Theriogenology 80 (2013) 73–76

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Prevalence and effect of uterine luminal free fluid on pregnancy and litter size in bitches

S.L. Freeman, M.J. Green, G.C.W. England*

School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, UK

ARTICLE INFO

Article history:
Received 4 February 2013
Received in revised form 19 March 2013
Accepted 19 March 2013

Keywords:
Bitch
Ultrasonography
Endometrial hyperplasia
Fertility
Uterine fluid
Mating-induced endometritis

ABSTRACT

Free fluid within the uterine lumen has been identified ultrasonographically as a transient finding in normal bitches in the period immediately after mating, and delayed clearance of the fluid has been documented in bitches with endometrial hyperplasia. This study used B-mode ultrasonography to investigate the prevalence of free fluid in the uterine lumen in normal bitches (n = 53) and bitches with endometrial hyperplasia (n = 10) on Days −7, 0, +5, and +14 in relation to estimated ovulation. For normal bitches without endometrial hyperplasia, 47 (86.6%) bitches become pregnant, whereas for bitches with endometrial hyperplasia, 3 (30%) become pregnant. In both the groups, the presence of uterine fluid on Days −7 and 0 was not associated with the probability of a bitch becoming pregnant, whereas the presence of uterine fluid on either Day +5 or +14 was significantly associated with a reduced likelihood of pregnancy. In pregnant bitches, uterine luminal fluid present on Day 0 was associated with a smaller litter size. This is the first study to establish the prevalence and effect of uterine luminal free fluid in bitches. We postulate that as the uterine luminal free fluid may be detected in normal bitches before mating and has no effect on the pregnancy rate or litter size in either group, this fluid is "physiological." However, it seems that the later in estrus the uterine fluid is present, the more likely it is to have an adverse effect on fertility. In some cases, this is manifest as a reduced litter size, while, importantly, the presence of fluid 5 to 14 days after ovulation is strongly associated with an absence of pregnancy, both in bitches with and without endometrial hyperplasia. We propose that ultrasonographic detection of uterine luminal free fluid after mating may be a useful prognostic indicator for pregnancy outcome, which may allow the targeting of treatments specifically to a population of animals that presumably develop mating-induced endometritis.

1. Introduction

Ultrasonographic examination of the uterus is a common component of breeding management in several species. Using this technology, in, for example, mares, small volumes of "physiological" uterine luminal fluid may be detected during early estrus associated with endometrial edema [1], whereas accumulation of luminal fluid after mating is a useful diagnostic criterion for mating-induced endometritis [2–4].

Very little is known about the impact of endometritis on fertility in dogs [5–7]; however, it has been recently established that clearance of fluid from the uterine lumen may be impaired in bitches with ultrasonographically detected endometrial cysts presumed to represent endometrial hyperplasia [8]. Furthermore, it has been demonstrated that there are greater numbers of polymorphonuclear neutrophils (PMNs) in uterine lavage samples collected from bitches with endometrial hyperplasia compared with normal bitches, and that in these

* Corresponding author. Tel.: +44 (0)115 951 6411; fax: +44 (0)115 951 6412.
E-mail address: gary.england@nottingham.ac.uk (G.C.W. England).

0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.03.024

former bitches a substantially larger PMN influx occurs after semen deposition which appears to be associated with reduced fertility [9]. These findings indeed suggest that mating-induced endometritis may occur in bitches, specifically in bitches with endometrial hyperplasia. The interesting features of delayed uterine fluid clearance reported in bitches with presumed mating-induced endometritis appears to be similar to that observed in mares [10], but there are no reports of routine examination of bitches for the presence of uterine luminal fluid before or after mating. In clinical practice, small volumes of uterine luminal free fluid are commonly detected in estrous bitches that are otherwise ultrasonographically normal and become pregnant, and as previous work suggests that bitches with mating-induced endometritis have persistence of uterine luminal free fluid after mating [8], we hypothesize that detection of uterine luminal free fluid after mating may be a useful prognostic tool for absence of pregnancy. Therefore, the purpose of this prospective study was to determine the prevalence of uterine luminal free fluid at different stages of the estrous cycle in normal bitches and bitches with endometrial hyperplasia and to evaluate whether the presence of fluid at different stages of the estrous cycle was associated with a detrimental effect on pregnancy.

2. Materials and methods

2.1. Ethics

Ethical approval for the procedures was granted by the School of Veterinary Medicine and Science, University of Nottingham; all animals were examined with the owner's consent.

2.2. Study population and animal management

Bitches from an actively managed breeding program producing assistance dogs were allocated to normal or endometrial hyperplasia group based on a detailed transabdominal ultrasound examination of the uterine body and distal uterine horns. Ultrasound was performed using a 10-MHz transducer (Pie Veterinary Ltd.) conducted 152–195 days before the onset of proestrus, which is defined as the first observation of a serosanguinous vulval discharge. Normal bitches had a homogenous endometrium with no cystic structures, and only a single central echogenic line representing the luminal interface was obvious. Bitches with endometrial hyperplasia had two to seven thin-walled anechoic (fluid-filled) cystic structures that were 0.5–2.5 mm in diameter present within a homogenous endometrium where the luminal interface was obvious [8,9]. Plasma progesterone concentrations were measured three times per week commencing 5 days after the onset of proestrus using an ELISA (Ridgeway Science, Gloucestershire, UK). Bitches were mated naturally on Days +2 and +4 after the progesterone concentrations first exceeded 5.0 ng/mL (Day 0). All male dogs that were used for breeding had achieved a pregnancy in the previous 2 months. In addition, all male dogs had their semen collected and evaluated between 1 month before to 2 weeks after mating demonstrating >72% progressive motility and 76% morphologically normal live spermatozoa using the standard techniques [11].

2.3. Identification of uterine fluid on different days of the estrous cycle and relationship to pregnancy outcome in normal bitches and healthy bitches with endometrial hyperplasia

The uterine body and distal uterine horns of 53 normal bitches (33 Labrador Retrievers and 20 golden retrievers bitches aged 2–7 years) and 10 bitches with endometrial hyperplasia (six Labrador Retrievers and four golden retrievers aged 4–7 years) were examined using a 10-MHz sector transducer in both the longitudinal and transverse plane at approximately Day 5 after the onset of proestrus (retrospectively this day was found to be between 5 and 9 days before ovulation and for convenience was termed Day −7), and on Days 0, +5, +14, and +28. On each occasion, a careful examination of the central region of the uterine body and distal uterine horns was undertaken to identify the anechoic regions that could be attributed to fluid within the uterine lumen. Fluid was recorded as present or absent at each examination. The actual depths of the fluid were recorded only when >2 mm. On Day 28, uterine ultrasonography was performed to record the presence of uterine fluid, or to confirm pregnancy. The number of puppies born was confirmed by the owner.

2.4. Statistical evaluation

Data collation and exploration were conducted using Microsoft Excel (version 14.0.6129.5000, Microsoft Corp., 2010) and Minitab (version 16.2.2, Minitab Inc., 2010). To evaluate the association between presence of uterine fluid at different time points and a successful pregnancy, a conventional binary logistic regression model was used [12]. The binary outcome variable was successful pregnancy (1 = yes, 0 = no) and the explanatory variables considered were as follows: presence or absence of uterine fluid on Days −7, 0, +5, and +14 with respect to estimated ovulation; age of the bitch at mating; and presence of endometrial hyperplasia in the bitch. Explanatory variables were considered as being significant at a level of $P < 0.05$.

A secondary analysis was conducted on the subset of bitches that became pregnant to assess the factors that affected litter size. A linear model was used with litter size as a continuous outcome and the explanatory variables considered were as follows: age of bitch at mating; presence of endometrial hyperplasia (yes or no); and the presence or absence of uterine fluid on Days −7, 0, +5, and +14 with respect to estimated ovulation.

3. Results

The age of the bitches in the study ranged between 2 and 7 years. All of the bitches had an apparently normal estrous cycle, ovulated, and were mated, and all bitches identified as pregnant on Day +28 subsequently whelped. The mean age of the normal bitches and bitches with