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HOW I INVESTIGATE BITCH INFERTILITY

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In comparison to the last 10 or 20 years, veterinarians are now frequently requested to solve fertility problems in the dog, mainly due to the increased popularity of purebred dogs as well as for sentimental or financial reasons. In fact, breeders may be concerned about different kinds of problems which include but are not limited to true infertility (their bitches produce no pups after mating or Artificial Insemination), anoestrus or a low prolificity rate.

The normal fertility rate in the canine species is difficult to determine with certainty. Data published by national kennel clubs probably underestimate the rate. For example, the annual report published by the French kennel club (Société Centrale Canine) states a fertility rate of approximately 50% which seems very low. In fact, their data include cases such as a bitch locked together with a male for one hour without any human control which was supposedly bred. Actually, as fertility may also vary tremendously among different breeds, such data do not provide very useful information. A study conducted in France concluded that the fertility rate of pure breed bitches which underwent natural mating or AI under controlled conditions (vaginal cytology and progesterone assays) was 75.4%, with a mean litter size of 5.6 pups (15). For this reason, we recommend that a bitch be considered infertile when she does not produce any pups or in insufficient number after two successive matings.

Many different problems can lead to infertility including hormonal problems, infectious diseases, congenital or acquired defects of the genital tract. However, few techniques unfortunately exist which can be utilized in the investigation of the reproductive functions in bitches. Therefore, when investigating the cause of infertility in the bitch, we recommend that veterinarians follow a very strict progression, commencing with the most frequent cause to the most uncommon cause.

Still, most bitches cannot be totally classified as fertile or infertile. Such a definite classification would be too simple, and many bitches may just be "hypo-fertile". First, several factors can act together causing the bitch to be infertile at a certain period of time. As in humans, the cause of infertility rarely is rarely due exclusively to the male part or the female part. Breeders often try to breed middle-aged bitches following their career in exhibition shows with relatively old males which have already produced valuable pups and which, as such, have demonstrated to be of good genetical or commercial value. Therefore, it is not surprising that breeders experience a lower than expected fertility than if more fertile young dogs had been used (or the opposite situation: young bitches were bred to old males).

In the bitch, things become even more difficult when we realize that apparent infertility can be due to very different situations which prove difficult to distinguish, such as lack of fertilization (no union between eggs and sperm) and early embryonic

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death. Therefore, when investigating a fertility problem, veterinarians must check with precision not only the ovulation period but also the early gestational phase.

In practice, many bitches belonging to dog breeders may have been empirically treated with hormonal or non-hormonal drugs. The first step in the examination of an infertile bitch consists of conducting a very precise survey of the general and reproductive history of the bitch (12, 31).

1. MISTIMED BREEDING

According to the vast majority of scientific publications, mistimed breeding represents by far the most common cause of infertility in the bitch. The incidence may vary between 40% to 80% of infertile bitches (12, 15, 19, 31). This aspect will be detailed by Dr. Lindy Vaughan elsewhere in this congress. The physiology shows that bitches should ideally be mated or inseminated 2 to 4 days after ovulation, in order to obtain the maximal litter size, although successful breedings with often reduced litter size have occurred as early as 3 days before and as late as 5 or 6 days after ovulation (10^{bis}).

Although the following belief has been clearly proved erroneous in approximately 30% of bitches (19), many breeders are still convinced that a bitch will conceive when mated around the 12th day of the heat period. Actually, it has been clearly demonstrated that a bitch may ovulate as early as the 3rd or 4th day after the onset of pro-oestrus and as late as the 30th day of the heat period. Normally, the beginning of estrous behavior, i.e. acceptance of mounting, begins near the onset of the LH peak, around 48 hours before ovulation. But strong clinical evidence shows that this rule undergoes some variations that surely explain that the acceptance of a bitch to be mated does not automatically coincide with the best fertility period.

Clinical factors such as the increased swelling of the vulva and the decreasing bleeding of the genital tract at the time of the ovulation period may help veterinarians determine if bitches are obviously mated at a wrong time. But, these criteria are in no way precise enough.

Nowadays, numerous available techniques may be utilized to investigate the “optimal time for breeding”: vaginal cytology, endoscopic appearance of vaginal folds and progesterone assays are the most commonly used methods. Ovarian ultrasonography is especially indicated in infertile bitches, as it represents the most accurate way to determine the precise date of ovulation. This method also helps quantify the number of growing follicles as well as follicles undergoing ovulation and as such, it helps to evaluate the fertility potential of the bitch. Some slides will be shown during the lecture.

Recent data also show that the optimal breeding period may vary among successive cycles in the same bitch. This explains why an infertile bitch has to be carefully checked for the best mating time with each new heat period.

2. MALE INFERTILITY

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After mistimed breeding, male infertility is a common cause of conception failure in bitches presented with infertility (19). If a bitch has remained infertile after a correct determination of the mating time, it is important to check the quality of the semen of the male. We personally consider that this is the second step in investigating an infertile bitch.

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3. OTHER CAUSES OF INFERTILITY

This represents the third step of investigation in front of an infertile bitch. As many other authors, we suggest that veterinarians should first consider if infertile bitches show regular inter-oestrus intervals or irregular ovarian cycles.

3.1. INFERTILITY WITH PROLONGED INTERESTROUS INTERVALS.

The length of interestrous intervals may be to a large extent influenced by the **breed**, with a heritability of 35% (19). According to our personal clinical experience, it is not surprising to witness interestrous intervals longer than 8 to 10 months in breeds like Labradors, Collies, Greyhounds or other racing dogs. The African Basenji dog is well known for exhibiting only one heat period per year.

The apparent prolongation of interestrous intervals occurs in dogs with a **silent heat**, defined as ovarian activity in the absence of overt physical and behavioral changes characteristic of canine estrus (12,13,19). Some bitches showing heats once a year (every 12 months or so) may in fact undergo two heat periods, one being clinically normal and the other being a silent heat (personal unpublished data).

The veterinarian's attention should be heightened when an infertile bitch that underwent previous normal interestrous intervals starts to experience prolonged interestrous periods. The underlying cause is often hormonal and is more or less of the same origin as in anestrus bitches.

Among the causes include hypothyroidism, hyperadrenocorticism, hyperprolactinemia, or bitches treated with hormonal compounds such as progestagens, androgens (racing dogs) or anabolic steroid compounds. **It is why we always investigate an anoestrous bitch in at least assaying progesterone and thyroxin.**

Ovarian cysts that secrete progesterone may cause prolonged interestrous intervals (12). In such a case, the bitch remains in a prolonged "diestrus" state, which is not associated with any clinical sign. The diagnosis is based on demonstrating a prolonged secretion of serum progesterone over the basal line (i.e. > 2 to 5 ng/ml) during 9 or 10 weeks. Two blood samples at 4 to 6 week intervals are often necessary to distinguish a normal luteal secretion after silent heats and a luteal dysfunction due to a cyst. The surgical removal of the cyst is often the best solution. Hormonal attempts using prostaglandins are not well documented in bitches.

Bad health, due to systemic diseases for example may interact with cyclicity (13,31). Bitches housed in very bad environmental conditions (including a high concentration of animals, low luminosity, low quality food) may undergo prolonged interestrous intervals. Surprisingly, it is often found that estrus may be delayed by 1 to 2 months after a pregnancy has occurred, but the underlying reason is unknown (13,19).

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According to some authors, stress could lead to infertility, but it seems anecdotal (2). A long stressful travel for mating could decrease fertility (31).

When an anoestrous bitch shows no hormonal disturbance and when we can be sure that she didn't undergo silent heats, inducing heats using hormonal treatments may be considered. In our everyday practice, when treating purebred bitches, we personally always try at first the antiprolactinic compound cabergolin, given orally once daily during 2 to 3 weeks, only on bitches with a prolonged anoestrus (> 7 months). Other close protocols have been published using oral cabergolin (29^{bis}). Other trials have used bromocriptin (32) or metergolin in anoestrous bitches. A bad fertility rate is more likely to occur if heats are induced too early (in dioestrus for example).

3.2. INFERTILITY WITH SHORTENED INTERESTROUS INTERVALS

As already stated for increased interestrus intervals, some breeds are well known to exhibit shortened interestrus intervals without any incidence on fertility. For example, German Shepherds and Rottweilers often show small interestrus periods of 4.5 to 5 months (12, 31).

It is when these small interestrus periods are associated with infertility that bitches are presented to their vets. Roughly, this type of infertility may be divided into two major causes: bitches suffering from ovarian hyperstimulation (mainly follicular cysts or Granulosa cells tumor), and/or bitches showing a premature decline in progesterone during diestrus.

3.2.1. Ovarian cysts or tumors.

The excess secretion of estrogens by ovarian cysts or tumors frequently lead to shortened interestrus intervals with prolonged heats, and often even to permanent (persistent) estrus. It is important to diagnose and remove these hormonally active cysts or tumors as quickly as possible for at least two reasons. First, it is necessary to cure them and try to restore fertility. Second, the secretion of high quantity of estrogens may act on the uterus as a potential factor towards the cystic endometrial hyperplasia – pyometra complex, but also on the bone marrow in creating progressive non regenerative anemia.

For these reasons, we strongly recommend breeders to note carefully the time of the appearance of successive heats periods in the same bitch and to quickly see a vet if the interestrus intervals are shortened, even before a bitch remains infertile.

Follicular cysts are thin-walled structures containing clear, serous fluid. Follicular cysts may be single or multiple; if multiple cysts are present in one ovary, the cysts do not communicate. The ovarian cysts in the bitch may be present in only one or both ovaries. In one study, the incidence of bilateral ovarian follicular cysts was reported to be 32%

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(19). In the bitch, it is not known if there is a hereditary influence on the incidence of follicular cysts, as has been shown in cows.

If the pathogenesis of follicular cysts (often follicles that did not ovulate after the LH peak) is unclear, the granulosa cells contained in the follicular wall may produce a high quantity of estrogens, leading to infertility with prolonged estrus. Sometimes, some luteal cells may be present as well.

Estradiol assays during the pro-estrus period may be useful for practitioners who do not perform ultrasonography. The patterns are often modified in two ways. First, the maximal level of estradiolemia may be increased from what is usually found at the peak of estradiol, 24 hours before the LH peak. In a French study, values as high as 300 pmol/l were found as normal bitches in heat never exceeded 200 pmol/l (personal data). In order to survey the level of estradiol, veterinarians are encouraged to take a blood sample every other day during pro-estrus. Often, the suspicion of an ovarian pathology will arise at the end of the heat period, when the estradiol plasma level will not drop at a basal level after the rise of progesteronemia (personal data).

Still, when possible, it is much more valuable to perform ovarian ultrasonography. Follicular cysts appear as focal hypoechoic to anechoic structures. It is not always easy to distinguish between normal follicles and pathological follicular cysts. Usually, fully mature follicles, just prior to ovulation, do not exceed 0.9 cm in size. Many authors consider that anechoic structures greater than 1cm are supposed to be cystic structures, which does not automatically imply that they are hormonally active (19).

The treatment of choice of ovarian follicular cysts is ovariectomy or ovariohysterectomy when the uterus is damaged. In bitches with valuable reproductive potential, one should consider the possibility of unilateral ovariectomy or even removal of the cysts only when cysts are present only in one ovary and when the uterus does not seem to suffer from cystic endometrial hyperplasia (31). Some authors recommend induction of luteinization of the cystic follicles, using GnRH or hCG (19). We personally do not recommend such protocols, as they increase the progesterone plasma level after a prolonged period of estrogen secretion. Based upon our clinical experience, this treatment very often leads to the occurrence of pyometra in the following weeks. It is our view that such treatments should be reserved only for bitches that will be neutered immediately after treatment; in such a case this treatment is not performed to cure the cysts but to cease the heat period in order to decrease blood irrigation of the genital tract and to perform the surgery in more secure conditions.

Aspiration of a cyst with a fine sterile needle may be tried under laparotomy or ultrasonography. In one case (personal unpublished data), a Golden Retriever bitch that suffered from a prolonged pro-estrus and failed to ovulate conceived 9 pups after aspiration of the cyst. Still, data are insufficient to know the exact success that one could expect from such a treatment. In all cases, breeders should be informed that pyometra may still appear when the cyst is just emptied and not removed, as it often reappears later.

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Granulosa Cells tumors (GCT) represent 23 to 52 % of the ovarian tumors (19). They occur mainly in bitches in the second part of life, with a mean age of diagnosis of 7.2 years. These tumors are mainly unilateral, but bilateral cases have been published. Local invasion is not frequent, and metastasis is reported in 10 to 20 % of the cases, mainly in omentum, mesentery, diaphragm, liver, kidney, bladder and intra-abdominal lymph nodes.

Granulosa cells produce estrogens; and therefore, it is not surprising that GCT often lead to infertility with prolonged heat periods. But sometimes, they also secrete quite a high level of progesterone, which produces rather an anoestrus clinical profile.

Other ovarian tumors like cystadenomas and adenocarcinomas may also secrete high quantities of estradiol, leading to the same kind of infertility (31).

Good clinical examination, including palpation, estradiol assays and ovarian ultrasonography are the major steps towards diagnosis. Treatment always consists of the surgical removal of the tumor. Ovariectomy could be unilateral in breeding bitches, but as previously stated, an evolution towards pyometra is always possible. Chemotherapy for treatment metastatic GCT has not been described.

3.2.2. Premature decline in progesterone

For unclear reasons, it is often noticed that when the period of progesterone secretion after ovulation is reduced, the occurrence of the following heat period will be sooner than expected (19, 31). Clinicians often notice this after having performed a medical abortion, for example with anti-progestin compounds such as aglepristone. Therefore, infertile bitches with shortened interestrus intervals do not all suffer from ovarian cysts or tumors.

Split heats are defined as successive short proestrus signs, at intervals of 2 weeks to 2 months, associated with short interestrus intervals (12,31). This pattern is more often observed in young bitches and leads to no real infertility in the rest of the genital life of the bitch.

Anovulatory cycles are not frequent in bitches. In one study, they were reported in 11 of 1152 bitches (1%) (19). It has been showed that some bitches may well have normal ovulatory cycles later in their life. In such anovulatory cycles, serum progesterone level never increases above 3.5 to 6 ng/ml (12, 19). This explains why the following heat period will often occur earlier than usual.

From our point of view, and according to human medicine, veterinarians should remember that anovulation may occur in different cases. First, the estradiol level may be too high to allow the LH peak to occur, due to pituitary inhibition. In such a case, it is often noticed that vaginal cytology has shown high cornification for many days and still

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ovulation has not occurred. This could be due to the existence of ovarian follicular cysts or GCT, and it has always to be checked first. Increased estradiol levels could occur for unclear reasons without the presence of cysts or tumors. In such cases, it could be valuable to try to induce ovulation using anti-estrogenic compounds like clomifene acetate. From our point of view, GnRH or hCG should be used carefully, recalling that the uterus has previously been under prolonged estrogenic influence.

Sometimes, it is found that anovulatory cycles occur in bitches with a prolonged proestrus phase. Vaginal cytology is not well cornified and ovarian ultrasonography, when performed before the end of the heat period, shows small immature follicles. It is therefore easy to understand that hCG will be of no value in the sense that it will at the most induce ovulation of immature oocytes that will quickly degenerate. In some cases, we have used eCG (PMSG), one or two injections IM at 48 hour intervals in order to improve follicular maturation. Such treatments should only be used when it is sure that anovulation is due to the lack of follicle maturation, as using eCG in normal bitches may induce an increased serum estradiol concentration leading sometimes to induction of a pyometra !

Bitches may also suffer from **hypoluteoidism**, which is the lack of progesterone secretion during pregnancy which makes the pregnancy impossible to maintain (19). It is usually considered as an ovarian problem in the bitch, but other factors like pituitary problems or hypothyroidism could promote this pathology. Some breeds are well known to express hypoluteoidism, like Rottweilers and German Shepherds. We have also experienced this problem in many Newfoundlands and Bernese bitches. In fact, the minimum level to maintain pregnancy is unknown. It has been demonstrated that if serum progesterone concentration falls to less than 2 ng/ml for more than 48 hours, abortion will occur with certainty. In practice, veterinarians and breeders prefer to not wait until such an extreme point, and usually bitches will be given progesterone supplementation much earlier. In our practice, we often give exogenous progesterone when serum level falls under 10 ng/ml before the end of the first month of pregnancy.

In infertile bitches, assay progesterone is strongly recommended when performing the early pregnancy diagnosis between 21 to 30 days of pregnancy. If the bitch is not pregnant, plasma progesterone concentration should still be quite high. If this is not the case, it means either that a luteal failure has occurred or that the bitch has underwent an early abortion (or fetal resorption). During the following cycle, blood samples should be drawn at least once a week (twice is better) in order to detect the defect in advance. Progesterone supply can be given parentally (progesterone in oil: 2 mg/kg every 3 days; allyl-trenbolone...) (19). In France, veterinarians often use oral micronised progesterone which is currently given to women. A recent study demonstrates that the best rate of daily administration is oral tablets 3 tid at a dosage of 10 to 20 mg/kg each time (28).

Of course, blood progesterone concentration should be checked regularly and at least once a week after the beginning of supplementation. Usually, it is recommended to stop the supplementation 58 to 61 days from ovulation, 56 to 59 days after mating or 55 days from the first diestrous vaginal smear (19, 28). Otherwise, the end of pregnancy may be

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delayed and sometimes the delivery of pups does not occur or the incidence of stillbirths may be increased. Bitches receiving ally-trenbolone may have poor milk production in the early postpartum period (19).

In our point of view, progesterone supplementation should not be given empirically during pregnancy, as it can promote malformations in the fetus such as cryptorchidism.

A “**short anoestrus syndrom**” has been described in Rottweilers and German Shepherds (13,31). It is not well defined and concerns animals which show normal estrus and ovulation, but have shortened interestrus intervals. The basis of infertility is unclear and could involve an insufficient duration of anestrus to allow for endometrial repair. It could also be associated with hypoludeoidism.

Early embryonic or fetal death remains most of the time impossible to detect (31), as no vulvar swelling occurs in general. Possible causes include endometritis, cystic endometrial hyperplasia, embryonic defects and possibly inbreeding (31). Our clinical data seem to show that it is often associated with a decline in blood progesterone concentration, which becomes clearly lower than in a normal non-pregnant bitch. This could be due to the release of uterine prostaglandins that could induce luteolysis. However, early embryonic or fetal loss does not always change the frequency of the heats of the bitch.

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3.3. INFERTILITY WITH NORMAL INTERESTROUS INTERVALS

3.3.1. HORMONAL PROBLEMS

Hormonal problems, such as hypothyroidism, hypoluteoidism, anovulation, follicular cysts, ovarian tumors do not always alter cyclicity. Sometimes, the interestrous intervals will remain unchanged at least for a certain period of time. Therefore, hormonal defects may be suspected also in bitches with regular interestrous intervals, and veterinarians should control the hormonal status of the bitch during the heat period and also during pregnancy.

3.3.2. INFECTIOUS DISEASES

Many infectious agents have been suspected to induce infertility in bitches. However, a paucity of studies has been conducted in this field. It is usually thought that the incidence of infectious diseases on canine infertility is greater in large breeding kennels than in small familial breeding kennels or in pet dogs.

Infectious diseases could promote infertility by various pathogenic ways. In the vagina, they could have a spermicidal activity, act on sperm motility, in creating ciliostasis for example (21) or emphasize the penetration of infectious agents into the uterus during proestrus and estrus (29). Uterine infection leads to lymphogenic infiltration of the uterine wall, creating a hostile environment for the sperm and eggs (21), interference with the development of the zygote or early embryonic death (13, 29). Later during pregnancy, endometritis and/or placentitis or placental infection may occur, leading to fetal resorption (29).

Several **viruses** have been shown to play a potential role in canine infertility. **Canine Herpes Virus (CHV)** is well known to have a pathogenic action on neonate pups. Still, it has been clearly demonstrated that transplacental infection could occur at mid to late pregnancy. Several elements suggest however that CHV may well act on infertility in the bitch. In a study published in 1971 concerning a group of breeding Alsatians, the fertility rate was halved after infection with CHV. In a recent publication, it was clearly shown that the pregnancy rate was highly improved in infected kennels after the use of a specific vaccine against CHV (25). Regular epidemiological surveys tend to show that the serological prevalence of this virus among breeding kennels has increased significantly during the past 10 years in all of the European countries. In a recent study conducted in France in 84 breeding kennels, the overall prevalence was 30.6% (16). In Belgium, a study stated a much higher prevalence above 80% ! There seems to be a strong effect of the size of the kennel, as seroprevalence of CHV is much higher in large breeding units (16,25). However, it is difficult to know if CHV plays a major role in canine infertility. Some clinical observations tend to show that in some cases, there could be a concomitant role of other infectious agents, such as mycoplasmas, and that CHV may act as a promoting local immuno-depressive agent.

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Tranplacental infection by **Canine Distemper Virus** has been shown in experimental conditions (20). This virus could act in the field as an immuno-depressive agent in association with other causes, such as Toxoplasmosis for example (10). Still, it surely plays a minor role towards infertility. Recently, some papers have pointed out the potential incidence of a parvovirus **Minute Virus of Canines (CPV1)** on resorption during the first half of pregnancy. Experimentally, oro-nasal inoculation leads to resorption in 3 out of 8 pregnant bitches.

The incidence of **bacterial infections** on canine infertility is better documented. **Canine Brucellosis**, which is well known as an abortive agent during late pregnancy, could also generate early embryonic or fetal death through endometritis (12, 31). Therefore, infertile bitches should always be checked for Brucellosis. Fortunately, recent epidemiological studies in Europe tend to show that the prevalence of this disease is low, between 4 to 8.5% (22).

Other **specific bacterial diseases** have been suspected to act on canine infertility. The bibliography stresses the potential role of *Campylobacter jejuni* (6), *Salmonella*, sp., *Listeria monocytogenes* (27), *Leptospira interrogans* (5), *Coxiella burnetii*, *Rickettsia rickettsii* (26) and *Chlamydia* sp (14). At present, there is still an obvious lack of studies and data to confirm these elements of suspicion.

However, **usual genital bacteria** may play a real role on infertility. **Canine Mycoplasmas and Ureaplasms** are commonly isolated in the genital tract of fertile and infertile bitches (21). But it has been shown that there is a higher incidence of these agents in the vagina of infertile bitches (9). It has been suggested that, as in women, their pathogenic role may depend on bacterial concentration. Reproductive problems may occur above 10^4 to 10^6 Colony Forming Units (CFU)/ml (23). Still, the exact incidence of Mycoplasmas and Ureaplasms on infertility is unclear. It may well be underestimated due to the lack of demand for a specific isolation. These agents may also act as opportunistic agents in immunodepressed bitches, in association with other bacteria or viruses.

Many bacteria are commonly isolated from the uterus and the vagina of normal fertile bitches (30). Bacteria found in the uterus are usually also found in the cranial vagina, where 60% of normal bitches harbor bacteria (19). Numerous bacteria are found in bitches with no reproductive problems (1,3,29), belonging to **aerobic flora** (*Streptococcus* sp., *E. coli*, *Pasteurella multocida*, *Staphylococcus* sp., *Proteus* sp., *Corynebacterium* sp....) but also to **anaerobic flora** (*Bacteroidacea* were isolated in 55% of vaginal swabbings in normal bitches, *Lactobacillus* sp., *Bifidobacterium* sp., *Clostridium* sp. *Corynebacterium* sp....). Usually, mixed cultures are predominant in normal fertile bitches: very often 2 to 4 germs are cultured after a vaginal swabbing (4,17).

Several studies barely demonstrate any difference in the composition of the vaginal flora between fertile and infertile bitches (3). But it has been shown that in case of vaginitis, there are significant qualitative and quantitative variations (4,17).

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Streptococcus sp., E. coli and Pasteurella sp. were more often isolated, and very frequently pure cultures (or showing less than 2 or 3 germs) were obtained after vaginal swabbing (4,18). Therefore, strong evidence exists that bacteria causing vaginitis may lead to infertility (11). The prevalence of vaginitis in adult female bitches has been estimated around 0.7% (19). It may well have been underestimated due to lack of specific clinical signs and due to the difficulty of the clinical examination of the vagina of the bitch. **Personnaly, I sometimes treat infertile bitches with antibiotics, but only when they harbour one (or sometimes) 2 germs in high concentration in the anterior vagina.**

Fungic infections seem to be extremely rare in the bitch, according to the publications. On the other hand, the role of **parasitic infections** on infertility is better documented. Toxoplasma gondii has been shown to cause an experimental placental infection during pregnancy (10). It could act as an opportunistic agent together with bacteria or viral infections. Recent experimental data suggest that Neospora caninum could cause early fetal death in the bitch (10).

3.3.3. DRUGS INDUCED INFERTILITY

In practice, many breeding bitches may be treated with drugs that may contribute to the decline of fertility. Steroid hormones and anti-fungic compounds may create hormonal defects in pre-puberal or adult bitches. Abortive drugs such as prostaglandins, antiprogestins and antiprolactinic substances have to be avoided during pregnancy.

3.3.4. ANATOMICAL ABNORMALITY OF THE VULVA, VESTIBULE OR VAGINA

Some bitches do not manage to mate because of **congenital abnormalities** of the posterior genital tract (vulva, vestibule or vagina). The most common problems are circumferential strictures or persistence of a vertical septum in the vagina (13,19), as a result of a non fusion of the Müller's ducts. In France, vestibular and vulvar stenosis are frequent in two breeds, Collies and Bergers Picards. Sometimes, the hymen which is located at the junction between the vagina and the vestibule will not disappear. This can lead to a bitch which has silent heats, without any bleeding of the vulva. These congenital abnormalities often lead to chronic vaginitis or uro-genital infections.

Vaginal hyperplasia may occur during the heat period or at the end of pregnancy when the estradiol level increases. This occurs most frequently on brachycephalic breeds (Bullmastiffs, Boxers, Pugs...). Of course, it often prevents the penis of the male from entering the female genital tract. It also increases the incidence of vaginitis, which may also lead to infertility. This abnormality is most likely in part hereditary. It is often recurrent in the same female even after surgical removal.

Pseudohermaphroditism or true hermaphroditism can cause infertility (24). They are often easy to diagnose clinically, when the genital tract does not seem to be normal: enlarged clitoris... (19).

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Acquired diseases or abnormalities of the posterior genital tract (scars after a bad parturition, episiotomy, violent mating...) may also lead to the lack of copulation.

3.3.5. UTERINE PATHOLOGY

Bitches with **cystic endometrial hyperplasia** (CEH) are often infertile due to implantation failure after conception. Often, no abnormality is noticed during the heat period and ovulation. Somehow, ultrasonography usually permits the visualization of the luteal endometrium. Hysterography or uterine biopsy may also be used. CEH is often considered to be irreversible. One successful therapy has been described with mibolerone oral administration, 30 microgrammes per 25 lb body weight daily during 6 months.

CEH often leads to pyometra, which may be treated in many cases by a mixed treatment using prostaglandins and antiprogestins (aglepristone). A healing of the endometrium seems to occur, as many bitches may have successful pregnancies at their next heat period.

Congenital or acquired uterine abnormalities including the lack of one or two horns, oviductal aplasia, segmented uterus may happen but are not well documented in the bitch. Diagnosis requires hysterography and very often laparotomy. Uterine tumors are very rare in this species but may of course cause infertility, apart from other clinical signs like persistent genital bleeding.

Cervical stenosis has been described in the bitch (31). Although difficult to diagnose, suspicion occurs when it is impossible to cannulate the cervix.

3.3.6. ABNORMAL SEXUAL BEHAVIOR

Many psychological factors may influence sexual receptivity in bitches. Very dominant bitches often refuse to be mated with subordinate males (2, 19, 31). Tranquilization of such bitches is not recommended, neither is a forced mating, where the bitch is attached to remain still. An accident may occur and create a fracture of the penis bone or some other undesirable thing. It is much preferable to use Artificial Insemination.

Sexual preference has been observed in the bitch (2).

Some authors think that psychology may influence factors like ovulation or early embryonic death in the bitch (2).

3.3.7. MISCELLANEOUS CAUSES

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Sometimes (mainly in large breeds), due to the preponderant weight of the male, the bitch is not capable of supporting its **weight** during the mating. Once again, Artificial Insemination is the ideal solution.

Advanced age is often responsible for a decreased conception rate and a decreased litter size, together with longer interestrus intervals (19,31). Still, the reproductive cycle continues in old bitches.

Bitches with **systemic diseases** like diabetes mellitus, hyperadrenocorticism or renal insufficiency may likely be infertile (19,31).

Immunologic infertility has been suspected in the bitch. A case of an lymphocytic oophoritis, possibly immune mediated, has been described. (24).

Finally, breeders stress nutrition when their breeding kennel suffers from decreased reproductive results. Little is known in this regard. Obesity may lead to anoestrus or ovulation failure. On the contrary, a vitamin A deficiency or a lack of energetic supply may alter fertility to a certain extent. Nowadays, the use of industrial pet food has probably solved any nutritional problem that could act as a potential cause of infertility.

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