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Findings in uterine biopsies obtained by laparotomy from bitches with unexplained infertility or pregnancy loss: An observational study

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ABSTRACT

Surgical uterine biopsies (SUBs) were obtained by laparotomy from 14 bitches with unexplained infertility whose pregnancy was not confirmed (NCP group) and from 7 bitches that had experienced unexplained pregnancy loss (PL). SUBs were obtained during the luteal phase of the cycle in 16 of 21 bitches. Overall, the biopsied uterine tissues presented obvious alterations in 17 of 21 cases. In the NCP group, 11 of 14 bitches had varying degrees of uterine lesions. In this group, six of nine bitches with macroscopically normal uteri presented histopathologic changes in the endometrium. All bitches with macroscopic modifications (five of 14) presented endometrial changes. Cytologic examination of the uterine fluid revealed eosinophilic amorphous material, erythrocytes, and a low number of degenerated leukocytes. On histopathologic examination, the most common lesion was fibrosis with degeneration of the endometrial glands (FDEGs six of 11), followed by endometritis (four of 11), cystic endometrial hyperplasia (CEH) (two of 11) and pseudoplacentational endometrial hyperplasia (PEH) (two of 11). Other lesions were adenomyosis, mucometra, and an endometrial polyp (one each of 11). Mixed lesions were present in four of the 11 bitches. In the PL group, macroscopic modifications consisted of intraluminal uterine dilatation (seven of seven) and presence of ovarian cysts (three of 7). Cytologic examination of the uterine content revealed high leukocyte counts. Histopathologic endometrial changes were found in six of seven bitches and included endometritis (three of seven), PEH (two of 6), pyometra (one of six), and CEH together with mucometra (one of six). Research of infectious agents was performed in 20 of 21 cases. Surprisingly, no bacteria or viruses were isolated from the uterine lumen in any of the cases. When the SUB was performed in diestrus, a medical treatment with aglepristone or with prostaglandins was established to avoid the risk of postoperative-induced pyometra. One case of complete uterine stenosis was the only side effect observed in the bitches that were not ovariohysterectomized after the collection of a biopsy (17 of 21). In conclusion, the most common findings in our biopsy specimens were FDEGs, endometritis, and hyperplastic remodeling of the endometrium (CEH and PEH). SUBs might provide useful information regarding the cause of infertility or PL. This technique allows visual inspection and palpation of the whole uterus and ovaries before the selection of a precise site for the biopsy. The size of the tissue sample collected can be large enough for an accurate

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diagnosis and samples for research of infectious agents can be obtained with no risk of contamination. Risk of side effects seems low when SUBs are performed according to this protocol, including postsurgery treatment with aglepristone or prostaglandins.

1. Introduction

Mistimed breeding [1] and male infertility [2] are considered by most authors as the major causes of conception failure in the bitch. When mistimed breeding can be avoided by proper timing of ovulation and if anatomic abnormalities of the female genital tract can be excluded [3], the causes of infertility or pregnancy loss (PL) are difficult to identify. *Brucella canis, Campylobacter spp., Escherichia coli, Salmonella spp., Streptococci spp., Toxoplasma gondii Neospora caninum*, Canine herpesvirus, Canine distemper, Canine parvovirus type 1 (CPV-1) and *Mycoplasma spp.* and *Ureaplasma spp.* are often mentioned as infectious agents responsible for infertility although they are rarely found when investigated [4]. Genetic defects [5], previous treatment with steroid drugs [6], hormonal disorders such as luteal failure [7], hypothyroidism [8], hypoadrenocorticism [9], hyperadrenocorticism, and diabetes mellitus [10], or ovarian cysts [11] are other possible causes. Systemic diseases should be ruled out when other clinical signs are associated, although most infertile bitches and most bitches with a history of PL are apparently healthy.

Uterine lesions, whether inflammatory, hyperplastic or neoplastic, are regarded as potential causes of infertility in the bitch [3]. Endometrial alterations might block syngamy by modification of the uterine environment. Lesions might also induce implantation failure or defective embryonic or fetal development [12]. Moreover, uterine lesions such as endometritis, endometriosis, uterine fibrosis, atrophia or hypoplasia and degeneration of the endometrial glands are known to play an important role in fertility in other species like women, cows, and mares [13–15]. To our knowledge, only one previous study reported cytobacteriological investigations on uterine flushings in infertile bitches. In that study, it was concluded that 10 of 26 bitches with unexplained infertility suffered from endometritis [16]. Surgical and transcervical techniques to obtain a uterine biopsy sample have been described [17,18], however, we found no study in the literature reporting histopathologic findings in bitches with infertility or PL. This study was designed to investigate the histopathologic and cytopathologic changes on uterine biopsies obtained from such bitches and to identify suspected infectious agents responsible for unexplained infertility or PL.

2. Materials and methods

2.1. General procedure

Twenty-one bitches, mean age 4.8 years (2–8 years), of 19 different breeds, were included in this study. All presented a good health condition and were properly vaccinated and dewormed. Eleven of 21 were cases at the CERCA (Centre d'Études en Reproduction des Carnivores, Maisons-Alfort, France) between 2008 and 2009, and 10 were cases admitted in 2002 to 2008 to a private veterinary clinic specializing in small animal reproduction (Veterinary Village, Lomira, WI, USA).

Bitches included in this study had recently reported episodes of nonconfirmed pregnancy (NCP; Table 1) or PL (Table 2). They were all mated (eight of 21) or inseminated intravaginally (IVAI; three of 21), into the uterus with fresh semen, or transcervically (TCI; five of 21) in France, or surgically via midline laparotomy (five of 21) in the United States, 1 to 3 days after the date of ovulation as determined by progesterone assays (chemiluminescence-Elecsys 2010, Roche Diagnostics, Mannheim, Germany for bitches followed in France and MiniVidas, Biomerieux, Lombard, IL, USA for bitches followed in the United States). The males used were all confirmed stud dogs with recent histories of siring successful litters. Ultrasound pregnancy diagnosis was performed at approximately 23 days (range, 21–24 days) after the date of ovulation in France and from 23 to 28 days after ovulation in the United States. When pregnancy was confirmed, the estimated number of normal embryos and their location in the uterus were recorded. Embryonic resorption (ER) was defined when dead embryos were observed by ultrasound but remnants were not expelled. Abortion was reserved to cases with expulsion from the genital tract dead or live fetuses, or, of fetoplacental remnants [19]. The dates of detection of ER or abortion were recorded.

Whenever possible, surgical uterine biopsies (SUBs) were performed during diestrus as close as possible to the date of negative ultrasound pregnancy diagnosis or, in cases of PL, as close as possible to the date of complete ER or abortion. The objective was to let the biopsy coincide with the leukocyte inhibition and suppressed activity of cellular immunity primed by progesterone in the uterus [20] and to maximize the chances of identification of any infectious agent that might be responsible for the infertility or the PL.

2.2. Classification of groups

Bitches were classified in two groups. The NCP group included bitches in which the early pregnancy diagnosis by ultrasound was negative (14 of 21), and bitches were classified in the PL group when complete ER (five of 21) or abortion (two of 21) was observed.

2.3. Ovarian cysts and hypoluteoidism

Progesterone (P4) in peripheral blood was assayed at the time of infertility diagnosis or PL to rule out luteal insufficiency.

When ovarian cysts were detected by ultrasound, a vaginal cytology examination was performed at the time of diagnosis to determine if there was estrogenic influence. A sample of peripheral blood was collected into a sterile glass tube without gel (BD Vacutainer Plus Plastic Serum Tube) for further determination of estradiolemia.