Clinical approach to infertility in the bitch
[Abordagem clínica à infertilidade na cadela]

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Introduction

Although several techniques have become available to researchers and clinicians in small animal reproduction over the last 20 years, reproductive soundness is still often difficult to assess in the bitch mainly because of inaccessibility of the canine female reproductive tract. Laparoscopy allows direct observation of the canine uterus through a small abdominal incision, but the ovarian bursa must be incised in order to view the ovary, and possible negative consequences of bursal incisions on fertility prevent a routine use of such technique. Furthermore, biopsies should only be performed under laparotomy, as under laparoscopy it may be difficult to make sutures which may lead to prolonged bleeding. Ultrasonography of ovaries and uterus has allowed considerable advances in investigating canine infertility, and uterine normal and abnormal anatomy has been defined. However, ovarian ultrasound is still limited by the fact that normal, quiescent ovaries may be hard to visualize in medium to small size dogs. In order to explore canine ovaries one should use a 7.5 MHz probe, although a 5.0 MHz probe can be utilized in large size dogs. Cost and time required for training are limiting factors for a widespread use of canine ovarian ultrasound.

Importance of progesterone assay

The single best technique which has produced a major step forward in our understanding of the physiology of canine reproduction is assay of reproductive hormones. Recently, serum progesterone assay (P4) by immunoenzymatic technique has become available to veterinarians as a rapid test to be performed in a clinic situation. Accuracy of immunoenzymatic assay is generally not higher than 80%, which means that one should not rely entirely on P4 results but should rather interpret it together with information on vaginal cytology and receptivity to mating. Serum progesterone assay is important to follow the canine estrus cycle as the bitch progresses towards ovulation, to confirm that she ovulated and maintained a 60-day luteal phase, to gain more information on fetal survival in case of problems during pregnancy, as well as to decide whether or not to use prostaglandin F2a in pyometra cases.

Clinical management of infertility

Stages of the canine reproductive cycle include PROESTRUS (average 9 days, range 3-17), ESTRUS (average 9 days, range 3-21), DIESTRUS/PREGNANCY (60 days) and ANESTRUS (1.5-7 months). In the average bitch onset of male receptivity and ovulation occur on day 9 and 12, respectively. However, although this is true for a high percentage of dogs, veterinarians should consider that a high proportion of bitches with a presenting complaint of infertility probably ovulate before or after day 12. Also, onset of male receptivity may be delayed for several days after ovulation, which may complicate interpretation of clinical data.

The following data base should be recorded for all previous seasons for which information is available:
1. Date of onset of proestrual bleeding
2. Date of onset of first receptivity
3. Breeding(s): dates, out/inside tie, AI, fresh vs frozen semen
4. Date of first refusal of mating
5. Male fertility, age, semen culture
6. Brucella canis antibody status of the bitch and dog
7. Pregnancy status at 28 days
8. Previous normal whelping(s)/litter(s)
9. Previous signs of false pregnancy
10. Previous reproductive disease
11. Previous hormonal therapy

Information collected through history can be used by the clinician to decide whether or not the bitch is cycling normally, whether or not she was bred/inseminated at the appropriate time and whether or not reproductive disease is present. An algorithm (Figure 1) is enclosed to help diagnose causes of infertility in the bitch (Johnston, 1984). Information from the above database is used to enter the algorithm and proceed towards a diagnosis.

**Breeding Management**

Timing ovulation is of utmost importance in achieving good conception rate as well as in solving cases of canine infertility. Surveys made in several veterinary colleges in the US and Europe agree on the fact that choosing the wrong time for breeding is the main cause of infertility. Table 1 portrays a list of clinical cases of bitches with a presenting complaint of infertility, from the Theriogenology Service of the Small Animal Hospital at the University of Pisa in the period 1990-2000. Total incidence of cases of female infertility and relative incidence of causes of infertility are shown. In our experience mistiming of breeding/AI accounts for >50% of all the cases of infertility in the bitch. This is probably due to the fact that breeders/owners typically choose day 12 as the optimum day for breeding, which means that bitches ovulating early or late cannot conceive.

<table>
<thead>
<tr>
<th>Total n° of Cases</th>
<th>= 1238</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases of Female Infertility</td>
<td>= 376 (21%)</td>
</tr>
<tr>
<td>- Mistiming of breeding/AI</td>
<td>= 204 (54.2%)</td>
</tr>
<tr>
<td>- Reproductive tract pathology</td>
<td>= 118 (31.3%)</td>
</tr>
<tr>
<td>- Endocrine disease</td>
<td>= 33 (9.0%)</td>
</tr>
<tr>
<td>- Abnormal karyotype</td>
<td>= 1 (0.2%)</td>
</tr>
<tr>
<td>- Anestrus</td>
<td>= 20 (5.3%)</td>
</tr>
</tbody>
</table>

Table 1: Total incidence of cases of canine female infertility and relative incidence of causes of infertility in the period 1990-2000 (Theriogenology Service - Small Animal Hospital, Faculty of Veterinary Medicine, University of Pisa - Italy)

Canine ovulation is reported to occur as early as day 5 or as late as day 23 after proestrus onset. Although occasionally a day 12 breeding can cause infertility in bitches ovulating late in their cycle, in our experience early rather than late ovulation is a more frequent cause of infertility. One clinical case of early ovulation is described. A healthy and reproductively normal 6-year old German Shepherd female had cycled regularly every 6 months throughout her life, and had never conceived following breeding on four different occasions in the past, always on day 12 or 13 of her season. Using vaginal cytology she was demonstrated to ovulate on day 5, was bred accordingly and whelped 6 puppies.
Performing vaginal cytology as well as checking the bitch’s behavior to look for onset of male receptivity are the 2 most practical ways of determining the best time for breeding. Owners should be instructed to bring their bitch to a male dog to check her behavior regularly as soon as possible after proestrus onset as well as to have a vaginal smear taken from the veterinarian every 2-3 days. Breeding should be performed as soon as the bitch stand and/or as soon as her smear is fully cornified, in order not to miss early ovulators. However, behavior does not always correlate with vaginal cytology: some bitches will not stand to be mounted even though their smear is fully cornified. Provided that vaginal abnormalities (strictures, bands of tissue, hymen) are ruled out, serum P4 as well as using different male dogs (to rule out male preference) are helpful in such cases, although some bitches become receptive to mating only in mid to late estrus. One clinical case is described of a 3.5-year old healthy Pomeranian bitch who ovulated on day 9 but did not accept breeding from any of 6 different males until day 16 when she finally became receptive, was bred and whelped 4 puppies on day 57 post-breeding.

Ovulation should always be timed using serum P4 assay every 2-3 days and the bitch should be bred when a high P4 value is observed (>5.0 ng/ml). Vaginal cytology should also be used to confirm serum P4 data (serum P4 kits which use a semi-quantitative colorimetric system are only 80% accurate) during and (most importantly) after breeding until the first day of cytological diestrus (D1) is identified, which occurs 6-8 days after ovulation.

Pyometra

Apart from mismating, reproductive diseases play a big role in canine infertility. Uterine problems are the most common cause of failure to conceive, while ovarian and oviductal pathology are reported in the dog but poorly documented. Canine pyometra is a disease characterized by presence of cystic endometrial hyperplasia (CEH) and high serum P4 concentration. CEH develops normally during the canine luteal phase due to prolonged endometrial stimulation from high serum P4 concentration, and regresses at the end of diestrus. In the adult or ageing bitch CEH tends not to regress completely and therefore accumulates from one cycle to the following. Larger sections of the endometrium become cystic and/or hyperplastic and may therefore be unsuitable for embryonic development. CEH is often characterized by prolonged and/or abnormal vulvar discharge at the end of estrus due to excessive bleeding from a hyperplastic endometrium. Presence of excess blood within the uterus facilitates bacterial contamination, which may lead to pyometra. Open cervix pyometra should be treated with specific antibiotics (as indicated by culture and sensitivity test on material obtained from a cervical swab) and PGF2α (100-150 mg/kg) BID to be continued until the uterus is fluid filled and/or serum progesterone is ≥ 2.0 ng/ml. Table n° 2 shows dosages of prostaglandins commonly used in bitches and queens. Pyometra has a higher incidence of occurrence in bitches not whelping regularly if compared to those who breed regularly, as well as a higher incidence of recurrence in the non pregnant bitch. Therefore, once the bitch has recovered from a pyometra she should be treated aggressively at the onset of the following proestrus with specific antibiotics for about 15 days or until after ovulation, bred with good management to a proven stud, and then spayed as soon as reproductive life ends.

<table>
<thead>
<tr>
<th>Prostaglandin F2α</th>
<th>Daily dosage in the bitch/queen</th>
<th>N° treatments/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural PGF2α or Dinoprost</td>
<td>Bitch - 100 µg/kg (0.1 mg/kg)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Queen - 500 µg/kg (0.5 mg/kg)</td>
<td>2</td>
</tr>
<tr>
<td>Cloprostenol</td>
<td>Bitch - 1-5 µg/kg (0.001-0.005 mg/kg)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Queen - 5 µg/kg (0.005 mg/kg)</td>
<td>1</td>
</tr>
<tr>
<td>Alfaprostol</td>
<td>Bitch - 20 µg/kg (0.02 mg/kg)</td>
<td>2</td>
</tr>
</tbody>
</table>

Table n° 2 – Dosages of the most commonly used prostaglandins in bitches and queens

Antiprolactinic drugs can also induce luteolysis if administered during the second half of diestrus. Table n° 3 shows dosages of antiprolactinics commonly used in bitches and queens.
Antiprolactinic | Daily dosage in the bitch/queen | N° treatments/day
--- | --- | ---
Cabergoline | 5 µg/kg | 1
Bromocriptine | 10-30 µg/kg (*) | 2
Metergoline | 500 µg/kg (*) | 2

Table n° 3 – Dosages of the 3 antiprolactinic most commonly used in small animals. (*)
There is no scientific information available for the queen.

Progesterone antagonists act by blocking progesterone receptors, causing opening of the cervix and in most cases a resumption of miometrial contractility. They are reported to be very efficacious for closed-cervix pyometra. Reported dosages are 6mg/kg twice daily on the first day followed by the same dose once daily on days 2, 3 and 4. Some authors prefer to use a larger dose (10 mg/kg) once daily to be repeated after 48 hours. (Fieni,1998).

**KEY FACTS:**
1 - Canine ovulation may occur as early as day 5 days or as late as day 23 after proestrus onset.
2 - Onset of receptivity to mating in normal bitches may occur during proestrus (up to 11 days before ovulation) or at mid-estrus (up to 6-7 days after ovulation)
3 - Ovulation should be confirmed assaying serum P4 and using vaginal smear every 2-3 days until a high P4 value and the first day of cytological diestrus are observed
4 - Pyometra is characterized by cystic endometrial hyperplasia and presence of high serum P4 concentrations.
5 - Treatment includes specific antibiotic and - if the cervix is open - PGF2α to be administered BID in diestrus (to cause luteolysis) or once daily in anestrus.

**References**
Fieni, F. - Proceedings 1° EVSSAR Congress, Barcelona 1998, p 199
Figure 1 - Algorithm for clinical approach to infertility in the bitch