

**Diagnostic approaches to pregnancy loss**  
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**Abstract**

Pregnancy loss can occur from events closely associated with conception up to just prior to parturition. Death of the embryo or fetus can be the result of hormonal, toxic, environmental and/or bacterial or viral agents. In many species of animals embryonic losses are higher than perinatal losses. In the dog, pregnancy diagnosis occurs midway through the gestation period further complicating the diagnosis of embryonic losses. The bitch is luteal dependent throughout pregnancy so luteolytic events can result in fetal loss or abortion. Death of the fetus can result in mummification, maceration or abortion. Viruses are a common cause of mummification in dogs and cats. Aborted fetuses often are autolyzed due to death 24-28 hours prior to delivery. History including housing, nutrition, supplements, vaccination status, exercise, illness and medications including topical and parasite control products should be obtained.

**Keywords:** Pregnancy loss, infectious agents, progesterone, environment

**Bacterial agents**

**Brucellosis**

*Brucella canis* is a small, gram-negative intracellular coccobacilli. It is the most significant cause of pregnancy loss and infertility in the canine species and is an under diagnosed and under reported cause of pregnancy failure in the canine. Clients perceive that a canine cannot have *Brucella canis* if it sexually naïve. Brucella infection is spread both orally and venereally through mucosal contact with infected material.<sup>1</sup> Vaginal discharge, semen and fluid and tissues from abortion contain the highest number of organism but urine, blood, milk, saliva and feces also contain organisms. The bacteria can survive in both chilled and frozen semen. Infection can cause infertility, early embryonic death, fetal resorption and late term abortion. Infected bitches often do not display any signs of illness. Abortion often occurs after day to day 50 and is accompanied by voluminous vaginal discharge. *Brucella canis* has zoonotic potential and has been diagnosed in laboratory workers and a young child exposed to an infected puppy. All breeding animals should be screened for *Brucella canis* prior to breeding. Screening tests include the tube agglutination test, indirect fluorescent antibody, rapid slide immunodiffusion, and agar gel immunodiffusion using cell wall antigen (AGIDcwa). Definite diagnosis involves blood culture, agar gel immunodiffusion using cytoplasmic protein antigens (AGIDcpa). Vaginal swab polymerase chain reaction (PCR) is a good choice for brucellosis diagnosis in bitches suspected to be infected.<sup>2</sup> Incidence of *Brucella canis* has increased in the United States as a result of imported dogs and the United States interstate dog trade.

**Salmonella and *Escherichia coli***

Salmonella is a gram negative bacillus that is transmitted through the gastrointestinal tract via contaminated feed or water. In the United States Salmonella has been identified as a pathogen in dog kennels feeding a raw diet based on chicken. Salmonella has zoonotic potential particularly in the very young, the very old and the immunocompromised and can result in severe clinical disease in humans. Infection with Salmonella can result in abortion, stillbirth, and poor puppy survival. Definite diagnosis of Salmonella associated abortion requires culture of fetal tissues and membranes. Several cases have been associated with the feeding of raw diets including a case of feline stillbirth with *Salmonella thyphimurium*.

*Escherichia coli* while commonly identified in case of metritis, mastitis and pyometra has been identified in cases of abortion. *Escherichia coli* is the most common bacteria isolated from the canine vagina. *Escherichia coli* produces an endotoxin that has been shown to be an abortifacient in other species.

### Campylobacter species

Campylobacter are gram negative curved rods. While they have been isolated from the feces of normal dogs they have been associated with abortion in dogs. Campylobacter may require special identification techniques including culture of the organism from fetal or neonatal tissues including stomach or stomach contents as well as from the placenta and vaginal fluid from the bitch. Special incubation techniques are necessary to culture this organism so refer to laboratory guidelines for proper sample collection and sample handling. Campylobacter infection can be zoonotic. Infections can be animals to human or vice versa.

### Other bacteria associated with canine abortion

Beta hemolytic streptococci have been isolated from a group of bitches with a history of abortion, infertility and neonatal death. This bacterium has also been isolated from the vagina of normal bitches and queens. Group G *Streptococcus canis* is a zoonotic pathogen that has been reported to cause reproductive disease and abortion in dogs as well as toxic shock and neonatal sepsis in cats. Leptospirosis associated infertility and abortion in dogs is most commonly associated with serovar Bratislava. Dogs are considered a maintenance host for serovars icterohaemorrhagiae, canicola and grippothyphosa. Dogs are incidental hosts for infection with autumnalis, australis, tarassovi, ballum, bataviae and bratislava. Infection in an incidental host is typified by low susceptibility, severe pathogenic effects and poor transmission. Leptospire are often transmitted through urine or contaminated water. Infection occurs when the organism penetrates mucous membranes or abraded skin. Mycoplasma and Ureaplasma species are often implicated by breeders as cause of infertility resorption, stillborn puppies and neonatal death. These gram negative cell wall free bacteria are among the many bacteria part of normal vaginal flora in the bitch and have been cultured from the vagina from over 60% of bitches. Breeders often request enrofloxacin as a preemptory attempt to solve their perceived Mycoplasma induced reproductive issues. Arbitrary use of antibiotics is not warranted and contributes to multi-drug resistance.

### Protozoa

There is experimental evidence that *Toxoplasma gondii* can infect puppies in utero. There have not been reports of naturally occurring Toxoplasma infections causing abortion in the dog. *Neosporium caninum* is a widespread protozoan parasite that has been shown to be transplacentally transmitted. Seroprevalence of *Neosporium caninum* in dogs appears to be relatively high but it is unknown if the organism is an important cause of canine abortion. Raw diets can increase the risk of infection.

### Viral causes of pregnancy loss in the canine

Canine minute virus also known as canine parvovirus 1 (CPV1) has been proposed as a cause of early pregnancy loss.<sup>3</sup> The more commonly seen canine parvovirus (CPV2) has not been associated with reproductive issues in the bitch. Canine distemper virus and canine adenovirus can both cause spontaneous abortion in the bitch. Appropriate vaccination can prevent infection with either of the viruses.

Herpes virus of canines is a controversial cause of reproductive failure in the dog. Infection in healthy adult dogs is often unrecognized. Infection of a naïve bitch during pregnancy or a resurgence of the virus in a bitch may be associated with early embryonic loss, resorption, abortion, stillbirth or most importantly infection of the neonates during passage through the vagina. Puppies infected at birth often succumb to fulminant infections. The herpes virus is heat labile so clinical infections in the neonate may be lessened by providing a well heated environment as the virus cannot replicate at normal body temperature. Canine herpes virus has been demonstrated in populations of dogs throughout the world. Serologic testing has limited value as antibody to the virus is only detectable for about 100 days. A canine herpes virus vaccine is available in some countries but is not presently available in the United States.

### **Luteal insufficiency**

The bitch is dependent upon the production of progesterone in the corpus luteum throughout pregnancy. Plasma progesterone levels of 2-3ng/ml are required to maintain pregnancy. Progesterone levels below 2-3ng/ml for 24- 48 hours routinely result in loss of pregnancy. It is difficult to determine if the progesterone levels drop due to loss of the pregnancy itself due to other causes or if the loss of pregnancy is due to the low progesterone. Definite proof that luteal insufficiency is a cause of pregnancy loss has not been defined. There are limited data that prove that declining progesterone levels over multiple days is the cause of repeated pregnancy loss. In two bitches examined at this author's clinic there were two cycles each where the bitch suffered apparent luteal insufficiency that were successfully supported by supplementation with altrenogest at 0.088mg/kg beginning when progesterone levels decreased to approximately 5ng/ml and discontinued several days prior to expected whelping date. Other authors have recommended the use of progesterone in oil or micronized progesterone orally. Use of these medications can result in masculinization of female fetuses and an increase in cryptorchid male puppies. If the hypoluteoidism is associated with shortened interestrus intervals, a genetic basis for the issue may be suspected. Daughters of bitches with luteal insufficiency have been observed to also exhibit luteal insufficiency. The placental hormone relaxin rapidly decreases after death of all the fetuses in a litter so monitoring relaxin levels can aid in monitoring placental health.<sup>4</sup>

### **Uterine disease**

There are numerous publications implicating uterine pathology as a contributing factor to infertility and total or partial pregnancy loss in the bitch. In a study by Fernando et al a group of seven bitches with historic pregnancy loss had uterine biopsies by laparotomy.<sup>5</sup> All seven bitches had intraluminal uterine dilation; three bitches had ovarian cysts. Histopathologic changes were found in six of the seven bitches and included endometritis (three of seven), pseudoplacental endometrial hyperplasia (two of six), pyometra (one of six), and cystic endometrial hyperplasia together with mucometra (one of six). The uterine contents revealed high leukocyte counts in all of these bitches. No bacteria or viruses were isolated from the uterine lumen.

### **Other causes of pregnancy loss**

There is a report in the literature (Bartos) inferring that there is a sociobiological origin of pregnancy failure in domestic dogs associated with return of a mated bitch to her home where there is a male dog present who is not the sire of the litter. Bitches mated elsewhere but returned home to be housed in a home pack were four times more likely to remain pregnant than a bitch housed individually after returning home. The highest proportion of successful pregnancies occurred in bitches mated at home and staying home.

Hypothyroidism has never been proven to be a cause of pregnancy loss in the canine. Likewise, diabetes mellitus, hypoadrenocorticism or hyperadrenocorticism are more likely to interfere with becoming pregnant than maintaining pregnancy. Body condition is important for pregnancy maintenance and neither under or over conditioning is desirable. If energy and nutrients are at suboptimal levels pregnancy can be compromised. The pregnant bitch should be fed an all life stages food or one labeled for pregnancy and lactation once pregnancy is confirmed by ultrasonography. A food labeled for adult maintenance is not intended for pregnancy and can result in inadequate nutrition. A bitch with an average litter size for her breed should be expected to gain approximately one-third of her pre-pregnancy weight during her pregnancy. Supplements, vitamins, nutraceuticals and topical preparation should only be used in a pregnant bitch if the label indicates that it is safe. Androgens, estrogens, glucocorticoids, antifungals or antineoplastic agents should not be used in pregnant bitches. Parasiticides should only be used if the label indicates the product has been tested as safe for pregnant and breeding animals. Topical ophthalmic and otic preparations often contain very potent corticosteroid products and have been proven to have systemic effects including suppression of the hypothalamic-pituitary axis. Certain plastics such as bisphenol can serve as endocrine disruptors and as an estrogen source. Investigation of pregnancy failures

in a kennel situation should warrant a physical inspection of the housing and management practices in the kennel.

### Summary

The causes of pregnancy failure in the bitch are many. The value of history, physical examination of the bitch and the environment are vital to obtaining a diagnosis and a treatment plan designed to minimize the recurrence of such loss in a bitch

### References

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