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Canine and feline pregnancy loss due to viral and non-infectious causes: A review

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Abstract

Among the causes for pregnancy loss, viruses and non-infectious factors are among the most important. In both dogs and cats, research and clinical evidence provide proof that there is an increasing incidence of pregnancy loss associated with infectious diseases like herpesvirus, as well as the presence of toxicants or chemicals in the animal's diet and environment. Endocrine causes must be taken into consideration when dealing with pregnancy loss. This review will cover the most recent knowledge regarding viral and non-infectious of pregnancy losses in the dog and cat.

Keywords: Pregnancy; Pregnancy loss; Abortion; Dog; Cat

1. Introduction

Recent developments in the field of small animal reproduction in the last few years have allowed for new and substantially improved strategies regarding the prevention and treatment of infertility, including reproductive loss in the bitch and queen. Reproductive failure and infertility are often complex and multifactorial, where the first objective is to determine the cause of the problem, followed by providing specific treatments (if available), and finally suggesting preventative measures for future pregnancies.

Infertility is defined as a reduction in the ability to produce young. Pregnancy loss includes all causes of termination of pregnancy, including embryonic death, fetal resorption, abortion at any stage of pregnancy, and stillbirth. Abortion accounts for only a small component of all pregnancy loss and is defined as the delivery of one or more fetuses that cannot survive outside the uterus (whether they are alive or dead at the time of delivery).

Older (>6–7 y) animals are observed to cycle less frequently, and to have reduced pregnancy rates and decreased litter size compared to younger animals. Infertility may reflect a problem with the male, the female, or both, and can be associated with normal or abnormal estrous cycles, or failure to mate. Etiologies are numerous and include improper management, as well as abnormal behavior, development, and abnormal anatomy or function of the reproductive system. In addition, infection, neoplasia, or iatrogenic causes may also be involved. Pregnancy loss may be considered a component of infertility, but is more specific in its etiology and/or pathogenesis.

The proper investigation of an animal with infertility involving pregnancy loss includes obtaining a complete history, performing a detailed clinical examination, and

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then using specific diagnostics as indicated to reach the proper diagnosis. This paper will focus on viral and non-infectious causes of pregnancy loss in the bitch and queen, including abnormalities of the pre- and post-implantation periods, embryonic development, fetal development, as well as hormonal causes such as hypoluteoidism.

Viral and non-infectious causes for pregnancy losses include:

a. A number of common viruses, with consequences on reproduction, including canine herpesvirus (CHV1)
b. Trauma
c. Neoplasia
d. Drugs
e. Endocrine abnormalities
f. Fetal defects and congenital abnormalities

Fetal and neonatal death related to dystocia or post-partum conditions will not be covered.

2. History, physical examination findings and general considerations of diagnostic testing

Pregnancy loss is easily recognized in animals that have been previously diagnosed pregnant in the early stages of gestation, but fail to deliver puppies or kittens at the expected time. Some animals may clinically have a decrease in abdominal girth, display vomiting and/or diarrhea, have poor appetite, are depressed, dehydrated, or febrile. Bloody or purulent vulvar discharge, abdominal straining, and discomfort may also be noted. However, in many cases, signs of pregnancy loss are inapparent or go unnoticed and pregnancy losses are only observed during a pregnancy follow-up examination or at the time of parturition.

When abortion occurs, the animal should be presented to a veterinarian as soon as possible for a complete physical examination and collection of samples for diagnostic testing. Only animals that have timely evaluations have a good probability of obtaining a definitive diagnosis, which in some cases may allow adequate time for treatment to salvage impending pregnancy loss. It is essential to remember that in most cases, these evaluations and diagnostic procedures help identify the cause and allow for appropriate supportive medical therapy and management practices which may help prevent future recurrence of the problem. Financial considerations must be discussed with the owner, as many of the diagnostic and therapeutic procedures are expensive.

2.1. History

In chronological order, starting with the first estrus, the following HISTORY should be recorded (ideally also including dates from previous cycles):

Date/age at the time of prior estrous cycles. This allows for calculation of the interestrus intervals, helps determine if the problem(s) has occurred repeatedly, and helps clarify at what stage of pregnancy the problem(s) occur. This history taking also allows the clinician to ascertain the competency level and experience of the owner. Good owners have good record keeping habits and have a clear overview of the problem. This helps provide clear interpretations, observations, and real facts, as opposed to impressions.

The vaccination history of the bitch or queen and the results of any prior serologic testing should be obtained.

Physical and behavioral characteristics of estrus and pregnancy should be documented. Information on previous vaginal cytology, vaginoscopy, progesterone assays, mating behaviors, numbers, and types of matings should be reviewed. These data provide objective information concerning the cycle and any abnormalities which may be related to the actual cause of pregnancy loss (i.e. abnormalities of spontaneous ovulation, abnormal CL development, presence of a cystic follicle(s), or prolonged bleeding).

Breeding management of the dog/cat and the kennel/cattery should be investigated, including: breeding dates, whether inside/outside ties occurred, if the stud was proven, if the same stud was used each time, the dates of any pregnancy diagnosis, and how pregnancy diagnosis was performed (i.e. palpation, ultrasonography, or relaxin assay).

The general health, records and management of the animals and colony should be evaluated. Any changes in diet should be noted, since some cases of colony infertility have been observed following a nutritional change. Overall management and hygiene should be discussed.

Food-related infertility problems can be observed in different animals from the same or different breeding colonies, especially in cases where phytoestrogens are detected in the diet. There are growing concerns relating to the careless use of holistic, organic, ethnic/traditional, and homeopathic supplements that are being used without any substantial scientific basis, by an increasing number of breeders. Many of these include substances of proven toxicity for reproduction.

The presence and date of onset of lactation or nesting behavior in the absence of whelping (overt false pregnancy or pseudocyesis), any body temperature

decrease, or any other abnormality at the time of expected parturition should be noted.

Any signs of past or current illness and any treatment(s) administered (in addition to reproductive disease) should be noted and are important, as concurrent diseases may indirectly induce pregnancy losses. Important aspects of the general history include vaccination and anthelmintic programs. The use of any past or present medications, including glucocorticoids, anabolic steroids, sex hormones, or thyroid replacement medications, may affect pregnancy.

2.2. Prior laboratory data

Whether related to reproductive problems or not, prior laboratory data can be useful to exclude other diseases which may impact reproduction (i.e. hypothyroidism, diabetes mellitus, Cushing's disease). The reproductive history of any closely related animals should be obtained, including any history of infertility, even if not directly correlated. Infertility problems may be related to poor hygiene in the colony, or to errors in general management.

2.3. Physical examination

A complete physical examination should be performed. The physical examination itself is routine, but specific attention should be given to conditions suspected to interfere with reproduction.

A thorough general exam for acquired diseases, as well as defects that are known to be inherited (i.e. atopy or hypothyroidism) should be performed. Further diagnostic examinations should be discussed with the client at this time (i.e. radiographs, ultrasonography, biopsy, serology, or microbiology).

A reproductive examination includes abdominal palpation of the uterus, digital examination of the vulva and vagina (if possible), and transrectal palpation of the vagina and bony pelvis.

2.4. Laboratory data

Laboratory data should be obtained and are useful in the overall investigation of the animal's health. These data should include vaginal cytology and culture, vaginoscopy, ultrasonography, radiography, endocrine tests (thyroid hormone, progesterone, and estradiol), as well as a standard hemogram and blood biochemical tests.

Vaginal cytology and vaginoscopy will help determine the stage of the estrous cycle, and concurrently enable visualization of vaginal abnormalities and subclinical lesions. Presence of numerous superficial cells, as well as red blood cells, may indicate the presence of cystic follicles. It is unlikely that vaginitis alone results in pregnancy loss, but when it is associated with other abnormalities, it may help clarify reproductive issues associated with maintenance of pregnancy. Uterine infections cannot be definitively diagnosed by vaginal cultures, as mixed cultures generally only reflect normal vaginal flora [1]. However, overgrowth of high numbers of a single organism may be suggestive of an etiologic agent. Diagnosis of uterine infection can be definitely diagnosed either by an exploratory laparotomy (or laparoscopy) or, much more easily, using the transcervical endocscopic catheterization technique (TECT), which allows for acquisition of uterine swabs and cultures. Biopsies can also be obtained using this technique [2,3]. However, specialized equipment and expertise is required for this method. This procedure is reserved for referral and specialty centers.

Blood chemistry and complete blood count provide an overall screening of the animal and are needed to exclude the possibility of adrenal, renal, hepatic, or metabolic dysfunctions, which may be responsible for the pregnancy loss. Changes in the complete blood count may give additional support for a diagnosis of estrogen excess in animals suspected to have ovarian cysts (non-regenerative anemia).

Urinalysis will allow for the exclusion of any concurrent urinary tract diseases or infections potentially complicating the diagnosis (i.e. leptospirosis, nephritis, or diabetes mellitus).

Serology for *Brucella canis*, leptospirosis and canine herpesvirus is required, particularly for imported animals or animals coming from regions where specific diseases are endemic. The surge in the international import and export of dogs makes this an absolute necessity.

Ultrasonography and radiography are essential diagnostic tools, with ultrasonography being the most useful, as it allows for superior evaluation of the uterus and ovaries during all stages of pregnancy.

Thyroid hormone testing and T4 tests (total T4 [TT4], free T4 by equilibrium dialysis [fT4-ED], and thyroid stimulating hormone [cTSH]) are required for exclusion of hypothyroidism.

Sex hormone assays may be obtained, depending upon the stage of the cycle when the animals are examined. Progesterone is a necessity if pregnancy loss is diagnosed during pregnancy, and the animal is presented during this period. Estradiol may be useful if the animal has been diagnosed not pregnant after proper

breeding management and still has substantial numbers of superficial cells or is still bleeding days after the end of estrus.

Relaxin can be tested if ultrasonography is not available to confirm pregnancy. However, it is noteworthy that plasma relaxin concentrations may remain elevated for several days after loss of a pregnancy [4,5].

2.5. Supplementary data

Supplementary data are often needed and can be of great help in the diagnosis and treatment of the reproductive failure.

Adrenal stimulation or suppression tests to rule out Cushing's or Addison's diseases.

Cytologic, virologic, and microbiologic evaluations of all discharges and expulsed tissues or fetuses should always be performed. Indeed, one of the most important but often overlooked diagnostic procedures is the examination of aborted fetuses and their associated membranes. In many cases, the bitch may consume all expulsed fetal tissues and membranes, but when they are available, they should be collected, placed in a sealed plastic bag, cooled and maintained at 4–5 °C (refrigerated, not frozen) and submitted as soon as possible to a diagnostic laboratory.

Chromosomal (karyotyping) and DNA testing of selected fetal tissues may be recommended to identify the cause of the pregnancy loss (chromosomal abnormalities, PCR, and in situ hybridization).

3. Etiopathogenesis of pregnancy loss in the dog

The establishment and maintenance of pregnancy is dependent on many biological interactions between the embryo or fetus and the pregnant female. In the dog, for approximately 12 d after fertilization, the development of free-floating embryos is dependent on the environment within the oviducts (first 3–5 d) and uterus (next 4–6 d) [6]. If this environment is inappropriate (inflammation, hormonal imbalances, infection, diet, etc.), the embryos may not survive. Death of embryos during this period often goes unnoticed, because the embryos are resorbed before pregnancy can been detected (the earliest that ultrasonography can be performed is approximately 8–22 d after the LH surge [7,8]). Most embryonic losses occur during this period or at implantation, when attachment to the uterus takes place. These losses are collectively referred to as early embryonic deaths [9,10].

After implantation, embryos depend almost entirely on nutritional exchanges with the dam; these can be substantially compromised by inadequate adjustments to the physical requirements and demands of pregnancy. Factors that lower the odds for survival include fetal or maternal abnormalities (including congenital defects), nutritional deficiencies, endocrine disruption, environmental stresses, or infectious causes [10–18]. Infectious agents are the most common causes of canine abortion [19]. When infectious agents are responsible for fetal losses, this may be due either to a direct effect on the fetus, as in fetal death by either viremia, bacteriemia, septicemia, or toxic agents, or due to an indirect effect on fetal development following placental infection (placentitis) and/or impaired feto-maternal exchanges.

3.1. Viral causes of pregnancy loss in the dog

3.1.1. Canine herpesvirus 1a

The involvement of herpesvirus in naturally occurring infertility and pregnancy losses [20] in the bitch is controversial; however, there is more and more evidence regarding the implication of this agent in cases of infertility [21–24]. The virus has been associated with reproductive failure if the dam is infected or reactivates during either estrus or the pregnancy. Reproductive failure due to herpesvirus is characterized by infertility (early unnoticed embryonic loss), resorption (non-expulsed fetuses before ossification/calcification), abortion (expulsion of well-developed and generally calcified fetuses not expected to live outside the uterus), stillbirth (expulsion of well-developed near term fetuses that would be expected to survive outside the uterus), delivery of poorly developed and compromised neonates, or neonatal death [24–29].

Infection in adults is generally inapparent, and is associated with latency [30–32], with the animal remaining a healthy carrier which can reactivate and restart shedding following stress or heat [33,34], thus further spreading the virus. In these animals, self-limiting lesions may occur on the genitalia of sexually active animals with typical bullous vesicles observed either in the vestibule or vagina, and on the prepuce [24]. Early neonatal infections result in fulminant sepsis and death [35–40], with survivors sometimes suffering from neurologic and cardiac defects. Survivors may have no clinical signs and remain inapparent healthy carriers until puberty when they may start shedding the virus [29].

Canine herpesvirus type 1 occurs in canine populations throughout the world, with serological studies

demonstrating an incidence of up to 60–80% depending on the country [22,24,41–44]. This incidence is probably underestimated, as serological testing only detects animals either recently infected or having reactivated the virus. Antibodies to CHV1 have been reported to persist for approximately 100 d [27,29].

Whereas one episode of reproductive failure due to canine CHV1a does not preclude future successes in pregnancy, it also does not imply definitive protection, as generally observed after immunization or infections with other viruses.

3.1.2. Canine distemper virus and adenovirus (infectious hepatitis)

These viruses may cause spontaneous abortion, with or without fetal infection. Abortion often results from the stress of the clinical disease [45,46].

3.1.3. Canine parvovirus (or minute virus)

Canine parvovirus (CPV1) has been proposed to cause early pregnancy abortion [47].

3.2. Non-viral causes of pregnancy loss in the dog (non-bacterial)

3.2.1. Immunological infertility

Clinical cases of auto-immune infertility have not been documented as naturally occurring in the bitch. In other species, such antibodies, naturally occurring or induced, can cause infertility and have indeed been the target for specific research concerning contraception [48–52]. In rats, monkeys and humans [53–55], immune-mediated infertility is associated with production of anti-sperm antibodies which have also been detected using immune-precipitation in some dogs [Verstegen J. Unpublished data]. Canine anti-sperm antibodies have also been demonstrated by indirect immunofluorescence [56]. At the present time, however, the significance of these antibodies in the dog are unknown.

3.2.2. Hypoluteoidism

Hypoluteoidism resulting in inadequate plasma concentrations of progesterone is a poorly documented cause of embryonic/fetal loss in bitches. Experimental studies suggest that in the bitch, a plasma progesterone concentration of at least 6–9 nmol/L (2–3 ng/mL) is required to maintain pregnancy and that concentrations below this threshold for more than 24–48 h result in loss of pregnancy [57–59].

In experimental studies, dogs have maintained pregnancies with plasma progesterone concentrations as low as 2–5 ng/mL without problems [60]. However, in the clinical setting, it is generally safer to anticipate potential pregnancy loss when plasma progesterone concentrations are decreasing, by initiating progesterone supplementation. This action is usually taken without actually demonstrating that in absence of supplementation, the pregnancy would have terminated [61]. Clinicians and breeders are unwilling to take the risk of waiting until progesterone decreases to 2–3 ng/mL to start supplementation, as the pregnancy may be unsalvageable at that point. Even though primary hypoluteoidism is difficult to document clinically, it cannot be excluded and has been suggested as the cause for pregnancy loss in dogs [61–63]. In cases where pregnancy loss is clearly associated with low plasma progesterone concentration at the time of the event, it is unclear whether the failure was due to the low plasma progesterone concentration itself, or if the failure was due to another cause, secondarily leading to CL failure.

When described, hypoluteoidism generally occurred at approximately Days 25–35, when progesterone secretion directly or indirectly increased in pregnant animals following a still unclear mechanism linked to placentation [63]. For unexplained reasons, either the required increased production of progesterone of ovarian origin was not detected, or progesterone metabolism was increased, leading to insufficient plasma progesterone concentrations to maintain pregnancy. There is only anecdotal evidence that habitual abortion occurs in the bitch due to hypoluteoidism. There is a paucity of data to clearly demonstrate that a persistent and declining plasma progesterone concentration is the cause of repeated pregnancy loss. In some reports, shortened interestrus intervals were noted. The increased incidence of shortened luteal phases and hypoluteoidism in some breeds suggests a possible genetic inheritance of the disease [64]. Some authors have suggested removing animals suspected of hypoluteoidism from the breeding pool, to avoid perpetuating this abnormality [64].

When progesterone concentrations are low and risk of pregnancy loss is imminent, progestin supplementation can be recommended, even if doses are still empirical. Progestin use should be restricted to those cases in which true luteal insufficiency has been diagnosed or is highly suspect. Improper use may lead to masculinized females and cryptorchid male puppies. Various progestins have been proposed and used, including megestrol acetate. The authors do not recommend the use of synthetic progestins, for at least two reasons: (1) their pharmacology and pharmacodynamics are poorly understood in dogs and they are

associated with longer durations of action; and (2) there are no means to evaluate the appropriateness of the dose administered. In that regard, no assays are commercially available to measure plasma concentration of these medications, preventing accurate monitoring. Therefore, when the need for supplementation arises, the authors recommend the use of micronized progesterone (Prometrium, Solvay Pharmaceuticals Marietta, GA, USA), which is a compounded product that is administered orally with good gastrointestinal absorption, as demonstrated in humans. Since this is a natural form of progesterone, plasma concentrations can be easily monitored using standard progesterone assays. The goal is to adjust the dose so that progesterone concentrations remain within the physiological ranges typically observed during the same stage of pregnancy. The standard dose used by the authors at the start of the treatment is 10 mg/kg p.o. q.d. Plasma concentrations are re-evaluated every second or third day for the duration of the treatment. The treatment is generally discontinued approximately 60–62 d after the LH surge. It is interesting to note that after administration of the same dose, plasma progesterone concentrations vary dramatically among animals, and need to be carefully monitored to maintain concentrations within the normal ranges observed during this part of pregnancy (Verstegen J. Unpublished data).

3.2.3. Uterine pathology

Uterine pathology can be responsible for pregnancy losses in bitches, although everything else appears normal. Cystic endometrial hyperplasia (CEH)/pyometra complex is more common in older females and has been associated with infertility (inability of the embryos to implant) if the condition is present prior to breeding and has been associated with partial or total pregnancy loss when the condition develops during pregnancy (for review see [65]). Predisposing factors are age and treatment with progestogens (for the postponement of estrus) or estrogens (for the early termination of pregnancy). The pathogenesis of this syndrome includes bacteria of vaginal origin ascending into the uterus during estrus when the cervix is open, resulting in uterine contamination and subsequent failure of clearance of these ascending organisms during diestrus. The diagnosis of CEH with macroscopic cysts can be made using ultrasonography. The return of fertility after treatment suggests that recovery of uterine integrity is possible.

3.2.4. Endometritis

The role of endometritis (apart from pyometra) as a cause of infertility is quite unclear. This reflects the difficulty in making a diagnosis of endometritis in cases other than those involving clinical signs that include a purulent vulvar discharge. In many species, acute endometritis is normal after breeding and represents a physiological process that cleanses the uterus of all debris, contaminants, and dead sperm cells introduced during mating [66–69] and also prepares the uterus for entry of the maturing embryo(s) from the oviducts. The presence of this process after mating in dogs has not yet been demonstrated. However, Ribeiro et al. [70] described leukocyte infiltration 10–12 h after AI with semen and seminal fluid or semen with extender. They did not detect any differences in leukocyte numbers between dogs inseminated with semen including seminal fluid or extenders and those that were not inseminated, indicating that seminal fluid provided immune modulating effects similar to that seen in other species. In dogs, the presence of post-coital endometritis appears less probable than in other species, due to the peculiar estrous cycle of the dog, which is characterized by prolonged estrus and multiple matings in a single cycle. If post-coital endometritis did occur, like it does in other species with shorter estrus and mating periods, it would likely be long enough to impair sperm migration, fertilization and early embryo development. Analyzing the presence of this ‘‘physiological’’ endometritis after mating has been a challenge due to the difficulty of gaining access to the uterus to obtain samples for microbiology and cytology. However, the development of the TECT will make these studies possible ([2,3,71], Verstegen et al., unpublished).

The presence of bacteria in the vagina is normal in dog and is not diagnostic of endometritis. When present, chronic endometritis certainly impairs the ability of the dog to get pregnant [72,73]. Structural abnormalities of the uterus, either developmental (hypoplasia) or acquired (scar tissue), may result in compromised placental function that is unable to meet the demands of the growing fetus(es). Recently, Günzel-Apel et al. [73] reported a case of prolonged fetal retention in a bitch, resulting in the development of a metritis responsible for infertility. Fertility was re-established after unilateral ovariohysterectomy.

3.2.5. Concurrent diseases

Pregnancy failure may be associated with disorders of other body systems, (i.e. poor body condition, debility, diabetes mellitus, hyperadrenocorticism or hypoadrenocorticism). However, it is generally believed that these diseases will be more responsible for the inability of the dog to become pregnant than for pregnancy losses to occur. Hypothyroidism has been

associated with increased danger of miscarriage in women, mice, rats, and dogs [74–82]. Lower than normal concentrations of circulating thyroid hormone have been proposed as cause of prolonged anestrus or infertility in the bitch [83–87]. However, in the clinically normal bitch without signs of the disease (poor coat, lethargy, poor appetite, low tolerance of cold, obesity, alopecia), the role of hypothyroidism is still highly controversial and questionable [82,84–86,88,89].

Recently Panciera et al. [88], did not detect significant effects of experimentally induced hypothyroidism on fertility in a model of short-term induced hypothyroidism in the dog. It did, however, prolong parturition in the affected animals and reduce pup survival in the peri-parturient period.

3.2.6. Aged gametes, chromosomal, developmental, and embryonic/fetal defects

These and related conditions have been associated with an increased incidence of early miscarriage in women. In the bitch, these factors could result in early embryonic death and infertility. Breeding at an inappropriate time may lead to aged eggs or sperm that have reduced fertility and this may lead to abnormal embryo development and death. Similarly, defects in genetic makeup may either be incompatible with embryo survival or associated with abnormal development of organ systems, which may in turn lead to embryonic or fetal death, resorption, or abortion.

3.2.7. Maternal environmental stress

These conditions can produce an adverse uterine environment that is incompatible with fetal development. Fetal death may occur at any stage of pregnancy, resulting in abortion.

3.2.8. Nutritional deficiencies

Energy and vitamin demands increase during pregnancy. If these are deficient, fetal survival may be compromised [90,91].

3.2.9. Iatrogenic exposure to drugs or other toxic compounds

Many drugs have been shown to potentially or clearly affect pregnancy [92,93]. Drugs which may prevent maintenance of pregnancy in the dog are numerous and include members of the following families: androgens, anabolic and estrogenic steroids, glucocorticoids, antimicrobials, antifungals, antiparasitics, antineoplastic agents, analgesics, anti-inflammatories, anesthetics, gastrointestinal, cardiovascular, anticonvulsant, and endocrine drugs. These drugs may, either directly or indirectly, affect the pregnancy by interacting with the neuroendocrine control of pregnancy (i.e. hormones, anesthetics), with the fetal or placental vascular system (anesthetics, anti-inflammatory drugs), or with fetal development itself (teratogenic effects). Ideally, no drugs should be administered during pregnancy, as they all have the potential to induce pregnancy loss [94–100].

3.2.10. Other diseases

Diseases such as extra-uterine ectopic pregnancy, uterine torsion and inguinal, perineal or abdominal hernias have been associated with increased risk of pregnancy losses [19].

4. Etiopathogenesis of pregnancy loss in the queen

Infertility and pregnancy loss in the queen have been poorly studied. The causes are likely similar to those observed in bitches, but some specific disorders are also described. Knowledge about infertility and pregnancy losses [101] can only be enhanced by careful and methodical reproductive examinations and good record keeping, using similar approaches as the ones described for the dog [102,103].

Diagnosis is also similar to that previously described for the dog and involves histopathology, microbiology, serology, hormone assays, and DNA analysis. Management of pregnancy losses in a cattery is essentially related to the hygiene of the cattery, the number of cats (population density), and vaccination.

4.1. Viral causes of pregnancy loss in the queen

Viral agents are the most commonly reported infectious causes of abortion in queens. Implicated viruses include feline panleukopenia virus (FPLV), feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline enteric corona virus (FECV), and feline herpesvirus type 1 (FHV1).

4.1.1. Feline panleukopenia virus

Infection may result in infertility due to early embryonic death, abortion of mummified or macerated fetuses, or queening of retinal, cerebellar or cerebral hypoplastic neonates. Feline panleukopenia virus has been shown to cause in utero infection, leading either to abortion or fetal resorption when it occurs early in pregnancy or to cerebellar hypoplasia and ataxia in kittens when infection occurs later in gestation [104–107].

Diagnosis is by isolation of the virus from fetuses or neonates submitted for necropsy, by documenting seroconversion in the dam, or clinically by the characteristic ataxia observed in the neonates. Vaccination of queens against FPLV prior to breeding is recommended [108,109].

4.1.2. Feline leukemia virus

Feline leukemia also produces various clinical signs and has been proposed to play a substantial role in the pathogenesis of the ''fading kitten syndrome''. In viremic queens (positive ELISA or IFA), FeLV induces pregnancy loss [110]. A pattern of fetal resorption in infected queens, with isolation of the virus from litters of unborn fetuses, newborn kittens, and from the uterus of viremic, pregnant queens, has been reported [110]. Although it appears that pregnancy loss is due to direct fetal infection, it has also been suggested that the virus disrupts the endometrium at the sites of placental attachment. Further studies are necessary to accurately define the occurrence and pathogenesis of abortion in FeLV-viremic queens.

Non-viremic queens (ELISA or IFA negative), who have overcome the initial infection but are latently infected in their bone marrow, can reproduce normally and do not appear to be at significantly increased risk for pregnancy loss [111,112]. However, the virus may be passed to the occasional kitten, that may then infect his or her litter-mates. Control of FeLV in the cattery is achieved by removing all ELISA positive animals from the cattery, and retesting all negative animals in 90 d [113]. Vaccination of negative animals may be beneficial.

4.1.3. Feline immunodeficiency virus

Feline immunodeficiency virus, first recognized in 1986, is a lentivirus similar to the human immunodeficiency virus (HIV), and has not yet been associated with reproductive failure. However, a recent study demonstrated that pregnant queens acutely infected with FIV can transmit the virus to their offspring [111]. In utero transmission [114] resulted in arrested fetal development, abortion, stillbirth, and birth of viable but infected kittens [115–118].

4.1.4. Feline infectious peritonitis virus (FIPV)

Feline infectious peritonitis virus is due to a mutated, disease-causing form of the common feline enteric corona virus (FECV). Historically, FIP has been cited as a major cause of pregnancy loss and kitten mortality in catteries. At the present time, this is less clear and it is currently an unproven cause of abortion. The majority of cats with FECV remain healthy, but in a smaller number of cases (less than 10%), FECV is the first in a chain of mutations leading to FIP. This mutation arises within the individual cat, and horizontal transmission is considered rare. Vertical transmission, however, is frequent and has been clearly demonstrated to be responsible for neonatal deaths between 1 wk and 6–10 mo of age in experimental studies [119,120]. In reality, its role in the pregnant queen appears to be less important than its consequences on the post-partum kittens. Mortality of 100% following birth of infected kittens is often observed, with mean survival times of approximately 57 d.

Three factors play a major role in the conversion of the virus from benign normal enteric virus to the aggressive form seen in FIP: genetic susceptibility, the presence of chronic FECV shedders, and a cat-dense environment that favors the spread and concentration of FECV [121–123]. Foley et al. reported the heritability of susceptibility to FIPV in purebred catteries to be very high (approximately 50%). It is likely a polygenetic trait and selecting for overall disease resistance is recommended, which requires removal of all suspected cats from the breeding programs. The same authors [121–123] demonstrated that persistent shedders of FECV, did not develop immunity, remained chronically infected, and were the source of infection for other cats. Careful identification of chronically infected, immunodeficient animals by PCR and removal from the colony would significantly reduce the risk of FIP. Currently, ELISA and PCR tests are commercially available [124]. Reduction in animal density in the cattery is recommended to reduce the spread of the virus and minimize horizontal contamination. Ideally, individual animals should be housed separately [124]. A licensed vaccine for FIP is commercially available (Primucell FIPTM, SmithKline Beecham Animal Health, USA). Although its use is still highly controversial, some publications seem to demonstrate that the risk associated with the use of this modified-live temperature-sensitive viral mutant vaccine is minimal [125,126]. Its use in pregnant queens is contraindicated, as it is a modified-live preparation.

4.1.5. Feline herpesvirus

Feline herpesvirus shares similarities with canine herpesvirus [127]. It appears to cause abortion due to the debilitating effect of upper respiratory infection in queens, and it also infects kittens during the neonatal period. In utero transmission of the disease has not been shown except under certain experimental conditions. In one study, pregnant queens infected intranasally aborted

following severe upper respiratory disease. However, no virus was isolated from the uterus, fetuses or placentas, nor were any histologic lesions seen [128]. A large epizootic infection of FHV1 in a large SPF cat colony of more than 690 queens was recently associated with only mild clinical signs of upper respiratory tract infection and only one partial abortion out of 51 queens. The queen aborted one dead kitten 3 wk prior giving birth to two healthy kittens. That queen was acutely ill, febrile, anorectic, and dehydrated at the time of abortion. However, 62% of kittens born from acutely affected mothers died within 1 wk after queening. [129]. As in dogs, many of the cats that recover from acute FHVl infection become persistent latent viral carriers, with possible reactivation occurring spontaneously or being induced by different types of stressors (illness, surgery, pregnancy, lactation, corticosteroid treatment) [130–132].

4.2. Non-infectious causes of pregnancy loss in cats

4.2.1. The cystic endometrial hyperplasia–pyometra complex

This complex is less often observed in cats than in dogs [133]. However, its frequency may be underestimated, as many cats do not clinically express the pathology. Clinical signs are few and in general no significant hematologic or biochemical changes are observed, except for a neutrophilia. Renal lesions are less common than in dogs. The etiology is less clear in cats and even if the relation with progesterone is generally proposed, this hypothesis is less easily demonstrable in cats. Since queens are induced ovulators [134–136], high and prolonged plasma progesterone concentrations occur only after ovulation, rather than during every cycle as in bitches. The recent description of a high incidence of pseudopregnancy in cats might be an explanation corroborating the possible role of the repeated and prolonged progesterone influence [137]. However, the observation in dogs that uterine reactivity to local stimuli (mechanical or biological) might be the first and real inductor of cystic endometrial hyperplasia is also certainly to be taken into account in the feline species. Further investigation is certainly warranted before the exact etiopathogenesis of the CEH-pyometra complex can be clearly understood. Hyperplasic development of the uterine tissues, whatever the origin, certainly leads or facilitates bacterial growth and pyometra. Cystic endometrial hyperplasia and endometritis are diagnosed by detecting uterine changes by ultrasonography. Definitive diagnosis may require uterine biopsy and culture of the uterine lumen. Endometritis can complicate CEH following bacterial contamination, persistence of mummified or incompletely resorbed fetuses, retained fetal membranes, or from post-partum metritis [138–141]. Feline pyometra can be treated like canine pyometra with good results and recovery of fertility [142,143].

4.2.2. Nutritional deficiency

This is an unlikely cause of pregnancy loss, since most cats are fed commercial, balanced diets, and other signs would be apparent. Nevertheless, special attention should be paid to vitamin A and taurine. Vitamin A deficiency has been associated with reproductive disorders such as anestrus, failure to conceive, early embryonic death, abortion and congenital defects. Queens fed a taurine deficient diet can appear clinically normal, yet suffer from reproductive failure [143–147]. Of 18 queens fed a taurine deficient diet, only six carried pregnancies to full term. Clinical findings included abortions, early embryonic death and malformations in the neonates. Queens were clinically normal, except for retinal degeneration detected by fundoscopic examination. Interestingly, affected neonates suffered from cerebellar dysfunction, due to a persistence of mitotic activity in the external granule cell layer of the cerebellum. Whereas the histopathologic lesions were different from those seen with panleukopenia virus infection, the clinical signs were similar and were indicative of cerebellar dysfunction. The kittens' hindlegs were abnormally developed, they frequently splayed out, and they often had rear-leg paresis.

4.2.3. Hypoluteoidism

The failure of the CL (exclusive source of progesterone during pregnancy in the cat) to secrete sufficient progesterone to maintain pregnancy, has not been definitively documented as a cause of pregnancy loss in the cat, although it is suspected in cats which habitually abort at approximately Days 50–58 of pregnancy. If present, similar treatment with natural progesterone is recommended, to prevent the fetal side effects associated with synthetic progestin treatments [148].

4.2.4. Fetotoxic drugs [149]

Fetal injury due to administration of fetotoxic drugs during pregnancy should also be considered as a potential source of fetal death. Nearly all drugs cross the placental barrier and reach significant concentrations in the fetuses, where they can induce teratogenic and lethal effects [92,93,150]. The specific drugs below are known

to cause fetal injury and should not be administered during pregnancy: antibiotics (trimethoprim-sulfonamides [151], quinolones, tetracyclines and gentamicin [152]), antifungals (griseofulvin [153]), anti-inflammatories [154,155], anesthetics [149], gastrointestinal drugs (misoprostol), anticonvulsants (phenytoin), steroids (testosterone and estrogen analogues), vitamin A analogues (isoretinoin) and mitotane (o,p'-DDD). Organophosphate insecticides, antineoplastic drugs, corticosteroids, and vaccination with modified-live vaccines (i.e. feline panleukopenia vaccine) should also be avoided in pregnant animals.

4.2.5. Genetic defects

Chromosomal abnormalities can cause fetal losses [156–158]. Many of the resorptions are not recognized, since they occur in early in pregnancy. Genetic abnormalities account for approximately 15% of infertility or pregnancy losses in abortion cases in domestic animals, including cats [159,160]. Genetic defects tend to occur when close inbreeding takes place. Although cats may have many genetic abnormalities, the Manx syndrome associated with the tailless condition is of particular interest. An autosomal dominant factor is responsible for the tailless condition and is associated with spina bifida, urinary and fecal incontinence, and locomotor disturbances of the pelvic limbs. These are all related to a disturbance affecting the development of the central nervous system in early embryonic life [161]. Analysis of aborted fetuses or stillborn kittens for genetic defects such as spina bifida or sacral dysgenesis should be considered when one or both parents are of Manx breeding.

5. Diagnosis of specific causes of pregnancy loss in the dog and cat

Differential diagnosis should include infections due to bacteria, viruses, protozoa, mycoplasma, ureaplasma, and all of the other non-infectious causes presented here.

Diagnosis will be based on clinical signs and will involve the use of appropriate laboratory techniques in an attempt to identify the cause. Viral and parasitic diseases require specific laboratory evaluations, including serology, microscopy, histopathology, and/or DNA isolation [162,163].

For CHV1, the infection can be suspected whenever compatible clinical findings occur. Hematologic and biochemical investigations are of limited value, since affected adults have non-specific changes. In clinically affected neonates, marked thrombocytopenia is uniformly present. Serologic testing is the traditional method of diagnosing CHV1. Specific ELISA tests are currently being developed, making the test easier to perform and more readily available [29,162,164]. The diagnosis, based on detection of specific antibodies against CHV1, is only valuable within a period of 2–3 mo following either an infection or reactivation. Thereafter, following the intial rise in titers, a dramatic reduction in titers will rapidly occur and antibodies will no longer be detectable, even if the animal remains a latent carrier. This is due to the herpesvirus being poorly immunogenic. However, in some cases, antibody titers may remain elevated for up to 2 y. When this occurs, it is typically seen in large infected kennels, where the infection pressure is elevated and reactivation or new infections occur frequently.

Any titer $>$1:16 is diagnostically significant and indicates prior infection. In many dogs, titer plateaus reach 1:64–1:640. To determine if the animal has had a recent or a prior infection, two tests at 1-mo intervals are suggested. When the titer is $<$1:2, the animal is obviously negative but may still be a carrier. To identify the status of a kennel, it is recommended some animals be followed longitudinally for two cycles. If the titers stay low, the kennel is probably free from infection. If the titer changes in one of the animals, the kennel is probably positive with latency. The other possibility, particularly in large colonies, is to test many dogs simultaneously. If some are positive, the kennel is probably infected, with some animals being latent carriers whereas others are either actively shedding or having been reinfected recently [27,29].

Following acute CHV1 infection, viral isolation is possible from infected tissues for 2–3 wk. In shedding adults, viral growth is restricted to mucosal surfaces of the oral cavity, genitalia, and upper respiratory tract. However, a recent study clearly showed that the likelihood of isolating the virus from secretory tissues (particularly vagina) is relatively low. Only 1 of 5 samples obtained from shedding animals is likely to be positive [29]. If virus recovery is the objective, considering the sensitivity of the virus to the environment, the samples should be refrigerated for storage and transport and freezing should be avoided. Virus recovery is positive confirmation of infection. In addition, PCR can be used and is less dependent on proper sample handling. Suitable samples in live animals include nasal and vaginal swabs [29].

From aborted fetuses and neonates, whole virus, viral antigen, or DNA is recoverable from the vascular endothelium, liver, adrenal glands, lungs, spleen, kidneys, and lymph nodes. Histopathological

examination of liver, lung and kidney reveal hemorrhages with necrotic foci and intra-nuclear inclusion bodies detected by immunofluorescence. Fresh-chilled samples of these tissues should be collected.

Some private companies propose specific DNA profiles, including DNA testing for most common pathogens causing abortion in dogs. HealthGene Laboratory offers two Canine Lost Pregnancy Profiles (*Brucella canis*, *Staphylococcus aureus*, *Streptococcus* spp., *Mycoplasma spp*., *Ureaplasma* spp., *Canine herpesvirus*, and *Chlamydophila* spp.). They also offer a canine semen profile using blood and semen samples to evaluate the possible role of the sire in the pregnancy loss; this profile includes DNA testing for *Brucella canis*, *Mycoplasma* spp., *Ureaplasma* spp., *Chlamydophila* spp., *Canine herpesvirus*, and *Leptospira* spp. Furthermore, DNA testing of selected fetal tissues may be also recommended and performed.

6. Treatment and prevention of pregnancy loss in the dog and cat

Affected dogs and cats should be hospitalized to permit close observation, diagnostic evaluation and supportive therapy, and to confine and isolate them while the cause of the abortion or pregnancy loss is being determined. Intravenous fluid therapy may be required to help stabilize a critically ill animal. Blood, urine, and culture samples should be taken immediately. Antibiotics should be administered if the white blood count and/or rectal temperature are consistent with the presence of infection. The patient should remain hospitalized while being treated and should be monitored with radiography or ultrasonography for complete emptying of the uterus.

An attempt can be made to salvage the remaining live fetuses in bitches and queens that have had partial abortions. Treatment for hypoluteoidism has been discussed above.

Uterine evacuation following an abortion can be accomplished by either removal of the whole uterus (ovariohysterectomy) or, if future breeding is important, by administration of a prostaglandin/dopamine agonist combination. The type and amount of vulvar discharge should be evaluated daily; resolution of the condition being indicated by a decreasing amount of discharge and by an increasing amount of mucus in the discharge. Uterine evacuation should also be monitored using ultrasonography. Blood parameters and serum chemistries should be checked regularly to monitor for major complications such as pyometra, renal diseases, or disseminated intravascular coagulation (DIC), which commonly accompanies chronic inflammatory diseases [165].

Treatment of CHV1 affected dogs remains problematic. Neonates can be protected on a preventative basis, but once infection is clinically apparent, treatment is not very rewarding, due to the fulminating nature of the disease. Prevention includes isolation of affected animals, optimal hygiene, increasing the environmental temperature to over 38 °C, passive intra-peritoneal immunization of the pups with 1–5 mL of serum from hyper-immunized animals, and/or administration of Acyclovir. The latter is a guanosine analog, commonly used in treating herpesviral infections in humans. It targets steps in virus replication, specifically the action of viral DNA polymerase. There have been anecdotal reports of maternal therapy for neonatal prophylaxis, as well as treatment of affected litters. Once clinical signs are present, however, infected neonates have a grave prognosis and will either likely have residual neurologic and myocardial deficits, or will remain latent carriers if they survive.

In Europe, dams can be protected using a vaccine for active immunization of dogs (Eurican Herpes 205, Merial, Lyon, France). This vaccine has been registered to protect against neonatal disease by active immunization of the dam [27,166]. The vaccine has also been shown to improve birth weight, weaning rate and litter size, suggesting a protective effect on the fetuses. The recommended vaccination scheme includes a first vaccination at approximately the time of breeding, and a booster 6–7 wk later to achieve maximum antibody titer around whelping. However, an alternative immunization scheme has been tested and proposed by the author to not only protect the neonates, but also try to protect the dam around the period of viral reactivation observed in estrus. This protocol entails a first vaccination within 1 mo before expected estrus, followed by a booster at approximately the time of mating. The antibody titer is then followed by serum neutralization or ELISA at mid-pregnancy and if the titer is not high enough (>1:128) a third injection is given. High levels of protective antibodies are then present during all the critical periods of the estrous cycle and pregnancy and are passed to the neonates via colostrum after birth.

7. Conclusions

Pregnancy losses in both dogs and cats are important problems for breeders. Epidemiological studies, as well as diagnostic approaches, have resulted in substantial improvements in the last 10 y, providing veterinarians

with better approaches to diagnosis and treatment. Research is still warranted to provide further improvements and reduce the incidence of pregnancy losses in dogs and cats.

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