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New approaches to semen improvement in dogs

Novas abordagens na melhoria de sêmen em cães

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Abstract

The present abstract provides an overview over the causes of infertility in male dogs and ways to improve semen quality. Problems jeopardizing male fertility can grossly be divided into infectious and non-infectious diseases. Causes for a decrease in semen quality are bacterial and more seldom viral infections of the prostate gland, scrotum, testicle and epididymis. In these cases, *B. canis* should be additionally considered. Non treated acute inflammations of the testicles may become chronical, in these cases testicular fibrosis, obstruction of testicular ducts and at last atrophy leading to irreversible infertility may result; autoimmune orchitis should be considered when the result of a bacteriological examination is negative. Hormonal problems like hypothyreoidism may occur, however seldom cause fertility problems. In all cases, a clear diagnosis and fast treatment of the causative agent is required; different treatment options are enrolled. Different oral supplementations have been investigated for their ability to improve semen quality and daily application of the antioxidant vitamin E proved to be useful. After semen collection, the semen can be improved by centrifugation and dilution, addition of different antioxidants can be beneficial.

Keywords: dogs, fertility, hormones, infection, semen quality.

Resumo

O presente resumo fornece uma visão geral das causas de infertilidade em cães machos e formas de melhorar a qualidade do sêmen. Problemas que podem comprometer a fertilidade masculina podem ser grosseiramente divididos em doenças infecciosas e não infecciosas. As causas de uma queda em qualidade do sêmen são infecções bacterianas e mais raramente virais da glândula da próstata, escroto, testículo e epidídimo. Nestes casos, B. canis deve ser considerado. Inflamações agudas dos testículos quando não tratadas podem se tornar crônicas, e nestes casos, fibrose testicular, obstrução dos dutos do testículo e finalmente atrofia, levando a uma possível infertilidade irreversível. Orquite auto-imune deve ser considerada quando o resultado de exame bacteriológico é negativo. Problemas hormonais como hipotireoidismo podem ocorrer, mas raramente causam problemas de fertilidade. Em todos os casos, um diagnóstico claro e tratamento rápido do agente causativo é necessário, e diferentes opções de tratamento são usadas. Diferentes suplementações orais foram investigadas por suas habilidades de melhorar qualidade do sêmen e a aplicação do antioxidante vitamina E provou ser útil. Após coleta de sêmen, o sêmen pode ser melhorado por centrifugação e diluição, e a adição de antioxidantes diferentes pode ser benéfica.

***Palavras-chave**: cães, fertilidade, hormônios, infecção, qualidade do sêmen.*

Introduction

Maintenance and improvement of reproductive fitness in male stud dog is a field of permanent interest. Many older stud dogs with proven ability to pass their excellent genes to their progeny, develop problems that in many cases can be treated successfully if recognized in time. If not, chronical diseases especially of the gonads may cause irreversible infertility. The present abstract shall provide an overview over the manifold causes of infertility in male dogs and possible ways to improve semen quality.

Infections of the prostate gland

Problems jeopardizing male fertility can grossly be divided into infectious and non-infectious diseases. A frequent cause for a sudden decrease in semen quality is the bacterial infection of the prostate gland. Involved bacteria are mostly Streptococcus spp, Staphylococcus spp, E. Coli, *Proteus vulgaris*, Pseudomonas spp, Klebsiella spp, *Pasteurella multocida*, *Mykoplasma canis* und *Ureaplasma canigenitalium* (Bjurström and Linde-Forsberg, 1992; Zöldag et al., 1993; Kustritz et al., 2005). The only species-specific agent is *Brucella canis* causing destruction of the epithelial cells and purulent inflammation (Serikawa et al., 1984; Hollet, 2006). Benign prostate gland hyperplasia (BPH), prostatic cysts, squamous metaplasia and neoplasia mostly occurring

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in older males, may predispose to the infection. Acute prostatitis causes depression of the general condition, fever, painful abdomen, vomiting, and dysuria and in advanced cases tenesmus due to constipation. Semen quality is severely decreased, inclusive a decrease in motility, an increase in morphologically abnormal spermatozoa and an admixture of erythrocytes and leucocytes (Smith, 2008; Nizanski et al., 2014). Diagnosis is made by sonographical examination, collection of urine, aspiration of prostate gland secretions and if necessary FNA of the prostate gland (Lopate, 2010; Levy et al., 2014). Bacteriological examination of the urine or prostate gland secretion will reveal the causative agent, however, treatment with a broad spectrum antibioticum should be started immediately (Lopate, 2012). Effective antibiotics are trimethoprim, clindamycin, chloramphenicol and erythromycin, furthermore fluorochinolones like enrofloxacin or marbofloxacin (Johnston et al., 2001b; Smith, 2008). Treatment should be proceeded according to the sensitivity testfor at least 3 weeks. An important measure is the application of antiandrogens to quickly decrease the prostate gland size. The success of the antibiotic treatment should be evaluated one week after the last application, the semen quality 8 weeks after the end of the treatment. This is only possible, if special antiandrogens not decreasing the dog's libido are used (Nizanski et al., 2014). Recommendable for stud dogs in acute and chronical cases is osaterone acetate (Ypozane®, Virbac, F), a competitive androgen receptor inhibitor. Recommendable dosage is 0.25-0.5 mg/kg p.o. once daily for one week. The size of the prostate gland will decrease significantly within 2 weeks while the libido will be maintained. Semen quality will be slightly decreased. The effect will last for 5-6 months (Tsutsui et al., 2000, 2001). Small intraprostatic cysts may diminish during this treatment, while for larger cysts and abscesses ultrasound guided percutaneous drainage eventually with alcoholization of the cavity (Bussadori et al., 1999; Boland et al., 2003) is recommendable. Semen quality will normalize after a complete spermatogenic cycle. In case of chronical prostatitis, clinical symptoms are less severe, usually without depression of the general condition. Semen quality is severely decreased with a massive decrease in motility and increase in morphologically abnormal cells as well as admixture of erythrocytes and inflammatory cells. Since the blood prostate barrier is not broken as in the acute cases, antibiotics do not penetrate easily and have to be applicated for 4-6 weeks. Treatment based on culture and sensitivity results is therefore mandatory. In addition, treatment with an antiandrogenic compound like osaterone acetate or finasteride is recommendable. The 5α reductase inhibitor finasteride converting testosterone to DHT in the prostate gland tissue has to be given over 5-15 weeks until a significant decrease of the size of the gland occurs (Iguer-Ouada and Verstegen, 1997). Finasteride was successfully used at a dosage of 0.1-0.5 mg/kg once daily over 16 weeks (Johnston et al., 2000) and 1 mg/dog over 3-21 weeks (Iguer-Ouada and Verstegen, 1997). At last it has to be emphasized, that neoplasia of the prostate gland may cause very similar symptoms and cannot be excluded sonographically. In case of ineffective treatment, prostate gland biopsy will therefore be necessary.

Infections of the scrotum, testicle and epididymis

The causative agents are the same causing infectious prostatitis; if case history reveals that the dog had mated abroad or had contact with a probably infected dog, *B. canis* should be additionally considered (Wanke, 2004; Hollet, 2006). Recently, an increasing number of infections in breeding kennels has been reported (Corrente et al., 2010; Gyuranecz et al., 2011; Hofer et al., 2012). Serology should be done twice two weeks apart and if positive or questionable, more samples should be drawn and at least a bacteriological culture performed in a suitable laboratory. Control of *B. canis* in a kennel requires strict measures, which in some countries are statutory (Johnston et al., 2001a; Hollet, 2006). Acute orchitis may cause transient depression of the general condition. Local findings are increase in testicular size, increase in temperature, painful palpation and occasionally fluid accumulation in the scrotum, pyrexia, purulent preputial fluor and atrophy of the unaffected testicle (Dahlbom et al., 1997; Johnston et al., 2001a). Diagnosis should be made clinically and by bacterial culture inclusive resistency test. Samples can be obtained by semen collection, if this is not tolerated by testicular FNA (Romagnoli et al., 2009; Gouletsou et al., 2011). Antibiotic treatment for at least 2 weeks according to the resistency test should be applied. As in case of prostatitis, success of the antibiotic treatment should be evaluated one week after the last application, the semen quality 8 weeks after the end of the treatment. In cases with negative outcome of the bacterial culture, autoimmune orchitis should be considered. Non treated infections may become chronical, in these cases testicular fibrosis, obstruction of testicular ducts and at last atrophy leading to irreversible infertility can result (Lein, 1977; Johnston et al., 2001a). In case of azoospermia, if retrograde ejaculation can be excluded, measurement of alkaline phosphatase in the seminal fluids will proof, whether incomplete ejaculation is the cause (Johnston et al., 2001a; Schäfer-Somi et al., 2013). Thereafter a FNA should be performed to assess, whether the testicular tissue is still able to produce spermatozoa (Dahlbom et al., 1997, Romagnoli et al., 2009).

Benign prostate gland hyperplasia

In older dogs, the benign prostate gland hyperplasia (BPH) affecting 90% of dogs older than 9 years, is a common reason for a decrease in semen quality. For diagnostic approach see (Levy et al., 2014), for treatment options Nizanski et al. (2014).Antiandrogenic compounds like osaterone acetate and finasteride have been used

successfully in dogs and are recommendable in stud dogs with BPH. In clinically still normal stud dogs, this problem coincides with a decrease in motility and an increase in morphologically abnormal cells which will normalize 62 days after antiandrogene therapy. The findings of a bacterial culture of the semen should be critically evaluated. Even in healthy and fertile stud dogs, a physiological mixed flora can be found. However, during a recent study (Schäfer-Somi et al., 2009), we found in cases of BPH a higher percentage of dogs with bacterial contamination than in dogs without BPH which mightpredispose to infections. When semen quality is decreased and high grade monocultures of bacteria and/or mycoplasmas are detectable, the dog should be treated according to a resistency test.

Non-infectious testicular problems

In older dogs, atrophy of the testicular tissue may be a cause for irreversible infertility. The size of the testicles decreases and the testicular tissue becomes smoother. In advanced cases, repeated semen collection will typically reveal oligozoospermia, decreased motility and an increase in morphologically abnormal spermatozoa. The autoimmune orchitis, sporadically coinciding with other autoimmune disorders, or caused by trauma or inflammation, can be diagnosed by assessment of the presence of antisperm IGG antibodies (ASA) on the spermatozoa surface (Attia et al., 2000; Romagnoli et al., 2009). Experimentally induced autoimmune orchitis revealed that orchitogenic antigens in testicular tissue and spermatozoa are the cause for the delayed type immunoreaction, and that tumour necrosis factor participates in the pathogenesis of the inflammation (Yule and Tung, 1993). Even though very seldom diagnosed, it should be considered in cases with decreased semen quality and azoospermia, when bacteriological findings are negative. Romagnoli et al. (2009) reported successful treatment of one dog with autoimmune orchitis. Prednisolone was given orally over weeks (25 mg tablets, 1/2 capsule SID for 15 days, then 1/4 capsule once daily for 15 days, further 1/4 capsule alternate days for 15 days); the dog ejaculated normally thereafter and produced litters again.

Hormonal insufficiencies

In male dogs with idiopathic failure to ejaculate, alack of libido may be treated with a GnRH injection (1-2 µg/kg s.c.) 2-3 h prior to semen collection. This improved libido and facilitated ejaculation (Purswell, 1994). To increase sperm output when no teaser bitch is present, natural PGF2α (50-100 µg/kg i.m.) can be administered once, 15 min prior to semen collection. This will affect via contraction of the smooth muscles of the reproductive tract. Disturbances of the thyroid gland may affect fertility in female and male dog, however, very seldom (Panciera et al., 2007; Segalini et al., 2009; Sontas et al., 2014). In male dogs, hypothyreoidism can decrease libido, semen quality and ejaculate volume (Johnson et al., 1997). However, Segalini et al. (2009) measured T4 and TSH in 204 dogs of different breeds, all prone to hypothyreoidism. They found that most male animals were normothyroidic, only in the population of male Dogue de Bordeaux 70% of hypothyroxinemic individuals were assessed. None of the true hypothyroid animals had reproductive disorders. Authors conclude among others, that evaluation of thyroid function requires more than measurement of T4 and TSH.

Effect of oral supplementations on semen quality

Improvement of semen quality can be supported by daily application of the antioxidant vitamin E (400-600 mg/dog). Vitamin E is able to pass sperm membranes and to reduce free radical formation by inhibition of lipid peroxidation (Suleiman at al., 1996). In one study, fertile dogs and dogs with decreased fertility (low sperm count and increased percentage of morphologically abnormal sperm) received vitamin C and E orally (500 mg daily) for 60 days. No effect on DNA peroxidation was assessed (Lopes-Santiago et al., 2012). However, in another study (Hatamoto et al., 2006), 500 mg vitamin E/dog/day was given orally to 18 dogs for 10 w to neutralize the detrimental effect of dexamethasone treatment. Authors found that supplementation of vitamin E significantly improved ejaculate volume and progressive motility, and decreased total sperm pathology.

Similarly, daily oral supplementation of 8 dogs with a diet consisting of fatty acids and vitamin E for 60 days significantly increased ejaculate volume and cell vigour and decreased the number of morphologically abnormal spermatozoa (Rocha et al., 2009).

Semen improvement after collection of semen

There are several possibilities to improve semen quality after collection. In cases of atrophy, the sperm output should be increased by using a teaser bitch or injection of natural PGF2α (50-100 µg i.m., 15 min prior to collection). Repeated semen collection may be necessary. The seminal plasma should then be removed by centrifugation (750g 5 min; Rijsselaere et al., 2002) and replaced by a diluent before insemination.

In cooled semen, addition of diluents containing antioxidants proved to be beneficial. Addition of B16 (0.1 mM), taurine (0.2 mM), catalase (100 u/ml), *N*-acetyl-L-cysteine (NAC; 0.5 mM), or vitamin E (0.1 mM) to

a semen sample diluted with TRIS-Citric acid-Fructose extender, preserved progressive motility significantly better for 72 h than addition of vitamin C (0.5 mM), or no antioxidant ($P < 0.001$). Addition of B16, taurine, vitamin E, and vitamin C (0.5 mM) conserved sperm viability better than the other antioxidants or no antioxidant. At last vitamin E and B16 proved to be best, they in addition gave best result during the HOST ($P = 0.016$) and decreased the production of reactive oxygen species ($P < 0.0005$; Michael et al., 2009). Beccaglia et al. (2009) found that dilution of semen with an extender containing 0.4% lecithin and catalase (150 IU/ml) preserved sperm motility and viability for at least 48 h. Kmenta et al. (2011) increased the lecithin amount to 0.8% and conserved motility and viability for 8 days, however, only when catalase (150 IU/ml) was added. Wittayarat et al., 2012 investigated the effect of vitamin C and green tee polyphenol supplementation on motility and viability of canine chilled spermatozoa. Addition of 0.5 mM of vitamin C plus polyphenol (0.75 mg/ml diluted semen) proved to optimally conserve motility and viability during cooled storage for 4 weeks (Wittayarat et al., 2012). However, it should be considered, that motility and viability are not the best parameter for the prediction of fertilizing capability; for this purpose a zona binding assay or fertilization of a bitch would be necessary.

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