# CANINE THERIOGENOLOGY CONFERENCE


**December 10, 2000**

**presented by**
**The Ohio State University**
**College of Veterinary Medicine**
**Columbus, OH**

# Speakers

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## Canine Theriogenology Conference

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# ANATOMY OF THE BITCH AND STUD

**Cassia Orlandi, DVM**

## MALE GENITAL ORGANS

The male gonad migrates outside the abdominal cavity through an opening in the inguinal region of the abdominal wall. This location provides a suitable temperature for the spermatozoa development. The penis must be external for copulation.

Prostate gland - single accessory sex gland located within the pelvic canal.

Scrotum - skin pouch located between the thighs to house the two testes. Its surface is creased by a median raphe. Internally, a medium septum creates a separate chamber for each testis.

- Dartos - smooth muscle and fibrous tissue layer that acts as cutaneous muscle and forms the septum. Contraction of dartos muscle fascicles causes the skin of the scrotum to wrinkle, thereby reducing exposed skin surface and shifting scrotal contents toward the body.

  Function: minimize the rate of heat loss from the scrotum.

Testis - each testis is ellipsoid and lies within a scrotal cavity with its long axis directed craniocaudally and tilted so its cranial end is most ventral.

- Tunica albuginea - thick fibrous capsule.

  Spermatozoa are produced by **Seminiferous tubules** within the lobules; the tubules empty into a network of ductules, called rete testis, within the mediastinum.

Epididymis - long tube that stores and transports maturing spermatozoa

It is divided into a head, body, and tail.

Head - caps the cranial end of the testis
Body - lies on the dorsolateral surface of the testis
Tail - attached to the caudal end of the testis
The tail transforms gradually into ductus deferens

Ductus deferens - ascends as a component of spermatic cord and enters the abdominal cavity through the inguinal canal, and it has a mesentery called mesoductus deferens.

Spermatic cord

- Ductus deferens ( deferential vessels and nerves and visceral vaginal tunic)
- Testicular artery, sympathetic nerve plexus, and visceral vaginal tunic surrounding these structures.

Together the artery and veins constitute a counter- current system for simultaneously conserving body heat and keeping the testes cool.

Inguinal canal - the opening in the abdominal wall through which the spermatic cord, vaginal process, internal spermatic fascia, cremaster muscle, external pudendal vessels, and genitofemoral nerve pass from inside to outside the abdominal cavity.

The canal consists of-

- deep inguinal ring
- inguinal ligament
- superficial inguinal ring

Cremaster muscle

When the cremaster muscle contracts, the testes and other contents of the fibrous tunic are held closer to the body where they are less pendulous and vulnerable during physical exertion.

Prostate gland

A medium septum that divides the gland into right and left lobes, which are divided by septae into lobules.

Capsule and septae- contain smooth muscle fascicles that contract to expel prostatic fluid during ejaculation.

Surrounds the proximal cranial end of the urethra, been caudal to the neck of the bladder and positioned between the rectum and symphysis pelvis. Disseminated prostate gland is present in the submucosa of the pelvic urethra.

Pelvic urethra

- prostatic urethra: surround by the prostate (lacking in smooth muscle, but rich in elastic fiber)
- postprostatic: retroperitoneal (thick coat of striated muscle- urethralis muscle, which is necessary for ejaculation).

Penis

- Bone (surrounding the penile urethra)
- Erectile tissue

Numerous venous sinuses enclosed within a fibroelastic capsule termed tunica albuginea. Engorgement of the venous sinuses with blood increases internal pressure which stretches the fibroelastic wall, rendering it and the penis turgid.

Bulb glandis - caudal portion of the glands. It continues to swell following copulation, and its slow collapse after ejaculation, which is responsible for the prolonged "tie" common between mating dogs.

- Paired retractor penis muscles: Run superficially to the midline along the caudal ventral surface of the penis and insert on the distal end of the body of the penis.

Prepuce

- External layer (skin)
- Preputial cavity (opens at the Preputial orifice and houses the enclosed penis)
- Internal lamina of the Preputial wall

Erection

Penis protrudes through the preputial orifice.

Internal lamina pulls away from the Preputial wall and coats the caudal end of the free penis like skin. Fascicles of cutaneous trunci, called preputial muscle, leave the parent muscle and insert in the lateral and ventral wall of the preputial to assist return of the penis into the prepuce.

## FEMALE GENITAL ORGANS

The external genital structure of the bitch:

Vulva-Positioned caudoventral to the ischial arch

Consists of: Pudendal labia, vulvar cleft (Rima pudenda) and clitoris

- Left and Right Labium (fat connective tissue/smooth muscle/ striated constrictor vulva muscle) The dorsal and ventral labial commissures are the fusion lines of the labia
- The Rima pudenda is the opening common to both urinary and genital systems
- Clitoris (homologue of the male penis)

Located ventral (cranial) to the labia end vestibule

- Glans (erectile tissue)

  - Body (fat within a fibrous capsule)
  - Paired crura (fibrous tissue surrounding a small core of erectile tissue)
  - Each crus attaches to the ischial arch and is covered ventrally by a small ischiocavernosus muscle.

*Fossa clitoridis – caudal extent of the clitoris, located at the ventral labial commissure and visible when the vulva cleft is parted (not to mistake the fossa clitoridis for the urethral opening.)*

Urethral Opening-located cranially in the vestibule.

Vestibule-Common urogenital chamber, extends from the vulva cleft to the vagina.

- Wall of vestibule (striated constrictor vestibule muscle)
- Minor vestibular glands
- Bilateral vestibular bulbs (joined by a ventral isthmus)
- Vestibular bulbs are masses of erectile tissue that are homologous to the bulbs of the penis.

The Constrictor Vestibuli and Constrictor vulvae are two muscles of considerable importance in the bitch. These striate muscles that encircle the vestibule and vulva comprise the Bulbospongious Muscle.

Constriction of the penile veins by the constrictors vulvae and vestibuli enhance erection in the dog.

Vagina - Located between the vestibule and the uterus

The vagina extends cranially for a considerable distance; longitudinal and transverse folds (RUGAE) in the vagina allow for its expansion.

Fomix of the vagina - cranial extent of vaginal lumen

Cranial wall - cervix of the uterus (the external uterine orifice is normally small)

Uterus - (cervix, body and two uterine horns)

- Cervix - short, greatly thickened by accumulation of circular smooth muscle. Normally only a small diameter cervical canal connects the lumen of the vagina with that of the body.
- Body of the uterus - short
  - caudal wall (cervix)
  - cranially (body bifurcates into two long uterine horns)
- Uterine Horn - houses fetus during pregnancy and courses along the lateral abdominal wall. The intercomual ligament connects the two cornua near this junction with the uterine body.

Bordered medially by the descending colon (left side) and the jejunum (right side)

Uterine tube - small diameter tube ( fertilization takes place)

Serves to convey ova released by the ovary to the uterus. Also, the uterus conveys spermatozoa to the tube, so that fertilization usually takes place within the uterine tube.

- Infundibulum - funnel shaped dilatation that opens into the ovarian bursa near the slit between bursa and peritoneal cavity.
- Fimbriae -extremely small finger-like processes on the free edge of the infundibulum that partially protrudes from the bursa into the peritoneal cavity.

Ovary - Located just cranial to its corresponding uterine horn, and close to the caudal end of its respective kidney, involved by an ovarian bursa.

- Ellipsoid in shape
- Surface appearance varies with the reproductive status of the animal

*Ligaments attach to the ventral border of the ovary*
- Proper ligament of the ovary (connects the ovary to the uterine horn)
- Suspensory ligament of the ovary (extends to the dorsolateral abdominal wall at the level of the last rib.

*Broad ligament – connection between visceral peritoneum and parietal peritoneum*
- Mesometrium (extends from the uterus and cranial vagina to the dorsolateral abdominal wall and lateral pelvic wall)
- mesosalpinx (lateral fold of mesovarium extends ventral and medial to the ovary)
- mesovarium (connects to the ovary, cranial border-suspensory ligament of the ovary)

The broad ligament allows considerable movement of the uterus.

ATLAS OF CORRELATIVE IMAGING ANATOMY OF THE NORMAL DOG (Daniel A. Feeney, DVM, MS; Thomas F. Fletcher, DVM, PhD; Robert M. Hardy, DVM, MS) 1991 W.B. Saunders Company.

CANINE ANATOMY-A SYSTEMIC STUDY (Donald R. Adams) 1986 The Iowa State University Press, Ames, Iowa.

# Spermatogenesis

**Robin Wulf**

**Spermatogenesis** -the production of sperm
Occurs along the basement membrane of the seminiferous tubules
Ends when the spermatozoa are released into the lumen of the seminiferous tubules
**Spermatocytogenesis** - process of cell division that result in a sperm cell with one half the chromosomal material. During this process, a spermatogonium differentiates into a spermatid
**Meiosis** - process by which genetic chromosome number is halved. The purpose of this process is to produce a sperm which, following fertilization, produce offspring with a normal amount of genetic material. This is also the time when all genetic variation occurs.

## Importance:

1. Genetic variation2. Chromosomal abnormalities such as XXY (Klinefelter's) sex reversal, and hermaphrodism

Spermiogenesis - phase during which morphologic change occurs with the maturation of spermatids to spermatozoa. Changes include:

1. condensation of nuclear chromatin and elimination of excess cytoplasm
2. formation of flagella .
3. development of acrosome

Types of cells lining the basement membrane of the seminiferous tubules:

1. Sertoli cells (nurse cells) – from the basement membrane to the lumen of the seminiferous tubule. Provides the blood-testis barrier and creates a suitable environment for spermatogenesis to occur.
2. Spermatogenic cells – also in the seminiferous tubule.
    a. spermatogonia – primitive germ cells resting on the basement membrane that replicate by mitosis – 2N
    b. primary spermatocytes – larger cells derived from the spermatogonia that move away from the basement membrane – 2N
    c. secondary spermatocytes – cells resulting from the first meiotic division of the primary spermatocytes – N
    d. Spermatids- smallest cells are located the closest to the lumen of the tubule – N
    e. Spermatozoa – most differentiated cells that result in the development of a head and tail. These tails extend into the lumen of the tubule.
3. Leydig cells – found outside of the seminiferous tubules in the interstitium. They are closely associated with blood vessels and lymphatic vessels.

Spermatogenesis is a continual process.

- Initiation of differentiation is staggered in time and in place

- At any given location within the seminiferous tubule, germ cells will be in varying specific maturational associations
- A given length of tubule is usually surrounded by lengths of tubules either proceeding or following in differentiation – "spermatogenic wave"

- Spermatogenesis therefore is a continual process.

Possible interruptions of spermatogenesis:

- Chemotherapy agents such as vinblastine
- Nitrofurantoin and amphotericin (antibiotics) arrest spermatogenesis

**Endocrine Control**

1. Testosterone - produced by Leydig cells

-Creates an appropriate environment for spermatogenesis. It is also essential for the development of accessory sex glands, normal behavior, and secondary sex characteristics.

2. Luteinizing hormone - produced by the pituitary gland

-Stimulates Leydig cells to produce testosterone
-Regulated by negative feedback by testosterone

3. Follicle Stimulating hormone - produced by the pituitary gland
-Stimulates Sertoli cells to produce androgen binding protein
4. Androgen Binding Protein - produced by the Sertoli cells
-Maintains high levels of testosterone within the seminiferous tubule

5. Inhibin - produced by the Sertoli cell
-Inhibits the secretion of FSH

**Blood-testis barrier** - prevents the seminiferous tubules from being exposed to blood and lymph components. Sertoli cell tight junctions prevent large proteins from entering the lumen of the tubule as well as excluding harmful chemicals. They create an environment in which meiosis can occur.

**Importance** - disturbances in hormonal regulation can cause decreased spermatogenesis. Examples:

1. Anabolic/Androgenic steroids - prevent LH release by negative feedback, which decreases the amount of testosterone production.

2. Progesterone - can cause decreased quality and release of sperm quality by decreasing plasma testosterone concentrations.

3. GnRH against chemicals - inhibit release of both LH and FSH

4. Cimetidine and glucocorticoids - inhibit LH release .

5. Ketoconizole - inhibits testosterone production

**Spermiation** - release of spermatozoa into the lumen of the seminiferous tubule.

# ESTROUS CYCLE OF THE BITCH

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## Puberty

Dogs generally attain puberty 2-3 months after reaching adult body size. Average 6 to 12 months age- range 4 to 24 months. First estrus can be abnormal (split or variable). Occurs sooner if young bitches are housed with cycling bitches.

Smaller breeds reach adult size earlier and consequently reach puberty sooner than large breeds.

Estrous cycle interval averages 7.5 months (range 4 to 24 months). Variable with breed and pregnancy and lactational status. Can lengthen interval by several weeks. Basenji, dingo and wolf - 12 months.

Cycles begin at all times of the year, but there is a small, yet significant increase in the occurrence of estrus in the late winter and spring months.

## Stages of the Estrous Cycle

Clinical Evaluations to determine the stage of the estrous cycle: Behavior; Vulvar appearance; Vaginal appearance; Vaginal cytology; Uterine tone; Progesterone concentrations; Luteinizing hormone concentrations.

1. Proestrus:

Several weeks prior to the onset of proestrus: Increase in appetite; Increase tolerance of male: Aggressive early and "sits down" late proestrus.

Immediately before proestrus: Inappetent; Listless; Nervous.

Average duration: 9 days; range: 3-17.

Period from onset of a sanguineous vaginal discharge and initiation of vulvar swelling which increases until late proestrus.

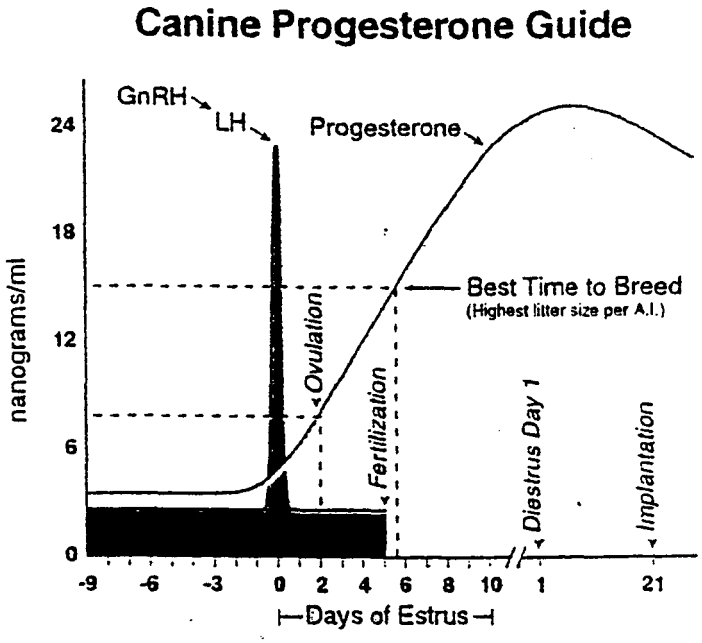
Estrogen levels increase beginning 3 to 4 weeks prior to onset of proestrus -correlated with follicular development.

Follicles begin to luteinize before estrogen secretion is maximum and prior to ovulation. This causes progesterone concentrations to increase prior to ovulation.

With the luteinizing hormone (LH) surge, luteinization occurs rapidly following ovulation. Thus, ovulating follicles rapidly develop into corpora lutea.

2. Estrus:

Average duration 9 days (range 3-18)
Bitch accepts mating – standing and flagging
Onset coincides with burst of LH.




Excellent uterine tone.

Estrous vulva is softer and smaller than during proestrus.

Vaginal discharge may remain sanguineous or appear straw-colored into estrus; in some bitches it remains red without appearance of red blood cells observed on vaginal cytology.

Serum estrogen levels drop dramatically at the end of proestrus.

Ovulation occurs within 24 to 72 hours following the LH surge.

Time of ovulation can be predicted through the examination of cytological changes observed in anterior vaginal samples and with the aid of serum progesterone concentrations.

The dog is unusual in that ova are released prior to formation and elimination of the first polar body (1st meiotic division). First polar body is shed from ovum in the oviduct.
This occurs within 24 to 48 hours post ovulation.
Fertilization occurs following shedding of the polar body because sperm penetration cannot occur until after its formation and elimination.

Fertility in the normal dog is usually not dependent on a precise breeding time because of the longevity and fertilizing capability of dog sperm (i.e., 7 days).

3. Metestrus I:

Time from ovulation until the CL is full functional - 5 to 6 days.

4. Diestrus (Metestrus II?):

Average duration 60 days.

Luteal activity reaches a peak about 25 days post ovulation and slowly declines there after.

Endometriai glandular formation increases significantly during the luteal phase under the influence of progesterone.

The prolonged periods of progesterone exposure as occurs during diestrus, together with the responsiveness or sensitivity of the endometrium, may result in cystic endometrial hyperplasia. The sensitivity of the endometrium to progesterone following estrogen sensitization is the basis for the relatively high incidence of pyometra in the dog.

Diestrus ends with a drop in serum progesterone to less than 1 ng/ml, which may not be distinguished clinically from anestrus.

5. Anestrus:

Duration approximately 4 months; breed dependent.

No reproductive hormonal activity until near the end of this stage.

Clinical Examination of the Bitch for Prediction of Estral Stage

I. Physical

- Uterus
  - Palpate uterus - uterine tone increases during proestrus.
- Vulva
  - Inspect vulvar conformation
  - Determine vulvar edema - disappearance of vulvar wrinkles during proestrus.
- Vagina
  - Digital
    - Determine physical and behavioral resistance.
  - Speculum
    - Observe anterior vagina for mucosal wrinkles; similar to vulvar changes.

II. Vaginal Cytology

A. Clinical Uses

To estimate progression toward first day of estrus in bitch that is being shipped for breeding, bred away from home, artificially inseminated with frozen, shipped, or fresh semen, or one which owners wish to confine until fertilization is no longer possible.

To correlate endocrinologic, physiologic and behavioral states in the bitch that has infertility problems.

To determine if an abnormal condition exists which may interfere with conception or pregnancy maintenance.

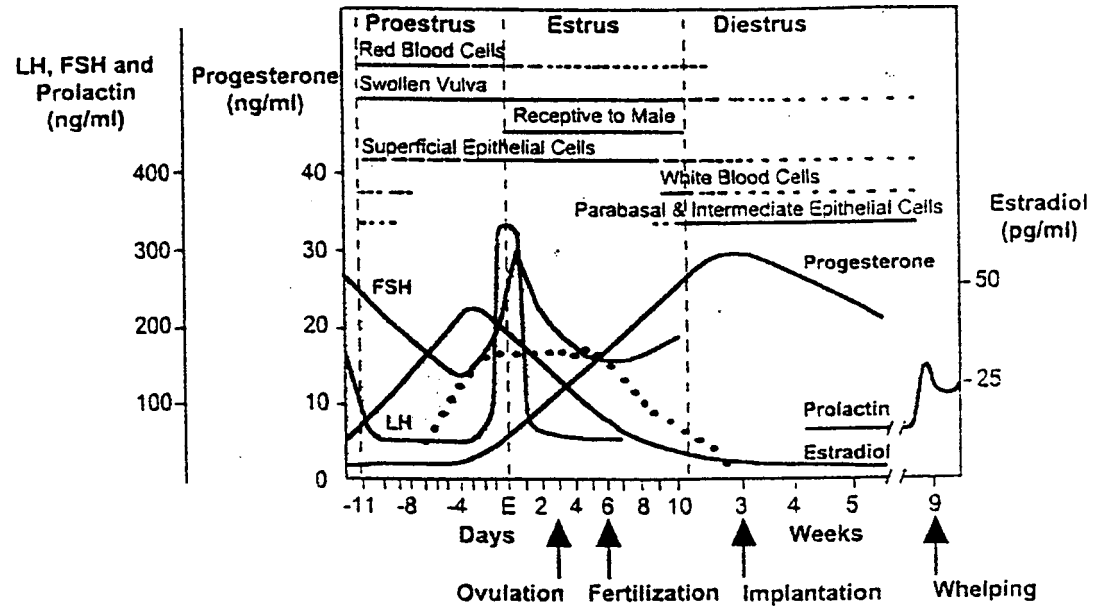
B. Interpretation

All the cytologic changes excluding the leukocytes are a reflection of the changing estrogen and progesterone concentrations which characterize the estrous cycle.

Vaginal epithelium is stimulated to undergo mitosis at the basal lamina due to estrogens. Therefore, cells being progressively moved further away from the blood supply and toward the lumen of the vagina undergo degeneration as they are displaced.

**Relationship of hormonal concentrations to vaginal cytology and behavior.**

## Concentrations of Hormones in Serum, Behavioral Events, and Physiologic Changes During the Canine Estrous Cycle




Cell Types

1. Noncornified Cells

Para basal and intermediate cells are considered non-cornified - close to blood supply.

a) Parabasal Cells: The smallest epithelial cells normally seen; they are round or elongated, stained more darkly than intermediate cells, and contain large, well-stained and well defined nuclei.
b) Intermediate Cells: Round or oval-shaped cells with abundant cytoplasm and large, well-defined vesicular nuclei; they may be subdivided into small and large intermediate cells.
c) Metestrum Cells: Modified parabasal cells which contain neutrophils within their cytoplasm.
d) Foam Cells: Modified parabasal cells containing prominent cytoplasmic vacuoles.

2. Cornified Cells

Superficial and anuclear cells are considered cornified – close to lumen.

The largest percentages of anuclear and superficial cells will be present within a day or two after the LH surge – i.e. , synchronous with ovulation.

Epithelial cellular changes:

1. become larger
2. border becomes irregular
3. cytoplasm density changes
4. nuclei disappear
    A) Superficial Cells: Large cells with angular shaped borders; cytoplasm starting to change toward a nonuniform staining appearance; and nuclei are small, darkly stained, and pycnotic.
    B) Anuclear Squames: Large cells resembling superficial cells; evidence of cytoplasm degeneration; irregular border; and the nuclei do not take up stain, so they appear anuclear.

3. Leukocytes

The few WBC's normally present disappear from the vaginal smear within a few days of the onset of proestrus due to the increased thickness of the vaginal epithelial wall.

4. Erythrocytes

Vascular system of endometrium develops under the influence of estrogen and RBC's appear in the vaginal smear during proestrus due to diapedesis.

**Vaginal swab for cytologic sample obtainment**

Saline soaked sterile cotton swab
Anterior vagina
Roll onto 2 slides

**Staining method we utilize (others available).**

1. alcohol fix – 15 to 30 seconds
2. eosin – 15 seconds
3. new methylene blue – 30 seconds

**Cytologic characteristics at different stages of the cycle are:**

Proestrus (early): Intermediate and superficial cells, red blood cells and white blood cells. Debris and mucus will be present.

Proestrus (late): Superficial cells and anuclear squames, red blood cells and not white blood cells. Debris and mucus will be less.

Estrus: More than fifty percent anuclear squames, superficial cells, fewer red blood cells. No white blood cells until possibly near the end of estrus. Debris and mucus will be absent.

Diestrus (early): More than fifty percent intermediate cells, superficial cells, squames, metestrum cells and foam cells.

As diestrus proceeds, the smear becomes completely noncornified with neutrophils appearing in varying numbers but never excessive unless abnormality exists.

Anestrus: Small numbers of parabasal cells and intermediate cells, with minimal neutrophils present.

**Pregnancy Diagnosis**

| | |
|---|---|
| 21 days | Reprocheck (Synbiotics) |
| 21-30 days | Palpation |
| 21 days | Ultrasonographic examination |
| | Should not be used to determine fetal number |
| 43 days | Radiograph |
| | Can be used to determine fetal number |

# DETERMINATION OF WHEN TO BREED

## GENERAL CONSIDERATIONS

Sperm: reach the mid to distal oviduct within 25 seconds of ovulation, can survive and be motile up to 11 days after natural breeding, survivability with high fertility is estimated to be up to 5 to 7 days, and capacitation (required for fertilization) requires approximately 7 hours.

Insemination, either natural or artificial, early in estrus *should* result in viable sperm being present at the time viable fertilizable oocytes are present.

Bitches bred 4 or 5 days before the ovulation produced pups from 96% and 90% of ovulated oocytes respectively. Better conception rates occur if the bitch is bred from 4 days before to 3 days following ovulation. Best litter size is obtained by breeding the bitch 2 days following ovulation. Because of this and other research, the recommendation is to breed bitches on the first day of standing estrus and again 2 and 4 days later in the absence of other diagnostic tests to increase the number of pups produced from eggs ovulated. Recommendations include the breeding of the bitch every 3 days from the onset of estrus if no other methods to predict estrus progression are utilized.

The preferred method is to determine the day of ovulation and breed the bitch two days later.

Although counting the days from the onset of proestrus is most commonly used to determine the day of ovulation, it is the poorest of the methods available.

Progesterone concentrations can also be utilized to predict the time to breed by waiting until the progesterone rises above 2 ng/ml. This is the second most effective and accurate method to predict ovulation and is frequently recommended for artificial inseminations. These assays can be performed in the hospital or clinic with no additional equipment available and results are available within ten minutes.

The most accurate method of predicting ovulation is the use of serum or urinary luteinizing hormone. Test kits are available to owners and veterinarians with results available within 10 minutes.

## DETERMINATION OF OVULATION

Reasons: To minimize breedings; Infertile bitches; chilled semen breeding; and frozen semen breeding

Behavioral Estrus:

Can be used to determine the time of breeding, but is the most variable of the four methods described.

Some bitches in proestrus will accept the male and breeding, therefore, occurs well in advance of the ideal fertilization time.

Many bitches will accept the male past the period of high fertilizability.

Some bitches will not accept the male at the proper time or at any time during the estrus.

For these reasons, the behavioral signs of the bitch are the most unreliable and other diagnostic aids are recommended.

Vaginal Cytology:

Cytologic samples taken of the anterior vagina can provide an indication of the progression of proestrus and estrus.

These samples should be repeated at two to three day intervals through proestrus and at the time of breeding.

The changes indicative of breeding include no red blood cells, no white blood cells, no mucus, no debris and 80 percent or more cornified superficial epithelial cells.

Due to the variation in this bioassay reflecting the changes in estrogen and progesterone, it is more precise to measure luteinizing hormone or progesterone directly.

At least one vaginal cytology should be performed on every bitch to determine the leukocyte numbers in order to establish the general health of the reproductive tract.

Progesterone Assay:

Progesterone concentration increases generally begin the day before the luteinizing hormone peak.

There is variation in this rise and, therefore, progesterone concentrations are used as an estimate of the luteinizing hormone peak.

Concentrations of less than 1 ng/ml indicate the bitch is in anestrus or early to mid proestrus.

A gradual increase to 1 to 2 ng/ml indicates a slight rise in luteinizing hormone and the probability that peak luteinizing hormone will occur the following day.

Concentrations of 2 to 3 ng/ml indicate the luteinizing hormone peak has occurred and concentrations of over 4 are indicative that ovulation has occurred.

Radioimmunoassays are more precise in quantitating the progesterone, but require more time than ELISA assays. ELISA assays (available in kit form for “dog” slide utilization) are only qualitative, but provide results within 10 to 15 minutes.

Luteinizing Hormone Assay:

Luteinizing hormone peaks approximately 48 hours before ovulation.

The oocytes are mature and capable of being fertilized at approximately 48 hours following ovulation.

The oocytes remain fertilizable for 48 to 72 hours following maturation.

The optimal time to breed a bitch is 4 to 6 days following the luteinizing hormone peak.

Blood or urine samples must be determined daily since luteinizing hormone peak lasts only 24 hours.

Breeding Recommendations:

Natural Service:

Breed at 2 days following the luteinizing hormone peak;
breed at 2 days following the day progesterone concentration reaches 2.5 ng/ml;
breed at 2 and 5 days following vaginal cytologic cornification reaching 80% or more with few to no red blood cells, no white blood cells, no mucus and no debris; or
breed at 2, 4 and 6 days following the first acceptance of the male.

Chilled Semen Inseminations:

Breed at 4 days following the luteinizing hormone peak;
breed at 3 and possibly 5 days following progesterone concentration above 2.5 ng/ml;

breed at 3 and 6 days following previously described vaginal and cytologic changes; or,
breed at 2, 4 and 6 days following acceptance of the male.

Frozen Semen Inseminations:

Breed at 5 days following the luteinizing hormone peak; or
breed 5 to 6 days following the progesterone concentration above 2.5 ng/ml.
Breed following acceptance of the male and following previously described vaginal and cytologic changes is not acceptable.

Sperm Survival in Bitch:

Fresh – 5 to 7 days following collection and immediate insemination or following natural breeding if the male is normal.
Chilled – 48 to 72 hours
Frozen – 24 hours

**Semen Collection, Handling, and Artificial Insemination**

Robin Wulf

Semen Collection

Reasons for collection

1. Evaluation for breeding soundness exam
2. Artificial insemination
3. Chilled/Shipped semen
4. Preservation of genetics through frozen semen

Preparation

Males should be placed in a quiet room without disturbances. Some males need to be in the presence of a bitch or even a bitch in heat in order to cause sufficient stimulation to permit semen collection.

Materials

1. Artificial vagina – plastic or latex is acceptable. The latex AV is sold as the small inner liner of a bull artificial vagina. Latex is preferred because it is easily sterilized and used again. There has been evidence that latex can be detrimental to semen but this does not seem to be significant due to the short contact time with the semen. It is important that there not be any soap or detergent residue left on the AV during cleaning.
2. Graduated tube – connected to the end of the AV. A graduated tube is superior to a nongraduated tube because it enables calculation of volume without transferring semen sample. This information is necessary to calculate concentration of semen in the ejaculate.
3. Sterile nonspermicidal lubricant – used in minimal quantities to prevent injury to the penis.

Collection

1. Penis should be prolapsed outside of the prepuce prior to complete erection. This reduces the possibility of pain associated with complete erection inside the prepuce.
2. Erection is obtained by grasping penis proximal to the bulb and applying pressure by placing fingers and thumb around the penis in this area.
3. Once erect, penis is firmly squeezed in this area and then relaxed.
4. This is repeated until ejaculation occurs.
   **Note-it is not necessary to vigorously rub the penis. To do so may result in damage to superficial blood vessels in this area.
5. Once the urethra begins to pulse, the first several jets of semen are allowed to go to the floor to allow flushing of the urethra.
6. The collection device is then placed over the penis and fluid collected until the seminal plasma becomes clear.

Evaluation

1. Volume is recorded
2. Motility is assessed immediately because this parameter is most likely to change. Evaluation of motility requires the use of a microscope.

3. Morphologic examination – a small drop of semen should be taken at this time and a stain made. The stain used most frequently here is eosin-nigrosin. This slide can be evaluated after the semen is evaluated.
4. Concentration – can be assessed either with a densometer or Unipette system with hemocytometer to count.

*Complete semen evaluation is necessary in a stud older than 5 years or in a stud that has not had a litter in more than a year.

Semen Handling

It is important to handle the semen sample carefully. Although many dogs have sufficient numbers of healthy sperm to result in conception despite poor handling, a dog with low sperm concentration or poor motility needs every advantage. Wide changes in temperature should be avoided. When motility is evaluated, slide should be warmed to near body temperature.

Chilling of Semen

*Important to use an extender that works well with dog

1. Extended semen is cooled in container to approximately 40°C
2. Semen shipped in sealed tube that has been placed in plastic bag
3. This is put in Styrofoam box with ice packs
4. Include paperwork on male inside cooler (including semen evaluation)
5. Also include instruction on insemination technique.

Artificial Insemination using Chilled or Fresh Semen

1. Warm up semen prior to insemination (only with chilled semen)
2. Draw sample containing sufficient number of sperm into Air-Tite brand syringe. (This syringe is superior because it does not have spermicidal effects on the semen and the air in the syringe prevents the retention of sample inside the syringe.
3. The bitch is elevated with her hindquarters 18 inches above her head.
4. The insemination rod is inserted into the anterior vagina of the bitch.
5. The bitch's hindquarters should remain elevated for approximately 10 minutes.
6. A finger may be used to feather the dorsal wall of the vagina, which is believed to cause release of oxytoxin and enhance transport of semen.

Freezing of Semen

*Again, it is important to use a freezing extender that works well with an individual animal.

1. Extended semen is cooled gradually. Semen is then allowed to equilibrate to the new temperature for several hours.
2. Semen can either be frozen in straws or in pellets. Freezing must be rapid and requires the use of frozen nitrogen.
3. Semen is stored in nitrogen tanks

There are many acceptable protocols for the freezing of semen. Many commercial extenders will provided instructions for their product. It is important to realize that freezing semen requires special equipment such as liquid nitrogen and requires some initial investment.

Insemination with Frozen Semen

Correct timing is critical when using frozen semen. Due to the reduced capability of the semen to pass through the cervix, surgical insemination is recommended. The most common procedure is a ventral midline approach with direct deposit of semen into the uterus. More recent techniques include laproscopy and catheterization of the cervix by flexion of the bitch. Surgical insemination is still the most frequently used technique.

# PARTURITION

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Preparturient changes in the bitch include relaxation of the vulva, increased mammary development with the presence of colostrums, and increased maternal behavior including nest building in many bitches. Rectal temperature of the bitch has been utilized by many breeders to determine the approximate time of whelping. The body temperature will decrease to less than 100°F and many time to less than 99°F. This drop in temperature occurs approximately 24 hours before the delivery. The decrease in temperature is believed to be related to the decrease in progesterone which precedes the temperature change by 12 hours. Progesterone is known to be thermogenic in other species and apparently in the bitch. It has its action by re-establishing the hypothalamic thermostat controlling body temperature. When progesterone drops the hypothalamic thermoregulatory mechanism must readjust in order to compensate for the reduction in a substance that has the capability of elevating temperature. Parturition in the bitch will occur until the progesterone concentration decreases below 2 ng/ml. This also can be used clinically to determine the approximate time of whelping. Cortisol concentrations decrease prior to parturition but the relationship of progesterone control to this decrease is not known.

As with other species stage I of parturition begins with increased uterine contractions and dilation of cervix. The bitch will become restless and appear apprehensive. The bitch's heart rate and respirations will increase and the later exhibited as panting. She will stay close to or in the nest. The length of a normal stage I labor is between 6 and 12 hours. The end of stage I labor occurs at the time of rupture of the first chorioallantoic membrane. This may not be as apparent as in other species due to the lesser amount of chorionic fluid in the bitch.

Stage II of labor involves the expulsion of fetuses from the reproductive tract. The duration of this stage is usually between 3 to 6 hours but may require up to 24 hours. As the fetus enters into the cervix, its presence creates a stimulatory stretching and resultant release of oxytocin. This phenomenium has been referred to as the Ferguson reflex. The oxytocin will increase uterine contractions and this will cause further abdominal contractions to aid in fetal expulsive. Although the bitch is usually recumbent during this stage of gestation it is not uncommon for her to move about within or outside the nest. Fetuses are delivered approximately every 45 minutes although variations of 2 to 120 minutes are possible. Approximately 60% of the fetuses are delivered in anterior presentation. The remaining posterior presentation deliveries do not create a problem as they would in the unipara due to the bullet shape and short extremities of the fetus. the bitch can rupture the chorionic membrane with her teeth and will also sever the umbilical cord and eat the placenta. The severing of the cord or the consumption of the placenta does not appear to be detrimental to the bitch or the neonate. It is considered to be an abnormal delivery if the bitch has not expelled a fetus within 5 hours of the beginning of stage II or if a fetus is not delivered within 2 hours of the previous fetal delivery.

Stage III is characterized by expulsion of the placenta and the beginning of uterine involution. The individual placentas are passed following delivery of each fetus or immediately after the delivery of a second fetus. This generally occurs when two fetuses are expelled from opposite uterine horns before the first fetal placenta is

passed; then both placentas may be passed at one time. If the pups are alive and well there is no need to administer oxytocin to the bitch postpartum to enhance uterine involution. If the fetuses were delivered dead or the pups die shortly following delivery, the administration may be indicated in order to assure normal involution. The nursing stimulus of the fetuses is an adequate stimulus for oxytocin release.

Uterine involution continues for 90 days following delivery. The vulvar discharge which occurs immediate following whelping is a red, brown, or greenish color with a consistency of seromucus. The normal uterine discharge should not be confused with uterine abnormalities since the misdiagnosis may result in unnecessary uterine therapy or the erroneous recommendation of ovariohysterectomy. Uterine disease discharge will be more watery in consistency and will have a foul smell. Furthermore, the bitch may appear systemically ill with severe uterine disease but may not with endometritis or nonseptic metritis.

# OBSTETRICS

Examples of breed related parturition abnormalities:

Dachshund - Susceptible to primary inertia.
Scottish Terrier – Maternal pelvis is compressed dorso-ventrally and fetal head is disproportionately sized.
Racing Greyhound – Lethal factor that may result in delivery of dead or nonviable neonates.
Brachycephalic breeds – Large fetal heads in relationship to the bitch's pelvis plus inability of normal abdominal pressure slows or causes failure of delivery.

**Approach to Dystocia**

History

Accurate breeding records
First signs of whelping and the exact sign of labor.
Appetite and urination and defecation history.

Examination

Abdominal palpation - if possible
Vaginal examination – Thoroughly cleanse the perineal area and use sterile gloves.
Feathering during the vaginal examination will often stimulate uterine contractions.
Oxytocin administration at a dosage of 0.5 to 1.0 cc dependent upon body size will stimulate uterine contractions.
No more than two doses of oxytocin should be administered before vaginal examination reveals fetal movement through the vaginal canal.

Anesthesia

Best is epidural anesthesia

General Principles

1. If the dam has been in labor, she may be hypotensive (dehydrated), hypoglycemic, and hypocalcemic. Correct these things, if possible, before anesthesia.
2. All anesthetic drugs cross the placenta. The time between induction of anesthesia and removal of the fetuses should be as short as possible. Clip and prep the patient prior to anesthesia. Use light sedation if necessary to accomplish this.
3. Epidural anesthesia is the most optimal way to provide anesthesia for the newborn.

Site L7-Sl
Anesthetic – 2% Lidocaine
Dose lml/6kg (3mg/kg) body weight over 1-2 minutes
Be certain CSF and/or blood is not coming from needle (1/2 dose).
Needle
1 inch – 20 to 22 gauge

Sedation

Oxymorphone

0.05 to 0.1 mg/kg IM

Acepromazine

0.05 to 0.1 mg/kg IM (3mg maximum)

4. Oxygenation is important. If using epidural anesthesia, may want to apply a face mask.
5. Inhalant anesthetics rapidly cross the placenta and should be used sparingly until the newborns are removed from the uterus, then their concentration can be increased.
6. The number one goal for the newborn is to get it to breathe. Minimize the use of drugs that produce significant respiratory depression or use drugs that are reversible (opiods).

Compromised patient:

Slight sedation (diazepam or oxymorphone). Oxygen. Line block in ventral midline with 2% lidocaine. Maximum dose 1 ml of 2% lidocaine/4lbs. body weight.

Tractable animal:

Diazepam/ketamine induction (1ml/20 lbs of a 50:50 vol:vol mixture), endotracheal intubation, oxygen, inhalant when the puppies are removed.

Intractable animal:

Aceptromazine (0.1 mg/lb IM up to 4 mg total dose) in combination with oxymorphone (0.03-0.05 mg/lb IM) or butorphanol (0.1 mg/lb IM). Induce with thiopental (2-5mg/lb IV to effect) or propofol (2-5 mg/lb IV). Intubate. Oxygen. Inhalant when the puppies are removed.

Maternal Dystocias

Pelvic abnormalities
Vaginal cystocele
Uterine torsion
Hormonal dysfunction

Uterine inertia

Primary uterine inertia is a lack of uterine tone due to deficiency of exercise, overweight conditions of the bitch, overstretching of the uterus, debilitating disease, dysfunction of the posterior pituitary, deficiency of oxytocin production, release or a failure of the presence of normal receptors. Treatment for this condition is the administration of oxytocin and assistance with the delivery by traction or a cesarean.

Secondary uterine inertia is due to extreme attempts to deliver a fetus with the uterus becoming eventually fatigued and unable to respond to the endogenous oxytocin. The treatment choice for this cause of dystocia is usually cesarean.
Calcium supplementation may be beneficial for this condition.

Fetal Dystocias

Oversize of fetus

Oversize of the head
Irregularity of the fetal limb
Irregularities of the fetal head
Fetal anasarca

Common types of fetal dystocias
- Anterior or posterior dorso-sacral position
  - Rotate fetus and then extract
- Lateral head displacement (flexion).
  - Use finger or forceps to redirect head.
- Posterior presentation
- Normal

# REPRODUCTIVE DISORDERS IN THE BITCH

## Failure of corpus luteum formation or function

- Bitches with the history of infertility should be examined for pregnancy at 25 days following the last breeding.
- Ultrasonographic examination or palpation.
- Serum progesterone concentration should be determined.
  - Bitches which are candidates for this test are all of those which are nonpregnant and pregnant ones when there is reason to believe resorption or abortion has occurred in the past with no other apparent reason.
  - Progesterone concentrations in nonpregnant bitches below 5 ng/ml indicate possible luteolysis has occurred.
  - Concentrations less than 1 ng/ml indicate ovulation did not occur or very early luteolysis did occur.
  - In bitches where premature luteolysis is suspected or differentiation between the two conditions is necessary, serial progesterone concentrations at 5 to 7 day intervals through day 25 or further into gestation is recommended.
  - Ovulation failure is an unlikely cause of lowered progesterone if estrous behavior, luteinizing hormone concentrations, vaginal cytology and/or vulvar discharge and swelling are normal.
- Progesterone supplementation of bitches during pregnancy may induce masculization of canine fetuses.

## Estrus Suppression

- Ovaban
  - Not indicated or recommended for use in breeding bitches.
  - Progestational compound and as such has an increased probability of pyometra or cystic endometrial hyperplasia developing.
- Cheque drops
  - Androgenic hormone used for estrus suppression.
  - Has the potential as do all androgenic steroids of inducing hepatotoxic lesions, its primary side effects include increased lacramation in toy breeds and clitoral hyperplasia.
  - Not approved for use in breeding bitches.
    - The author routinely uses it for suppression of estrus for 6 to 10 months and had a nonbreeding animal on medication for 12 years with no adverse side effects.

## Cystic Ovaries

- Prolonged proestrus and estrus
  - Signs of both occurring repeatedly multiple times during the same estrus, may be diagnosed as split heat or cystic ovarian disease.
  - Based upon information available in other species, the prolongation of a mature oocyte within the follicle reduces the probability the oocyte will be fertile and that ovulation will be normal.
- For this reason and because this condition can persist for 6 to 8 weeks if untreated, the author prefers to

treat accordingly with human chorionic gonadotropin. The success of this therapy is extremely high with over 90% of the animals responding to a single treatment.
The bitches cease the proestrus or estrus within 5 to 7 days.
Recommended dosage is 500 to 1000 international units administered intramuscularly.

Since the proestrus and estrus were prolonged it is advisable to always examine these animals at 25 to 35 days following treatment for the possible development of pyometra.

GnRH has also been used at a dosage of 50 ug administered intramuscularly.

Uterine subinvolution (subinvolution of placental sites)

Cause – unknown. Endometrial vessels do not thrombos and occlude

Signs – Bloody discharge 4 to 16 weeks, no systemic signs, spherical uterine enlargements

Considerations

No elevated progesterone
No infection present
No dystocias involved
No problems with fetuses
No effect on future fertility

Differentials

Any condition involving lumen of reproductive tract, metritis, vaginitis, vaginal neoplasia

Diagnosis

History
Vaginal cytology – Many RBC's no WBC's

Treatment

Nonbreeder

Ovariohysterectomy

Breeder

Ergonovine
Oxytocin
Prostaglandin F2 alpha

<u>Mismating</u>

First consideration-if the bitch is not to be used for breeding an ovariohysterectomy should be recommended within the first four weeks following mismating. This will obviously eliminate future problems with unwanted pregnancies.

Second consideration – examine the vaginal cytology for the presence of sperm.

Treatments

Estrogens:

Have been used for the termination of unwanted pregnancy.
Given before the embryo passes into the uterine lumen.

The purpose is to retard the transport of the embryo by causing contraction of the isthmal sphincter of the oviduct.

Also causes a degeneration of the embryo and an alteration of the endometrium.

Estradiol cypionate (E.C.P.) has been administered at the dosage of 0.25 to 1mg per bitch.

Diethylstilbestrol given at a dose of 1mg/lb up to a maximum of 25mg has been administered once intramuscularly.

Recommended times to administer are:

immediately after the breeding occurs in order to prevent normal development within the oviduct and again 7 days later to create an undesirable environment within the uterus in case fertilization and development did occur.

Disadvantages of the administration of the estrogens:

Extends the length of time the bitch is exhibiting signs of estrus.

Increased predispositon to pyometra occurring during diestrus.

Increased possibility of bone marrow suppression (bone marrow aplasia).

This may be non reversible and result in death of the bitch

Diethylstilbestrol appears to be less potent in the production of bone marrow suppression.

Prostaglandin F2 alpha

Not approved for use in the canine but is frequently utilized because of its excellent results.

Determine bitch is pregnant at 25 to 30 days before using this treatment.

Prostaglandin F2 alpha works best when given after day 18 of gestation.

Several doses of frequencies of administration are recommended in the literature.

Recommendation:

First time you use it:

Give 50 to 100 micrograms/kg SQ in a 10 to 20 ml final volume.

Give 100 to 150 micrograms/kg SQ in a 10 to 20 ml final volume.

Give 250 microgram/kg SQ in a 10 to 20 ml final volume.

Administer this dose repeatedly until embryos are gone.

This may require 5 to 10 days of treatment depending on the day of gestation treatment was started.

If you have used PGF2 alpha and are comfortable with it, start at the 250 microgram/kg dosage SQ with expanded volume.

Side effects:

Increased salivation
Increased heart rate
Increased respiration
Increased defecation
Increased urination
Gagging
Vomiting
Ataxia
Depression

These side effects begin approximately 20 minutes following administration and end approximately 20 minutes after initiation.

Bitches can be aborted starting treatment at 53 days of gestation using this procedure.

Advantage:
- Most physiologic
- No long term effects on the bitch
- No short or long term effects on reproductive health

Pyometra

Pyometra initiated by an interaction of:
- hormonal influences and
- uterine bacteria.

Endometrium physiologically altered due to repeated exposure to progesterone in some bitches.

Results in an eventual atypical response to this hormone.

No reported differences in the occurrence of pyometra with regard to estrous cycle irregularities, abnormal estrus and pregnancy characteristics. No reported difference between lesions seen in bitches having had a pseudopregnancy and those which did not.

Pyometra has been associated with the presence of corpora lutea, cystic corpora lutea older age of bitch and the first four weeks following estrus.

Death of the bitch if not treated promptly after systemic signs are present.

Predisposing factors include age, cystic endometrial hyperplasia, uterine bacteria, diestrus, estrogen administration and progesterone administration.

Progression of pyometra
- introduction of bacteria into the fluid within the uterine CEH lumen.

Pyometra has been observed to occur only during or immediately after diestrus.
- Elevated progesterone has been suggested as being an important part of the disease process.

Exogenous estrogens or progestogens
- 75 percent of affected dogs with pyometra less than three years of age had been given Estradiol cyprionate or megestrol acetate within the previous six months.

Exogenous Estradiol or progesterone

Uterine cultures
- E. coli was present in 66 percent of the uteri, Klebsiella sp. in 11 percent; Pseudomonas sp. in 6 percent; Proteus sp. in 6 percent; Staphylococcus aureus in 6 percent; Hemophilus sp. in 4 percent; and Serratia sp. in 2 percent.

Gram negative bacteria release a biologically harmful endotoxin (lipopolysaccharide (LPS)) during the disease process and following the use of bacteriostatic or bacteriocidal antibiotics.

Endotoxic substances remain active for up to two weeks in tissue.
Normal bitches have circulating LPS concentrations of 0.053 + 0.004 ng/ml.
Pyometra bitches had concentrations of LPS ranging from 0.091 to 0.956 ng/ml
Lethal range of concentrations being 0.7 to 1.0 ng/ml.
LPS possesses many capabilities: detrimental vasoactive actions and neuroendocrine actions.
Detrimental influence on complement, kinin, and the hemostatic systems.

Vaginal cytology

Increased numbers of white blood cells would be indicative of genital tract abnormality; not specifically uterine disease.

Taken immediately following the obtainment of an anterior vaginal culture using a guarded culture rod.

Cultures are most useful for the determination of antibiotic sensitivities for proper selection of antimicrobial agents.
Vaginal speculum examination has been found to be useful in the determination of the origin of vulvar discharges.
It also proves useful for differentiation of discharges originating from vaginal tumors.
Prostaglandin F2alpha (PGF2alpha) has been very successful in treating pyometra.

Currently appears to be the medication of choice.
Not approved for use in the canine in the United States.
Widely used for treatment of pyometra in this species.
Actions of PGF2alpha include:

Contraction of the myometrium
Relaxation of the cervix.

These two actions aid in physical expulsion of the pyometra fluid from the uterine lumen.

Recommended use of PGF2alpha in animals with pulmonary or cardiovascular disease varies due to the effect of this hormone on these systems.

Although no deaths were directly attributed to the PGF2alpha treatment, caution should be taken when treating animals with these conditions.
Side effects to be expected commenced within five minutes following PGF2alpha administration include: restlessness, increased respiration, increased lacramation, increased frequency of defecation, increased frequency of urination, abdominal pain, increased body temperature and vomiting. These side effects usually last for 20 to 30 minutes.
Systemic antiobiotics should be administered concurrent with PGF2alpha. Ten percent of treated bitches reportedly have a positive blood culture.
Anti-lipopolysaccharide (LPS): Plasma enriched with anti-lipopolysaccharide (LPS) (endotoxin) immunoglobulins alone or in combination with conventional therapy has been used for treatment of pyometra.

<u>Hypothyroidism</u>

Decreased or reduced function of the thyroid gland. Primary effects are on growth, reproduction, hair and weight.

Two most common causes of hypothyroidism are: iodine deficiency and inherited low production of thyroid stimulating hormone, simple autosomal recessive gene.

Reproductive system- diminished function – female; anestrus, irregular cycles, galactorrhea, stillborn pups. Male; azoospermia and lack of libido.

Because thyroid supplementation has few side effects there is a tendency for veterinarians and breeders to use L-thyroxine as a panacea for reproductive failure in the dog.

History: Infertility with irregular cycles.
Information obtained thus far with fT4 and fT3 assays in dogs indicated that these determinations do not appear to offer any added information over that given by total T4 measurement alone.

It is possible that the free thyroid hormone assays commonly used may not be accurately estimating the true free fraction in serum of dogs, especially in those with concurrent nonthyroidal illness or those given certain drugs.

An accurate means to estimate fT4 in all dogs (healthy dogs, euthyroid dogs with clinical signs of hypothyroidism, and dogs with hypothyroidism) would likely be a useful diagnostic tool.

Recent evidence suggests that some commercially available fT4 assays may be close to reaching this objective.

# REPRODUCTIVE DISORDERS IN THE MALE DOG

Testicle

1) Tumors
   a) enlargement of testicle(s)
   b) palpation of testicular mass
   c) ultrasonographic examination indicating density change
   d) other signs
      i) Feminization
      ii) Alopecia
   e) sertoli cell tumor
      i) feminization
      ii) attraction of male dogs
      iii) loss of libido
      iv) alopecia
      v) decreased sperm number and quality
   f) Seminoma
   g) interstitial cell tumor
      i) fertility affected by pressure necrosis and endocrine aspects
   h) treatment
      i) hemicastration
         (1) bilateral tumors are very common
2) Testicular Degeneration
   a) common in older dogs (over five years)
   b) diagnosis
      i) testicles palpate softer than normal
      ii) possibly smaller
      iii) semen
         (1) increased primary abnormalities
         (2) decreased concentration
   c) treatment
      i) hormonal therapy
         (1) FSH
         (2) HSG
3) Cryptorchidism
   a) believed to be inherited in the canine due to the genetic factors existing in other species
   b) if bilateral, there may be some difficulty in the confirmation of the condition if the historical information regarding castration is lacking
      i) observation of the scrotum for surgical scars
      ii) best method to diagnose the presence of one or both testes remaining in the canal or abdomen
         (1) use hCG stimulation test
            (a) retained testicle is incapable of the production of sperm
            (b) testicle retains capability to produce androgens
         (2) blood sample obtained prior to the injection of hCG and a second sample 4 to 24 hours later

(3) diagnostic for the presence of a retained testicle if there is a two or more-fold increase in testosterone

c) treatment
  i) castration
  ii) medical – questionable procedure
    (1) question being whether an inherited defect should be corrected hormonally
    (2) however, since the treatment of the condition is usually unsuccessful, it greatly reduces the concern regarding treatment
    (3) administration of 50 ug of GnRH or 500 to 1000 international units of hCG intramuscularly every three days for two to four weeks has been recommended
      (a) if the testicle is in the inquinal canal or located subcutaneously dorsal to the scrotum, testicles may descend on their own
      (b) testicles located within the abdominal cavity after the first few weeks of life are probably destined to remain there due to closure of the internal inquinal ring.
  iii) surgical – unethical procedure and should not be performed
    (1) surgical placement of prosthetic material (Neuticals) into the scrotum to replace a retained testicle should also not be performed

4) Epididymis
  a) always think Brucella!!
  b) diagnosis
    i) palpation
    ii) Ultrasonographic examination
    iii) pain may be present
    iv) localized area involved
  c) treatment
    i) hemicastration

5) Spermatic Cord
  a) Torsion
    i) Uncommon
  b) diagnosis
    i) severe pain
    ii) swelling
    iii) palpate tail of epididymides
  c) treatment
    i) surgery
      (1) correct position and suture
      (2) hemicastration if testicle ischemic
  d) AV Shunt
    i) uncommon
    ii) diagnosis
      (1) swelling in the scrotum above testicle
      (2) differentiate from hernia
    iii) treatment
      (1) hemicastration

6) Penis
  a) persistent penile frenulum

b) fracture of os penis
c) balanoposthitis
d) phimosis
e) paraphimosis – persistent erection
    i) thromboembolism of cavernous venous tissue
    ii) lumbar disc lesions
    iii) normal excitable male!

7) Prostate
a) three major conditions
    i) prostatitis - benign hyperplasia
    ii) prostatitis – bacterial
        (1) infectious
            (a) acute
            (b) chronic
    iii) neoplasia
    iv) hyperplasia
        (1) age related
            (a) usually over five years of age
            (b) males have highest fertility between two and five years
        (2) signs
            (a) blood drips from penis
            (b) not sick
            (c) no pain on palpation
            (d) blood in ejaculate
            (e) no bacterial growth
            (f) infertility
            (g) may not ejaculate completely
            (h) decreased sperm motility
        (3) treatment
            (a) castration
                (i) prostate regresses in three months
            (b) medical
                (i) 5-alpha-reductase inhibitors
                1. stop conversions of testosterone to dihydrotestosterone
                2. Ovaban – ¼ mg/lb/day for minimum 30 days
                3. Proscar – Merck
                    a. Finasteride
                    b. 1 mg/kg for minimum 30 days
b) infectious prostatitus – acute
    i) signs
        (1) vomiting
        (2) diarrhea
        (3) bloody discharge
        (4) temperature 104
        (5) slight dehydration
        (6) prostatic pain

(7) WBC increase
(8) Urine-occult blood
(9) most won't ejaculate
(10) have to collect seminal fluid
(a) polypropylene catheter into bladder to empty it
(b) then pull catheter back to prostate and palpate + aspirate for two minutes

c) infectious prostatitus – chronic
i) signs
(1) recurrent bouts of prostatitis
(2) breeding (fertility) problems
(3) prostate enlarged
(4) decreased motility of sperm
(5) may not ejaculate
(6) blood in urine and ejaculate
ii) infectious – treatment
(1) antibiotics
(a) Baytril
(b) Chloromycetin
(c) Trimethoprim sulfa
(2) drain abscesses (?)
(a) drainage – penrose tubing
(b) many, many short and long term complications
(3) castration
(4) hormonal therapy
(a) estrogen – no
(b) progesterone (Ovaban) – yes

d) Cancer
i) less than two percent of cases
ii) diagnosis by
(1) Ultrasonographic examination
(2) biopsy
iii) treatment
(1) castration
(2) chemotherapy

# CANINE PYOMETRA

1) Pyometra initiated by an interaction of:
   a) hormonal influences
   b) uterine bacteria
2) Endometrium physiologically altered due to repeated exposure to progesterone in some bitches
3) Results in an eventual atypical response to this hormone
4) Increased endometrial growth and glandular secretion
   a) stimulated by elevated progesterone concentrations following ovulation
   b) may predispose the uterus to pyometra during the luteal phase
5) decreased activity of the myometrium under the progestational influence combined with the accumulation of glandular secretion within the uterine lumen may enhance the growth of intrauterine bacteria
6) terminology used to describe a pyometra
   a) open or closed depending on the presence or absence of a vulvar discharge
   b) may be quantitative rather than qualitative
      i) 39 of 40 bitches in one report had a purulent discharge visible from the cervix upon vaginoscopy
7) descriptions of the various types of pyometra encountered based on histologic classification has been presented by Dow
   a) Type I – uncomplicated cystic endometrial hyperplasia. Clinical sign present, if any, a mucoid vulvar discharge during metestrus
   b) Type II – cystic endometrial hyperplasia plus a diffuse plasma cell infiltrate. Vulvar discharge found only between day 40 and 70 following estrus
   c) Type III – acute endometritis and cystic endometrial hyperplasia present. Animals were clinically ill and the uteri were distended. White blood cell number (WBC) was between 19,000 and 145,000 per cu mm. Signs were present at 30 + 14 days following estrus.
   d) Type IV – chronic endometritis with systemic illness. Severity was dependent on degree of cervical opening. Lesions were observed between 55 and 90 days following estrus.
8) no reported differences in the occurrence of pyometra with regard to estrous cycle irregularities, abnormal estrus and pregnancy characteristics. No reported difference between lesions seen in bitches having had a pseudopregnancy and those which did not.
9) death of the bitch if not treated promptly after systemic signs are present
10) predisposing factors
   a) age
   b) cystic endometrial hyperplasia
   c) uterine bacteria
   d) diestrus
   e) estrogen administration
   f) progesterone administration
11) cystic endometrial hyperplasia (CEH) related to increased progesterone. Pyometra has been experimentally induced without progesterone supplementation at any stages of the reproductive cycle by placing E. coli into the uterine lumen
12) CEH
   a) mucometra
   b) begins during the luteal phase

c) endometrium becoming thickened and containing many irregular cystic elevations
d) mucus present within the uterine lumen initially does not contain inflammatory cells
e) becomes inflammatory under the continued influences of progesterone

13) Progression of pyometra
a) introduction of bacteria into the fluid within the uterine CEH lumen

14) Pyometra has been observed to occur only during or immediately after diestrus
a) elevated progesterone has been suggested as being an important part of the disease process
b) no alterations of estrogen or progesterone in bitches with pyometra

15) exogenous estrogens or progestogens
a) 75 percent of affected dogs with pyometra less than three years of age had been given Estradiol cyprionate or megestrol acetate within the previous six months.

16) cystic endometrial hyperplasia experimentally produced by:
a) prolonged administration of progesterone
b) induction of artificial estrus using stilbestrol and progesterone combinations
c) administration of progesterone and diethylstilbestrol.

17) cystic endometrial hyperplasia produced solely with prolonged progesterone administration suggested that estrogen was not involved with the onset of this condition

18) progesterone determines:
a) the degree of cystic change
b) the extent of subsequent inflammation
i) inflammation did not occur until a progesterone threshold concentration was reached

19) no correlation between increased endogenous progesterone concentrations and the development of pyometra

20) no increased or prolonged concentration of progesterone in bitches which have pyometra was found above that of nonaffected bitches

21) no significant difference in endogenous progesterone concentrations in bitches with:
a) closed pyometra (4ng/ml)
b) open pyometra (3.77 ng/ml)

22) repeated exposure to progesterone may explain why several cycles may be necessary (older bitch) prior to the onset of the naturally occurring disease.

23) estrogens enhance the stimulatory effect of progesterone on the endometrium

24) exogenous estrogens significantly increase the probability of the occurrence of pyometra

25) intact bitches should not receive exogenous estrogens for any purpose including the termination of an unwanted pregnancy

26) prostaglandin F2a (PGF2a)
a) logical alternative to estrogen administration for abortion induction.

27) No difference in bacterial populations in the anterior vagina of bitches with pyometra and those without. “Chance invasion” by opportunistic pathogens into a uterus under progesterone influence is responsible for the development of pyometra.

28) Endotoxic substances remain active for up to two weeks in tissue.

29) Endotoxic substances remain active for up to two weeks in tissue.

30) With the bacterial destruction occurring due to antimicrobials, the LPS concentration may increase up to 2,000 fold.

31) Reported systemic complications of pyometra are:
a) brain thromboembolisms following septicemia, sepsis, renal function abnormalities, hypoglycemia, hepatocellular injury, cardiac arrhythmias, and clotting abnormalities.

32) Other changes observed in bitches with pyometra include those of: electrolytes, acid-base values, hemotologic values, intrahepatic cholestasis, respiratory alkalosis with metabolic compensation, and metabolic acidosis.
33) History
    a) Two types of history involving bitches with pyometra
34) Exceptions to the value of diagnostic tests
    a) exemplified by the blood urea nitrogen, serum creatinine concentration, and urine specific gravity as well as the white blood cell count, normal in a dog which was dehydrated, depressed and presumably toxic as a result of pyometra.
35) Vaginal cytology
    a) increased numbers of white blood cells would be indicative of a genital tract abnormality; not specifically uterine disease.
        i) taken immediately following the obtainment of an anterior vaginal culture using a guarded culture rod.
    b) Cultures are most useful for the determination of antibiotic sensitivities for proper selection of antimicrobial agents.
    c) Vaginal speculum examination has been found to be useful in the determination of the origin of vulvar discharges.
    d) It also proves useful for differentiation of discharges originating from vaginal tumors.
36) Prevention and Treatment
    a) Bitches not intended for breeding purposes should be ovariohysterectomized as early as possible following that decision
    b) Seventy-two dogs ovariectomized only and followed for a 10-year period had no reported cases of pyometra.
    c) Bitches exhibiting systemic signs of pyometra should receive supportive therapy consisting of fluid and antibiotic therapy.
    d) Appropriate and rapid stabilization of the bitch prior to ovariohysterectomy greatly decreases morbidity and morality.
    e) Removal of the uterus and ovaries can be performed as described in the literature or modification of these techniques can be made to suit the surgeon.
37) Complications of the surgical approach include:
    a) anesthetic risks, hemorrhage, peritonitis, inappropriate ligature placement involving ureters, and incomplete removal of ovaries.
38) Postoperative complications can also occur in the form of:
    a) incisional swelling
    b) dehiscence
    c) trauma to ureters
    d) uterine stump pyometra
    e) fistulous tracts
    f) urinary incontinence.
39) Surgical drainage of lavage of a uterus with pyometra without removal of the uterus has been reported and used more frequently in breeding bitches prior to the introduction of prostaglandin F2a
40) Surgical drainage of the uterus without removal has been reported with one of three bitches producing offspring following treatment.
41) Catheter placement into the uterine lumen by vaginoscopy to permit only drainage over an average of approximately 10 days has been described.

42) Nine of twelve bitches responded favorably with five of six bitches bred conceiving.
43) Attempt to medically treat pyometra have involved:
    a) antibiotics, locally or systemically
    b) androgenic steroids
    c) Ergonovine maleate
    d) estrogens
    e) oxytocin
44) All of these treatments had minimal to moderate success in eliminating the pyometra condition.
45) Broad-spectrum antibiotics are frequently used since antibiotic sensitivities are not available.
46) Use of aminoglycosides should be cautioned due to the reportedly high incidence of renal dysfunction with this condition.
47) Testosterone propionate at a dosage of 25 mg administered intramuscularly twice weekly irrespective of the animal's size resulted in an 80 percent elimination of the pyometra within three weeks.
48) Prostaglandin F2a (PGF2a) has been very successful in treating pyometra
49) Currently appears to be the medication of choice.
50) Not approved for use in the canine in the United States
51) Widely used for treatment of pyometra in this species
52) Actions of PGF2a include:
    a) contraction of the myometrium
    b) relaxation of the cervix
53) These two actions aid in physical expulsion of the pyometra fluid from the uterine lumen.
54) The luteolytic effect of PGF2a may be somewhat important by decreasing the progesterone concentration.
55) Decision to utilize PFG2a is based primarily on the owner's desire to have a breeding animal over that of an ovariohysterectomized pet.
56) Recommended use of PGF2a in animals with pulmonary or cardiovascular disease varied due to the effect of this hormone on these systems.
57) Although no deaths were directly attributed to the PGF2a treatment, caution should be taken when treating animals with these conditions.
58) Concern by many is the use of PGF2a in bitches with closed cervix and the possible resultant pyometra fluid being expelled into the peritoneal cavity through the oviducts or through ruptures occurring with the uterine wall while the uterus is under the influence of the hormone.
59) Use of estrogen for cervical dilation is not recommended due to the increased blood flow the uterus accompanying its administration and the potential for increased endotoxin absorption.
60) Prostaglandin F2a has been administered in low doses for treatment of pyometra. 20 ug/kg administered three times per day for five to eight consecutive days induced "expulsion of uterine contents", nine of twelve dogs responding by elimination of the pyometra, 6 of 7 bitches bred whelped.
61) Dosages between 41.6 ug/kg and 250 ug/kg included bitches with pyometra plus at least one of the following characteristics:
    a) neutrophilia (>12x109/L)
    b) left shift (>0.3x109 immature neutrophils/L)
    c) purulent discharge
    d) diestrus phase of cycle
    e) lethargy
    f) anorexia
    g) palpable uterine enlargement
    h) polydipsia and/or polyuria

62) Dosages of 250 to 500 ug/kg given intramuscularly or subcutaneously have also been used successfully in the treatment of pyometra.
63) Multiple treatments appear essential to the success of therapy as was reported with the use of 250 ug/kg of PFG2a daily for five days, or with 250 and 500 ug/kg of PGF2a administered subcutaneously daily for three days.
64) Four of ten bitches produced a litter within one year.
65) Systemic antibiotics should be administered concurrent with PFG2
66) Ten percent of treated bitches reportedly have a positive blood culture.
67) Success of treatment appears to be related to duration of therapy necessary to obtain the desired result
68) Bitches treated more than six days with PGF2a did not whelp following therapy. However success has been reported following two five-day treatment periods.
69) Reasons observed for treated bitches failing to whelp following breeding included:
    a) failure to conceive
    b) embryonic absorption
    c) abortion
70) It was suggested that these may be due to a persistent bacterial infection in the uterine lumen or endometrial changes.
71) Vulvar discharge is usually present following therapy. May not be seen due to the bitch cleaning the vulvar area.
72) Success of treatment is primarily base on:
    a) a decrease in uterine size
    b) increase in uterine tone
    c) vulvar discharge changes to one characterized by a sero-sanguinous or serous nature.
73) Re-evaluation of the bitch is essential and recommended between ten and twenty days following the last PGF2 treatment. The need for additional treatment is determined at that time.
74) Side effects to be expected commenced within five minutes following PFG2a administration include:
    a) restlessness
    b) increased respiration
    c) increased lacramation
    d) increased frequency of defecation
    e) increased frequency of urination
    f) abdominal pain
    g) increased body temperature
    h) vomiting

    These side effects usually last for 20 to 30 minutes.
75) It has been suggested that animals be walked for 20 to 40 minutes following treatment to reduce the signs and to permit someone to be readily available to watch the dog.

Summary

If a bitch with pyometra is not to be used for breeding, an ovariohysterectomy should be performed.

If the bitch is a poor surgical risk, conservative therapy should be initiated to re-establish more normal body functions prior to surgery.

Therapy may include consideration of the use of anti-LPS plasma.

Time available from presentation of animal with severe signs until surgery will vary with each case and may be from minutes to days.

Preparation for surgery should include administration of fluids to establish renal function and antibiotics to control bacteremia if present.

If a bitch is to be treated medically and maintained for breeding purposes, it is still imperative to maintain adequate kidney function during therapy.

PGF2a appears to be the preferred medical treatment for this condition and should be used in a repeated manner regardless of the dosage selected.

Systemic antibiotics should be incorporated into the medical treatment plan due to the possibility of septicemia.

Bitches should be bred on the estrus following treatment as recurrence has been reported.

The use of systemic broad spectrum antibiotics at the time of that breeding may be indicated to reduce the possibility of intrauterine bacteria.

# POSTPARTUM BLEEDING

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Uterine subinvolution (subinvolution of placental sites)

Cause – unknown
endometrial vessels do not thrombos and occlude

Signs - bloody discharge 4 to 16 weeks
no systemic signs
spherical uterine enlargements

Considerations
No elevated progesterone
No infection present
No dystocias involved
No problems with fetuses
No effect on future fertility

Differentials
Any condition involving lumen of reproductive tract
metritis, vaginitis, vaginal neoplasia

Diagnosis
History
Vaginal cytology – many RPC's, no WBC's

Diagnostic Aids (?)
Anterior vaginal culture
CBC – anemia, systemic infection

Treatment
Nonbreeder
- Ovariohysterectomy

Breeder
- Ergonovine
- Oxytocin
- Prostaglandin F2 alpha

# DNA – WHAT IS IT?

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The Ohio State University
Professor and Head
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DNA stands for deoxyribonucleic acid and it is the blueprint for what the dog will genetically be and what ancestors before it have been.

## Background

Every dog has its own identifying DNA. DNA analysis can indisputably determine the identity of any dog. The only dogs having the exact same DNA are those which are identical twins. Relatives have similar DNA but not identical. The DNA of a dog never changes. It is more precise than fingerprints in the human. DNA analysis is accurate to one in 100 billion dogs.

## Source of DNA for testing

Every cell in the dog's body contains the identical DNA.

## How DNA functions in the body

A comparison as to how the function of DNA occurs is:

The sequence of four amino acids (adenine-A, thiamine-T, guanine-G and cytosine-C combine to be called nucleotides. The sequence or order of these amino acids determines the variation that exists within each nucleotide and, therefore, within the DNA. Sound simple! However, the sequence of those letters can change each time they are repeated on the "string" of DNA present.

Therefore, since the nucleotides (each can assume a different sequence in the chain) are repeated approximately 150 million times in each animal. The variation is enormous! If the chain only consisted of two nucleotides, the possible combinations would be (4x4) 16 and if five nucleotides were present, the possible combinations existing would be (4x4x4x4x4) 1,024. Imagine the possibilities with 150 million nucleotides!!

If the genetic make-up of the dog is compared to a library (all existing genetic information is archived in the DNA) which is very appropriate, the shelves of the library are called the chromosomes. The books would be considered the genes. The genes consist of words which are the nucleotides and the words are made up of letters (A, T, G and C) which represent the four amino acids. The dog has 39 pairs of chromosomes. One of each of these pairs comes from the dam and one from the stud. The material contained in these genes is called the genotype.

## Uses for DNA

Permits identification of lost dogs.

Validates parentage and pedigree.
Identifies which pups in a litter potentially sired by two males belong to each.
Is admissible in legal cases.

Potential uses for DNA

Identify genetic traits of an individual.

Sample obtainment

Current testing is based on cheek mucosal samples. A small brush is inserted into the cheek area and gently rotated. The brush is placed into an envelope and mailed to the laboratory. This technique can be performed by owners. DNA information CANNOT be released to anyone without the written consent of the owner of the dog.

Laboratory telephone number 1-800-995-2473.