New approaches to treatment of infertility in the bitch

Novas abordagens ao tratamento de infertilidade em cadelas

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Abstract

Infertility in the bitch has manifold causes. Bacterial endometritis was found to be causative in most cases and since the incidence of *B.canis* infection in breeding bitches is increasing, serology for *B.canis* should always be performed. In subclinical or low grade cases of other bacterial infections, conservative treatment may be successful with the repeated application of the antiprogesterone aglepristone and synthetic prostaglandins. Another frequent cause is primary anoestrus or secondary anestrus. Both conditions can be caused by multiple diseases, organ dysfunctions and even medicaments. Treatment of the cause is mostly successful, however, induction of oestrus can be successful in single cases. The GnRH implant deslorelin proved to be useful when implanted in anestrus; resulting pregnancy rates excceded 70%; however, as a side effect, luteal failure may occur. Cystic degenerative and inflammatory conditions of the uterus may cause infertility, resorption or abortion. Diagnosis can be improved by cytological, bacteriological and histological examination of uterine specimens but the risk of endometritis and pyometra has to be considered. Treatment with the antiprogesterone aglepristone and broad spectrum antibiotics is recommended. Abnormalities of the vulva, vestibule and vagina as well as genetical disorders of sexual development are very seldom but should be excluded by a thorough gynaecological examination prior to first breeding. Hormonal imbalances like luteal deficiency and hypothyroidism can be primary or caused by other diseases and should be considered in case of repeated failure of pregnancy. During pregnancy, dietary substitution of folic acid is important to prevent neonatal cleft palate. However, feeding tyrosine during oestrus did not improve copulation behaviour.

Keywords: anestrus, endometritis, hormones, infertility, uterine disease.

Resumo

Existem diversas causas de infertilidade em cadelas. Endometriose bacteriana tem provado ser a causa na maioria dos casos e como a incidência de infecção com B. canis em cadelas de reprodução está aumentando, a sorologia para B. canis sempre deve ser realizada. Em casos subclínicos ou de baixo grau de outras infecções bacterianas o tratamento conservador pode ter sucesso com a aplicação repetida de antiprogesterona aglepristone e prostaglandinas sintéticas. Outra causa frequente é o anestro primário ou secundário. Ambas as condições podem ser causadas por diversas doenças, disfunções em órgãos e até mesmo medicamentos. O tratamento da causa geralmente tem sucesso, porém, a indução de estro pode ser bem sucedida em casos individuais. O implante GnRH deslorelina provou ser útil quando implantado no anestro, resultando em taxas de gestação acima de 70%; porém, como efeito colateral, falha lútea pode ocorrer. Condições císticas degenerativas e inflamatórias do útero podem causar infertilidade, reabsorção ou aborto. O diagnostico pode ser melhorado por exame citológico, bacteriológico e histológico de amostras do útero, mas o risco de endometriose e piometria deve ser considerado. O tratamento com antiprogesterona aglepristone e antibióticos de grande espectro é recomendado. Anormalidades da vulva, vestíbulo e vagina, além de distúrbios genéticos de desenvolvimento sexual são raros, mas devem ser excluídos através de exame ginecológico antes da primeira reprodução. Desequilíbrios hormonais como deficiência lútea e hipotireoidismo podem ser primários ou causados por outras doenças e devem ser considerados no caso de falha repetida de gestação. Durante a gestação a substituição de ácido fólico na dieta é importante para prevenir fenda palatina neonatal. Porém, a alimentação com tirosina durante o estro não melhorou o comportamento reprodutor.

***Palavras-chave**: anestro, doença uterina, endometrite, hormônios, infertilidade.*

Introduction

Infertility in the bitch, the incapability to produce progeny, has manifold causes; systemic diseases as well as organ dysfunctions, infectious diseases and hormonal imbalances are just a few to mention. Especially in young breeding bitches with excellent genes, this diagnosis is disastrous, but also in older, proven bitches which otherwise would be fit enough to give birth to another litter. The diagnosis often is a challenge, especially in cases of hormonal imbalances and when sampling is difficult like in case of chronical degenerative diseases of

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the uterus. The treatment should aim to eliminate the causative problem; hormonal treatments and manipulation of the oestrus cycle should be restricted to defined cases.

Since the causes for infertility are manifold, a thorough case history is obligatory; it should comprise signalment, environment, general health, reproductive history inclusive pregnancies, parturitions, resorptions, abortions, furthermore previous diseases, treatments (especially hormone applications),and the breeding history of the male (Johnston et al., 2001). For a good diagnostic approach, it is advisable to follow a systematic diagnostic pathway (Wilborn and Maxwell, 2012).

Infectious causes

Bacterial endometritis

Bacterial endometritis was found to be causative in 70% of bitches in one study; *Pasteurella multocida*, Groupe G *Streptococcus*, *Staphylococcus intermedius*, *Escherichia coli* and *Proteus mirabilis* were isolated from the uterus in cases of infertility and the bacteria were supposed to originate from the cranial vagina (Fontaine et al., 2009). The only species-specific agent is *Brucella canis* causing infertility, resorption, late abortion and weak puppies or normal puppies that may spread bacteria (Hollet, 2006). Even though *B.canis* is seldom diagnosed in breeding bitches, single cases in breeding kennels have been reported during the last decade all over the world (Corrente et al., 2010; Gyuranecz et al., 2011; Hofer et al., 2012). Diagnosis for infertility should therefore comprise serology for *B.canis* and if the outcome is doubtful, in addition a culture (blood or other secretions/excretions; Wanke, 2004; Hollet, 2006; Hofer et al., 2012). Conservative treatment may be successful in subclinical or low grade cases, however should not be performed in pyretic or hypothermic bitches with suspected sepsis and peritonitis (Fieni et al., 2014). Fieni et al. (2014) recommends the application of the antiprogesterone aglepristone (Alizine, Virbac, F; 10 mg/kg s.c. on two consecutive days). Furthermore the synthetic prostaglandin cloprostenol s.c at 1 µg/kg daily from day 3-7 days. The author of this article recommends 1 µg/kg every second day which was sufficient in all cases treated (Schäfer-Somi, 2015; Vetmeduni Vienna, Austria; unpublished data). Antibiotic treatment should consider the presence of both Gram+ and Gram- bacteria but mainly haemolytic E Coli, furthermore that no nephrotoxic medicaments should be used. According to literature, fertility was successfully restored in up to 87% of treated bitches (Feldman and Nelson, 2004). Bitches should be mated in the following oestrus since pregnancy and parturition were shown to prevent recidives for up to 4 years (Niskanen and Thrufield, 1998)

Non-infectious causes

Primary and secondary anoestrus

Primary anoestrus, i.e. the lack of oestrus by 24 months of age, may be caused by multiple diseases, organ dysfunctions as well as treatments (Johnston et al., 2001; Wilborn and Maxwell, 2012). The diagnosis should be made by a thorough case history and exclusion of silent heat, genetic disorders of sexual development (DSD), hypothyroidism and systemic diseases (Johnston et al., 2001). Recently, a case of primary anoestrus due to diet induced hyperthyroidism was reported (Sontas et al., 2014). Secondary anoestrus coincides with prolongation of the interestrus interval of 10-18 months (Johnston et al., 1994).

Treatment of the cause in most cases will help; however, in some cases, induction of oestrus may be useful. Many protocols for induction of oestrus including synthetic oestrogens, dopamine agonists, GnRH agonists, exogenous gonadotropins and opiate antagonists are available (Johnston et al., 2001; Kutzler, 2007); however, success rates are highly variable. Induction of oestrus almost without side effects proved to be possible in 75% of cases with the dopamine agonist cabergoline (Arbeiter et al., 1988; Jöchle et al., 1989), due to an increase in pulsatile secretion of FSH (de Gier et al., 2008). Induction of oestrus with GnRH agonists has been tried by using manifold protocols and synthetic derivates; The GnRH implant deslorelin (Ovuplant®, Ayerst Laboratories; Guelph, Ontario USA) has been used successfully for induction of oestrus in anoestrus bitches (Kutzler et al., 2001). Application beneath the vestibular mucosa improved conception rates even though pregnancy rates were similar to those obtained after subcutaneous application; however, premature luteal failure was observed in 4 out of 14 bitches, even though the implant was removed after the onset of oestrus (Kutzler et al., 2009). In another study (Fontaine et al., 2011), 24 bitches were implanted subcutaneously in the umbilical region with 4.7 mg deslorelin (Suprelorin®, Virbac, F) in anoestrus. Oestrus occurred in all bitches 2-7 days after implantation, ovulations were observed in 87.5% and pregnancy in 78.3% of bitches. Authors observed 3 cases of luteal failure (progesterone drop below 10 ng/ml prior to 50 days post-ovulation) and emphasized the necessity to measure the progesterone concentration once weekly following mating. In another study, the same medicament was used for a clinical trial with 16 bitches in anoestrus and similar results were obtained. All bitches came into oestrus 1-10 days after implantation and 81.3% ovulated. Pregnancy rate was 68.8% and average litter size 7.6 puppies (von Heimendahl and Miller, 2012). Even though these results are promising,

luteal failure may occur and owners have to be informed before the treatment that progesterone substitution during pregnancy might be necessary.

Cystic conditions of the uterus

Cyclic bitches tend to develop proliferative and cystic degenerative diseases of the endometrium, most frequently cystic endometrial hyperplasia (Dow, 1959; De Brosschere et al., 2001; Feldmann and Nelson 2004; Smith, 2006). This disease is believed to be mainly caused by the periodical local effect of oestrogen priming and progesterone on the endometrium, aggravated by delayed down regulation of oestrogen receptors, decreased uterine perfusion, accumulation of fluids, inflammation and delayed uterine clearance after mating. Furthermore local irritations caused by bacteria may cause the same histological findings (Nomura et al., 1990; De Bosschere et al., 2001; England et al., 2012). Infertility may appear despite regular cycles and normal sonographical findings; the inability to conceive, resorption or abortion is possible consequences of the progressive cystic degeneration. Manifold similar degenerative diseases of the endometrium have been described. In one study, fibrosis with degeneration of the endometrial glands (FDEGs), pseudoplacentational endometrial hyperplasia (PEH), CEH and endometritis were the lesions most frequently found in bitches with infertility (Mir et al., 2013). To improve diagnosis, new approaches like cytological, bacteriological and histological examination of uterine specimens taken after transcervical cannulation have been evaluated (Watts et al., 1996; Günzel-Apel et al., 2001; Fontaine et al., 2009). These techniques may be helpful however, the risk of endometritis and pyometra due to the manipulation should be considered. Treatment should aim to reduce intraluminal fluids by application of the antiprogesterone aglepristone (Alizine®, Virbac, F; 10 mg/kg s.c. on two consecutive days). Additional application of prostaglandins accelerates emptying of the uterus and improves healing in pyometra cases (for review: Fieni et al., 2014); however, in cases of uncomplicated CEH is usually not necessary. According to England et al. (2012), application of broad spectrum antibiotics improves fertility in bitches with CEH; treatment according to a resistency test, and considering *mycoplasma canis* and *ureaplasma canigenitalium* would be desirable. Very important is the breeder information about what may happen when the bitch conceives. Spontaneous resorptions/abortions may occur and the worse the endometrial changings, the higher the probability of pregnancy loss. In case of multiple endometrial and ovarian cysts as well as thickened endometrium, breeding should not be performed.

Congenital abnormalities

Abnormalities of the vulva, vestibule and vagina of the bitch like circumferential vaginal strictures may lead to infertility due to the inability to mate normally. According to Root et al. (1995) and others, the incidence of infertility in these cases is very low (~1%). As a prophylaxis, thorough gynaecological examination prior to first breeding should be recommended. Vaginal septa can then be removed surgically/endoscopically (Root et al., 1995; Arlt et al., 2012). The incidence of genetic disorders of sexual development (DSD) inclusive sex reversals is even lower (Christensen, 2012).

Hormonal imbalances

In some bitches, hypoestrogenism has been described to cause prolonged oestrus. Clinically, non-receptive behaviour is typical, and the oedematous swelling of the vulva and vaginal mucous membrane will be below average. Oestrogen and progesterone values usually are below the average concentrations described for normal bitches. Treatment can be tried with FSH 25-100 I.U s.c., i.m. or i.v. every other day until occurrence of oestrus; mostly two injections will be sufficient (Arbeiter and Dreier, 1972).

Hypoluteoidism is defined as luteal deficiency during pregnancy, when serum progesterone concentration drops below 5 ng/ml during week 4-5 of gestation causing resorption or abortion (Root Kustritz, 2005). Primary and secondary hypoluteoidism can be differentiated, the first without any obvious cause, the second being caused by any infectious or non-infectious disturbance during pregnancy and causing fetal distress. Diagnosis of primary luteal deficiency is therefore performed by excluding causes of secondary luteal deficiency and it has been questioned whether primary luteal deficiency due to abnormal corpora luteal function exists (Johnston et al., 2001). The case history is important, repeated pregnancy failure may indicate luteal deficiency but also hypothyroidism. Clinically, a brownish-bloody vaginal fluor may occur in the 3rd week of gestation, while the general status of health is undisturbed. Sonographically, fetal death can be seen. For diagnosis, serum concentrations of progesterone as well as thyroxin (T4) and thyroid stimulating hormone (TSH) should be measured. Vaginal swabs for cytology and bacteriological examination should be taken. Serology mainly to exclude parvovirus, herpesvirus and *B. canis* infection as well as a blood picture can be helpful. Infectious causes should always be considered before treatment with natural or synthetic gestagens. In case of disturbance of the general status of health, fever and smelling vaginal discharge, the bitch should not be treated with gestagens. Otherwise, natural progesterone in oil should be applied during the first half of pregnancy to avoid

disturbances of embryo organogenesis since synthetic gestagens are teratogenic. Different dosages and application schemes have been recommended such as 2 mg/kg i.m. every 72 h (Purswell, 1991) or 1 mg/kg i.m. once daily. From day 35 on, oral gestagens can be applied and successfully prevented abortion in different studies. Medroxyprogesteroneacetate (MPA) has the advantage that the course of natural progesterone can be monitored; a dosage of 0.1 mg/kg once daily until day 40-58 dependant on clinical findings and serum progesterone concentration is recommendable (Görlinger et al., 2005; Günzel-Apel et al., 2012). Since diagnosis will take a few days, bitches treated with gestagens should receive broad spectrum antibiotics.

The impact of manifest hypothyroidism on fertility is debatable. A negative effect on conception rate, periparturient mortality and puppy birth weight has been observed in one study (Panciera et al., 2012). Furthermore abortion and stillbirth have been described (Johnson et al., 1987). Other authors did not find an increased incidence of reproductive disorders in hypothyroxinemic dogs compared to normal dogs (Segalini et al., 2009). However, measurement of thyroxin and thyroid stimulating hormone should be performed in each case of repeated fetal resorption/abortion; the author of this abstract observed at least two cases of beginning resorption, combined with hypothyroxinemia, which could be successfully stopped by hormone substitution (personal communication). It must be emphasized that disturbances of the thyroid gland function can be caused by other organ or systemic diseases. Furthermore, even though most cases are acquired, cases of congenital hypothyroidism have been described (Dodgson et al., 2012).

The impact of food substitutions

Breeding bitches have an increased demand for certain vitamins, the requirements changing during pregnancy. However, while the substitution of folic acid during early pregnancy was proven to significantly decrease the percentage of neonatal cleft palate (Elwood and Colquhoun, 1997), the effect of the amino acid tyrosine to increase fertility in breeding bitches is controversially discussed. Tyrosine (100 mg/kg/day) is recommended in some german textbooks (Wehrend, 2010) due to a supposed effect on GnRH secretion, consecutively increasing gonadotropin secretion (Gibson et al., 1982). Recently, Spankowsky et al. (2013) orally administered either 100 mg tyrosine /kg/day or a placebo to an experimental and a control group of 25 dogs each. The impact on mating behaviour and oestrogen/progesterone serum concentration was assessed. They did not find any significant difference between groups and therefore concluded that feeding tyrosine during oestrus does not exert a positive effect on copulation behaviour or estradiol-17ß concentration.

Conclusions

The search for the causative agent in cases of infertility is a challenge and should begin with a comprehensive case history followed by a thorough clinical examination. New diagnostic approaches include cytological and bacteriological examination of uterine specimens taken after transcervical cannulation. In some cases, new conservative treatments like oestrus induction with GnRH implants can be successful, however, have to be carefully considered with regards to side effects.

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