Infertility in the male dog - A diagnostic approach  
[Infertilidade no cão - Abordagem clínica]

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Introduction

Infertility in the male dog can which has a normal libido and is able to mount can be due to lack of or incomplete ejaculation or to poor semen quality. Infertility due to inability to mount or to low libido may or may not be a reproductive issue (it is often an orthopedic or a behavioral problem) and will not be discussed here.

Ejaculation problems

Failure of or incomplete ejaculation may occur if the coital lock is not adequate because of fright or discomfort during mating or at semen collection. Ejaculation may sometimes occur retrogradely into the bladder if there is an incompetence of the internal urethral sphincter muscle.

Retrograde ejaculation - The ejaculatory process is coordinated by sympathetic and parasympathetic nervous activity, and is divided into seminal emission (the deposition of semen from the vasa deferentia and accessory sex glands into the prostatic urethra) and ejaculation (passage of semen through the urethra and outside through the external urethral orifice). During ejaculation the bladder neck contracts, thus playing an important role in preventing a retrograde flux of spermatozoa into the bladder. Vasa deferentia and bladder neck are primarily under the control of the sympathetic nervous system. Alfa-adrenoceptor stimulation causes contraction of the vas deferens, while beta-adrenoceptor stimulation mediates relaxation of the vas deferens. The use of alfa-adrenergic agonists increases seminal emission: for example, administration of xylazine (alfa-2 adrenoceptor agonist) in the dog causes increased contraction of vasa deferentia and decreased urethral pressure, thereby facilitating passage of spermatozoa into the bladder (not associated to ejaculation). The bladder neck has a rich cholinergic and adrenergic innervation. Stimulation of the adrenergic component causes contraction of the bladder neck and relaxation of the bladder body. Administration of fentolamine (an alfa-adrenergic antagonist) in the dog 5-10 minutes prior to semen collection increases the (physiologic) retrograde flux of sperm into the bladder.

Diagnosis of retrograde ejaculation requires confirmation of aspermia = lack of ejaculate or presence of minimal amounts (≤ 0.2 cc) following normal erection and urethral contractions, followed by observation of presence of high numbers (5-50 millions) of spermatozoa in the bladder. Treatment consists of oral administration of 5 mg/kg pseudoephedrine 3 and 1 hour prior to semen collection. Retrograde ejaculation in the dog is considered a rare event. However, aspermia will obviously go unnoticed at natural matings, and presence of spermatozoa in the urine after ejaculation is not regularly assessed. Three cases of canine retrograde ejaculation have been diagnosed at the Department of Veterinary Clinical Sciences of the University of Pisa during the 1991-2000 period.

Poor semen quality

From the classification standpoint, poor semen quality can be defined as azoospermia (absence of spermatozoa in the ejaculate), oligozoospermia (low number of spermatozoa in the ejaculate), asthenozoospermia (presence of a high percentage of spermatozoa which do not show a normal progressive motility) or teratozoospermia (presence of a high percentage of spermatozoa with morphological defects).
Poor semen quality can be due to poor quality of spermatozoa or may reflect abnormal seminal plasma. Poor quality of spermatozoa can be due to congenital defects such as testicular hypoplasia, the immotile cilia syndrome, chromosomal abnormalities (XXY syndrome, XX males), mono- or bilateral cryptorchidism, anomalies of the duct system (cysts or other developmental anomalies of the epididymis, the vas deferens or the rete testis). Hypogonadism is a poorly characterized disease in the dog. A recent survey showed >70% of 314 cases of canine hypogonadism as being of idiopathic etiology. A familial tendency is suspected in some breeds (Bull Mastiff, Bernese mountain dog, Beagle, Welsh Corgi, Cocker Spaniel). Abnormal seminal plasma may be due to prostatic disease or to inflammation of the testis or of the epididymis.

**Prostatic hyperplasia**

A complete discussion of prostatic diseases is beyond the scope of this paper. However, benign prostatic hyperplasia can predispose the dog to infertility, especially if it evolves into prostatitis. Therefore, prostatic hyperplasia will be briefly outlined.

Canine prostatic hyperplasia is the most common canine prostatic disorder, with almost 100% of intact dogs developing histologic evidence of prostatic hyperplasia with aging. It is characterized by an increase in epithelial cell numbers (hyperplasia) as well as an increase in epithelial cell size (hypertrophy), but the increase in cell number is more marked. It begins as glandular hyperplasia in dogs as young as 2.5 years of age. Intraparenchymal fluid cysts may develop in association with hyperplasia. Such cysts are variable in size and contour, contain a thin, clear to amber fluid and, if intraparenchymal, may communicate with the urethra thus leading to intermittent haemorrhagic or clear, light yellow urethral discharge.

Hyperplasia is associated with an altered androgen:estrogen ratio, and requires the presence of the testes. Dihydrotestosterone (DHT) within the prostate gland probably serves as the main hormonal mediator for hyperplasia. The hyperplastic prostate is highly vascularized and therefore the gland bleeds easily, which explains the common clinical sign of blood from the tip of the penis or blood in the urine. Blood loss in the prostatic urethra can be so intense that the ejaculate may appear completely red. Although presence of blood in the semen is typically considered to be a cause for infertility, dogs with some blood in their ejaculates may sometimes be fertile. However, prostatitis or abscessation are likely consequences of presence of blood in the prostate.

**A clinical approach to the infertile dog**

If infertility must be ruled out, the following steps need to be carefully followed: a) collect a thorough history; b) perform an accurate clinical exam of the external genitalia; c) evaluate one or more semen samples.

Information needed in order to collect a meaningful reproductive history - The first and most important information to be collected is a complete list of all bitches mated, starting from when the dog was still fertile, so as to be sure to understand when the problem began. Furthermore, clinicians should try to put together the following information:

- a) If and how was fertility assessed for mated bitches
- b) If and how was ovulation determined in previous breedings
- c) If some of the bitches bred did whelp, it is very important to calculate the exact duration of pregnancy. Gestations of 57-59 days mean that the bitch had already ovulated when she was mated, while gestations of 67-72 days mean that the bitch ovulated 2-9 days following mating. Gestations whose lengths is shorter or longer than the interval 60-66 days may be characterized by a small litter size, but this is more likely due to wrong timing of breeding rather than to poor male fertility.
- d) If, how and when (after breeding) was pregnancy diagnosis carried out on mated bitches
e) Whether any drugs were used which could depress testosterone production (such as androgens, glucocorticoids, oestrogens, progestogens, cimetidine or ketoconazole) or act on the ejaculatory process (such as xylazine or fentolamine).
f) Whether the dog has suffered from any disease, accidents or injuries which can be relevant to reproduction.

The clinical exam - Following a complete physical exam (to rule out systemic disease directly or indirectly affecting reproduction), scrotum, prostate and penis should be carefully inspected and palpated. Normal testicles are freely movable within the scrotal sac, have the same shape and consistency and their size must be average for the breed. Epididymides are palpated on the dorso-lateral aspect of the testicle to check for the presence of nodules, keeping in mind that palpation can only exclude the presence of macroscopic lesions. The prostate is examined using the index finger of one hand per rectum to locate the symmetrically bilobed spongy structure, whose caudal half can be felt with the fingertip cranial to the pelvis; the other hand should locate the prostate abdominally and push it backward towards the rectally located index finger. Finally, penis and prepuce are examined to rule out presence of lesions as well as to make sure that the penis can be normally extruded from the prepuce.

Semen Evaluation - Sperm motility, concentration and morphology, presence of white blood cells in the sperm sediment, seminal plasma pH and alkaline phosphatase should be assessed. Sperm concentration can be measured by loading a haemocytometer with the right amount of semen and the right dilution rate according to manufacturer’s instruction for the haemocytometer being used. The number of cells in the central square millimetre \( \times 10^6 \times \text{sperm volume} \) gives the total number of spermatozoa/ejaculate (normal is 200-2000 million sperm/ejaculated). Sperm morphology should be evaluated on at least 100 cells (we normally evaluate 200 sperms in infertility cases). The number of white blood cells (WBC) can be calculated by counting the number of WBC in the 4 large corner squares of the haemocytometer and multiplying by 250 (normal is < 2000/ml). Seminal plasma alkaline phosphatase (ALP) is produced in the epididymides. Low concentration or absence of ALP indicates incomplete ejaculation or bilateral obstruction of the epididymides or of vasa deferentia. Seminal plasma ALP is measured by laboratory equipment routinely used to measure the enzyme in serum. Laboratory technicians should be advised to centrifuge the semen sample (some sophisticated equipments may be damaged by spermatozoa) and also to dilute the centrifuged sample as seminal plasma ALP concentrations are typically very high (5,000-40,000 IU/L), and the result of the undiluted sample could be so high that might be not readable. Sperm bacteriology and virology tests should also be performed.

Endocrine Testing - **Testosterone** – The most reliable method to measure testosterone is to do a stimulation test using GnRH (25-50 mcg/dog, or 2.0 mcg/kg, IM) or hCG (44 IU/kg). Basal and 1-hour (GnRH) or 4-hour (hCG) post-stimulation blood samples should be taken. Serum testosterone is 0.1-4.0 ng/ml prior to stimulation, and 3.0-7.0 ng/ml after stimulation. **Gonadotropins** – Because of the difficulty to get specific antibodies against canine gonadotropins, it is very difficult to find laboratories which can provide canine LH or FSH radioimmunoassays as a service. Commercial semi-quantitative LH assays can be used if the testosterone concentration following stimulation is low, in order to distinguish between a hypothalamic-gonadal problem and a true gonadal problem. However, since there are no specific studies on LH levels measured in ipofertile dogs using semi-quantitative assays, interpretations of such tests should be cautious.

**Thyroid hormones** – Although hypothyroidism has been related to infertility in the dog, a clear link between low thyroid function and reproductive disease has not been established yet. Nevertheless, hypothyroidism should be ruled out in hypofertile dogs.

**Ultrasonography** - Prostate and testicles are best evaluated in the sagittal and transverse planes using 5.0 or preferably 7.5 MHz scanners. An enema should be administered prior to
scanning the prostate to eliminate colonic contents which may mimic peripheral prostatic disease. Conditions such as cysts or abscesses are visualized easily. Other less distinct but echogenically complex areas may indicate neoplasia or areas of infection within the gland. Ultrasonography of the testicles may reveal non palpable neoplasms, cysts, abscesses or areas of cavitation.

Research tests - Measurement of anti-sperm antibodies can be of interest in hypofertile male dogs. Canine anti-sperm antibodies have been demonstrated both by direct immunofluorescence and gelatin agglutination. Acute testicular inflammation can damage the integrity of the seminiferous tubules, exposing spermatozoa to the immune system, thereby causing development of antibodies directed against spermatozoa, spermatogenic cells and tubular basement membranes. Autoantibodies have been demonstrated in 2 dogs with infertility and focal inflammatory lesions of the testes. Also, epididymitis-induced autoimmune disease of the testis has been postulated in minks with secondary infertility. Unfortunately, by the time infertility is noted by the client much time has elapsed, the cause of testicular inflammation is very difficult to establish and prior traumatic or infectious etiologies are generally not identified.

Treating the infertile male dog - There are very few fertility problems of the male dogs which can be solved using specific treatments. Chronic bacterial orchitis, epididymitis or prostatitis should be treated with long-term (4-12 weeks) specific antibiotics. Drugs with low protein binding and high lipid solubility should be selected such as chloramphenicol or fluoroquinolones. Determination of prostatic fluid pH (usually neutral or slightly acidic) prior to treatment can be of help since weak base antibiotics (i.e. erythromycin or trimethoprim-sulpha) will achieve a higher prostatic concentration.

Low testosterone and high LH/FSH levels indicate primary testicular failure, for which there is currently no treatment. Dogs with low testosterone and low gonadotropins should be investigated for pituitary neoplasm. Low gonadotropin function will result in spermatogenic dysfunction, therefore it is advisable to try to stimulate gonadal secretory activity using hCG at 500/dog IU SC twice weekly (to cause Leydig cell function) and PMSG at 20 IU/kg SC 3 times weekly (to cause Sertoli cell function). Treatment should be continued for approximately 3 months.

When dealing with oligozoospermic dogs or when semen quality is unknown, semen evaluations should be planned at least one week prior to the expected day of breeding, as gonadal reserves may take several days to be restored when spermatogenic function is abnormal. Clomiphene and tamoxifen have been used with success in oligozoospermic men in non-controlled clinical studies, while the synthetic androgen mesterolone has been used in a small number of dogs at the dosage rate of 0.75-1.5 mg/kg.

When semen quality is below normal, ovulation must be timed very accurately and artificial insemination performed intravaginally on day 4, 5 and 6 post-LH peak if total count is >100.000.000 spermatozoa. If this approach fails or if total count is >20.000.000 spermatozoa, artificial insemination should be performed intrauterine on day 5 post-LH peak.

Diagnosing and treating prostatic hyperplasia - An enlarged, hypertrophic prostate may cause blood dripping from the tip of penis, or it may grow and expand in the rectal canal, causing tenesmus and sometimes difficult defecation. Other than the above signs, affected dogs are usually normal and the prostate on palpation is non-painful, symmetrically enlarged and with variable consistency. Urine may contain blood (gross or microscopic). If hyperplasia is accompanied by urethral discharge, this is typically haemorrhagic or clear but not purulent. Prostatic enlargement may be visualized on abdominal radiography as causing dorsal displacement of the colon and cranial displacement of the bladder. On retrograde urethrocystography the prostatic urethra may be normal or narrowed and undulant with mucosal irregularity, and the urethroprostatic reflux may be normal or greater than normal. On ultrasound, the prostate may appear diffusely hyperechoic with parenchymal cavities (which means that intraparenchymal cysts have developed).

A definitive diagnosis of benign prostatic hyperplasia is only possible by biopsy. However, biopsy is not necessary to institute a therapy if clinical signs are present. If the dog is asymptomatic owners should be advised to watch for the development of clinical signs in order to
start treatment as soon as possible. The most effective treatment is castration, following which prostatic size may decrease as much as 50% in 3 weeks and 70% over 9 weeks. As post-castration involution begins within days of surgery, clinicians should palpate the dog’s prostate 3 weeks post-operatively to make sure the involution rate is normal thus ruling out a more serious prostatic disease such as neoplasia or abscessation. When castration cannot be considered, drugs such as estrogens, antiandrogens, $5\alpha$-reductase inhibitors (finasteride) or progestogens can be used. Estrogens act indirectly by reducing androgen concentrations through an inhibition of gonadotropin secretion/release by the pituitary. Prostatic size is thus decreased through a reduction of cellular mass. Size and number of intraparenchymal cysts may not be affected. Because of the potential risk of serious bone marrow side effects (anemia, leukopenia, thrombocytopenia, pancitopenia) as well as because of the risk of growth of the fibromuscular stroma of the prostate, metaplasia of the prostatic glandular epithelium and secretory stasis resulting in prostatic enlargement and predisposition to cyst formation, bacterial infection and abscessation, the clinical use of estrogens to treat prostatic hyperplasia is currently not advised.

Flutamide is a human antiandrogen which can cause a significant decrease in prostatic size as detected by ultrasonography within 10 days. When administered to research dogs at 5 mg/kg/day PO for 1 year, there was no change in libido or sperm production. In most countries Flutamide is not approved for use in veterinary medicine.

Finasteride is the $5\alpha$-reductase inhibitor (the final enzyme in the synthetic pathway for dihydrotestosterone), approved for use in men, which can produce a dose-dependent decrease in prostatic size in dogs. We use it at the daily dose of 1.5 mg (approximately 1/3 of a 5.0 mg pill) for dogs ≤15 kg body weight, 2.5 mg (approximately half pill) for dogs of 15-30 kg body weight, and 5.0 mg for dogs of >30 kg body weight. Finasteride is well tolerated and can be used for long periods of time. However, as soon as it is discontinued the prostate will start growing again.

Progestagens can also be used. Megestrol acetate at the dose of 0.55 mg/kg/day PO for 4 weeks resulted in resolution of clinical signs of prostatic hyperplasia with no decrease in sperm production. In most countries this drug is not approved for use in male dogs. Medroxyprogesterone acetate can also be used.

References