

CANINE REPRODUCTION SEMINAR

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GENERAL

There is a single follicle wave in a bitch's cycle (as in mares, women, pigs, hamsters etc.) but the wave is protracted when compared with other animals and follicles only mature every three to eight months.

Bitches are not really inactive between estrous periods. Their follicles are just small and produce small amounts of steroids. In fact, if a bitch is ovariectomized during the months between estrous periods, pituitary gonadotropin (LH and FSH) concentrations rise dramatically, illustrating the recent effect of steroid feedback and providing proof that the ovaries were not inactive at all. In these bitches urinary sphincter tone also diminishes significantly within days after the removal of the ovaries.

Although the inter_estrus period varies between three and eight months and is quite variable between cycles, some breeds are known to have consistently shorter inter_estrus periods than others, e.g., German Shepards _26, versus Collies 36 weeks. Also, as bitches grow older, their inter_estrus periods tend to become longer. In fact, inter_estrus intervals of nine to 12 months seem to be normal for some old bitches. Unfortunately most of these reports are anecdotal and the specifics of this trend are not known.

SEASONAL EFFECTS

In one Beagle breeding colony it was

noticed that there were twice as many estrous periods in the spring and summer than during the second half on the year. This appears to be reflected by the incidence of reproduction cases in practice as well, i.e., more cases relating to breeding seem to occur in the spring and summer than later in the year. Such findings are not surprising because wolves & foxes are naturally spring-time breeders. Interestingly, wild canidae (and perhaps domestic bitches too) are probably not responding to increasing photoperiod, but to the delayed effect of decreased photoperiod of the previous fall because they become reproductively active even before day length starts to increase in late December.

Note: Basenjis's (a barkless' breed native to Afghanistan) are different to all other canids, breeding most often during the period of shortening day length, i.e., the fall.

PREDICTING THE TIME OF OVULATION.

See this web site for a breeding chart: <http://www.upei.ca/~lofstedtopence/wait.html>

Besides being the best method of achieving optimum fertility, the time of ovulation must often be predicted accurately to test_breed apparently "infertile bitches" under ideal conditions. (Interestingly this usually reveals that they are normal!). It is also used for AI with cooled_shipped or frozen_thawed semen. In the former case, air freight costs can be cut to a minimum. In the case of frozen semen where surgical insemination is

There is a single follicle wave in bitches.

Wild canidae are spring breeders.

most often done, only a single insemination is performed and therefore, it must occur at the best possible time for conception.

Another advantage of knowing the time of ovulation is that the time of whelping can also be accurately predicted; it consistently occurs 65 days \pm 1 day after the LH surge. This is much more accurate than monitoring the behaviour of the bitch in late gestation looking for a pre-partum decrease in body temperature. The probable time of whelping is valuable information for owners but is also useful to veterinarians to determine the time for pregnancy diagnosis (as early as 18 days after the LH surge) or elective C-sections (the last three days of gestation).

Detection of the LH surge is the diagnostic cornerstone of breeding in dogs.

It is now quite easy to detect the LH surge in routine veterinary practice because of the advent of rapid and easy patient-side progesterone and LH assays (see opposite). In contrast, radioimmunoassay and ELISA tests for progesterone are widely available but radioimmunoassays for LH are specialized and not performed routinely.

Ovulation occurs during the first four days after the LH surge but canine oocytes ovulate in an earlier state of meiosis than other domestic animals. Therefore they have to complete the first, as well as the second reduction division of meiosis before fertilization can occur.

A brief review of meiosis follows for

those who feel they need to know the inner workings of the process:

During meiosis I, oocyte DNA doubles to a 4n state and crossover of the chromatids occurs (to affect genetic diversity between parent and offspring) then the double DNA chromatids separate in two cells, completing the first stage of meiosis. In that process, an immature oocyte and the first polar body are formed. Both are haploid in genetic information but still contain twice the amount of DNA of a gamete. In most species and probably in dogs as well, when a sperm penetrates one of these newly ovulated, immature oocytes, it stimulates the onset of meiosis II, causing the chromatids to split again, forming haploid oocytes with half the amount of DNA and half the genetic code of its adult diploid form. The same process occurs irregularly in the polar bodies and up to three polar bodies may form in the first few days after ovulation.

With all these processes occurring, it takes several days for a canine oocyte to become fertilizable, at least four days after the LH surge. However, within two days after they have matured to a fertilizable state, the oocytes begin to degenerate. In contrast to sperm, they have a very short life. Therefore the fertile period in bitches is actually quite short, only three to four days during estrus. It is fortunate that sperm can last for long periods in the uterine tubes (Fallopian tubes) because they are usually still ready and able to fertilize when at last, the oocytes have matured. In fact, single fertile matings up to seven days before the LH peak are common, indicating that sperm can

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Detection of the LH surge is the diagnostic cornerstone of breeding dogs.

remain fertile in the female tract for up to 10 to 12 days after breeding.

DETECTING THE LH SURGE.

Vaginal mucosal shrinkage, vaginal cytology, vulva compressibility, vaginal electrical resistance, vaginal mucus “ferning” patterns and elevated serum progesterone concentrations are all correlated to the time of the LH surge. By themselves however, vaginal cytology, vulvar compressibility, ferning and vaginal mucosal shrinkage are too loosely correlated with the LH surge to be reliable indicators of the surge.

When it is essential that the time of the LH surge be accurately detected (surgical AI, shipped semen etc.) we use the following approach:

Vaginal cytology is only be relied upon during the first few days of proestrus and when there is obvious evidence of cornification (50% or more) daily blood samples are taken. To start, we suggest that progesterone should only be measured in every second serum sample. These can be run in your own practice using any of the commonly used progesterone ELISA assays. **Although cow-side milk progesterone assays are suitable, they are becoming difficult to obtain as demand has not be strong for them.** One that is made specifically for dogs is “Status Pro” (Synbiotics, San Diego, Ca). Most of these assays change color when the concentration of serum progesterone exceeds 2ng/ml. This is fortuitous because serum progesterone concentrations usually exceed

2ng/ml on the same day as the LH surge!

In our clinic we run radioimmunoassays for progesterone because it is possible to tell when progesterone probably began to rise above baseline concentrations. By contrast, when simple ELISA assays are used, it is impossible to determine how high the serum progesterone concentration is once the negative_ positive color change has occurred, i.e., the initial rise in progesterone could have occurred on the day of sampling or the day before. If an actual number is available for serum progesterone concentration, the initial rise in progesterone is easier to determine. For example, if the serum progesterone concentration is 7.8ng/ml., we know that the rise probably began the day before (progesterone concentrations rise rapidly). In the case of a sample with a progesterone concentration of 3.1ng/ml, the progesterone concentrations are probably rising above baseline concentrations on that very day. This is the day of the LH surge!

When an ELISA assay such as “Status Pro” is being used for progesterone assay, testing should occur every day.

Ideally, the serum LH concentration should also be measured so the day of the LH surge is verified beyond doubt. Therefore, when the approximate first_rise of progesterone is detected, the serum samples on that day and the day before should be tested using the “Status LH” test. The Status LH test is widely available in the US and recently, in Canada too.

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Occasionally, the LH surge cannot be detected because it is less than 24 hours in length and sampling intervals may straddle the surge. In such cases, one has to rely entirely on serum progesterone assays.

Even if the LH surge itself is not detected, detecting progesterone alone is far more accurate than performing vaginal cytology alone. With vaginal cytology, the first peak in cytology may occur three days before or after the LH surge, an unacceptable level of accuracy for the management of infertility, transported semen and certainly for the use of frozen_thawed semen. By comparison, serum progesterone concentrations can indicate the day of the LH surge to within a standard deviation of about one day.

Once the LH surge detected (either directly or indirectly) transported cooled semen can be shipped to the bitch owner. It is inseminated on days four and six after the surge. Therefore, two shipments are required for optimal fertility. Non surgical insemination of frozen_thawed semen should be done on the same days but in the case of surgical insemination, surgery is performed on day five after the LH surge.

The interval between the LH surge and the fertile period is very valuable in practice because it gives one plenty of time to obtain the results of hormone assays and make arrangements for shipping, anesthesia for surgical insemination etc. The corollary of this is that owners become nervous because their bitches are bred so long after they normally would have been bred, i.e., by sexual

receptivity alone. Some client education is usually in order.

Levels of fertility to expect

Data generated in more than 200 bitches indicated that about 95% of normal bitches should become pregnant if they are bred at the optimal time. Even in the so called "problem" bitches in that study, pregnancy rates exceeded 75%! In another large group of bitches, it was shown that the pregnancy rate increased by 4_5% if breeding occurred more than once. The number of puppies per litter increased as well, presumably due to the fertilization of a few late_maturing oocytes.

Other methods of detecting ovulation include:

LAPAROSCOPY:

Although developing follicles can be seen on the surface of the ovary about 10 days before ovulation using laparoscopy, the ovarian bursae normally cover the ovaries in bitches so ovarian structures can only be seen in surgically prepared bitches. Therefore, laparoscopy has no value as a routine clinical procedure to determine the time to breed a bitch.

ULTRASOUND:

The ovaries are situated caudal and ventral to the caudal poles of the kidneys therefore, the kidneys are primary landmarks to locate when one examines the ovaries. They are close to the abdominal wall in the standing bitch and caudo_lateral to the kidneys in recumbent bitches.

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Days 4 to 6 after the LH surge are the most fertile.

It is much easier to obtain an ejaculate when an estrual bitch is present;

The ovaries are generally anechoic until about five days before the LH peak and at that time, with good equipment, follicles can be recognized as discreet anechogenic structures. Follicles reach about 0.5 cm in diameter around the day of the LH surge and within four days after the LH peak (with echogenicity varying greatly from one animal to another) they change gradually from anechoic spheres to a mixed population of echogenic structures as they ovulate.

Corpora lutea can be seen for the first time as soon as one day after the LH surge but by day five after the LH surge, they are present in all bitches.

Ovulation is seldom seen as a distinct event in bitches because canine follicles do not luteinize abruptly as is they do in farm animals. Cystic corpora lutea are also common, giving the impression that some follicles have not yet ovulated. In many cases, the only sign of ovulation is gradual thickening of follicle walls due to luteinization.

Interestingly, the number of follicles that are visible on ultrasound does not correlate well with the number of oocytes that actually ovulate. This is because 20 to 30% of canine follicles are “poly_ovular oocytes.” This means that they contain more than one oocyte; the highest number recorded in one follicle being 17. Therefore, in theory at least, a whole litter of pups could be born from the product of a single ovulation!

Because of these challenges, we do

not use ultrasonography to monitor bitches for breeding. However, if you do wish to examine the ovaries, for example in cases of cystic ovarian disease, a “standoff” should be used so that the ovarian stroma is in the focal zone for single zone transducers (otherwise the optimal focal zone is too deep for optimal resolution of ovarian structures). Some transducers have built_in standoffs.

Although a 7.5 MHz transducer can be used to observe canine ovaries, a 9.5MHz transducer is optimal. However, even with a 7.5 MHz transducer, the uterus can be imaged fairly easily, especially during proestrus and estrus when a “starburst” pattern similar to that in mares is seen.

Ultrasonography is also very useful to demonstrate pregnancy, pyometra and cystic ovarian disease.

Artificial insemination with fresh semen

SEMEN COLLECTION

Ideally, several days of sexual rest should be allowed prior to semen collection and evaluation.

Canine semen is most easily collected using masturbation with a gloved or bare hand, although some operators find the process distasteful and prefer to use an artificial vagina (AV). However, most artificial vaginas are made of latex and most latex is spermicidal. In addition, these artificial vaginas cannot be gas sterilized because the residues of gas sterilization are also toxic to spermatozoa. If an AV is used, it

should be cleaned by washing and chemical disinfection then rinsed several times with distilled water and air dried. The artificial vagina and attached plastic tube are warmed to body temperature and lubricated with a small amount of sterile aqueous lubricant.

It is much easier to obtain an ejaculate when an estrual bitch is present; therefore owners should be asked to bring in teasers when appointment are made. Alternatively, a non estrous bitch of the same breed or size may be used. A commercially available pheromone (methyl paraben _ "Eau d'estrus, Synbiotics corporation at <http://www.synbiotics.com> , phone 1_858_451_3771, fax at 1_858_451_5719) may be used to stimulate the male but we have no experience in its use. It is sometimes very difficult to collect semen if no bitch is available at all can it can be done.

Optimally, the male and female are brought together on leashes in a quiet room with nonslip flooring. As the dog sniffs at the bitch's vulva or mounts her, the collector quickly moves the prepuce back, behind the bulbus glandis and directs the tip of the penis into the AV, held in the left hand. Once the artificial vagina is slipped onto the penis, the right hand is used to hold the artificial vagina onto the penis while exerting firm pressure around the back of the bulbus glandis. Once this occurs, the dog will usually show pelvic thrusting and normal ejaculation. An AV is certainly not essential to collect semen from dogs. Excellent ejaculates can be obtained by hand

collection alone.

If a gloved or bare hand is used instead of an AV, the dog is masturbated rapidly for a few seconds until he gains a full erection. In the process, the prepuce is slipped behind the bulbus glandis. Masturbation ceases and the hand held behind the bulbus glandis using very firm pressure, until ejaculation is complete. The other hand is used to hold a plastic bag over the end of the penis.

Almost any warm receptacle can be used to collect the semen but most commonly sterile "Whirl_pak" bags are used. Syringe casings and other hard objects should be avoided as the penis is very easily traumatised during collection and substantial bleeding may occur into the ejaculate. This does not seem to decrease fertility in dogs (Cf horses) but it interferes with semen evaluation and of course, alarm owners.

Semen can be collected when the bulbus glandis expands within the prepuce but some dogs object to this. Therefore, it is usually best to be sure that the bulbus glandis is out of the prepuce before it expands.

The reader can see therefore than the term "masturbation" is perhaps, misleading. Most of the contact time consists of pressure exertion behind the bulbus glandis; a process identical to that used with an artificial vagina!

Ejaculation occurs intermittently over a variable period, perhaps five to 15 minutes, usually just long enough to deprive the collector (squatting on the floor) of all blood flow and feeling to the legs.

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If pressure is maintained firmly around the bulbus glandis, pulsations can be palpated in the urethra. The anus will also be observed to contract in a rhythmic fashion. The dog may stop ejaculating for several minutes then pulsations will resume.

Initially, a few drops (one to 3 ml) of clear to slightly cloudy pre sperm fraction are ejaculated, followed by a



Semen collection: Note how a firm grip is maintained behind the bulbus glandis. The penis is being deflected caudally.

whitish sperm_rich fraction (0.1 to 6.0 ml) but most often these fractions are mixed and only a homogeneous light grey_opalescent ejaculate is obtained. The collector should try keep one hand around the collection vessel to keep it near body temperature. This is easiest when a plastic bag is used as a collection vessel.

Soon after the dog begins to ejaculate, he will often lift his hind limb as though attempting to step into the rump_to_rump position that occurs during natural breeding. If this is observed, the collector should allow the dog to step over his/her arm so that the penis then extends out caudally from the dog.

Soon the clear, third fraction of the ejaculate (mostly prostatic fluid) is ejaculated increasing the volume to as much as 60 ml. If the semen is being collected for artificial insemination as well as evaluation, enough prostatic fraction is collected to bring the total volume to three to 10 ml so large numbers of sperm are not lost in the insemination process and the insemination volume is comfortable to work with. Frequently, only a few ml of semen are collected but total sperm numbers, not semen volume, is what is important in A.I.

After collection is complete, the male is observed until his erection subsides.

Paraphimosis may occur following collection, so the dog must never be kennelled or sent home until the penis is completely inside the prepuce. To prevent paraphimosis, one should lubricate the preputial opening liberally after semen collection.

SEMEN EVALUATION

Semen should be kept at a 35 to 37°C until progressive motility has been determined, after which it can be allowed to cool to room temperature. Semen volume is not important, but it is necessary to record the volume of the sperm_containing

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portion so that the total number of sperm per ejaculate can be calculated. Sperm cell motility, morphology, and concentration are determined in the conventional manner.

Normal prostatic fluid makes a good diluent if one is required for motility estimates. It can also be pooled and frozen for future use as a diluent for per vagina insemination of frozen-thawed semen (a relatively new approach to the use of frozen semen).

Normal ejaculates have these characteristics (also see chart on p. 18)

Colour: Opalescent to milky white with a clear prostatic supernatant or homogeneous greyish white.

Volume: Pre sperm fraction: 0.1 to 3 ml, Sperm-rich fraction: 0.1 to 6 ml Prostatic fraction: one to 50 ml Total volume: one to 60 ml

Progressively Motile Sperm: 60 to 90%

Number of Sperm per Ejaculate: 60 to 600 X 10⁶

Morphologically Normal Sperm: 70 to 90%

Bacteria: Many; usually more than 10,000/ml. However, only the presence of many white blood cells is an indication for bacterial culture of the semen.

The presence of epithelial cells, red blood cells, inflammatory cells, and germinal epithelial cells are noted under low magnification. All cells other than sperm (COTS) are easy to see if a smear is stained with

Wrights_Giemsa or Diff_Quick but difficult to differentiate using common sperm morphology stains.

Semen from males that have not ejaculated recently may contain more epithelial cells and debris than semen from a male that is used frequently but if large amounts of debris or dead sperm are present, a second sample should be collected 24 hours later.

ARTIFICIAL INSEMINATION

Artificial insemination is performed when behavioral problems prevent mating (especially female dominance) when mounting is difficult or unlikely due to orthopedic problems or inexperience, or when transported or frozen semen is used.

Semen is collected from the dog using masturbation or an artificial vagina and only the sperm-rich fraction and a small amount of prostatic fluid is used (total volume three to 10 ml).

Semen collection should not be more frequent than once every two days. Daily ejaculation results in very low concentrations of ejaculated sperm after five to seven days.

Artificial insemination of the bitch is most easily performed by depositing the semen in the cranial vagina with a Cassou sheath shortened to about 25 cm. These sheaths are normally used to cover the rigid A.I. Cassou rods used for inseminating cattle. They are available from any veterinary supplier or A.I. cooperative.

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Artificial insemination of the bitch is most easily performed by depositing the semen in the cranial vagina with a Cassou sheath shortened to about 25 cm.

They are soft and flexible and therefore, far superior to the rigid plastic cattle inseminating pipettes sometimes used. In addition, they fit directly on a syringe and do not require adapters like the rigid pipettes. The Osiris apparatus from France is recommended by some operators but its superiority to other methods has not been demonstrated.

In all cases, the vulva is washed, rinsed and dried. The pipette is then inserted into the vagina, first dorsally for several centimeters, then cranially until the cervix is reached. If resistance is felt, the pipette is withdrawn one or two centimeters, then reinserted at a slightly different angle. The vagina of the bitch is long and an insemination pipette may have to be inserted to a depth of more than 20 cm in large bitches.

The hindquarters of the bitch are then elevated so that the spinal column is at an angle of 45 _ 60 degrees and held there for as long as possible, up to 10 minutes. One excellent study demonstrated that this was advantageous for sperm transport. Some inseminators also stroke the dorsal wall of the vagina with a gloved finger ('feathering') or massage the clitoris for about one minute when the hindquarters are elevated. This may promote semen transport within the uterus but it has not been objectively studied.

After the hindquarters are released, the bitch should not be allowed to squat or jump for another 10 to 15 minutes.

It is usual to inseminate the total volume of undiluted ejaculate and

prudent to use the ejaculate as soon as possible after collection. Insemination doses should contain at least 200 million motile sperm because fertility decreases with ejaculates containing less than 50 million live cells. Single estrus pregnancy rates up to 90% can be achieved with A.I. using fresh semen in fertile dogs.

Optimal extension rates for dogs have not been studied but experience with horses suggests that it should be extended at a rate of between 1:1 to 1: 6 (semen: extender) for transport or if it is to be used more than an hour or two after collection. In most cases, a dilution rate of one part semen to two parts extender works well. It is also packed and transported in cooling containers like those used for equine semen transport.

The "Equitainer" (Hamilton Thorne; sales@hamiltonthorne.com) cools the semen at 0.3oC/minute maintaining better motility than other cooling rates. Perhaps more important, this container has the best insulation on the market, a consideration when semen is to be transported through various temperature extremes.

Various semen extenders can be used for canine A.I. with satisfactory results. However a simple and effective extender can be made for shipped_cooled semen by heating skim milk to about 95oC for ten minutes in a double boiler. It is then cooled to 37 o C for use. The heating step denatures a spermicidal albumin component in milk. It can be frozen in 50 ml aliquots for several weeks but its exact shelf_life is

The "Equitainer" has the best insulation on the market, a consideration when semen is to be transported through various temperature extremes.

unknown. A specific canine extender called "Fresh express" is available from the Synbiotics corporation at <http://www.synbiotics.com> (phone 1_858_451_3771 or fax at 1_858_451_5719). Commercial extenders used for equine semen transport can also be used; for example Kenney's or "E_Z mixtin" extenders (arssales@dupreeinc.com or mntubcan@execulink.com) but no data exist to show that any of these extenders are superior to the others.

VAGINAL ENDOSCOPY

Vaginal endoscopy (vaginocopy) is the examination of the vaginal mucosa using an endoscope. The procedure is well tolerated in the non sedated, standing bitch. The examination can take as little as two minutes, and valuable information may be collected quickly and with minimal expense.

DETERMINATION OF THE OPTIMAL TIME FOR BREEDING

Vaginoscopic assessment is based upon observation of mucosal fold contours and profiles, the color of the mucosa, and of any fluid present. During anestrus, the vaginal mucosa is relatively flat, dry and red in appearance. At the onset of proestrus and during early estrus, the mucosal folds are enlarged, edematous, and pink or pink/white.

These changes are due to thickening of the mucosal epithelium and oedema accumulation within the submucosa; both of which are effects

produced by estrogen. At approximately the time of the LH surge, there is progressive shrinking of the folds, accompanied by pallor. These effects are probably the result of an abrupt withdrawal of the water retaining effect of estrogen, during its preovulatory decline. Subsequently, mucosal shrinkage is accompanied by wrinkling of the mucosal folds which become distinctly angulated and a dense cream to white color.

The onset of the fertile period can be detected by observing mucosal shrinkage without angulation, whilst shrinkage with angulation is characteristic of the fertilization period. Mating or insemination should be planned during the fertilization period, or four to six days after first detecting mucosal shrinkage. The end of the fertilization period is marked by a decline or cessation of mucosal shrinkage, combined with sloughing of the vaginal epithelium.

Vaginoscopic changes are useful in clinical practice because they are progressive, therefore it is not necessary to examine the bitch each day.

ASSESSMENT OF REPRODUCTIVE TRACT PATHOLOGY

Examination of the vagina using a rigid or flexible endoscope may be useful for diagnosing some causes of reproductive pathology. For convenience the examination can be divided into three parts: examination of the vaginal vault, examination of the external urethral orifice, and examination of the cervix.



Abnormalities of the vaginal wall

Cases of vaginitis which may be primary or secondary to a specific cause such as a foreign body, and can be diagnosed by reddening of the vaginal wall and the overproduction of vaginal mucus. In many cases, the underlying cause is obvious.

Bleeding of the vaginal wall due to trauma or the presence of varicose vessels may also be diagnosed by direct observation. Many of these cases require nothing other than conservative treatment. In bitches that experience pain during coitus (dyspareunia) the presence of vaginal strictures or hymenal remnants may be documented using vaginoscopy. In certain cases the endoscope may be used to place ligatures around hymenal remnants to allow their sectioning. In other cases an episiotomy may be required.

Abnormalities of the urethral orifice

The presence of a colored discharge from the external urethral orifice may be useful, often confirming pathology is associated with the urinary tract rather than the genital system.

Abnormalities of the cervix

Observation of the cervix may document abnormalities that interfere with the establishment of normal pregnancy.

Non_patency of the cervix or severe adhesions or fibrosis may cause such problems. Observation of the

cervix may also be useful for investigation of bitches with a vulval discharge. During pregnancy, this may be associated with resorption or abortion, or bleeding from the marginal hematoma of the placenta. Documentation of the site and the nature of the discharge may influence the management of these cases.

In non_pregnant bitches, the nature of the discharge can be assessed and when possible, material should be collected for cytological and bacteriological screening.

For a more accurate assessment of endometrial disease it is necessary to catheterize the cervix, either to allow direct endoscopic visualization of the endometrial surface, or to enable to collection of material for cytological and bacteriological investigation.

CATHETERIZATION OF THE CERVIX

It is very difficult to place a catheter through the cervix because the vagina of the bitch is long and narrow (14 to 22cm) and the cervical opening is small, and placed at a downward facing angle in the vagina.

Those contemplating this procedure should see the accompanying website for illustrations of the canine vagina and cervix.

Using a rigid endoscope, the cervix can be seen and the cervical os can be located accurately. A fine catheter may then be placed through the endoscope toward the cervical os. It is often necessary to place a

guidewire inside the catheter to increase its rigidity.

Once the catheter tip has been placed into the os, the guidewire is withdrawn slightly, and the catheter is pushed cranially, using a rotating action.

The catheter can normally be introduced the full length of the uterine body. The technique requires training and practice before it can be achieved reliably. Catheterization is simplest in medium sized dogs, and most difficult in giant breeds which have very long vaginas. It is also difficult in toy breeds where the size of the catheter is often too large.

Some bitches need to be sedated because movement of the bitch makes placement of the catheter very difficult.

The cervix is easiest to catheterize during estrus but is also quite relaxed during proestrus and anestrus (the time between estrous periods). In the immediate postpartum period, endoscopes can often be passed directly through the cervical os. Cervical catheterization is most difficult during the luteal phase (the first 65 days after ovulation).

Catheterization during estrus is most commonly performed for artificial insemination. However, it is also used to collect material from the endometrial surface by aspiration. Small cytology brushes can also be passed through cervical catheters.

DOGS

INTRODUCTION

Dogs only have a single accessory sex gland, the prostate (Tom cats have both a prostate gland and a pair of bulbourethral glands).

CLINICAL HISTORY

A relevant clinical history is essential and is useful for constructing a list of differential diagnoses. It should include previous breeding history, previous successes as a sire, treatments, and a general medical history.

PHYSICAL EXAMINATION

As always, a full clinical examination is mandatory to eliminate generalized systemic disease. Rectal palpation of the prostate gland is also possible. It should be symmetrical and globe shaped with a dorsal median furrow. The position of the prostate gland is variable, although it is usually intra pelvic. An increase in size occurs normally with advancing age.

Testes should be examined and compared for their size and consistency. The epididymis should be evenly attached to the dorsolateral surface of the testis. Particular note should be made of the epididymal tail which is usually pea_sized.

The prepuce should be examined for abnormal discharges and the penis should be exposed for inspection.

INVESTIGATIVE TECHNIQUE

Several techniques can be used to investigate reproductive disease in the dogs. Semen samples provide a form of biopsy and diagnostic information about both testicular and prostatic function.

Conventional testicular biopsies may be obtained by incision or needle aspiration but are not advisable in breeding animals since they are followed by marked inflammatory responses. In particular, incisional biopsies may cause local inflammation with tubular degeneration, fibrosis and oligospermia.

The measurement of serum (or plasma, the two are practically interchangeable) hormones may be used to confirm testicular failure. Although resting serum concentrations of testosterone, LH, and FSH fluctuate markedly, serial sampling or the response of serum testosterone to the administration of hCG or GnRH may be valuable.

Imaging of the male reproductive tract is also possible radiographically (with the caveat that ionizing radiation causes testicular degeneration) and more recently, ultrasonography has also been shown to be valuable in prostate and testicle examination.

Further evaluation of the prostate gland may be achieved by needle aspiration or biopsy of the gland, especially using ultrasound guiding.

Prostatic cells may also be collected

by rectal massage and urethral washing. Initially, a urethral catheter is passed into the bladder to allow drainage of urine then flushing of the bladder with saline. Subsequently, the catheter is partially withdrawn until its tip is within the distal prostate gland, as determined by rectal palpation. The prostate is then massaged per rectum or trans_abdominally for one to two minutes. Five to ten ml of sterile saline is then introduced through the catheter whilst compressing the urethra, distal to the prostate with a finger per rectum. In this way, cells massaged from the prostate gland are flushed into the bladder. These cells are then recovered by advancing the catheter and aspirating the contents of the bladder.

REPRODUCTIVE ENDOCRINOLOGY

Interstitial (Leydig) cells are the source of testosterone production but interestingly, also produce estrogens in substantial amounts by aromatizing androgens. The production of steroid hormones is mainly stimulated by LH, while FSH stimulates spermatogenesis by supporting Sertoli cells (Sertoli cells lie adjacent to spermatogonia within the seminiferous tubules). Both testosterone and estrogens exert a negative feedback effect on FSH and LH, the release of which is governed by gonadotropin releasing hormone (GnRH). Serum concentrations of all these hormones, fluctuate markedly during the day, especially those of .FSH and LH.

SEMEN COLLECTION

Semen collection has been described elsewhere. Besides being essential for artificial insemination, it is of cardinal importance in breeding soundness examinations.

Warming and insulation of collection equipment is essential to ensure that samples are not temperature shocked. Also, many items of equipment are toxic to dog sperm. Therefore, all plastic tubes, syringes and pipettes should be tested prior to routine use.

THE LIMITATIONS OF SEMEN EVALUATION

Although semen evaluation is helpful, conventional analysis does not assess functional competence of spermatozoa. Breeding trials under carefully controlled conditions are the gold standard.

SPERM MOTILITY

Motility is temperature dependent. Samples should always be evaluated at a standard temperature on a heated stage. Conventionally, this is 37 to 39 degrees C, with the latter temperature being more appropriate as it is the average vaginal temperature in bitches.

Motility is evaluated in a subjective manner with the percentage of sperm expressed as a percentage of progressively motile sperm.

Alternatively, it can be categorized as follows:

O = immotile sperm; I = sperm that are motile but not progressive; II = sperm that have sluggish motility and poor progression; III = sperm with reasonable motility and moderate progression or finally, as IV = sperm with rapid forward progressive motility.

Only category IV spermatozoa have normal motility. Although it has been suggested that a lack sperm motility is currently the best predictor of infertility, there are as yet, insufficient studies to substantiate such a relationship.

Most fertile dogs have greater than 70% of sperm with category IV motility.

SPERM NUMBERS

The total number of spermatozoa per ejaculate is the most accurate measure of sperm production. Total sperm numbers are calculated by multiplying the sperm rich volume by its spermatozoal concentration. Most commonly, concentration is measured using a hemocytometer.

A relationship has been demonstrated between breed of dog and the number of sperm ejaculated; larger breeds generally produce more sperm. Surprisingly, for optimal fertility a total of only 200 x 10⁶ live normal spermatozoa are required.

SPERM MORPHOLOGY AND LIVE/DEAD STAINING

Sperm morphology is best examined using special morphology stains but conventional Wrights_Giemsa or Quick proprietary stains will often suffice. In fact, the latter stains may

when the percentage of live normal sperm declines below 60%, there is a statistically significant reduction in fertility. This does not mean that dogs with lower values are infertile, only that their fertility is sub_optimal.

Eosin-nigrosin stain can be obtained from the suppliers listed in the accompanying website entitled "Suppliers" Per-

Factor	Average value	Standard error of the mean	Range of values
% Progress. motile	86	+0.8	40-95
Volume	1.3	+0.05	0.4-3.2ml
Sperm concentr. mill/ml	318.4	+11.3	50-590
Total sperm	409.8	+16.6	36-650
% Live sperm	76.5	+1.1	52-92
<i>Unpublished data from 60 dogs that were fertile for six months prior to, and after these examination. G. England</i>			

be very valuable to study blood cells (neutrophils and red blood cells) when they are present. Through "vital staining" i.e. using a combination stain such as nigrosin_eosin, details of sperm morphology can be studied in both live (unstained) and dead (pink_stained) sperm. Although this practice is of questionable value in other domestic animals, it has been shown in dogs that

haps an even better stain is the special sperm morphology stain available from the Lane supply company in the United States

PRESENCE OF OTHER CELLS

Common hematology stains, e.g., Diff_Quik, can be used to identification of other cells other than sperm (COTS) in the ejaculate. Neutrophils are common, usually originating from the pre-

puce but large number of inflammatory, neoplastic, or red blood cells may indicate prostate disease. Such changes also result in the aggregation of sperm and reduced fertility.

MICROBIOLOGY

Many aerobic and anaerobic organisms are frequently isolated from the prepuce of the dog and the bacterial flora is usually mixed. These bacteria, including beta hemolytic *Streptococcus* spp., are now considered to be commensal organisms.

ARTIFICIAL INSEMINATION

Whilst vaginal insemination is easy to perform, there is usually no physiological response from the bitch; vaginal and uterine contractions do not occur, and there is poor transportation of sperm into the uterus. This is particularly important if semen of poor quality is used, for example, frozen_thawed semen. In such cases, fertility can be low following vaginal insemination. Therefore, techniques have been developed to allow uterine insemination.

Vaginal insemination

Semen is deposited into the vagina close to the cervix using a long inseminating pipette, and the pipette is flushed with warmed physiological saline. Some workers use the third fraction of the ejaculate to flush the catheter, whilst others use physiological saline. The vagina should be stimulated using a finger in an at-

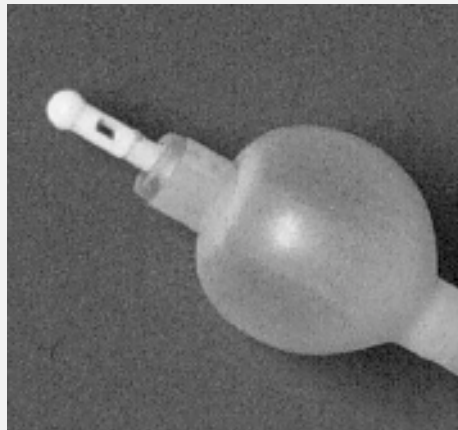
tempt to initiate vaginal contractions and assist in sperm transport to cranial portions of the reproductive tract.

It is common to raise the hindquarters of the bitch to ensure that the semen runs cranially and pools around the cervix. One elegant study showed that this practice increased sperm numbers in the cranial tract significantly in comparison to control bitches.

Uterine insemination

It is very difficult to place a catheter through the cervix because the vagina of the bitch is long and narrow and the cervical opening is small, and placed at an angle to the vagina. Several methods have therefore been developed to achieve uterine insemination:

(i) Foley _ Osiris technique: A Foley catheter ("Osiris" catheter, manufactured by IMV, France) may be used



The tip of an Osiris catheter. Note the central catheter tip with a side discharge port. The rounded tip of the inner catheter is pulled back after insemination, blocking retrograde flow of semen into the vagina .

Essential knowledge:

In the Egyptian story of the Trinity, Horus was magically conceived by Isis and the dead god, Osiris. Osiris was cut up into fourteen pieces and scattered all over Egypt by his brother. Isis found thirteen of the fourteen dismembered pieces; all but the phallus.

In the form of a bird, she put Osiris together and resurrected him by blowing life into him with her wings. Even without a physical member, Osiris inseminated Isis, and she conceived Horus.

for effective uterine insemination.

The catheter has two components, an outer sheath with the Foley bulb positioned at its tip, and a second catheter with a side exit port which is inserted through the outer sheath. The outer catheter is inserted and the balloon is inflated. The inner catheter is then advanced and the semen is deposited, after which the inner catheter is withdrawn inside the Foley thereby closing the exit ports. By using this catheter, semen can only run forwards through the cervix and does not drain back along the vagina. The device is left in place for up to 15 minutes to ensure that a pool of semen is located next to the cervix. The Foley bulb may also simulate the copulatory tie and result in vaginal contractions.

The principal of the technique is excellent, but there is often leakage of semen around the balloon and retrograde, through the catheters.

(ii) Norwegian technique.

This device (available from Dr. J. Ougner, Norway) consists of an outer plastic sheath and inner metal catheter with rounded, bulb-ended tip. The outer plastic catheter is inserted into the vagina to the level of the cervix. The cervix is then palpated trans-abdominally and realigned so that it is continuous with the direction of the vagina. The inner catheter is pushed forwards to the cervix and manipulated through the cervix.

This technique works well but requires training and may be difficult to perform in large, obese or nervous bitches.

(iii) Endoscopic technique.

This method employs a rigid endoscope which is inserted into the vagina and advanced to the level of the cervix. An inseminating catheter is inserted through the endoscope and directed into the cervical os. The technique also requires training before catheterization can be achieved reliably. As expected, some bitches also need to be sedated for the procedure.

(iv) Surgical technique:

This technique has gained wide acceptance and is often advocated since it requires no special training. Consideration should however be given to the ethics of performing a surgical procedure simply to achieve a pregnancy.

A small caudal ventral midline laparotomy incision is made and the uterine body is lifted into the incision. A 22_gauge over_the_needle intravenous catheter is placed into the lumen of the uterine body after a small puncture wound has been made with the blunt end of a suture needle. Padded bowel clamps are placed across the cervix to prevent escape of the semen into the vagina. The semen is introduced slowly into the uterus and allowed to run proximally into the uterine horns. Following removal of the catheter, pressure is applied to the injection site to provide hemostasis. No uterine sutures are required and the abdominal incision is closed routinely.

SEMEN PRESERVATION

Fresh semen

This is also discussed elsewhere. Fresh semen insemination may be useful in a number of different circumstances including when the bitch is unwilling to stand to be mated, when mating is difficult because of differences in size, or when the male is unable to mate normally because of age, debility or non inherited disease.

Semen may be inseminated immediately after collection, or can be stored for three or four hours by allowing it to cool to room temperature. For longer periods, semen should be diluted and cooled.

In most cases vaginal insemination is suitable but if the semen quality is poor it may be necessary to perform uterine insemination. The success rate with fresh semen insemination depends upon its quality and the fertility of the bitch. Assuming that both are normal and insemination occurs at the optimal time pregnancy rates of approximately 85% should be expected.

Chilled Semen

Semen can be chilled and stored for up to 48 hours and in some cases up to 72 hours. Chilled semen is increasingly used for transportation between countries and in general, fertility is greater than with frozen_thawed semen. Semen is diluted in an extender to protect the sperm during the cooling and re warming process. The extender also

provides energy and buffers pH changes during storage. Several extenders are used commonly with the choice of extender being based on semen storage tests conducted with each dog.

Besides the skim milk extender mentioned elsewhere, the following common extender can also be used:

Non fat dry milk (2.4 g) glucose (4.9 g) sodium bicarbonate (0.15 g) and sufficient de ionized water to make the volume up to 100 ml. Penicillin 150 000 IU and Streptomycin 150 000 mg may also be added if the semen is highly contaminated or if long storage times are anticipated. The semen is diluted and cooled slowly to five degrees C. As mentioned elsewhere, an Equitainer is well suited to semen cooling. In the absence of an Equitainer, chilled semen can also be transported in a small thermos flask containing crushed ice.

Semen deteriorates each day that it is stored and after two or three days, its fertility is approximately the same as for frozen_thawed semen.

When the semen is to be inseminated, it is warmed slowly to body temperature and its quality is reassessed. The semen can then be inseminated. Cervical or uterine insemination is used depending upon the length of time stored and the original quality of the sample.

For samples inseminated within two days of collection the pregnancy rates may be approximately 65% for fertile bitches that are inseminated at the optimal time.

Frozen_thawed semen

Semen that is required for long term storage must be frozen. Although frozen semen can be thawed as required, fertility is almost always lower, and associated costs are higher.

Many procedures have been described for freezing canine semen, a testament to the fact that none work well for every dog. Nevertheless, the following basic process can be used:

Semen is first diluted at room temperature with an extender containing Tris buffer (6.06 g) fructose (2.5 g) citric acid (3.4 g) and deionized water q.s. to 184 ml. To this, glycerol and egg yolk are added to a final concentration of 8% and 20% respectively. Penicillin and streptomycin are frequently (used as described above) to prevent bacterial growth while the semen is undergoing the cooling and thawing process. Antibiotics are not required at minus 196 degrees C, the temperature of liquid nitrogen.

The diluted semen is slowly cooled to 5 degrees C, allowed to equilibrate for up to four hours, and is then frozen, most commonly in 0.5 ml straws.

For practical freezing, straws are placed in a wire basket four cm over the level of n liquid nitrogen. They are held in the vapor for five minutes then lowered into the liquid nitrogen and after a few minutes, transferred with forceps to the canes used at storage units in liquid nitrogen containers.

Factors such as the height of the freezing basket over the liquid nitrogen and the number of straws frozen in each batch have been shown to be important in other species but are seldom addressed in literature on dogs.

One should standardize ones' freezing procedure and document all steps accurately making gradual changes as required. In the absence of substantial fertility data, changes can only be based on post thaw motility, which unfortunately is poorly correlated to fertility.

Thawing rates are important to cell survival, and straws of frozen semen are frequently thawed at specific rates by plunging them into a water bath at temperatures defined by the person who froze the semen. In the absence of specific thawing suggestions, the semen should be thawed in water at body temperature for at least 20 seconds. It should then be used as soon as possible.

The freeze_thaw process causes a considerable decrease in sperm morphology, motility and longevity. For this reason fertility rates are highest when uterine insemination is performed. Average international conception rates are approximately 40% per cycle.

TIMING OF INSEMINATION

As described in detail elsewhere, the timing of insemination is important but never so important as when frozen_thawed semen is used. Surgical insemination should occur

on day five after the first day that the serum progesterone concentration increases above 2ng/ml. If the initial rise in progesterone cannot be detected, the first (non surgical) insemination is planned when progesterone concentrations are 10 ng/ml and the second when values are 18_25 ng/ml (usually 24 _ 36 hours later).

NUMBER OF SPERM INSEMINATED

In general, when fresh semen is used, the whole ejaculate is collected and inseminated. The situation is similar for chilled semen except that the semen has been diluted at a ration of 1:1 to 1:2 (semen: extender) and consequently the volume increases.

When frozen semen is used, it is most sensible to thaw one straw after freezing so that the semen quality can be reassessed and the minimum number of straws can be established for successful insemination. Usually, a total of 150 to 200 million motile spermatozoa is inseminated on two to three occasions. Obviously, this cannot be done surgically.

DIAGNOSIS OF PREGNANCY

The peculiarities of the estrous cycle of the bitch and queen mean that endocrinological methods of pregnancy diagnosis cannot simply be adapted from other species. However, imaging technologies such as diagnostic B_mode ultrasound, and radiography are equally applicable to

all domestic species.

The diagnosis of early pregnancy in the bitch can be confusing because of the difference between the actual and the apparent length of pregnancy. Whelping actually occurs within a three_day period, about 60 days after oocytes become fertilizable.

Recall that oocytes are fertilizable when they become haploid, about five days after the LH surge. However, matings that occur six to seven days before the LH surge may be still be fertile. Also, oocytes can still be fertilized for two to three days after they have become haploid. Therefore, the apparent length of pregnancy can vary between 58 and 71 days. This information is important because bitches are frequently examined during early pregnancy and the examination may be undertaken before a positive diagnosis could be made, resulting in a false negative diagnosis. Incidentally, this problem does not occur in cats which are induced ovulators. In that species, pregnancy length can be estimated from the mating date.

In the following discussions, the day of the LH surge is used as a benchmark for staging pregnancy. Practitioners should attempt to do this as well, using a combination of vaginal cytology, serum progesterone and even LH assays. This will be of use when scheduling pregnancy examinations but will also be useful if the need for elective cesarian section arises.

The detection of the LH surge also allows one to predict the time of

whelping (65 days + 1 day SD after the LH surge) more accurately than any other method.

ABSENCE OF ESTRUS

In many species, the absence of estrus at 21 days can be used as an indicator of pregnancy. In the bitch, however, the inter_estrus interval is identical in pregnant and non_pregnant cycles.

BEHAVIORAL CHANGES

Both pregnant and non_pregnant bitches can exhibit behavioral changes such as mammary gland enlargement and nesting as serum progesterone concentrations decline, 30 days or more after the LH surge. This so-called "pseudopregnancy" is associated with the inverse relationship between serum progesterone concentrations and serum prolactin concentration. It also occurs when bitches with active corpora lutea (high serum progesterone concentrations) are ovariectomized.

Food intake usually increases by approximately 50% in the second half of pregnancy. However, it is common for pregnant bitches to have a brief period of reduced appetite approximately three to four weeks after mating.

PHYSICAL CHANGES

Pregnant bitches commonly develop a slight mucoid vulval discharge approximately one month after mating, but this can also be seen in non_pregnant animals. Its origin is obscure.

Body weight increases throughout pregnancy but becomes noticeable from day 35 onwards, and eventually increases by 50% of normal. Abdominal enlargement becomes noticeable from day 40 but may not be obvious in primigravida, in muscular bitches, or bitches with small litters.

Mammary gland enlargement is also usually obvious from day 40, at which time serous fluid can be expressed from the glands. Colostrum is present in the teats in the last seven days of pregnancy. Mammary changes vary considerably between primigravida and multigravida, and care should be taken when assessing changes in mammary size and secretion because similar features are common in pseudopregnant bitches.

ABDOMINAL PALPATION

Abdominal palpation can be accurate but problems can be encountered in muscular breeds, obese or nervous bitches.

The optimum time for the diagnosis of pregnancy is approximately one month after mating, at which time the technique is nearly 90% accurate in the hands of an experienced clinician.

By 25 days, the conceptuses have formed small, tense, spherical swellings with diameters varying between 15 and 30 mm. These are readily palpated in relaxed bitches.

After day 35, the conceptuses be-



A 28 day canine pregnancy. The fetal units are clearly palpable.

come elongated and begin to lose turgor, making them more difficult to palpate.

After day 45, the uterine horns fold upon themselves, resulting in the caudal portion of each horn being positioned against the ventral abdominal wall, and the cranial portion of the same horn being positioned dorso_cranially.

After day 55, the foetuses can often be identified especially if the fore-quarters of the bitch are elevated and the uterus is manipulated caudally toward the pelvis.

It is difficult to accurately count the number of conceptuses by palpation except when performing an examination at approximately day 28 in a relaxed and thin bitch.

IDENTIFICATION OF FETAL HEART BEATS

In late pregnancy it is possible to auscultate fetal heart beats in the bitch (and queen) using a stethoscope. They are not difficult to detect, since the heart rate is usually more than twice that of the dam. Fetal hearts may also be detected by recording a fetal ECG. Both of these methods are diagnostic of pregnancy.

RADIOGRAPHY

Uterine enlargement can be detected from day 30 when the enlarged uterus is readily identified in the caudal abdomen. Originating dorsal to the bladder and ventral to the rectum; it frequently causes cranial displacement of the small intestine. However the early pregnant uterus has only soft tissue opacity

and it cannot be differentiated from other causes of uterine enlargement, such as pyometra which occurs at the same stage of the estrous cycle.

A definitive radiographic diagnosis of pregnancy is not possible until after day 45 when partial mineralization of the fetal skeleton is detectable.

It is unlikely that the fetuses will be damaged by the ionizing radiation after day 45, but if sedation or anesthesia of the dam is required, those are potentially greater risks. In late pregnancy, the number of pups can be estimated by counting the number of fetal skulls. Although this is usually an accurate method, one study showed that radiographic estimation of fetal numbers was sometimes incorrect, presumably because of fetal overlaying on radiographic views.

ENDOCRINE TESTS

Serum concentrations of progesterone are not useful for the diagnosis of pregnancy in the bitch, and while there may be pregnancy-specific changes in serum and urinary estrogen concentrations, these have not been adequately evaluated to provide clinically useful pregnancy tests.

There is a significant elevation of serum prolactin in pregnant bitches compared with non-pregnant bitches, and it is possible that prolactin assays may, in the future, become useful as methods of pregnancy diagnosis. Currently, they are not widely used.

Measurement of relaxin is diagnostic of pregnancy from approximately 25 days of pregnancy onwards. Relaxin is produced mainly by the placenta. Therefore it gives an indication of fetal viability and fetal numbers, as well as being a sensitive method of detecting pregnancy. A commercial ELISA test has recently become available for the measurement of relaxin (ReproCheK _ Synbiotics Corp. <http://www.synbiotics.com>)

ACUTE PHASE PROTEINS

Acute phase proteins are released by the liver in response to inflammation and include C-reactive protein, haptoglobin, fibrinogen and acid glycoprotein. Of these, only fibrinogen is currently used as a test for pregnancy detection.

An acute phase response occurs in pregnant bitches at approximately the time of implantation. Amongst domestic animals, this response appears to be unique to the bitch, and measurement of fibrinogen is a sensitive marker for pregnancy. The initial rise of fibrinogen occurs from day 20 onwards with a peak at approximately day 40 but false positive diagnoses may result from inflammatory conditions such as pyometra which occurs at the same stage of the estrous cycle.

Fibrinogen is the basis of a commercial pregnancy test in Europe (Serono Diagnostics). This is not available in North America. In the absence of a commercial test, clinical lab measurements of fibrinogen greater than 280 mg/dl between day

25 and 50 after the LH surge are highly suggestive of pregnancy.

ULTRASOUND EXAMINATION

Diagnostic B_{mode} ultrasound can be used for early pregnancy diagnosis in both the bitch and the queen. It is a non_{invasive} imaging modality, safe for both the operator and the patient. The most accurate time to perform an examination is generally one month after the last mating.

Technique

One should use at least a 5MHz transducer to image early pregnancies, preferably a 7.5MHz or higher.

The homogeneous uterus can be identified dorsal to the bladder. Uterine enlargement occurs during the luteal phase whether the bitch is pregnant or not. In pregnant bitches, embryos can be seen as early as 15 days after ovulation, at which time they appear as spherical anechoic structures approximately 2 mm in diameter. The anechoic areas seen at this time are yolk sacs.

During early pregnancy, the embryo is located adjacent to the uterine wall and cannot be seen but by day 20, the conceptus is approximately 7 mm in diameter and 15 mm in length and can be imaged using ultrasound. The presence of the embryonic heart beat can be detected from approximately 22 days after ovulation.

The developing allantois initially appears as a nearly spherical structure within the conceptus. It subse-

quently increases in size and surrounds the yolk sac. The amnion is only seen later in pregnancy because it is in such close apposition to the fetus.

The most rapid growth of the fetus occurs between days 32 and 55. During this time, limb buds become apparent and there is clear differentiation of the head, trunk and abdomen.

The zonary placenta can usually be easily identified from this stage of pregnancy onwards.

The Fetal skeleton becomes evident from 40 days onwards when fetal bone becomes hyperechoic and cast acoustical shadows. The heart is also easily identified and its hyperechoic valves can be seen moving. The large arteries and veins can also be seen cranially and caudally. The lung tissue is hyperechoic with respect to the liver, and the region of the forming diaphragm can easily be identified.

From 45 days onwards, it is possible to identify the fluid_{filled} (anechoic) stomach, and a few days later the bladder can be imaged.

In late pregnancy, the head, spinal column and ribs produce intense reflections and are easily identified. In the last 20 days of gestation the kidneys can be seen and in late pregnancy small intestines may also be detected.

In several domestic species and women, ultrasound is used to determine the gestational age. This is also valuable in bitches with multiple

or uncertain mating times. Unfortunately fetal size at known gestation lengths has only been established for a few breeds. Therefore, an alternative approach is to use the time that specific organs can be imaged using ultrasound. **For example, the fetal bladder is usually only imaged during the last 20 days of gestation and the kidneys in the last seven days of gestation.**

Accuracy

Ultrasonography is very accurate, especially after day 20, but early examinations may be inaccurate if they are done in relation to the time of mating and not the time of ovulation. This may result in a false negative diagnosis. Other causes of inaccuracy include overlooking a conceptus and acoustic artifacts produced by gas or fecal material hiding a conceptus.

False positive diagnoses may be the result of confusion of empty loops of small intestines with early pregnancy. Embryonal death may also produce a disparity between the number of conceptuses imaged and the number of offspring born.

Although fairly accurate at the first examination one month after mating, the subsequent accuracy of ultrasound for detecting fetal numbers is poor. Most commonly, the number of fetuses is underestimated and the accuracy is reduced as litter size increases.

PHARMACOLOGICAL CONTROL OF REPRODUCTION IN DOGS AND BITCHES

PROGESTOGENS

Progestogens include progesterone and all are compounds with progesterone-like activity. Progesterone is produced by the corpora lutea of the bitch. It suppresses spontaneous myometrial activity, stimulates endometrial growth, and is also responsible for mammary gland development. Progesterone also has a feedback effect upon the hypothalamus and pituitary gland and that is the principal reason why they are so widely used for the control of reproduction.

By extrapolation from other species where the action of progestogens is well understood, it was initially thought that the negative feedback of progestogens was due to decreased LH secretion. However recent evidence suggests that whilst there is a reduction in circulating FSH concentration, there is no change in serum LH concentration, only a decreased responsiveness to GnRH. Therefore, the effects of progestogens on the control of the estrous cycles of bitches remain unclear.

Progesterone and progestogens are commercially available in a variety of formulations including oral therapy that must be given daily, and oily suspensions and implants that provide a slow progestogen release over several weeks or months.

GENERAL

ADVERSE EFFECTS OF PROGESTOGENS

Many transient effects may follow the administration of progestogens. Due to the fact that progestogens stimulate growth hormone secretion their use is often associated with increased appetite and weight gain. Progestogens also decrease the sensitivity of some tissues to insulin and are inherently diabetogenic. This is why women often become diabetic during pregnancy. Cats appear to be particularly sensitive to the diabetogenic effects of progestogens but the acromegalous effects of growth hormone are seldom obvious in these cases.

Due to their close relationship to corticosteroids, most progestogens also have sedative and anti-inflammatory actions but these effects are seldom a problem although mild lethargy is occasionally reported by owners. Again, because of structural similarity to corticosteroids, prolonged progestogen therapy can also produce adrenocortical suppression.

When progestogens are withdrawn, serum prolactin increases and occasionally lactation may be seen. Hair and coat changes have also been associated with steroid imbalances so it is not surprising that hair discoloration and local alopecia can be seen with subcutaneous administration of some progestogens, especially the depot preparations. All these effects vary in their incidence between different progestogens.

SPECIFIC ADVERSE EFFECTS IN THE BITCH

Progestogens may result in the development of cystic endometrial hyperplasia and pyometra. The risk is greater with some progestogens than others and also depends on the amount administered, and the duration of treatment. This action appears to be potentiated by estrogen. For that reason, in Europe, medroxyprogesterone acetate (e.g., Depot_Provera) is not licensed for use when the bitches are in proestrus, when estrogen concentrations are elevated. For the same reason, this progestogen should probably not be used to suppress estrus. This product is not licensed for use in companion animals in Canada.

Interestingly, other depot progestogens (proligestone and delmadinone acetate) have been shown to be safe when administered at practically any stage of the estrous cycle. Unfortunately, neither of these products is available in Canada. Oral therapy with megestrol acetate (e.g., Ovarid) has been shown to produce only a low incidence of adverse effects.

No preparations are recommended for suppression of the first estrous period in peri-pubertal bitches.

Benign mammary nodules can be induced by progestogen therapy and it has been suggested that progestogens may induce mammary neoplasia but this has not been substantiated.

Progestogens should not be used

during pregnancy as they may delay, or prevent parturition and can produce masculinized female puppies and cryptorchid males.

SPECIFIC ADVERSE EFFECTS IN DOGS

Although both megestrol acetate and chlormadinone acetate (two progestogens known for their 5 α -reductase blocking activity) do not suppress sperm production when used to treat prostatic hypertrophy, high doses of progestogens may decrease semen quality, mainly via a direct effect upon the epididymides.

CLINICAL USE OF PROGESTOGENS IN BITCHES

Control of estrus

In bitches (both not other domestic animals) progestogens inhibit FSH secretion and this may be how they block estrous cycles. If they are given when there is follicular activity (early proestrus or even early estrus), ovulation is inhibited and the bitch returns to anestrus.

Progestogens are generally administered in one of four regimes to control estrus in bitches. A large number of agents and formulations are available and the reader should consult specific data sheet recommendations for doses and treatment regimes.

a. Subcutaneous administration of depot preparations during anestrus.

Depot progestogens (medroxyprogesterone acetate, delmadinone acetate, and proligestone, depending on country) are administered subcutaneously during anestrus and with regular dosing at four to six month intervals can be used to prevent estrus on a long term basis.

Medroxyprogesterone acetate and proligestone are licensed for this purpose in Europe but not North America. Delmadinone acetate is not licensed for this purpose in the UK and is used on the recognition of the veterinarian. It has to be administered more frequently than other depot preparations because of its shorter half life.

Providing that a breakthrough cycle does not occur, prolonged prevention appears to have few adverse effects, especially when the more recently developed progestogens are used. Indeed it has been reported that the incidence of pyometra and mammary tumors are reduced compared with untreated females. When therapy is stopped, most females cycle normally, although the time to return of estrus is variable.

b. Oral administration of progestogens during anestrus.

Low doses of orally active progestogens (megestrol acetate, medroxyprogesterone acetate, altrenogest, norethisterone acetate, depending on country) can be used to prevent estrus for as long as administration continues.

Megestrol acetate (e.g., Ovarid) and medroxy-progesterone acetate (e.g., Depot Provera) are licensed for this purpose in the bitch in the UK but not North America. In North America, megestrol acetate (e.g., Ovarid) is only licenced for periods of administration up to 40 days

The drugs are best given during late anestrus but should the bitch enter proestrus during the first few days of treatment, the dosage can be increased. A period of anestrus usually follows therapy so the animal does not return to estrus immediately after treatment stops.

c. Oral administration of progestogens during proestrus.

High doses of orally active progestogens (megestrol acetate, medroxyprogesterone acetate, altrenogest, norethisterone acetate, depending on country) may be given during proestrus to suppress the signs of estrus.

Megestrol acetate and medroxyprogesterone acetate are licensed for this purpose in the bitch but only megestrol acetate (e.g., Ovarid) in North America.

Usually the signs of proestrus or estrus disappear within approximately five days but a reducing dose regime administered from the first signs of proestrus and continued for up to 16 days is usually efficacious and will prevent the recurrence of estrus soon after treatment ends. In North America however, megestrol acetate is only licensed for the suppression of estrus using an eight-day treatment so extended

treatment is used on the recognition of the veterinarian. This regime is frequently followed by a variable period of anestrus, and estrus will return between four and six months after treatment.

d. Subcutaneous administration of depot preparations during proestrus.

In Europe, administration of new depot progestogens, e.g., proligestone to bitches in early proestrus is being used to suppress estrus. Older progestogens such as medroxyprogesterone acetate which have potent effects upon the uterus are not recommended due to an increased risk of uterine disease, especially pyometra. Signs of proestrus or estrus disappear within approximately five days and following the depot progestogen, anestrus can vary from three to nine months.

Treatment of pseudopregnancy

Administration of progestogens to bitches with clinical signs of pseudopregnancy causes decreased prolactin secretion and clinical signs rapidly disappear. Serum prolactin concentration and the signs of pseudopregnancy are suppressed for the duration of progestogen administration, but may increase if progestogen therapy is withdrawn rapidly.

Progestogens may be administered either orally daily (megestrol acetate, e.g., Ovarid, 2 mg/kg/day) or by depot injection (proligestone 20 mg/kg or delmadinone acetate 1.0 mg/kg depending on country), although some of these products are not

licensed for this purpose. Ovarid is licenced for this purpose in North America.

Medroxyprogesterone acetate (e.g., Depot Provera) is not recommended since it can result in the development of pyometra.

In general, depot progestogen therapy works well, since the progestogen concentration gradually reduces in the circulation. By contrast, oral therapy is often associated with relapses of the clinical signs when therapy is terminated too quickly. **Pseudopregnancy can occur once the serum concentrations of the progestogen falls, stimulating a further increase in prolactin production. In some cases, this see-saw effect has prolonged pseudopregnancy for months or even years! This can usually be prevented by reducing the dose over a period of approximately seven to ten days.**

Care must be taken to ensure that the bitch is not pregnant especially if depot progestogens are to be used since these can delay or prevent parturition.

It is likely that the return to estrus will be delayed following the administration of progestogens.

Treatment of habitual abortion

There is only anecdotal evidence that habitual abortion occurs in the bitch; in many alleged cases, pregnancy has never been confirmed by a reliable method. Most are probably non_pregnant bitches that are repeatedly mated at an inappropriate time. An abnormal uterine environ-

ment such as cystic endometrial hyperplasia or chronic endometritis may result in repeated pregnancy failure but there is no evidence that primary progesterone deficiency is a cause of repeated pregnancy loss.

It is not appropriate to supplement bitches with progestogens to prevent habitual abortion unless a persistently low serum progesterone concentration has been documented. Progestogen supplementation during pregnancy may produce masculinized female pups, cryptorchid male pups, and may possibly impair or delay parturition resulting in fetal death. Progestogen use should be restricted to those cases in which a true luteal insufficiency has been diagnosed and in these cases oral therapy is most appropriate.

CLINICAL USE OF PROGESTOGENS IN DOGS

Antisocial behavior and other behavioral problems

In some cases, problems such as aggression, roaming, territory marking, copulatory activity, destruction and excitability exhibited by both entire and castrated dogs may be controlled by progestogen administration.

The action of progestogens in these cases relates both to their antiandrogenic effect and their central sedative action.

Depot progestogen therapy may need to be repeated between every month for the shorter acting preparations (delmadinone acetate 1.0 to 2.0 mg/kg) to six months for the

longer acting preparations (medroxyprogesterone acetate 3.0 mg/kg and proligestone 20 mg/kg). Neither product is licensed for this use in North America and must be used on the recognizance of the veterinarian. Oral therapy has also been shown to be effective and has the advantage that the dose may be adjusted to the effect. Commonly oral therapy commences with an initial high dose for two weeks and a reduction over several months.

Behavior modification training is an essential adjunct to progestogen therapy, and castration should be considered when dogs are still entire.

Benign prostatic hyperplasia

Prostatic enlargement may encroach upon the structures within the pelvis and produce clinical signs of dysuria or fecal tenesmus. These signs are often preceded by hemospermia, which progresses to hematuria. Growth of the prostate gland is dependent on dihydrotestosterone (DHT) and not testosterone.

A related steroid, finasteride (e.g., Proscar or Propecia) exerts a specific suppressive effect on 5 α hydroxylase, the enzyme responsible for converting testosterone to DHT. It is currently a drug of choice for treating benign prostatic hypertrophy in dogs and men. Progestogens have a similar effect to finasteride and cause a rapid reduction in the clinical signs associated with regression of the prostate gland. Depot therapy usually causes remission of clinical signs within four days, although a second treatment may be

necessary after a short time in some individuals.

I

In stud dogs, oral progestogens should be used because of their sparing effects upon sperm production. Delmadinone acetate (1.0 to 2.0 mg/kg where licensed) is also used because of its short period of action.

In dogs not required for breeding, castration in the treatment of choice.

Prostatic neoplasia

Some clinical improvement may be seen in dogs with prostatic neoplasia following the administration of progestogens. The effect is however usually only short-term, and progestogens offer little relief in metastatic disease.

Perianal adenomas

The antiandrogenic effect of progestogens given in depot form, e.g., delmadinone acetate 1.0 to 2.0 mg/kg, or as oral preparations are useful in causing temporary reduction of the size of perianal adenomas. Tumors with central necrosis may not respond well, and the remission time is related to the duration of action of the progestogen used. In dogs not required for breeding, castration is the treatment of choice.

Contraception

Long term administration of high doses of progestogens alone may cause reduced fertility but concurrent administration of depot androgens appears to be more efficacious and

allows the progestogen dose to be reduced.

Castration is the method of choice in dogs that are not required for breeding.

ESTROGENS

Many different estrogens are produced by ovarian follicles in bitches, Leydig cells in dogs and in smaller amounts, by the adrenal glands of both males and females.

Estrogens cause the development of the female sexual characteristics and the production of pheromones.

Because they are highly mitogenic and cause Na⁺ retention by tissues, they also cause uterine growth, swelling of the vulva, thickening of the vaginal mucosa, secretion from the cervical glands, and mammary development.

Estrogens are necessary for normal secretion and functioning of the uterine tubes, and the increase in estrogen concentration in late proestrus cause the LH surge via a positive feedback effect, resulting in ovulation. In late pregnancy, serum estrogen concentrations are also increased and (as for other species) may be involved in the initiation of parturition and mammary gland growth. In males, estrogens are involved in the negative feedback control FSH and LH.

In both male and females, high doses of exogenous estrogens cause a negative feedback at the hypothalamic-pituitary axis and subsequent suppression of gonado-

tropin secretion, but at low doses estrogens enhance the release of FSH as well as LH. The latter point is important when one considers how exogenous estrogens can induce fertile estrus in bitches.

Estrogens increase osteoblastic activity and cause the retention of calcium and phosphorus by stimulating the release of thyrocalcitonin. They also produce an increase in total body protein, increase metabolic rate, and affect skin texture and vascularity.

ADVERSE EFFECTS OF ESTROGENS

General

Estrogens are known to produce dose-related and often irreversible bone marrow suppression. This can result in severe anaemia, thrombocytopenia and even death. There is considerable individual variation in the toxic dose, and for some bitches this may even lie within the manufacturers' recommended dose range. Toxic effects are dose-related and toxicity is less likely if low doses are given over a long period of time. Oral therapy should be prescribed whenever possible so that administration can cease if adverse effects are noted.

In nine bitches aged 2-7 years given a single i.m. injection of estradiol cypionate, doses of 0.2 mg/kg or more produced a depressive effect on the bone marrow with leukopenia and thrombocytopenia. Doses of 0.75 and 1 mg/kg produced a hemorrhagic syndrome, with death in one case.

As mentioned previously, steroids often produce dermatological effects. Prolonged estrogen therapy can produce non pruritic, bilaterally symmetrical alopecia and skin hyperpigmentation.

Specific adverse effects in bitches

One trial showed that estrogens alone did not produce cystic endometrial hyperplasia or pyometra in ovariectomized bitches, but did potentiate the effect of progesterone on the uterus. In addition, they can cause cervical relaxation and probably allow vaginal bacteria to enter the uterus. For these reasons estrogen treatment during the luteal phase can result in the development of cystic endometrial hyperplasia and pyometra.

Large doses of estrogens may stimulate signs of estrus in both entire and ovariectomized bitches and during estrus, estrogen treatment will prolong behavioral estrus.

If estrogens are administered during pregnancy, they may cause cervical relaxation and abortion, possibly as a result of the inhibition of LH which is a major luteotrophic factor in bitches. Estrogen administration during pregnancy may also cause congenital defects in the developing fetuses including abnormalities of phenotypic sex.

Specific adverse effects in dogs

The antiandrogenic effects of estrogen administration may, after prolonged therapy, result in abnormalities of semen quality with a

resultant reduction in fertility. Prostatic size initially decreases during estrogen therapy, although subsequently reversible prostatic metaplasia may result, causing the prostate to increase in size therefore estrogens are not recommended in cases of prostatic hyperplasia.

CLINICAL USE OF ESTROGENS IN BITCHES

Misalliance, Unwanted pregnancies.

Estrogens alter zygote transport time and impair implantation when administered soon after mating and may also produce a short luteal phase by interfering with LH support of the corpora lutea.

In clinical practice, diethylstilbestrol (DES. Oral DES is not available in the USA) estradiol cypionate (e.g., ECP) estradiol benzoate, and mestranol (Mestranol is not licensed for animal use in North America) have been widely used to treat unwanted matings.

Neither estradiol nor DES is licensed for this use in bitches in Canada. Only oral formulations of DES are available for veterinary use in Canada and only estradiol benzoate is licensed for the treatment of unwanted mating in the UK. Estradiol benzoate is administered at a low dose (0.01 mg/kg) three and five (and possibly also seven) days after mating. The low dose regime has been advocated in an attempt to reduce the possibility of adverse effects. Similarly, with widespread use of estradiol cypionate in North America, the current recommenda-

tion is that bitches should be treated on days three and five after misalliance with 0.01mg/Kg ECP IM. If there is a suspicion of re_breeding it can be given again on day seven. No significant side effects have been reported with this treatment.

Pseudopregnancy

Both parenteral and oral DES and parenteral estradiol benzoate have been used to suppress prolactin secretion but neither is specifically licensed for this purpose in the UK. A preparation of ethinyl estradiol (0.8 :g/kg/day) combined with methyltestosterone (0.7 mg/kg/day) is available in the UK and produces a good clinical response, although the dose suggested seems arbitrary. Recurrence of the clinical signs may follow abrupt termination of treatment, and a reducing dose regime may be necessary especially in bitches with recurrent or persistent clinical signs. Where available, specific prolactin inhibitors such as the ergot derivatives (bromocryptine, cabergoline etc.) should be used instead of steroids.

Juvenile vaginitis

A mucoid vulval discharge, which may become purulent, is commonly seen in bitches from eight weeks of age onwards. Signs may also include frequent licking and attractiveness to male dogs. The condition usually regresses after the first estrus and usually does not warrant treatment, however if the clinical signs are severe some control may be affected using low doses of oral estrogens daily for five to seven days. Estrogens are not licensed for

this purpose in the UK or North America and should probably not be given to bitches required for breeding. An alternative therapy is the topical application of estrogen containing creams.

Estrus_induction

Low doses of estrogen probably increase FSH secretion and also stimulate the formation of LH receptors on granulosa cells. The result is follicular growth and ovulation. The induction of estrus with DES has been used clinically for some time. Good results can be achieved using 0.3 mg/kg/day orally for up to 10 days. Treatment is stopped one or two days after the onset of a hemorrhagic vulvar discharge. The cycle is then monitored using conventional methods.

Attractiveness in ovariectomized bitches

Some bitches may become attractive to male dogs several years after ovariectomy. These bitches do not have an ovarian remnant (although this should be eliminated as a differential diagnosis) but instead have low grade vaginitis. Bacterial examination usually reveals commensal organisms and is generally unrewarding. A good clinical response may be achieved using low doses of DES (0.06 mg/kg/day) or other orally active estrogens such as ethinyl estradiol (0.06 mg/kg/day) for up to seven days, or the topical application of estrogen containing creams.

Urinary incontinence

The expected incidence of urinary incontinence after ovariectomy in bitches is about 11-20% according to various authors. In a study of 412 bitches, about 20% became incontinent after having been spayed; some within and few days, others after several years. The tendency in that study was for larger bitches to become incontinent. It was also shown that urethral pressure profiles fell almost immediately after ovariectomy in most bitches but incontinence did not occur in many of the bitches.

Although most cases of urinary incontinence are treated with sympathomimetic agents such as phenylpropranolamine and ephedrine, some cases respond to estrogen therapy alone. A combination of treatments may be required in some cases. Oral DES (0.03 mg/kg/day) is licensed for this purpose in Canada but oral ethinyl estradiol (0.03 mg/kg/day) is also efficacious. Daily therapy is administered for up to three weeks with a response being observed after a few days in most cases. The length of treatment is kept to a minimum required to produce a clinical effect and treatment is repeated as necessary.

CLINICAL USE OF ESTROGENS IN DOGS

Antisocial behavior and other behavioral problems

The antiandrogenic actions of estrogens may be useful in dogs which have antisocial behavior.

However, due to the potential adverse effects of long term estrogen therapy, and the superior action of progestogens, estrogens should not be used for this purpose in dogs.

Benign prostatic hyperplasia

Repeated administration of estrogens will result in a reduction in prostatic size and amelioration of clinical signs.

The effect of estrogens upon semen quality and fertility have not been documented and prolonged estrogen therapy may result in prostatic metaplasia and an increase in the size of the prostate gland. Therefore, low dose progestogens are the treatment of choice. Alternatively one should castrate dogs that are not required for breeding.

Prostatic neoplasia

Clinical signs of prostatic neoplasia may be controlled in the short term by the administration of estrogens (DES, 0.03 mg/kg/day). Progestogens may also be useful in the short term. In men antiandrogens and GnRH antagonists are widely used.

Perianal adenomas

The antiandrogenic effect of estrogens may be useful to cause a decrease in the size of these tumors but progestogenic treatment is probably safer. Castration is the treatment of choice in dogs that are not required for breeding.

ANDROGENS

Testosterone, dihydrotestosterone and other androgens are produced by the interstitial cells of the testes and the adrenal glands.

In male, androgens mediate the development and maintenance of primary and secondary sexual characteristics and play an important role in the initiation and maintenance of spermatogenesis.

It is clear that androgen administration can antagonize estrogenic function, probably by blocking estrogen receptor sites. Androgens also have a negative feedback effect upon the hypothalamic_pituitary axis and usually suppress both LH and FSH secretion.

Many synthetic androgens are available, and the duration of their activity is related to the nature of the synthetic ester. Androgens have either primarily virilizing actions, or primarily anabolic actions. The virilizing effects include the development of the secondary sexual characteristics, including physical changes and the promotion of libido and (within special system that exists in the testicle) spermatogenesis. Actually, spermatogenesis is usually suppressed by parenteral administration; probably via its suppressive effect on LH secretion. Anabolic effects of androgens include the stimulation of appetite, promotion of protein synthesis and muscle deposition, and the retention of certain elements including nitrogen, potassium, phosphorus and calcium.

ADVERSE EFFECTS OF ANDROGENS

General

Androgen therapy may produce virilizing effects such as aggression and in prepuberal animals premature epiphyseal growth plate closure may occur. Actually this is a potential problem with the use of all sex steroids in prepuberal animals. Hepatic dysfunction has also been reported following androgen administration.

Specific adverse effects in bitches

Prolonged androgen administration in bitches can result in clitoral hypertrophy, and rarely, even the development of an os clitoris. Repeated or prolonged androgen therapy may also result in persistent vaginitis, and severe urogenital abnormalities may develop in female fetuses if androgens are administered to bitches during pregnancy.

Specific adverse effects in dogs

High doses of androgen produce severe suppression of spermatogenesis and are generally contraindicated in any male used for breeding.

CLINICAL USE OF ANDROGENS IN BITCHES

Control of estrus

Androgens are often administered to bitches in anestrus to prevent a return to cyclical activity. This is especially common in racing Greyhounds although androgens are not

licensed for this purpose.

The exact mechanism of action has not been determined, although it is likely to be similar to that of progestogens in suppressing gonadotropin secretion. Androgens are not useful for suppressing estrus in bitches that are already in estrus so administration must commence in late anestrus, at least 30 days before the onset of proestrus. When androgens are used in this manner, they do not mimic the luteal phase, and there is no subsequent anestrus. Therefore, cyclical activity tends to return soon after the end of treatment.

In the UK, androgens are most commonly given during anestrus either as a prolonged release implant or as a depot injection of mixed testosterone esters. It is common for depot therapy to be supplemented by daily oral therapy. For example, mixed testosterone esters (25 mg/kg) may be given intramuscularly every four to six weeks, and methyltestosterone (0.25 to 0.5 mg/kg) is given orally daily.

In the US but not Canada, an orally active synthetic androgen, mibolerone (Cheque_Upjohn) is available. This is effective for the long term prevention of estrus in bitches but has adverse effects typical of other androgens, including clitoral hypertrophy, vaginitis, and behavioral changes. In addition, anal gland abnormalities, offensive body odor and obesity have also been recorded.

Pseudopregnancy

High doses of androgens may be used to produce an inhibition of prolactin secretion in bitches with pseudopregnancy. This results in a rapid resolution of the clinical signs. Androgens may be more useful than either progestogens or estrogens since they do not have any adverse effects on the uterus. Oral methyltestosterone or parenteral testosterone esters are effective but in the UK, an orally active product containing methyltestosterone (0.7 mg/kg/day) combined with ethinyl estradiol (0.8 µg/kg/day) produces a good clinical response.

Anabolic therapy

Various androgens both as depot or repeated oral therapy are used for their anabolic effect in aged or debilitated dogs. For example, parenteral therapy (mixed testosterone esters, 25 mg/kg) is usually given every one or two weeks, or oral therapy (methyl testosterone 0.25 to 0.5 mg/kg) is recommended daily.

CLINICAL USE OF ANDROGENS IN DOGS

Poor libido

There is no evidence in the dog that poor libido is caused by low circulating androgen concentrations. The condition is more likely to have a psychological background or to be the result of musculoskeletal pain during copulation.

Although both oral and depot androgen administration have been advocated for the treatment of poor libido,

androgens should probably not be used since they can produce significant suppression of spermatogenesis.

Poor semen quality

There are many causes of poor semen quality in the dog but those related to inadequate endocrine support have not been fully elucidated in males of any species. It is not appropriate to administer androgens without a definitive diagnosis. Nevertheless, androgen supplementation is commonly used often with disastrous effects on fertility.

An analogue of dihydrotestosterone, mesterolone, is available for human use and is unusual because it is not aromatized to estradiol and for this reason it is presumed, does not significantly suppress the release of pituitary gonadotropin secretion. It has been used in dogs to stimulate spermatogenesis (1.5 mg/kg/day) but its effects have not yet been fully evaluated.

Contraception

Long term administration of high doses of androgens may be useful for reducing semen quality by a negative feedback suppression of spermatogenesis. However, androgen treatment with concurrent administration of progestogens appears to be more efficacious. Castration is obviously the method of choice in dogs that are not required for breeding.

GONADOTROPINS

The gonadotrophins FSH and LH are secreted by the anterior pituitary gland in response to GnRH production by the hypothalamus.

In bitches, FSH stimulates the growth of small to intermediate sized follicles. LH stimulates the terminal stages of follicle growth and is also the trigger for ovulation. LH is probably also the principal luteotrophic agent in the bitch, more so than in the noncarnivorous domestic animals. In males, FSH stimulates the division of spermatogonia, the youngest cells in spermatogenesis. Only later in spermatogenesis does testosterone stimulation become important.

As mentioned elsewhere, LH stimulates the (interstitial) Leydig cells to produce testosterone and this is made available to the Sertoli cells which lie close by, within the seminiferous tubules. Sertoli cells produce androgen binding protein which allows high concentrations of testosterone to accumulate within those cells.

Neither FSH nor LH of canine origin are available for use in the dog, because the molecules are complex and cannot be synthesized easily as is the case for steroids and other, smaller molecules. However equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) are commercially available. eCG is produced in the mare during pregnancy and is mainly FSH-like in activity, but it does have some LH activity. hCG is extracted from the urine of first trimester pregnant

women and is primarily LH_like in its effect.

ADVERSE EFFECTS OF GONADOTROPINS

General

Antibody formation to these large glycoprotein molecules is probably a significant consideration. This is known to occur in other humans, horses and monkeys. Usually however, antibodies to these hormones only render the product ineffective.

Occasionally, the FSH_like or LH_like product can be so close to endogenous hormones in the animal being treated, that antibodies may form against the animals' own endogenous hormones and affect fertility. This has not been demonstrated in domestic animals but is known to occur in primates given hCG. There is only a slight risk of anaphylactic reactions.

Specific adverse effects in bitches

The administration of a single dose of either gonadotropin to anoestrous bitches is unlikely to have any effect. However, eCG if given to estrous bitches may cause luteinization of follicles, causing "follicle capture" and preventing ovulation. This effect has also been documented in women. Excessive stimulation of the ovary may also result in persistent estrous behavior and persistently elevated estrogen concentrations may cause estrogen toxicity.

Specific adverse effects in dogs

hCG administration causes an endogenous rise of serum testosterone. This effect is short lived but in a small number of cases temporary changes in temperament may occur.

CLINICAL USE OF GONADOTROPINS IN BITCHES

Induction of estrus

When repeated doses of eCG (20 IU/kg for five days) are given to anoestrous bitches follicular growth is stimulated and ovulation may occur spontaneously, following an endogenous surge of LH. Ovulation has also been induced using hCG (25 IU/kg on day 5). This treatment is more effective in late anoestrous bitches than in early anestrus. Owners sometimes request this treatment for bitches when a breeding opportunity has been missed.

Occasionally, endogenous hyperestrogenism may occur and result in inhibition of implantation, bone marrow suppression and death. Low doses of gonadotropins probably produce more physiological serum estrogen profiles than higher doses. Insufficient progesterone production and short luteal phases are common after gonadotropin_induced estrus. It has been suggested that this may be due to high endogenous or exogenous estrogen concentrations, suppressing the release of LH, an important luteotropin.

Delayed puberty

Although the majority of bitches reach puberty before 18 months of

age, some bitches, especially those of the larger breeds reach puberty much later, sometimes as late as 2.5 years. Therefore, treatment is not warranted until after this age.

Therapy to induce estrus may then be attempted using an eCG / hCG regime such as that used for inducing estrus as described above.

Hastening of ovulation

Bitches that repeatedly fail to conceive are sometimes given hCG at the time of mating on the assumption that ovulation has not occurred or that early development of the corpora lutea is inadequate. There is no evidence for this and such blind therapy cannot be recommended. However some bitches may have prolonged proestrus where bleeding has persisted for three weeks or more. In such cases, the administration of hCG (25 IU/kg) may possibly hasten ovulation when it administered after 90% of vaginal epithelial cells become anuclear, or when a slight rise in serum progesterone concentration has been detected. Premature administration of hCG may result in follicular luteinization, oocyte capture and failure of ovulation.

Identification of ovarian tissue

It is often difficult to determine whether a bitch has been ovariectomized or ovariohysterectomized. Administration of hCG (25 IU/kg) to bitches with ovaries will result in an increase in serum estrogen concentration but the value of this technique has not been fully evaluated.

CLINICAL USE OF GONADOTROPINS IN DOGS

Detection of testicular tissue

In dogs with no scrotal testes the presence of testicular tissue can be confirmed by performing an hCG stimulation test.

Serum testosterone concentration is measured before and 60 minutes after the intravenous administration of 50 IU/kg hCG. A significant rise in testosterone concentration is diagnostic of testicular tissue. In stallions, the same test is used and at least a doubling of basal testosterone concentrations is expected if a testicle is present.

Dogs with functional testicular tissue, whether intra_abdominal or extra_abdominal generally have a higher resting serum testosterone concentration than castrated dogs but large fluctuations in serum testosterone throughout the day make an hCG stimulation test the preferred method of diagnosis.

GnRH.

This small molecule is stable, non allergenic and appears to have the same structure in all mammals. Therefore it is equally active in all mammals and can be used whenever LH or FSH stimulation is required. However, GnRH is released at a pulse frequency rate from the hypothalamus that is specific for the stage of the cycle, the hormone produced (FSH or LH) and for each species of animal. Exceeding this rate or "flooding" the adenohypophysis with GnRH will result in a refrac-

tory state with complete absence of gonadotropin secretion.

More than one thousand analogs of GnRH have been produced, some very active with respect to gonadotropin secretion and others suppressive. As the molecule is so small, many of these are even active when used intra nasally or sprayed on other mucous membranes.

GnRH can be used in dogs or bitches at total doses of between 10 and 100 micrograms, depending on body weight. However its effects will remain unpredictable until the pulse frequencies at all the stages of the canine estrous cycle are determined

PROSTAGLANDINS

Prostaglandins are small, relatively simple molecules produced by virtually all body tissues. In the reproductive tract, we know that they are synthesized in the endometrium, are luteolytic and cause uterine contraction. They also cause contraction of smooth muscle throughout the body, especially that of the digestive and respiratory systems. This accounts for the increases respiration rate and defecation that are almost invariably seen when bitches are treated with prostaglandins.

Although endogenous prostaglandin has not been shown to be involved in natural luteolysis in cycling bitches, exogenous prostaglandin is strongly luteolytic. Prostaglandins are also involved in parturition.

No naturally occurring or synthetic prostaglandin analogues have been licensed for use in bitches. Neverthe-

less, the use of the native hormone, prostaglandin F₂α, and analogues such as cloprostenol and luprostirol are widespread. When administered to bitches 25 to 30 days after the LH surge they cause lysis of the corpora lutea and the termination of pregnancy. Earlier than this, repeated therapy may be necessary. Prostaglandin administration will also cause uterine contractions making it useful for treating pyometra.

GENERAL ADVERSE EFFECTS OF PROSTAGLANDINS

Prostaglandin administration may be followed by restlessness, excessive salivation, vomiting, abdominal pain, pyrexia, tachycardia, ataxia and diarrhoea. These effects may develop quickly after administration and usually persist for up to 60 minutes. The therapeutic index of prostaglandins in bitches is low and high doses can be lethal.

Specific adverse effects in bitches

Prostaglandin administration to pregnant bitches may cause fetal death or abortion. Although uterine rupture is frequently cited as a potential result of prostaglandin use, especially when the cervix is not dilated, close examination reveals that not cases have been reported.

CLINICAL USE OF PROSTAGLANDINS IN BITCHES

Termination of pregnancy

Although it has been shown that a four day treatment with dinoprost

(150 to 270 :g/kg) could cause luteolysis when corpora lutea were less than three weeks old, these results are not consistent and early treatment is not usually recommended.

Low doses of dinoprost (150 :g/kg) or cloprostenol (0.025 mg/kg) given daily, or twice daily, for several days can be used to produce luteal regression. Repeated low doses cause lysis of the corpora lutea and eventually induce abortion, especially when given later than 23 days after ovulation. Earlier treatment may not be efficacious since developing corpora lutea are more resistant to the effects of prostaglandin. However, recent studies have shown that daily treatment with high doses (250 :g/kg) of dinoprost, beginning five days after the onset of the luteal phase may cause luteolysis although adverse side effects are common. A better therapeutic option is to use a combination of prostaglandin (dinoprost, 5.0 :g/kg every other day for 10 days) and a prolactin antagonist such as bromocriptine or cabergoline (5.0 :g/kg/day for 10 days), although the latter agents may also be used alone. Again this is more effective as the corpora lutea become mature.

Treatment of pyometra

Cystic endometrial hyperplasia and pyometra occur in older bitches during the luteal phase of the estrous cycle.

Progesterone_dependant cystic endometrial hyperplasia usually precedes the development of pyometra which is associated with bacte-

rial invasion of the uterus during estrus.

Cases are described as "open_cervix" or "closed_cervix" pyometra. In the former cases, there is usually a copious vulval discharge, and following its diagnosis, prostaglandins may be used to induce uterine emptying and to remove the stimulatory effects of progesterone. Dinoprost given at low doses (150 :g/kg) twice daily for five days, combined with appropriate antibiotic and fluid therapy is usually a successful treatment. Following treatment, approximately 20% of bitches return to fertility. The long term complications of pyometra include recurrence, failure to conceive, and abortion.

In cases of "closed_cervix" pyometra, vaginal cytology should be performed to determine if the cervix is sufficiently open to allow treatment. In the experience of some clinicians, many cases of "closed_cervix" pyometra are in fact cases where the cervix is open but the bitch is fastidious and has kept her vulva clean.

PROLACTIN ANTAGONISTS

Together with LH, prolactin is a primary luteotrophic factor in both pregnant and non_pregnant bitches. In fact, prolactin was originally known as LTH, or luteotopic hormone. Canine corpora lutea initially appear to be autonomous but from day 20 onwards, the use of prolactin antagonists causes a rapid decrease in prolactin concentrations. This results in destruction of the corpora lutea and termination of the luteal phase. Interestingly, the administration of

prolactin antagonists during anestrus causes a return to estrus sooner than anticipated but the mechanism of this function is unknown.

Bromocriptine and cabergoline are ergot derivatives and competitive dopamine agonists. This means that they act like dopamine, a sympathetic system neurotransmitter known to inhibit prolactin release from the adenohypophysis.

Cabergoline appears to be a more specific agent than bromocriptine, having fewer side effects attributable to dopaminergic stimulation of the central nervous system. Bromocriptine is widely available but cabergoline is only licensed for use in dogs in Europe (e.g., Galastop) and is not available in North America.

ADVERSE EFFECTS OF PROLACTIN ANTAGONISTS

General

Bromocriptine often causes nausea and vomiting but lethargy and occasional constipation may also be noticed. These side effects can be reduced by using the minimal effective dose and mixing the drug with food. It has been suggested that the anti emetic metoclopramide should be used to prevent vomiting but this drug actually a dopamine antagonist and promotes prolactin release! Therefore whilst clinically useful, its administration does not make pharmacological sense. By contrast, side effects are uncommon with cabergoline.

Specific effects in bitches

Administration of prolactin antagonists during pregnancy may cause abortion. Repeated administration will result in a shortening of anestrus and a return to estrus.

CLINICAL USE OF PROLACTIN ANTAGONISTS IN BITCHES

Pseudopregnancy

Suppression of prolactin causes a rapid resolution of the signs of pseudopregnancy. Bromocriptine (20 :g/kg/day) has been used for this purpose for some time but cabergoline (5.0 :g/kg/day) is now widely available in Europe (e.g., Galastop). It has a longer duration of action and produces fewer side effects than bromocriptine. Unfortunately cabergoline is not available in Canada.

Termination of pregnancy

When canine corpora lutea are 20 to 30 days old, repeated administration of the prolactin antagonists produces a reduction in serum progesterone concentration, fetal death and abortion. Before this time they are usually ineffective.

Abortifacient efficacy is increased when prolactin antagonists and prostaglandins are given simultaneously (see prostaglandins above). One regime is to use a combination of prostaglandin (dinoprost, 5.0 :g/kg) every other day for ten days and a prolactin antagonist such as cabergoline (5.0 :g/kg) daily for ten days. It can be seen that abortion is

not rapid but may take more a week to complete.

Induction of estrus

Numerous experiments have shown that the interval between estrous periods can be shortened to less than half its normal length by treating bitches during, or after the luteal phase. For example, in four dogs treated with 20 µg of bromocryptine per kg body weight daily for five days, (started one to five days after ovulation) the inter-estrous period was about 100 days shorter than for 10 untreated controls (123.3±23.1 and 245.9±8.8 days respectively).

Although the inter-estrous period can be shortened if treatment is started soon after ovulation poor conception rates are obtained. It is preferable to start treatment after the end of the luteal phase and to continue daily treatment until proestral bleeding begins. One day later, treatment is stopped. This gives excellent results and bitches are usually very fertile.

The usual time from the start of treatment to the beginning of proestral bleeding is six to eight weeks.

PROGESTERONE ANTAGONISTS

Mifepristone (RU486 – “The French Death Pill”) is an orally active progesterone receptor site antagonist marketed for use in bitches in France. In most other countries it is not available because it is a potent abortifacient in humans as well. A similar progesterone antagonist (RU46534 or RU534 _aglepristone) has recently been, and is available

as an injectable solution in some countries but not North America.

As expected, administration of these agents produces no initial change in serum progesterone concentration, but binding of progesterone to its receptor sites is blocked and pregnancy terminates.

Onapristone and lilopristone are similar compound; also not yet available for animal use.

ADVERSE EFFECTS OF PROGESTERONE ANTAGONISTS

General

Neither RU486 nor RU534 has been associated with adverse side effects in bitches. However, women describe nausea, vomiting and uterine cramps as side_effects with the use of RU486.

Specific adverse effects in bitches

Administration of progesterone receptor site antagonists will result in resorption, abortion or premature parturition depending upon the time of administration.

CLINICAL USE OF PROGESTERONE ANTAGONISTS

BITCHES

Pregnancy termination

The administration of RU486 at 28 days or more after ovulation produces fetal death and abortion within

five days of treatment. Earlier administration is only effective after a longer treatment period. In later pregnancy, mifepristone administration results in abortion, or premature parturition. By contrast, RU534 (4.0 mg/kg) can be used to terminate pregnancy at any stage. In fact, it may even be used to prevent conception by administration immediately after ovulation.

Pyometra

RU534 has also been used in bitches with pyometra. Administration on day one, three, five, eight and 16 after presentation was successful for the treatment of both open and closed_cervix pyometra in the majority of bitches. Treatment also produced no adverse side effects, and emptying of uterine fluid occurred quickly. Some of the bitches returned to fertility after treatment.

Progesterone synthetase inhibitors

Epostane (Sterling Winthrop/Upjohn) is a competitive inhibitor of the enzyme which catalyzes the formation of progesterone from pregnenolone. Apparently Epostane may also alter corticosteroid synthesis. It is highly abortifacient in bitches but is not yet available for clinical use in North America. It has been shown to be effective at a parenteral dose of 14 mg/kg. Although abscess formation was noted at injection sites, no other side effects were seen. It was also an effective abortifacient at oral doses varying from 25 to 300 mg/dog, given once daily for a week.

PREDICTION OF PARTURITION

Introduction

Prediction of whelping in a bitch can be difficult because the apparent (not true) length of pregnancy is so variable (58 days to as long as 72 days). If she has not been monitored during estrus, it can be difficult to predict the likely time of parturition.

Bitches monitored during estrus:

Parturition occurs 63days \pm 1 day from ovulation, 65 days \pm 1 day from the LH surge or about 60 days from the time of conception. Therefore parturition can be relatively accurately predicted in bitches that have been monitored by measurement of serum progesterone or LH during estrus. Vaginal cytology can also be helpful but is not as accurate as determining the time of the LH surge. The first maximum of superficial vaginal cells occurs 65 days \pm 3 days before whelping and metestrus vaginal cytology (a sudden reduction in the number of superficial cells and frequently, an influx of PMNs) is first detected about 56 days before whelping.

Examination of bitches in mid pregnancy

When bitches are examined during mid pregnancy an estimation of the likely date of parturition can be made by measuring fetal dimensions. However, normal values have only been established for a few breeds and data are not generally available.

Examination of bitches close to parturition

In parallel with the drop in serum progesterone concentration before whelping, there is a drop in body temperature from 39°C to less than 37°C. The mean interval from the drop in body temperature through 37.5°C to the onset of parturition is approximately 24 hours. This interval is fairly consistent, therefore it is a useful predictor of the time of parturition. In practice, the rectal temperature should be measured every 6 to 8 hours starting on day 62 to 63 after the LH surge and should be graphed to facilitate interpretation. Unfortunately, this is labor intensive and body temperature tends to fluctuate, making interpretation difficult.

Frequently owners believe that parturition is overdue. Progesterone assays are useful in such cases because low (<2ng/ml) progesterone concentrations indicate that whelping is close and that an elective cesarian section may be required. Rapid ELISA assays are valuable but radioimmunoassay results give precise serum progesterone concentrations and are more useful.

NOTES ON COMMON GENITAL SURGERY

Introduction

The common surgical techniques including ovariohysterectomy and castration are widely performed in general practice.

Remnant ovarian tissue

Return to estrus after removal of the

ovaries is not common, but it is a problem in the referral practices. Usually, the entire ovary (often the right ovary) has been left in situ because it has been covered with fat and was not identified at surgery. In a few cases, a portion of the ovary is left behind or a few cells have seeded onto the peritoneal surface. Care must be taken to distinguish this problem from low grade vaginitis which can cause bitches to become attractive to males.

Signs of estrus, vaginal cytology and hormone assay may be useful for the diagnosis of ovarian remnant syndrome. Serum progesterone concentrations can be measured two weeks after the clinical signs of estrus have disappeared. If other diagnostic attempts are not fruitful, a high concentration of progesterone at that time will confirm the presence of an ovarian remnant. Surgical exploration is best performed during estrus or in the early luteal phase when the ovary is at its largest size.

Ovariectomy

Ovariectomy is an alternative technique to ovariohysterectomy. The procedure is quick to perform, requires only a small incision, is minimally traumatic and has a rapid recovery time. There is also some evidence of reduced risk of urinary incontinence following ovariectomy compared with ovariohysterectomy. It has been shown that in the absence of exogenous hormone treatments, ovariectomized bitches have no problems associated with the remaining uterine tissue.

Peri vulvar skin fold ablation

Vulval skin fold ablation may be required in bitches that have a severe skin fold dermatitis surrounding the vulva. The condition is said to occur more commonly in bitches which have been neutered prior to puberty, although data supporting this contention is absent. A wide surgical margin is essential to ensure the success of this technique. This technique is illustrated at : <http://www.upei.ca/~lofstedt/opence/AVCfallcon.html>

include non-healing wounds and ulcers, tumors, persistent penile exposure, necrosis following paraphimosis, subsequent to fracture of the os penis and ulcerated/prolapsed urethra. Careful explanation of the post-surgical recovery to the owner is essential in these cases.

Episiotomy

Episiotomy may be necessary for the exposure of vaginal lesions including neoplasia and vaginal strictures. Careful preparation of the patient is necessary to ensure an accurate repair of the vaginal wall and closure of the episiotomy incision.

Vaginal sub-mucosal resection

In bitches with vaginal hyperplasia, resection of edematous hyperplastic vaginal tissue may be necessary. Urinary catheterization and strict attention to hemostasis is essential. The potential hereditary nature of this condition should also be considered.

Vasectomy

Vasectomy is rarely performed in dogs. It may be considered in working animals that are not required for breeding, where high levels of testosterone-stimulated activity may be considered essential.

Penile amputation

The indications for penile amputation

CASE STUDIES

CASE 1

You are presented with a bitch that is 68 days from breeding but has no signs of imminent parturition.

1. How would you investigate this case?
2. How could you establish if parturition was overdue?
3. What methods are available to predict parturition in the dog?

AN APPROACH TO CASE 1

1. How would you investigate this case?

There are a number of possibilities with this case. The bitch may not be pregnant, she may be pregnant and within normal gestational limits, or she may be overdue, having suffered primary uterine inertia.

Prediction of parturition is difficult since the apparent length of pregnancy can vary between 58 and 72 days. If the bitch has not been monitored during estrus it can be difficult to predict the likely time of parturition.

Investigation should include:

- making a diagnosis of pregnancy.
- establishing the number of days from mating.
- clinical and ultrasonographic examination to assess whether fetuses are alive and are normal

Note: Bradycardia is the normal fetal response to hypoxia. However, a heart rate of less than 100bpm indicates that a fetus is severely distressed and intervention is required.

2. How could you establish if parturition was overdue?

To establish if parturition is overdue, serum progesterone can be measured using a rapid ELISA method. Basal values of progesterone demonstrate that parturition has begun or is close to starting.

Where rapid radioimmunoassay (same day) is available, this should be used instead because exact progesterone concentrations make the time of whelping easier to predict than if an ELISA test (HI-LO values only) is used. Obviously, high values of progesterone indicate that parturition is not imminent.

3. What methods are available to predict parturition in bitches?

Parturition may be predicted by a number of methods:

- Bitches should be monitored during estrus for the first maximum of superficial cell index or better still, serum progesterone should be measured every second day after vaginal cornification becomes obvious.

Ideally, daily serum samples should be retained so that when progesterone rises, that sample can be tested for its LH content. If the concentration of LH is not elevated in that sample, LH should be measured in the sample from the day before. In this manner, the exact time of the LH surge, ovulation, fertilization and parturition will be known.

Rapid LH assays are not available worldwide and are not required in all cases. In most cases, vaginal cytology and progesterone assays will suffice.

Parturition occurs 63 ± 1 days from ovulation or about 65 days after the LH surge.

When breeding and ovulations have already occurred, one may have to

rely on the vaginal cytology alone. Whelping occurs about 56 days after a sudden reduction in the superficial cell index and the influx of numerous PMNs.

Examination of bitches in mid pregnancy

When bitches are examined during mid pregnancy, an estimation of the likely date of parturition can be made by measurement of fetal dimensions, although values are only carefully established for a small number of breeds.

Examination of bitches close to parturition.

Measurement of serum progesterone can be diagnostic.

There is also a drop in rectal temperature from 39°C to less than 37°C as whelping approaches. The mean interval from the drop in body temperature through 37.5°C to the onset of parturition is approximately 24 hours. This interval is fairly consistent, therefore it is a useful predictor of the time of parturition.

In practice, the rectal temperature should be measured every 6 to 8 hours starting on day 62 to 63 after the LH surge and should be graphed to facilitate interpretation. Unfortunately, this is labour intensive and body temperature tends to fluctuate, making interpretation difficult

CASE 2

You are presented with a bitch 24 hours after an alleged mating and the owners do not want the bitch to have puppies.

1. What are the treatment options available?
2. How might you further investigate this bitch to better consider the appropriate treatment?
3. Assuming the risk of pregnancy is thought to be low how would you proceed?

AN APPROACH TO CASE 2

1. What are the treatment options available?

There are several treatments available to terminate pregnancies. Some prevent or interfere with implantation while others cause fetal death and abortion. Ovariohysterectomy should also be considered if the bitch is not required for future breeding.

2. How might you further investigate this bitch to better consider the appropriate treatment?

Although blind treatment of all bitches presumed to be recently mated or pregnant may sometimes be appropriate, the adverse effect of any treatment should be considered.

It is preferable to examine vaginal cytology to establish the stage of the estrous cycle and to try to detect spermatozoa. In recently mated bitches, spermatozoa may be seen but they are rapidly cleared from the vagina and may not be seen more than 24 hours after mating.

Accurate detection of spermatozoa requires a special technique. A saline-moistened swab should be placed into the vagina for 1 minute then the swab tip should be placed in a small test tube with 0.5 ml physiological saline and allowed to stand for 10 minutes. The swab is then squeezed dry on the side of the tube and the saline is centrifuged at 600 to 700g ($g = 1.12r[.001 \times \text{rpm}]^2$ where r is the radius in mm) for 10

minutes. Collection of the sediment and examination after staining with Diff-Quick or Wright-Giemsa stain allows detection of sperm heads. Using this technique, spermatozoa are found in 100% of samples in which mating occurred within the previous 24 hours, and in 75% of samples in which mating occurred within the previous 48 hours.

Because spermatozoa are able to remain viable within the female reproductive tract for periods of more than 10 days, some early matings may result in a pregnancy. Therefore, the possibility of pregnancy cannot be dismissed if mating has occurred during early proestrus. These bitches should receive treatment, even if spermatozoa are not seen in the vaginal smear.

Bitches that are in cytological estrus (in these cases) are likely to conceive especially if serum progesterone concentrations are higher than 2ng/ml. These bitches should also receive treatment, even if spermatozoa are not seen in the vaginal smear.

Bitches that are found to be in cytological metestrus and have high serum concentrations of progesterone are likely to be pregnant and should be treated. Occasionally owners may believe that a bitch has been bred but vaginal cytology reveals that they are in diestrus and the serum progesterone is low. These bitches should not be treated.

3. Assuming the risk of pregnancy is thought to be low how would you proceed?

There are several treatment options: If the bitch is not required for breeding, the simplest option is ovariohysterectomy after the end of estrus.

A second option is to treat the bitch in an attempt to prevent implantation while the third option is to wait until a positive diagnosis of pregnancy can

Treatment options can be considered with respect to the risk of conception as laid out in the following table.

In low risk cases, the animal may be examined 28 days after mating for pregnancy. If pregnant, abortifacient treatment can commence.

3. If the risk of pregnancy is thought to be low, it is appropriate to wait until a diagnosis of pregnancy can be

Sperm	Serum Progesterone conc.	Vaginal cytology	Risk of conception*
No	Low	Proestrus	Low
No	Low	Estrus	Low
No	Intermediate to high	Estrus	Intermediate
No	High	Metestrus	Low
Yes	Low	Proestrus	Low
Yes	Low	Estrus	Intermediate
Yes	Intermediate to high	Estrus	High
Yes	High	Metestrus	Low
* This risk also depends on the time interval between presumed mating and the time of examination.			

be made. Then terminate pregnancy. In the last case, the bitch is best examined with real time ultrasound using 5MHz to 7.5MHz transducers at about 28 days after mating.

made and then to terminate by pharmacologically destroying the corpora lutea.

Prostaglandins

When administered to the bitch, prostaglandins lyse the corpora lutea and reduce the serum concentration of progesterone. The corpora lutea of the bitch are more resistant to prostaglandins than in other species, and repeated therapy is necessary to achieve luteolysis. Prostaglandins also cause contraction of smooth muscle which may be part of their abortifacient effect. The actions on smooth muscle also account for the adverse side effects of prostaglandins, including salivation, vomiting, pyrexia, hyperpnea, ataxia and diarrhoea. High doses may be lethal.

Prolactin antagonists

Prolactin antagonists such as cabergoline reduce serum concentration of progesterone especially when they are administered during the late luteal phase. The combination of cabergoline and cloprostenol, induces abortion from day 25 after the LH surge. Cabergoline can be administered orally daily (5.0 mcg/kg) for 10 days and cloprostenol (5.0 mcg/kg) subcutaneously, every other day. This treatment reduces the adverse effects of prostaglandin therapy alone, and increases the efficacy of prolactin antagonists.

When bitches are treated for approximately nine days, 100% of the pregnancies are aborted and there are generally no adverse effects. Unfortunately cabergoline is not available in Canada, so oral bromocryptine must be substituted at a dose of 20 g/kg/day.

CASE 3

You are presented with a bitch that was spayed two years previously but is presently attracting males at six month intervals.

1. How could you confirm the presence of ovarian tissue?
2. How does the presence of a clinical pseudopregnancy help the diagnosis?
3. How would you treat this condition?
4. What other condition may be responsible for this presentation?

AN APPROACH TO CASE 3

1. How could you confirm the presence of ovarian tissue?

Accurate diagnosis requires the examination of a vaginal smear when the bitch is still attracting dogs. When there is an ovarian remnant the vaginal epithelium will be cornified. Red blood cells will be absent if the uterus has been removed.

Serum/plasma progesterone concentrations can be measured about two weeks after the clinical signs of estrus have disappeared; a high concentration verifying ovarian presence and indicating that ovulation has occurred.

2. How does the presence of a clinical pseudopregnancy help the diagnosis?

Whilst clinical pseudopregnancy may be a useful sign when there is an ovarian remnant it should be interpreted with care because some bitches develop pseudopregnancy when ovariectomy is performed during the luteal phase. Other cases bitches (with other reasons for being attractive to males (see 4 below) may have been treated with depot progestogens resulting in signs of pseudopregnancy as the serum progestogen concentration decreases, and serum prolactin concentrations increase.

3. How would you treat this condition?

In most cases an entire ovary (often

the right ovary) has been left in position. Occasionally a portion of the ovary has been left behind or some ovarian cells may have seeded onto the peritoneal surface.

Surgical exploration is best performed during estrus or in the early luteal phase when the ovary is at its largest size.

4. What other condition may be responsible for this presentation?

Some bitches may become attractive to male dogs several years after ovariectomy. These bitches do not have an ovarian remnant (although this should be eliminated as a differential diagnosis) but instead appear to have low grade vaginitis. Bacterial examination usually reveals commensal organisms. A good clinical response may be achieved using low doses of DES (0.06 mg/kg/day) or other orally active estrogens such as ethinyl oestradiol (0.06 mg/kg/day) for up to 7 days, or the topical application of estrogen containing creams.

CASE 4

You are presented with a bitch that has been in estrus for five weeks. She appears clinically well and the owners do not want to breed from her.

1. What is the likely diagnosis in this case?
2. How would you confirm the diagnosis?
3. What treatments are available and in which order might you use them?

AN APPROACH TO CASE 4

1. What is the likely diagnosis in this case?

Several possible diagnoses:

- This bitch is probably experiencing prolonged proestrus and prolonged estrus.

This condition is especially common in young bitches and probably results from either inadequate production of LH or a poor ovarian response due to inadequate LH receptors.

Although she has been in proestrus for five weeks, she could still be normal. Normal intervals between the onset of vulvar bleeding and ovulation vary from 6 to 28 days. Most often, the serum LH surge occurs about 10 days after the onset of vulvar bleeding but many bitches experience prolonged proestrus and ovulate later in the cycle than anticipated. These bitches do not require treatment but do need careful assessment of the optimum mating time.

- Estrogen secreting follicular cysts may also cause persistent estrus in bitches.

Follicular cysts are rare and their causes are unknown although it has been suggested that they are associated with systemic disease such as diabetes mellitus and hypothyroidism.

These cysts are usually easy to see using ultrasound.

If the underlying cause cannot be determined and treated, ovariectomy is indicated.

- Estrogen producing ovarian neoplasms are rare but may occur. In these cases chronic elevation of serum estrogen can lead to bone marrow suppression with anaemia and thrombocytopenia.

- Estrogens secreting follicles that do not ovulate but are not enlarged or cystic. This condition is very rare and has been associated with an abnormal karyotype (77 XO)

- Persistent vaginitis causing production of a local pheromone which is attractive to males.

2. How would you confirm the diagnosis?

Ultrasonographic examination of the ovaries, elimination of systemic disease such as diabetes mellitus, vaginoscopy and vaginal cytology and finally chromosomal analysis (karyotyping).

3. What treatments are available and in which order might you use them?

The administration of hCG (300 to 500 IU/dog) may be useful to induce ovulation or luteinization, and a normal luteal phase. Signs of estrus end within five days of administration but often the treatment is not successful possible because of a deficiency of ovarian LH receptors. If there is no response suppression of the estrus using progestogens should be attempted. However, this has the potential for inducing pyom-

etra since progestogens are being administered when there is estrogen present.

An eight day course of megestrol acetate is usually curative.

GnRH treatment is usually to no avail because persistent pulsatile administration is usually required to induce ovulation.

In the case of ovarian neoplasia and cystic ovarian disease, should be performed. There is no treatment for karyotypic abnormalities but ovariectomy will stop some of the clinical signs associated with these problems.

CASE 5

You are asked to examine a bitch with a muco-purulent discharge, 21 days after breeding. A vaginal swab produces a heavy growth of beta-hemolytic streptococcus

1. What are the likely diagnoses?
2. How might you further investigate this case?
3. What is the significance of the bacteria that is isolated and what action does this require?
4. Which organisms are likely to cause vaginitis in the bitch?

AN APPROACH TO CASE 5

1. What are the likely diagnoses?

The presence of a mucoid discharge is common and normal in bitches in the early luteal phase. However it may also indicate the presence of a pyometra, vaginitis, early pregnancy failure, or a urinary tract infection (UTI.)

2. How might you further investigate this case?

A complete blood count and ultrasonographic examination should be used to rule out pyometra and embryonic death. In most cases, no other diagnostic procedures are required.

3. What is the significance of this organism, and what action would you take?

Beta-hemolytic streptococci are common commensal in bitches. Many species of bacterial are present in the vagina of normal bitches. Aerobic commensals commonly isolated include *E. coli*, staphylococci and streptococci. The majority of these probably originate from the skin and the digestive tract. Occasionally pure growths of a single bacterial species occur but pure growths may also be isolated from normal dogs. Therefore, it is not rational to exclude animals from mating on the basis of vaginal culture of common commensal bacteria.

Vaginal bacterial flora changes after mating these changes are not permanent.

4. Which organisms are likely to cause vaginitis in the bitch?

Specific infectious causes of vaginitis include *Brucella canis* and Canine herpes virus.

Canine herpes virus may cause vesicles in the vagina and vestibule. Severe vaginitis has been reported following experimental infection. Canine herpes virus infection in pregnant bitches may produce small litters, late abortion, stillbirths and the birth of weak puppies. Neonatal infection of puppies can also occur as they pass through the birth canal.

The etiology of many cases of vaginitis often remains obscure. In one study no causal factor was identified in 32% of cases

Apart from infectious organisms, vaginitis can also be caused by urine pooling, foreign bodies, neoplasia. Anatomical abnormalities of the vagina such as vestibulo-vaginal constrictions can predispose bitches to vaginitis. Surgical ablation of constrictions is sometimes required.

Diagnosis of vaginitis requires vaginal cytology (often overlooked as diagnostic tool) digital examination of the vestibule and vagina, endoscopic examination and contrast radiographic examination.

Usually, removal of the underlying cause rapidly results in a cure.

When there is no obvious cause for vaginitis, empirical treatment with systemic and topical antimicrobial agents may be of some value.

