or reproductive tract can be stated when more than 10 000 bacteria per milliliter of semen are cultured (Keenan, 1998). But it is also sometimes very useful to culture seminal fluid even if no leukocytes are present after centrifugation.

Cultures must be interpreted cautiously. Bacteria that are isolated may represent a primary infection causing infertility, but they may be contaminants (Feldman and Nelson, 1996). Bacteria isolated in semen may be different from those isolated from testicular tissue or prostatic cysts.

Hematospermia

Dogs with blood in the ejaculate are not necessarily infertile (Feldman and Nelson, 1996). Veterinarians should always check first if any lesion of the penis may bleed during erection. If so, a local antisepsis will be done, and sexual abstinence for several weeks will be recommended.

If no lesion of the penis is observed, a thorough clinical examination of the prostate, including ultrasonography, must be conducted because a prostatic problem is often the initial cause of hematospermia. The specific treatment will depend upon the nature of the prostatic disease.

Sometimes, the coagulation parameters should be checked if nothing is viewed either in the prostate or on the penis.

Failure to achieve erection

Diagnosis of the cause of failure to achieve erection requires the careful observation of copulation and/or semen collection. The veterinarian will notice if the dog experiences pain. The localization of the pain will direct further diagnostics. If no semen can be obtained, a blood sample should be taken to assay androgens and thyroxine levels.

Androgen therapy in order to improve libido should be avoided as it may, in fact, cause a decline of the semen quality secondary to its suppressive influence on GnRH and FSH secretion (Feldman and Nelson, 1996).

In some cases, electroejaculation under general anesthesia could be considered as a mean of obtaining semen from very recalcitrant dogs (Fontbonne et al., 1998; Keenan, 1998; Fontbonne, 1999).

Failure to achieve copulation

Non steroidal anti-inflammatory drugs in dogs with painful orthopedic conditions may allow limited use of such dogs (Keenan, 1998).

Dogs with idiopathic poor libido could be given GnRH injections prior to semen collection or breeding attempt, as described previously. If they admit semen collection, artificial insemination is often the best solution.

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