

## **I did everything right to breed my bitch—what went wrong!**

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### **Abstract**

There are many reasons for a bitch not getting pregnant after breeding. Breeding soundness examination (BSE) of the stud dog would establish if the dog used for natural service is infertile or sterile. Improper breeding management for artificial insemination (AI) is a major cause for a bitch not getting pregnant. Progesterone (P4) assay, vaginal cytology and other related tools are utilized for AI at the appropriate time. A bitch with a split heat shows proestral bleeding, vulvar swelling but does not ovulate. Affected bitches go out of proestrus and come back into proestrus again two to ten weeks later. The second half of the split heat is usually normal. Follicular cysts produce estrogen and cause prolonged proestrus or estrus. Ovarian follicles develop normally but do not ovulate. Luteal cysts produce P4. Persistent and prolonged P4 production may cause infertility or predispose the bitch to cystic endometrial hyperplasia and pyometra. Ovariohysterectomy is the treatment of choice for bitches not intended for breeding. Valuable breeding bitches with follicular cysts are treated with gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin. Hypothyroidism is the most common endocrine disorder, and is considered a hereditary condition in the dog. Dogs suspected of hypothyroidism are diagnosed by measurement of both thyroxine (T4) and thyroid stimulating hormone (TSH). The condition is treated by oral supplementation with thyroid hormone. Dogs without a confirmed diagnosis of hypothyroidism should not be given thyroid supplement. Early embryonic loss and resorption may be caused by infectious agents, including bacteria, parasites and viruses. Non-infectious causes of resorption include endocrine abnormalities, certain drugs given to the bitch during pregnancy and genetic factors. Hypoluteoidism, an endocrine abnormality, can cause embryonic death and resorption. Monitoring progesterone in a bitch with suspected hypoluteoidism once or twice weekly allows accurate diagnosis of this problem. Hypoluteoidism is treated by P4 supplementation of the bitch.

**Keywords:** Dog, breeding management, breeding soundness examination, early embryonic death, ovarian cysts

### **Introduction**

There are many reasons for a bitch not getting pregnant after breeding. It may be due to problems related to the male or the female. The male used for natural services may be infertile or sterile. For AI, improper breeding management and issues surrounding AI may be the problem. Other reasons for the bitch not getting pregnant may include split heat, ovarian cysts, and early embryonic death. This paper will discuss briefly each topic to understand possible reasons for a bitch to fail to conceive after breeding.

### **The male factor**

Whether natural breeding or AI is used, a stud dog with desirable breeding potential is important. Many dog owners and breeders assume that all males are fertile. Stud dog owners and breeders may invest a lot of time and money showing the dog and may receive awards, only to find that the stud cannot get a bitch pregnant. It is relatively inexpensive to have the stud examined for breeding soundness before putting him on the show circuit or using him for breeding.

### **Breeding soundness examination**

The author recommends having a BSE performed by a veterinarian. One must remember that the BSE is valid only at the time of examination. The BSE findings frequently cannot explain the reason for infertility and cannot guarantee the future performance of the stud. The examining veterinarian will ask for a general medical history which may include vaccinations and past illnesses. A detailed reproductive history would include the results of any previous semen evaluation if available and *Brucella canis* test

results. If the male was used for breeding, it would be important to know the results such as litter size, the number of bitches bred by the male, and the method of pregnancy diagnosis used.

If a bitch does not become pregnant after breeding, some dog owners and breeders assume the bitch was pregnant and reabsorbed the litter based upon weight gain and enlarged mammary glands. Bitches in diestrus or pseudopregnancy also show these changes, so these signs cannot be used to conclude that the bitch was pregnant.

Special attention is given to abnormalities which may interfere with the expression of male sexual desire (libido) or interfere with mating ability (e.g., lameness). The examination of the reproductive organs includes the testes, epididymides and prostate. Depending upon the temperament of the male, especially in the case of anxious dogs, it may be better to wait and do this part of the examination after semen has been collected when most of the studs are easily manageable.

Owners may be interested knowing about the hormones responsible for male reproductive functions including sperm production. The hypothalamus, which is located at the base of the brain releases GnRH, which stimulates release of two important reproductive hormones from the pituitary gland (located below the hypothalamus). These hormones have same names in males and females, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The testes produce sperm and the male sex hormone, testosterone. Both FSH and LH affect the functions of the testes.

#### Sperm production and sexual desire

As mentioned above, the testes perform a dual function—producing sperm and testosterone. At the time of ejaculation, sperm are transported from the epididymis to the pelvic urethra by the ductus deferens. The sperm are mixed with fluid (seminal plasma produced primarily by the prostate gland) and released from the penis during ejaculation.

It takes about 60 days to complete a cycle of sperm production (spermatogenesis), 45 days in the testes and 15 days to travel through the epididymis. In other words, the semen collected today was made about 60 days ago. This fact is important because if a semen sample collected today shows poor quality one must wait for about 60 days to collect semen containing a new ‘crop’ of sperm.

*Testosterone therapy for dogs with poor sexual desire.* Some dog owners request testosterone therapy for their dogs with poor sexual desire. It has been shown in horses that testosterone therapy decreases testicular size and increases sperm abnormalities in the ejaculate. This is due to negative feedback on the pituitary gland. Most experts agree that libido is inborn characteristic of the male.

#### Unique reproductive anatomy

Some unique features of dog reproductive anatomy include the presence of a bone in the penis (os penis or baculum) and the presence of a well-developed accessory sex gland, the prostate.

The author considers the presence of two palpable testes in the scrotum a requirement for a sound breeding dog. The absence of one or both testes is unilateral or bilateral cryptorchidism, respectively, which is an undesirable hereditary condition. Normal testes should be symmetrical and have a firm consistency. Even though there is limited information available regarding testicular size in different breeds, most experts agree that there is a positive correlation between testicular size and sperm production. In other words, larger testes are associated with more the sperm production, assuming the testes are normal and not enlarged due to the presence of abnormalities.

#### Semen collection and evaluation

The characteristics of normal canine semen are shown in Table 1. Semen can be collected by digital manipulation of the penis with the help of an artificial vagina (AV; a latex rubber cone attached to a 15 ml calibrated plastic centrifuge tube). Another technique is to utilize a gloved hand with semen collected into color-coded funnels. The author prefers the funnel technique because of the ease in collecting semen fractions separately. Most experienced dogs will ejaculate with simple hand

stimulation, whereas inexperienced or younger dogs may require a teaser bitch in heat. Some dogs do not care if the bitch is in heat or not—they just like the company.

The semen collection environment, the collector’s experience and the dog’s temperament influence the outcome of collection. A door mat or a carpet for good footing is helpful. Attempts to try to collect semen on a stainless steel examination table are almost always unsuccessful. The author gets on his knees on the floor at the dog’s level to collect semen. If a teaser bitch is used and is not familiar with the male, it is useful to give them some time to become acquainted with each other.

Most dogs ejaculate the pre-sperm (clear) and sperm rich fraction (cloudy) of semen during the time of most rapid pelvic thrusting, then dismount and lift one hind leg as though trying to step over the bitch and achieve a tie. At this time, the penis is rotated nearly 180 degrees and directed caudally while maintaining tight pressure around the bulbus glandis until the prostatic fraction of semen is ejaculated.

*Prostate examination.* The prostate is the only accessory sex gland present in the dog and it contributes its secretion as part of the ejaculate. A lubricated gloved index finger is inserted into the rectum to palpate the walnut-shaped, bi-lobed prostate on the floor of the pelvis. In medium to large dogs, the examiner has to push the ventral abdomen upward to facilitate feeling the whole prostate gland. Benign prostatic hypertrophy is a common condition, especially in older dogs.

*Semen evaluation.* The color of normal semen should be cloudy to milky white. A red or pinkish ejaculate may be indicative of a prostate problem. A yellow semen color indicates urine contamination or, less commonly, purulent exudate or other foreign debris originating from the prostate or epididymis.

Semen volume can be read from the calibrated collection tube. Semen volume varies with the breed, size of the dog and the amount of prostatic fluid collected.

Sperm motility is evaluated within a few minutes of semen collection by placing a drop of semen on a warm (37 °C) microscope slide, placing a glass cover slip over the drop and examining under the microscope. The percentage of motile sperm, those moving in a progressive forward manner, is estimated. Sperm moving in circles or wiggling in one spot can indicate chemical or cold shock. Even though sperm motility is a subjective test, it is a quick functional test of semen quality. Expensive automated semen analyzers and computer-based equipment are available to assess sperm motility. About 80% or greater sperm motility is expected in a normal dog ejaculate. Low sperm motility may be an indication of incomplete ejaculation, infectious diseases involving the reproductive system or increased temperature of the scrotum (e.g., fever).

The total number of sperm in the entire ejaculate is more important than the number of sperm per milliliter of semen because the total volume depends upon how much prostate fluid is collected. Again, expensive automated semen analyzers are available for this purpose, but the most common technique used is to count sperm using a hemocytometer.

Sperm morphology (normal and abnormal sperm) is assessed by examination of a stained slide of semen, and 100-200 sperm are examined. The cells are classified as normal sperm, those with primary abnormalities (e.g., head abnormalities, proximally coiled tails, proximal cytoplasmic droplets), or those with secondary abnormalities (e.g., detached heads, bent tails, distal cytoplasmic droplets).

Table 1. Characteristics of normal canine semen

Parameter	Unfractionated Ejaculate	Seminal Fraction		
		1	2	3
Color	Opaque to white	clear	Milky-white	clear
Volume (ml)	~30	0.1-2	0.1-4	1-25+
Sperm motility	>80%			
Morphologically normal sperm	>80%			

## The female factor

Improper timing of breeding is the major cause of conception failure in bitches bred by AI. Basic understanding of the reproductive cycle of the bitch is essential for proper breeding management and expectation of successful AI.

### Estrous cycle

Bitches cycle twice a year, with a minimum of a four month inter-estrus interval. Bitches with less than four months between successive cycles usually do not get pregnant. Some breeds (e.g., Basenji, wolf and wild-dog crosses) may cycle every 9-12 months with normal fertility. Bitches ovulate spontaneously, which means that they ovulate without any stimulus from breeding. The bitch starts cycling due to the action of hormones released from the hypothalamus and pituitary gland. Gonadotropin releasing hormone, released from the hypothalamus stimulates the release of FSH and LH from the pituitary gland. As the names imply, FSH is primarily responsible for growth of follicles on the ovaries whereas LH causes ovulation of the follicles.

The estrous cycle in the bitch is unique and is different from other domestic animals. It starts with proestrus. Most breeders refer proestrus as start of the 'season'. Bitch owners notice spotting or bleeding from the vulva. During proestrus, the bitch attracts males but does not allow mating. Estrogen produced by ovarian follicles causes a change in the epithelial cells of the vaginal mucosa, which will be discussed in detail below. Estrus or the time of receptivity follows proestrus and the bitch accepts mating. The average duration of proestrus and estrus cited in most textbooks is nine days each. This average may be correct but unfortunately many bitches do not follow the textbook and each phase may range from two to 21 days. During proestrus, the vulvar lips are turgid or firm and become soft and wrinkled as the bitch approaches the receptive phase of the cycle. The bloody discharge characteristic of proestrus usually changes to a straw color near estrus in most bitches but some bitches continue to bleed throughout estrus. A graph depicting the hormone pattern during the estrous cycle of bitches can be found at <http://www.peteducation.com/article.cfm?c=2+2109&aid=3201>.<sup>1</sup>

The production of P4 is also unique during estrous cycle of the bitch. The early increase in P4 during estrus is due to luteinization of follicles on the ovaries. This increase in P4 can be useful in breeding management and will be discussed in detail below. The bitch ovulates in response to LH. Ovulation occurs about three days after the LH peak. After ovulation, the follicles are replaced by corpora lutea (CLs) which secrete P4. The production of P4 continues throughout diestrus, the next phase of cycle. The duration of diestrus is the same as pregnancy, about 62-63 days after the LH peak. Even if the bitch is not pregnant P4 is produced for the duration of diestrus. It appears that there is no production of prostaglandin F2alpha (as in other domestic animals) from the endometrium (inner lining of the uterus) to cause luteolysis (CL regression). During diestrus, many bitches display variable signs of 'pseudopregnancy'. The bitch may experience weight gain and an enlarged abdomen and may show more overt signs of pseudopregnancy including mammary gland enlargement, nesting, adoption of toys (or shoes), and give the impression she is about to whelp. Pseudopregnancy (or diestrus) is considered a normal occurrence and may not require treatment.

The ova released during ovulation in the bitch are primary oocytes; the first polar body has not been shed, and sperm are not able to penetrate the ovum. Three days are required for the ovum to become a secondary oocyte and ready to be penetrated by the sperm. Dog sperm can survive up to ten days in a bitch's reproductive tract. It can be a diagnostic challenge for a veterinarian when a bitch's owner requests a cesarean section 62 days after breeding. Theoretically a pregnancy at 62 days after breeding could be as early as 52 days. A few tools are available to predict whelping. Hypothermia or a decrease in rectal temperature of two to three degrees F, caused by a decrease in P4 12 to 36 hours before whelping is a fairly reliable predictor of whelping. The indication of diestrus day 1 (D1) by vaginal cytology is another reliable way to predict whelping. Bitches whelp about 57-58 days after D1.

### Vaginal cytology in breeding management.

For successful vaginal cytology, the examiner needs to appreciate the unique reproductive anatomy of the bitch. The vagina of the bitch is very long, about 20 cm (~9 inches) in a medium size bitch. The cervix in the bitch is located in the abdominal cavity, whereas in other domestic animals (e.g., cow and mare) the cervix is located at the pelvic inlet. Therefore, in most bitches the cervix cannot be palpated or visualized with a speculum but can be visualized with a flexible endoscope with a light source. This becomes important when frozen-thawed semen needs to be deposited in the uterus via the cervix.

A clean cotton-tipped swab is commonly used to obtain a sample for vaginal cytology. It is most convenient for a right-handed examiner to hold the bitch's vulva with their gloved left hand and open the vulvar lips with the thumb and the middle finger, while using the index finger behind the vulva to support it. The swab is inserted with the right hand in a nearly vertical position into the vagina, avoiding the clitoral area, and directed up and over the brim of the pelvis. The swab is moistened with warm tap water before being inserted. If the swab sticks in the vaginal folds, pull it back slightly, redirect it and proceed. Once the swab has been inserted at least 6-10 cm (2-4 inches), the swab is rolled a few times in one direction (if rotated back and forth, the cotton may unroll and drop in the vagina) and removed. The author recommends inserting the swab as deep as possible, as there is less debris on samples taken from the cranial vagina. Getting into the habit of inserting the swab deep into the vagina is also helpful for AI. The swab is rolled on a clean microscope slide, air-dried and stained with Romanowsky's stain (Diff-Quick<sup>®</sup>, Dade Behring, Inc., Newark, DE). The author recommends making two slides as insurance against accidental breakage of a single slide. Other stains used by veterinary practitioners include methylene blue, eosin-nigrosin, gram stain, and others. Romanowsky's stain is suitable for staining epithelial cells and white blood cells. After the slides have been air-dried, they are dipped five to seven times in each solution, rinsed with tap water, air-dried and examined under a microscope. The author recommends an initial examination with the 10X objective to get an overall impression and distribution of the cells before moving on to the 20X lens to closely view the desired field.

Various theriogenologists (veterinary reproduction specialists), clinical pathologists and other veterinary practitioners interpret vaginal cytology differently. For this presentation, the author will use the definitions described by Olson, et al.<sup>2</sup> It is important to remember that the vaginal mucosa is responsive primarily to estrogen, thus vaginal cytology is useful only during estrogenic phase of the cycle. Parabasal cells are small and round with large and distinct nuclei. The total area of the cytoplasmic portion of the cell is smaller than the nucleus. These cells (along with red blood cells) are present during proestrus. Superficial intermediate cells are larger than parabasal cells with small nuclei and irregular or folded borders. Large numbers of these cells are observed during late proestrus to early estrus. Superficial cells, the anucleated cells also called cornified or keratinized cells, are the largest of the epithelial cells present during estrus. Under the microscope, the nuclei of these cells appear faded or absent. The cells also appear thin, multi-layered and have folded borders. The appearance of 80-90% of these cells in the smear is used as an indication to start breeding. Two observations are important here. First, the examiner must analyze a series of vaginal cytology samples to observe the progressive change in epithelial cells. A single smear is unreliable. Some bitches maintain the same type of epithelial cells for many days, where as others change within 24 hours. Second, in the author's experience many bitches reach 80-90% cornification during estrus but others never go above 70% cornification. Obviously, if one waits for 80-90% cornification to breed, the opportunity to breed will be missed in these bitches. Another tool help manage breeding of these bitches is to measure blood P4, which will be discussed below. If vaginal cytology is continued, the appearance of neutrophils (a type of white blood cell) is detected; this is considered to be the first day of the diestrus or D1. This finding is important because the bitch is expected to whelp 57-58 days after D1. It is recommended that vaginal swabs be taken after breeding every day, because in some bitches the change from cornified to superficial intermediate cells and the appearance of neutrophils may occur within 24 hours. Many breeders find it convenient to take vaginal

cytology samples at home. After a brief training, they prepare slides at home for the recommended time. The slides are then brought to the clinic, stained and evaluated.

As discussed above, in addition to the CLs, P4 also is secreted before ovulation by luteinized follicles on the ovaries. The early P4 rise can be used to determine ovulation in the bitch. Recall that ovulation takes place about three days after the LH peak, which is very short. Kits to measure LH are available, and it is recommended that LH be measured every day during the expected time of ovulation. Progesterone assays are commonly used for canine breeding management. Enzyme-linked immunoabsorbent assays are available to measure P4. These are qualitative tests based upon a color change and indicate a range of P4 concentrations. Many clinicians prefer to send the blood samples to an endocrinology laboratory for P4 determination by radioimmunoassay. Progesterone is the same hormone in all mammals, including humans; therefore blood samples can be analyzed for P4 in veterinary or human hospital laboratories.

Progesterone assays are a useful tool to determine the time of ovulation so breeding can take place at the appropriate time. This becomes very critical when using chilled transported or frozen-thawed semen for AI. Like vaginal cytology, P4 concentrations are monitored starting a few days after the onset of proestrus. Progesterone concentrations in the range of 2 to 2.9 ng/ml are indicative of ovulation in about two days; concentrations from 3 to 3.9 ng/ml indicate ovulation in one day, and those from 4 to 10 ng/ml are found on the day of ovulation.<sup>3</sup>

Other causes of failure to conceive

*Split heat.* Split heats are seen in bitches that have proestral bleeding, vulvar swelling and attract males, but do not progress to ovulation. In other words, the bitch has ovarian follicle growth, production of estrogen but ovulation does not take place. Affected bitches go out of proestrus, but then come back into proestrus again two to ten weeks later. The second half of the split heat is usually normal. The causes of split heat are not known and the condition is not associated with infertility. However, bitches with a split heat cause problems in breeding management.

*Ovarian cysts.* Ovarian cysts are characterized by prolonged proestrus or estrus and are seen mostly in younger bitches. The ovarian follicles develop normally but do not ovulate. As in normal follicles, estrogen is produced from the granulosa cells of the follicle lining. However, the granulosa cells do not go through the normal change to produce progesterone. Cystic follicles may be induced by estrogen injections. Bitches with ovarian cysts that produce estrogen have prolonged proestrus or estrus and may attract males. Less common are luteal cysts which secrete progesterone. Persistent and prolonged progesterone production may cause infertility or predispose the bitch to cystic endometrial hyperplasia and pyometra.

Follicular cysts may be diagnosed by series of vaginal cytology examinations as discussed above. Animals with follicular cysts produce estrogen and vaginal cytology would show cornification of the epithelial cells. Ultrasonography may detect fluid-filled cystic structures in the ovaries.

Ovariohysterectomy is the treatment of choice for bitches not intended for breeding. Valuable breeding bitches with follicular cysts are treated with GnRH, which stimulates the release of LH from the pituitary gland to cause luteinization or ovulation of the cystic follicles. Another option for treatment is human chorionic gonadotropin, which mimics the action of LH and causes luteinization or ovulation of the cystic follicles. Even though there is no documented evidence that ovarian cysts are hereditary in the bitch, ovariohysterectomy is recommended in those with reoccurrence of ovarian cysts. Ovarian cysts have been shown to be hereditary in dairy cattle.

*Hypothyroidism.* Hypothyroidism is a common endocrine disorder and is considered to be a hereditary condition in the dog. Even though dogs with hypothyroidism often have poor reproductive performance, an exact link between hypothyroidism and reproductive function has not been established. Reproductive disorders may include irregular cycles, small litter size and poor semen quality in males.

Dogs with hypothyroidism have a lower metabolic rate than normal dogs. Owners may notice less tolerance for exercise, weight gain, dry hair coat and eventually hair loss. It is recommended that both T4 and TSH be measured in dogs suspected of hypothyroidism. The condition is easily treated by oral supplementation with thyroid hormone. Dogs without a confirmed diagnosis of hypothyroidism should not be given thyroid supplement. Dogs receiving the supplement should be tested periodically to confirm appropriate supplementation. Excessive thyroid hormone supplementation may result in hyperthyroidism and can cause an increase in metabolic and heart rates and eventually weight loss. In humans, hyperthyroidism has been linked with reproductive dysfunction.

*Early embryonic loss.* Early embryonic loss and resorption of embryos can be a diagnostic challenge. Some owners assume a bitch was pregnant based upon outward signs of weight gain, but do not whelp. Pregnancy should be confirmed before suspecting a pregnancy loss. Pregnancy in the bitch can be reliably diagnosed by ultrasonography 25 days after the last breeding. If early embryonic death occurs, clinical signs are usually not observed. There are no abdominal contractions and embryos are not seen to pass. Embryonic death and resorption is caused by infectious and noninfectious agents. Infectious agents suspected causing embryonic death include bacteria (e.g., *Mycoplasma*, *Brucella canis*, *Campylobacter*, *E. coli*, and B-hemolytic *Streptococci*), parasites (e.g., *Toxoplasma gondii* and *Neospora caninum*) and viruses (e.g., canine herpesvirus, canine parvovirus type 1, and canine distemper virus). Non-infectious causes of resorption may include endocrine abnormalities, certain drugs given to the bitch during pregnancy and genetic factors.

*Hypoluteoidism.* Hypoluteoidism is an endocrine abnormality and can cause embryonic death and resorption. For pregnancy maintenance, P4 must be secreted from the CLs throughout the pregnancy. If luteal tissue fails to secrete progesterone at any stage, the pregnancy is lost. Monitoring P4 in a bitch with suspected hypoluteoidism once or twice weekly allows accurate diagnosis of this problem. Greater than 2 ng/ml P4 is required to maintain pregnancy. In a normal pregnancy, P4 reaches peak levels (15-90 ng/ml) by 15-30 days after the LH peak. During the last semester, P4 decreases (4-16 ng/ml) before dropping below 2 ng/ml about one day before parturition. Hypoluteoidism is treated by supplementing the bitch with P4.

## Conclusion

There are many reasons for failure to conceive after breeding. Infertile or sterile males used for natural service and improper breeding management for artificial insemination are the most common. Other causes may include split heat, ovarian cysts, and early embryonic death. Appropriate examinations and diagnostic tests performed by a veterinarian specializing in reproduction may provide answers.

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