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Normal and abnormal response to sperm deposition in female dogs: A review and new hypotheses for endometritis

G.C.W. England [a, *], T. Rijsselaere [b], A. Campbell [c], R. Moxon [a, c], S.L. Freeman [a]

[a] School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, UK
[b] Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[c] Guide Dogs, National Breeding Centre, Bishops Tachbrook, Warwickshire, UK

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ABSTRACT

In mammalian species there are significant physiological responses of the female reproductive tract to the deposition of sperm. These are particularly notable in species where sperm are deposited directly into the uterus, and function both to facilitate sperm transport to the sperm reservoir, and to eliminate introduced contaminants.

In the bitch, sperm are deposited into the vagina and are rapidly transported through the open cervix. Sperm are then distributed around the uterus by uterine contractions such that transportation to the tip of the uterine horns occurs within 1 min of the start of mating.

The main sperm reservoir appears to be the distal part of the utero-tubal junction which forms a pre-uterine tube reservoir. Sperm remain attached here by their heads to uterine epithelium and remain viable. In non-capacitating conditions sperm slowly detach from this site and this seems important to replenish the uterine tube reservoir, where sperm may re-attach to the epithelium. Post-ovulatory signals trigger capacitation changes and subsequent hyperactivated motility that is associated with detachment of sperm from both reservoirs; thus facilitating fertilization.

After mating, a physiological post-mating uterine inflammatory response occurs, evidenced by an influx of polymorphonuclear neutrophils, increased uterine contractions, an increased uterine artery blood flow and a decrease of the resistance index indicating a short-duration vasodilation.

Disturbance of this tightly regulated system has the potential to impact fertility by a failure of elimination of the introduced contaminants (such that a clinically-significant post-breeding endometritis ensues) but also by impairing sperm transport.

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1. Introduction

Inherent female reproductive tract factors are thought to be responsible for the 'passive transport' of sperm [1,2]. Species differences occur during the journey that sperm take following deposition in the female tract [3]. Vaginal deposition is often associated with contractions of the vagina which quickly propel the ejaculate to the cervix. In species, such as humans, where there is an initial harmful low vaginal pH and a significant cervical mucus barrier, the ejaculate coagulates close to the cervix and then liquefies later after vaginal pH has increased (and is no longer hostile), thus allowing sperm to swim actively and penetrate the cervical mucus. In several other species, such as the horse and pig, the cervix does not form a barrier to sperm transport as the ejaculate is deposited into the uterus [1,2,4].

Uterine contractions are involved in passive transport such that sperm are found within the uterine tube shortly after mating [5,6]. Uterine contractions may be influenced by a release of oxytocin at coitus [7–10] or the presence of prostaglandin or oestrogen within the ejaculate [11,12]; especially in species where there is uterine deposition of sperm. Additional physiological factors, such as presence of sperm in the uterus, can also contribute to the increase in uterine contractions noted at mating [13,14].

The rapid transport of sperm to the uterine tube, appears important because this site is often considered to form the functional sperm reservoir. Sperm that do not reach the reservoir are eliminated from the female tract (see later). Once within the uterine tube, sperm are rapidly distributed by active peristaltic contraction of the uterine tube itself [15]. This distribution allows

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* Corresponding author.
E-mail address: gary.england@nottingham.ac.uk (G.C.W. England).

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sperm to contact regions of the uterine tube epithelium in order to establish the uterine tube sperm reservoir, which is maintained by an intimate interaction of sperm heads with the epithelium. It has been known for some time that this transient epithelial contact is important for maintaining sperm viability [16] and fertilizing ability [17]. Whilst 'attached' by their heads, sperm maintain slow flagellar activity, however following an appropriate capacitation signal, sperm membrane destabilisation processes commence, fertilizing ability is achieved, and a proportion of the sperm become hyper-activated [17]. This change in motility may assist the sperm in 'detaching' from the epithelium, enabling the location of oocytes, following which binding to the zona pellucida, the acrosome reaction and oocyte penetration occur [16].

In many species there is a transient physiological uterine inflammatory response which occurs after sperm enter the uterus [18,19]. This is most prominent in those species in which sperm are deposited directly into the uterus and is characterised by increased uterine contractions, luminal influx of polymorphonuclear leucocytes (PMNs), increased uterine artery blood flow and uterine vasodilation [20]. The response is thought to remove excess and dead sperm, as well as bacterial, cellular and fluid contaminants. Importantly, mitigation against the effects of this inflammatory response is required to ensure that it does not negatively impact upon sperm and thus fertility. In horses, for example, seminal plasma from the stallion reduces sperm binding to PMNs and improves fertility [21].

Disturbance of the female response to the deposition of sperm may have an impact upon fertility. This may occur because (1) sperm transport is impeded, for example, purported abnormal cervical mucus quality preventing sperm entering the uterine lumen and leading to a failure of fertilization, or (2) because elimination of contaminants is not fully effective, leading to the development of a post-breeding clinical endometritis.

In veterinary clinical practice perhaps the most common abnormality is noted where ejaculation occurs directly into the uterus, for example in the mare, sow and camel. In these species, significant numbers of sperm, cellular material and bacteria directly enter the uterus when compared with species where there is a functional cervical barrier (such as primates and ruminants). Whilst uterine deposition of sperm results in rapid transportation to the utero-tubal junction, failure of elimination of the introduced cellular contaminants and, in particular, any micro-organisms may result in a clinical endometritis [22]. This differs from the previously described 'physiological' endometritis described above. Persistence of such a post-breeding (also called mating-induced) clinical endometritis [23] may create a hostile uterine environment that prevents survival of the developing embryos when they, at a later time, enter the uterus.

It is not surprising that the species in which uterine deposition of semen occurs are those in which post-breeding endometritis is a common cause of infertility. It has been suspected for some time that in the female dog there is vaginal deposition of semen but rapid transport through an open cervix [24], and knowledge of sperm transport has improved over recent years. Alongside this information is a growing body of evidence which suggests that perturbation of the normal physiological response of the bitch may occur (particularly in bitches with endometrial hyperplasia) which may impact the elimination of uterine contaminants and also potentially be detrimental to sperm transport, both of which would result in impaired fertility.

The aim of this review is to describe normal sperm transport in the female dog, to discuss the consequences when this is disturbed, and to propose new hypotheses for the development of endometritis.

1.1. Normal response to sperm deposition in the female dog

The female dog has an interesting biology compared with many other species. Unlike many mammals, dog ova are released at ovulation as immature primary oocytes [25]. Between one to three days after ovulation they have reached metaphase of the second meiotic division (when the first polar body is extruded), and they are considered fertilizable from this point onwards. Interestingly, oocytes may remain fertilizable for up to five or more days after ovulation [26]. The time over which mating may result in fertilization is further increased as sperm can survive for up to 11 days within the female tract [27], such that mating many days before ovulation may result in a pregnancy. The phenomena of prolonged survival of sperm within the female tract clearly points to the presence of a sperm reservoir.

1.2. Transport of sperm to the sperm reservoir

The ejaculate of the dog has three fractions of which the first and third originate from the prostate [28], and the second fraction is the epididymal sperm-rich portion. The sperm-rich fraction of the ejaculate is deposited into the cranial vagina and is flushed through the cervix by the large volume of the prostatic fluid during the protracted copulatory tie. Interestingly, an early study showed that when only a small volume of radiographic contrast medium was introduced into the cranial vagina this was rapidly transported into the uterine lumen without the requirement for flushing with prostatic fluid [29], perhaps indicating that the flushing action of the prostatic fluid is not essential. However, vaginal contractions are present during normal coitus, and can be stimulated in the estrous bitch by digital palpation/dilation of the caudal vagina. These contractions might function similar to those noted in other species, which force the ejaculate to, or through, the cervix. Contractions of the abdominal musculature occur in some bitches and may also contribute to passive sperm transport [24], although this contention is not proven.

It is clear that the bitch cervix opens approximately four days before ovulation allowing the passage of fluid, largely unobstructed, into the uterus [30]. The cervix closes approximately five days after ovulation once progesterone concentrations have increased [30–32]. Overall, during the fertilization period it would appear that the cervix of the bitch does not form a barrier to the passage of sperm. Indeed, Evans [24] found sperm at the fistulated tips of the uterine horns within 1 min of mating. That author also observed that during mating active abdominal contractions resulted in semen being forced in small jets up to 25 cm from the fistulae.

Basal uterine contractions have been identified in the bitch [33], and we were able (with some difficulty) to identify uterine contractions using M-Mode ultrasound [4]. Two specific types of contractions were evident; (1) spontaneous basal contractions which increased in frequency from approximately five days before until five days after ovulation, and (2) specific mating-induced contractions which had the greatest frequency from the day of ovulation until four days later. The variation in contraction frequency appeared to have some relationship with plasma hormone concentration; most noticeably an inhibitory effect of progesterone that coincided with the end of the fertile period [4] (Fig. 1). The increased number of contractions caused by mating could not be mimicked by teasing with a male, nor by tactile stimulation of the vagina or cervix during vaginal or trans-cervical insemination, however distension of the vestibule and manual stimulation of the dorsal wall of the vagina produced contractions that were similar to those noted during mating [34]. It is likely that these contractions are responsible for the rapid distribution of sperm throughout the

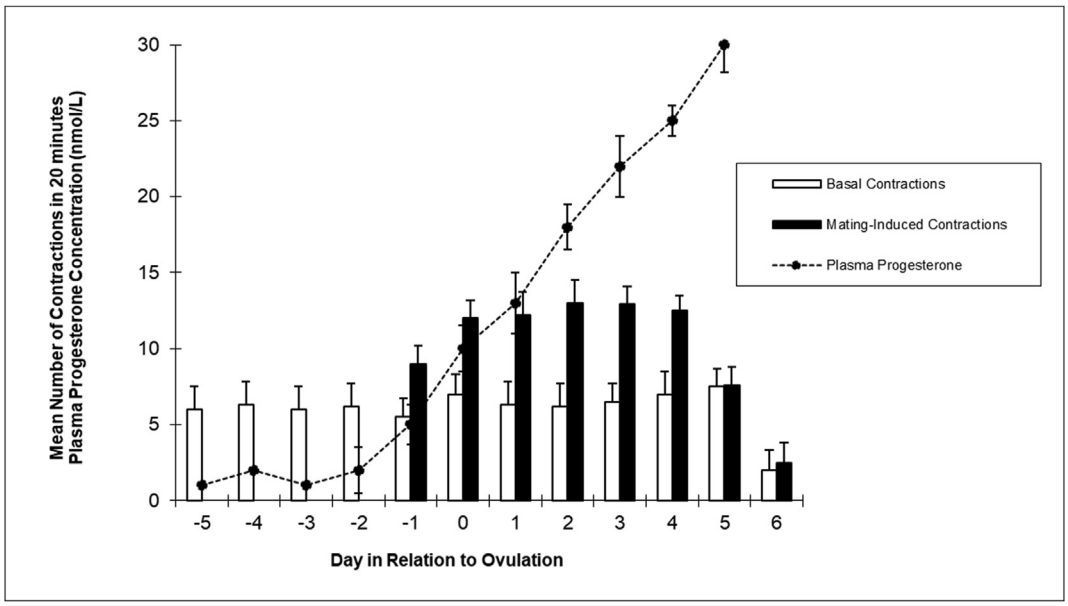

Fig. 1. Mean (+SEM) number of spontaneous and mating-induced uterine contractions per 20 min, and mean plasma progesterone concentrations for five bitches in relation to the estimated day of ovulation. Adapted from England et al. [4].

uterus including to the tip of the uterine horn [35]. They are probably also important for elimination of material from the uterus (see later), although the direction of contractions in the bitch has not yet been established.

In dogs, sperm transport and distribution was shown to be influenced by stage of the cycle at the time of sperm deposition. Greater percentages of uterine glands containing larger numbers of sperm were found one day after ovulation compared with before ovulation or at a time later after ovulation [36,37]. Similarly, higher numbers of sperm were found within uterine crypts when sperm deposition occurred during the ovulatory period [38]. The effect of stage of the cycle on sperm distribution may relate to hormone influences on uterine and/or uterine tube contractions as previously mentioned, or possibly on the degree of sperm phagocytosis, or chemotactic and chemokinetic activities exerted by ovulatory secretions [39].

1.3. Sperm storage

There is no information to suggest that storage of sperm occurs within the cervix of the bitch. It is clear, however, that storage of low numbers of sperm occurs throughout the uterus within the uterine glands [27]. In our early work we focussed on the potential of the uterine tube as a possible sperm store and identified that shortly after mating very low numbers of sperm were found within the uterine tube. Some of these sperm were free within the lumen whilst others were attached to uterine tube epithelium and had continual flagellar activity [40]. At this time, we presumed that this was the site of the sperm reservoir in the bitch. Given that only small numbers of sperm enter the uterine tube, it follows that the uterine tube sperm reservoir will ultimately be depleted especially when there is a long interval between mating and ovulation, as can occur in the bitch. Pregnancy data from dogs shows that mating many days before ovulation can be fertile with normal litter size [41], and we were therefore compelled to hypothesise that there must be replenishment of the uterine tube reservoir.

Examination of sperm distribution within the bitch reproductive tract at 6, 12, 24 and 48 h after mating (where mating occurred one day after ovulation) [42] showed that at 6 h sperm were widely distributed in the lumen of the reproductive tract as far as the distal utero-tubal junction and were attached to the epithelium of uterine crypts. At later time points (12 and 24 h after mating) the greatest number of attached and free luminal sperm were consistently present in the proximal uterine horns and the distal utero-tubal junction (Figs. 2 and 3). At 48 h after mating the highest number of attached sperm were found in the distal utero-tubal junction [42]. This site has a large surface area which facilitates sperm attachment to epithelium. Interestingly, we also showed consistently higher numbers of uterine crypts and glands with spermatozoa in the more proximal regions of the uterus and at the utero-tubal junction [42]. Rijsselaere [36,37] however found no significant differences in the percentage of uterine crypts with sperm between the proximal, middle and caudal uterine horn and the uterine body one day after artificial insemination. From days two to

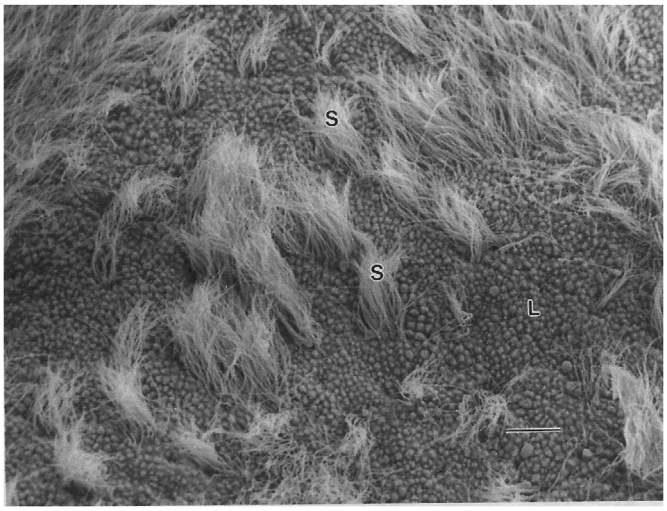

Fig. 2. Scanning electron micrograph of the proximal uterine horn of a bitch 12 h after mating. The luminal (L) epithelium of the uterine horn contains no sperm in some areas, whilst in other areas sperm (S) are attached in clumps with their tails protruding into the uterine lumen.

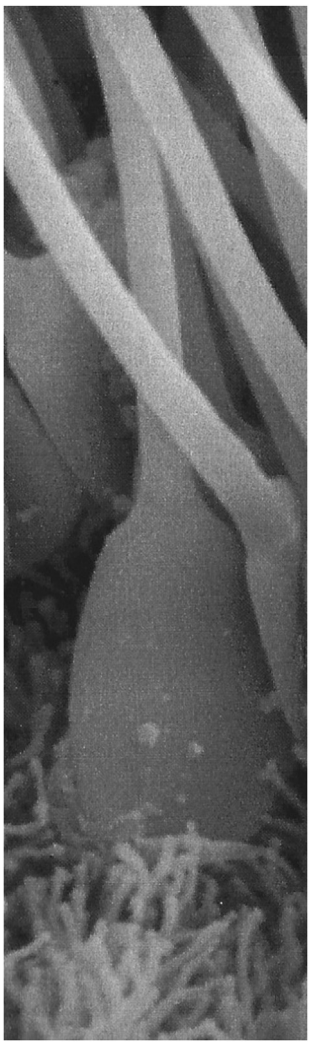

Fig. 3. Scanning electron micrograph of the proximal uterine horn of a bitch 12 h after mating. Sperm can be seen interacting with the microvilli of the uterine epithelial cells.

four after ovulation, a significant decrease was observed in the percentage of glands containing sperm, and was combined with a marked reduction in the mean sperm number present within the glands of the uterine horn and a decrease in the number of sperm present at the utero-tubal junction [43].

Subsequently, Freeman and England recognised [44] that in non-capacitating conditions (i.e. for matings that occur before ovulation) dog sperm attach to uterine epithelium at the distal part of the utero-tubal junction and maintain flagellar activity. In non-capacitating conditions there was a low level of continual sperm detachment and they hypothesized that the distal part of the utero-tubal junction provided a reservoir for the slow release of sperm to be available for movement within the reproductive tract, presumably to replace the depleting uterine tube reservoir. They also showed that the process of attachment to uterine epithelium slowed capacitation (and interestingly capacitated spermatozoa had reduced ability to attach to uterine epithelium). When conditions changed to being capacitating, increased numbers of sperm were found to detach and exhibit transitional and hyperactive motility. These detachment and motility changes could be induced by post-ovulation but not pre-ovulation uterine tube flush fluid and by components of follicular fluid and solubilised zona pellucida. It has been previously documented that addition of estrous plasma to dog sperm can cause initiation of hyperactive motility [45]. It seems plausible that sperm attached to either uterine or uterine tube epithelium respond to this post-ovulatory signal in a manner that allows large numbers to detach for the purpose of fertilization.

The concept that a post-ovulatory signal may influence transport and storage of sperm within the reproductive tract of the bitch has been previously noted, since Rijsselaere [36,37] found different outcomes dependent upon whether sperm were deposited before or after ovulation (as mentioned above). Importantly, we are suggesting here that in the absence of specific post-ovulatory signalling, the distal part of the utero-tubal junction provides a reservoir for the slow release of spermatozoa that are available for movement within the reproductive tract, to replace the depleting uterine tube reservoir [44]. This concept is supported by previous observations of the attachment and distribution of spermatozoa within the bitch's reproductive tract [42,43], and earlier work where disappearance of spermatozoa from uterine glands prior to the loss of spermatozoa from the uterine lumen also suggested that over time glandular spermatozoa supplement the luminal population [27].

1.4. Dealing with introduced contaminants

At the time of, and immediately after mating, it is possible to identify fluid within the uterine body and the uterine horns using real-time diagnostic ultrasound [46] (Fig. 4). The depth of the hypoechoic/anechoic fluid visible within the uterus was found to be between three and six mm. In the normal bitch the majority of fluid disappeared within 1 h, but took up to 24 h for all of the fluid to disappear. Presumably uterine contractions that have been previously mentioned are responsible for the expulsion of the ejaculatory fluid, cellular debris and dead and unwanted sperm via the open cervix [20,34]. Indeed, the frequency of uterine contractions was related to the rate of fluid clearance from the bitch's uterus after mating [34,46].

It is clear that in the bitch both mating and artificial insemination result in an influx of PMNs into the uterine lumen [20]. It would seem likely that this is part of the 'physiological' inflammatory response observed in other species and discussed earlier. The role of PMNs is likely to be phagocytosis of the introduced

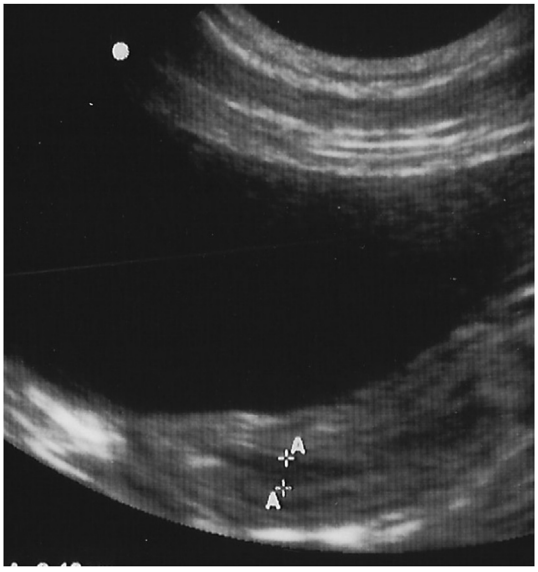

Fig. 4. Ultrasound image of a bitch in late estrus showing the uterine body positioned adjacent to the bladder. The lumen of the uterus contains anechoic fluid (markers).

contaminants [20]. Although the inflammatory response is important for removing uterine contaminants, PMNs also reduce the ability of sperm to attach to uterine epithelium [20] and therefore could reduce the sperm reservoir and have a deleterious impact upon fertility. Fortunately, the impact of PMNs in reducing sperm-epithelial attachment is ameliorated by both dog seminal plasma (SP) and prostatic fluid (PF), both of which appear to reduce the attachment of PMNs to sperm [20]. It seems likely that this is part of the mechanism of regulating the impact of the physiological inflammatory response. Interestingly, PF also appears to have a role in reducing uterine contractions, presumably to reduce the speed of elimination of sperm from the female tract, and, at least in bitches that are artificially inseminated (with fresh and frozen-thawed semen), PF significantly increases pregnancy rate and litter size [34]. PF clearly has a pivotal role in regulating the effect of PMNs on sperm-epithelial attachment, as well as regulating uterine contractions and ultimately influencing fertility.

Entrance of sperm into the bitch's uterus also results in a transient increase in uterine artery blood velocity and a reduction in the resistance index; changes which indicate a short duration vasodilation [20]. Interestingly, SP and PF produced a similar magnitude of effect. We hypothesized that vasodilation following breeding was largely induced by SP and PF which, together with PMN influx, was part of a normal uterine response to semen deposition.

1.5. Abnormal response to sperm deposition in the female dog

As mentioned previously in other species there are two methods by which fertility may be impacted when the female response to deposition of sperm is disturbed. This can be either by the impeding of sperm transport, or because elimination of contaminants is not fully effective. The latter is most commonly seen in species where sperm are deposited into the uterus, and the condition is most well studied in the mare where a significant uterine inflammatory response occurs when there is physical obstruction to the elimination of uterine fluid. In the mare, the result of this substantial post-breeding clinical endometritis is a hostile uterine environment which may impair fertility should the endometritis persist until the fertilized embryos enter the uterus [47].

Endometrial disease has been recognised in the bitch for many years. In most studies the condition investigated is endometrial hyperplasia often called cystic endometrial hyperplasia (CEH) or pseudo-placentational endometrial hyperplasia (PEH) [48]. A degree of endometrial development occurs normally during the luteal phase, where progesterone is thought to cause changes in the uterus in preparation for pregnancy. At the end of the luteal phase these changes regress and the uterus returns to a more normal state, although as bitches get older the regression does not appear complete, and age-related changes can be observed [49]. In CEH cases, the number and size of endometrial glands are increased, and there is disparity in the number and configuration of glands causing a thickened endometrium and increased secretory activity [50]. The condition may pre-dispose bitches at later cycles to develop a pyometra.

Cases of CEH commonly have the presence of endometrial glandular cysts, which can become large and may then be detected using trans-abdominal diagnostic B-mode ultrasound [51] (Fig. 5). Glands may be variably dilated and therefore have a different diameter. CEH may affect particular segments of the uterus, or sometimes a large proportion of the endometrial surface [48].

1.6. Abnormal female response: post-breeding endometritis

We have focussed some of our attention on healthy breeding bitches that have ultrasound-determined cystic endometrial hyperplasia [46,52] since it is our hypothesis that in these bitches there is some failure/delay/obstruction to the elimination of material introduced at the time of mating (although it will also be seen later that it is likely that post-breeding endometritis may also occur in bitches without CEH).

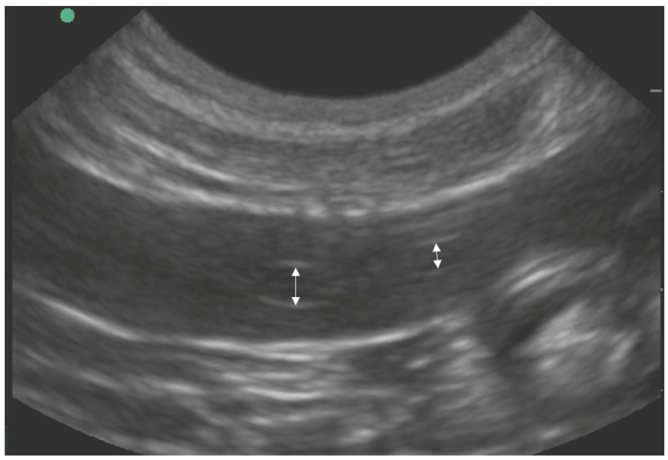

Fig. 5. Ultrasound image of the mid-uterine body of a bitch in the luteal phase, demonstrating 2 almost spherical endometrial cysts (arrows). In both there are relatively prominent dorsal and ventral specular echoes. The cyst lumen is anechoic.

Endometritis as a clinical disease has been rather overlooked in bitches, until relatively recent studies identified endometritis in a significant proportion of infertile bitches [53–56].

In our laboratory, we identified an age-related incidence of CEH in breeding bitches with approximately 7% of two-year old and 60% of six-year old bitches affected [57]. Importantly, bitches with CEH appeared to have lower fertility; those with CEH had significantly lower pregnancy rates (55.7 ± 36.4%) and litter sizes (6.4 ± 2.5 puppies) compared with normal bitches (90.9 ± 0.6%, 7.7 ± 2.5 puppies) [46,52].

We showed that in these bitches there was a delay in the clearance of uterine fluid after mating or insemination compared with normal bitches [46], and we described the development of a post-breeding endometritis. Importantly, Mir et al. [54] found that of 14 bitches that were recently mated but did not get pregnant, four (28%) had endometritis diagnosed histologically. We propose that the development of endometritis is due in part to delayed fluid clearance from the uterus, related to a physical obstruction of fluid movement because of the presence of luminal cysts, as well as reduced uterine contractions. These findings are consistent with observations from Dow [50] that acute endometritis was most common in the early part of the luteal phase.

We also found in these otherwise healthy bitches with CEH that, although there was a clear mating-induced increase in the number of uterine contractions, there were fewer uterine contractions than in normal bitches. The reason for this difference might relate to histological changes present within the uterus. Doppler ultrasonography showed that in normal bitches there was a significant increase in uterine artery blood velocity and a decrease in the resistance index after mating, indicating vasodilation. In bitches with endometrial hyperplasia the baseline resistance index was significantly higher than normal bitches, and furthermore, although there was a significant decrease in resistance index after mating, in the bitches with endometrial hyperplasia this was of a smaller magnitude than in normal bitches. These findings indicate lower baseline uterine perfusion, and a blunted vasodilation response to mating in bitches with CEH. These features may all contribute to the development of the post-breeding endometritis.

In a later study uterine artery waveforms were examined during estrus in old and young bitches without CEH [58], and it was discovered that bitches which failed to become pregnant were more likely to have (1) fewer waveforms with continuous diastolic flow, (2) lower end diastolic velocity, and (3) a higher resistance index. It is plausible that these factors during estrus could have affected fertility by interfering with normal changes in perfusion that occur at the time of mating.

Our studies also demonstrated a greater PMN influx post-insemination in bitches with CEH compared with normal bitches [52]. We postulate that reduced clearance of uterine fluid allows the establishment of a post-breeding endometritis and larger influx of PMNs as has been described in other species. Furthermore, the uterine fluid that accumulated in bitches with CEH (which contains greater number of PMNs than normal bitches) was shown to reduce sperm attachment to normal uterine epithelium, mediated principally but not solely by the presence of PMNs [59]. Interestingly, in that early study [52] we found that bitches with CEH appeared to have reduced fertility, and that this could be partially alleviated by the post-insemination administration of systemic antibiotic; an effect that was later confirmed [46]. This observation potentially provides some evidence that the condition was an endometritis associated with bacterial persistence within the uterus.

The findings were interesting since in bitches with CEH that were treated with antibiotic post-breeding, more became pregnant but litter size remained lower than bitches without CEH. This suggested that perhaps in some cases the uterine environment had not fully returned to a situation able to maintain the viability of all embryos. The authors do not consider routine treatment with antibiotic appropriate or good stewardship and have only used such regimes in dogs with evidence of CEH. More recently, in unpublished work, we studied the outcome of breeding in more than 50 healthy bitches with ultrasound-detected CEH. These cases were aged between 2.5 and 6.5 years at first diagnosis. All bitches were treated with oral amoxicillin/clavulanic acid administered twice daily for four days starting on the day of the last breeding. Pregnancy rate was 94% with a mean litter size of 8.1 ± 0.3 puppies (unpublished observations), similar to expected breed averages. This works confirms that fertility in bitches with CEH can be maintained by early intervention with antibiotics, presumably preventing or helping with the early resolution of post-breeding endometritis.

Overall, we conclude that bitches with CEH appear to have delayed clearance of fluid from the uterus after breeding, mediated by a physical obstruction to fluid flow and reduced uterine contractions. Furthermore, there are changes in uterine artery waveform and a blunted vasodilatory response to mating. These bitches also have a greater PMN influx following mating and lower fertility. We propose that these features, support the contention that bitches with CEH are susceptible to post-breeding (mating-induced) endometritis. Interestingly, the impaired pregnancy rate may be mediated by a hostile uterine environment that affects the embryos when they enter the uterus, since we showed that short-duration post-mating administration of systemic antibiotic increased pregnancy rates. This phenomenon is similar to that seen in the mare [60]. We suggest that the mechanism behind this 'rescue' of fertility by post-mating antibiotic is that, in the absence of uterine inflammation at the time of breeding, sperm are harboured 'safely' within the distal utero-tubal junction and/or the uterine tube, and that the endometritis develops subsequent to sperm deposition and largely involves the uterus. Sperm therefore remain available for fertilization, and as long as the endometritis is resolved prior to entrance of the fertilized embryos into the uterus the pregnancy will be maintained.

Work in the mare has also demonstrated that post-breeding administration of low doses of uterine ecbolic agents such as oxytocin can enhance clearance of fluid from the uterus and improve pregnancy rate [61]. In preliminary work we have limited evidence that post-breeding oxytocin may help clearance of fluid in bitches with CEH (Fig. 6), but further work is required to demonstrate any impact upon pregnancy rate.

Whilst much work has been undertaken to investigate the pathogenesis of post-breeding endometritis [46,52,59], both acute and chronic endometritis may occur in bitches that have not been mated and do not have CEH. Crucially, in different populations of apparently unmated dogs, histological endometritis was identified during the luteal phase in 54–63% of bitches [56,62]. The cause of endometritis in these cases is uncertain, but interestingly, the early work of Dow [50] found that acute endometritis was most common in the early luteal phase. It is feasible that bacterial contamination of the uterus occurs through the open cervix in the absence of mating. Dow [50] also showed that chronic endometritis was more common in the late luteal phase, and that would also fit this hypothesis.

1.7. Abnormal female response: impaired sperm transport

This review has so far considered the role of an abnormal female response in relationship to impaired elimination of introduced contaminants; effectively the establishment of an endometritis after breeding. It is also plausible that impaired sperm transport occurs in bitches that have endometritis prior to breeding. In such a case the uterine environment would be hostile at the time of sperm deposition, and sperm transport and storage would therefore be impaired and fertility reduced. Such a chronic endometritis may or may not be associated with CEH.

Bitches with CEH do have a reduced ability of spermatozoa to attach to the (abnormal) uterine epithelium *in vitro* compared to normal bitches [52]. It is probable that this effect may affect the capacity of the pre-uterine tube sperm reservoir and may lead to an impact upon fertility. Interestingly, in that study [52] it was found that attachment to uterine tube epithelium of bitches with CEH was unaffected so presumably the uterine tube reservoir would function as normal.

In the mare it has been established that endometritis may persist from one cycle to the next, such that when estrus begins there is exhisting hostile uterine luminal fluid [47]. In such cases uterine fluid needs to be removed and the endometritis must to be resolved prior to breeding otherwise in the face of a hostile uterine environment sperm do not survive and fertilization does not occur; post-breeding antibiotic does not allow establishment of a pregnancy.

It is clear in the bitch that endometritis may persist after estrus into the luteal phase and may be associated with infertility [53]. Persistence of endometritis through anestrus to the next estrus has not been described however a recent detailed case report documented fluid accumulation in this type of case [63]. These authors showed that pre-breeding treatment with systemic antibiotics, oxytocin and uterine lavage, was successful in removing the fluid, and that repeating these treatments post-breeding allowed establishment of pregnancy. These treatments are similar to those utilised in mares [60]. Other treatments such as NSAIDs have yet to be evaluated. We have also detected uterine luminal fluid using ultrasound examination of bitches in early estrus [64]; some of this was thought to be physiological and associated with endometrial edema. However, presence of fluid on the day of ovulation (which was prior to breeding) was associated with a lower litter size; presumably mediated by interference of sperm transport to the sperm reservoirs.

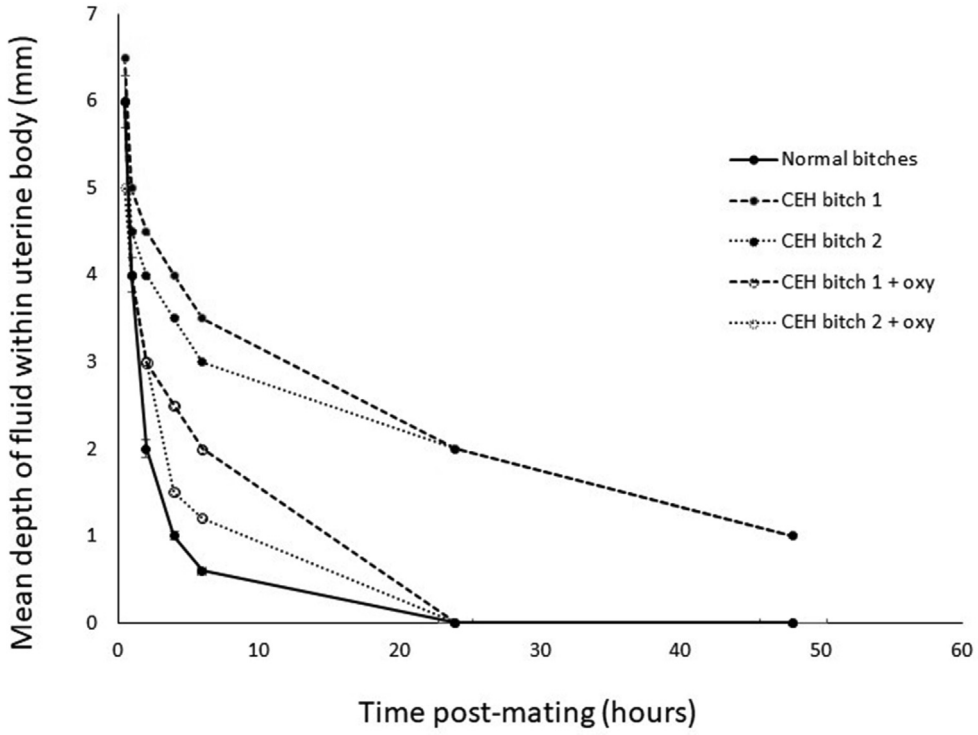

Fig. 6. Depth of uterine fluid identified within the body of the uterus after mating for two bitches with CEH that were mated on two consecutive days where 0.08 IU/kg oxytocin (oxy) was administered immediately after the second mating. Mean depth of fluid for six normal bitches is shown for comparison; data for the normal bitches is adapted from England et al. [46].

2. Conclusion

There is increasing awareness of endometritis and its association with infertility in bitches [53–56]. Importantly, however, there is no currently accepted understanding of the aetiopathogenesis of the condition. This is understandable since, although robust, previous work has (1) used different criteria for diagnosing endometritis, (2) examined bitches at different stages of the estrus cycle, (3) included bitches with an uncertain breeding history, (4) frequently reported no data of whether mating occurred at the most recent estrus, and (5) included studies conducted for different purposes including investigation of infertility [55] or surveillance [62]. It is noteworthy that in a recent substantial investigation of subfertile bitches [55] endometritis was identified histologically in 42% of cases, of which 48% were acute or subacute and 52% were chronic. Approximately 70% of these cases were diagnosed in the luteal phase, and it is certainly probable that some of these cases originated as a post-breeding endometritis, as discussed earlier in this review. Crucially though, in Gifford's study [55] and other work [56,62] it is apparent that endometritis in the luteal phase is not always the result of mating at the preceding estrus (as not all dogs in their studies were mated). It seems most likely then that endometritis may arise as a result of bacterial contamination of the uterus in the absence of breeding, possibly as a result of bacterial contamination of the uterus through the open cervix. A further possibility is that not all cases are initiated solely by bacterial contamination [54,65]. Additional work is warranted to elucidate any other instigating factors for endometritis that is not caused by mating. It is clear from the work of Gifford et al. [55] that underlying factors are not always CEH, since they found that only 44% of dogs with CEH had a concurrent histological diagnosis of endometritis (although it seems likely from their data that many of these dogs were not mated at the preceding estrus and it is plausible more cases might have occurred if they had been recently mated).

We have much to learn about the mechanisms of sperm transport in the female dog. It is apparent though that disturbance of this tightly regulated system has the potential to impact fertility, most likely by a failure of elimination of the introduced contaminants (such that a clinically-significant post-breeding endometritis ensues) but also by the impairment of sperm transport.

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References

[1] Hunter RHF. Ovarian endocrine control of sperm progression in the fallopian tubes. Oxf Rev Reprod Biol 1995;17:85–124.
[2] England GCW, Pacey AA. Transportation and interaction of dog spermatozoa within the reproductive tract of the bitch3. Centre for Reprod Biol; 1998. p. 57–84.
[3] Rickard JP, Pool KR, Druart X, de Graaf SP. The fate of spermatozoa in the female reproductive tract: a comparative review. Theriogenology 2019;137:104–12.
[4] England GCW, Burgess CM, Freeman SL, Smith SC, Pacey AA. Relationship between the fertile periods and sperm transport in the bitch. Theriogenology 2006;66:1410–8.
[5] VanDemark NL, Heys RL. Rapid sperm transport in the cow. Fertil Steril 1954;5:131–7.
[6] Kunz G, Beil D, Deininger H, Wildt D, Leyendecker G. The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. Hum Reprod 1996;11:627–32.
[7] Rowson LEA. The movement of radio-opaque material in the bovine uterine tract. Brit Vet J 1955;111:334–9.
[8] VanDemark NL, Heys RL. Sperm transport in the perfused genital tract of the cow. Am J Physiol 1955;165:674–9.
[9] Garcia-Villar R, Schams D, Alvinerie M, Laurentie MP, Toutain PL. Activity of the genital tract and plasma levels of oxytocin and cortisol at the time of mating in the Ewe. J Endocrinol 1985;105:323–9.

[10] Carmichael MS, Warburton VL, Dixen J, Davidson JM. Relationships among cardiovascular, muscular, and oxytocin responses during human sexual activity. Arch Sex Behav 1994;23:59–79.
[11] Bygdeman M. Effects of prostaglandins on the genital tract. Acta Vet Scand Suppl 1981;77:47–54.
[12] Claus R, Meyer HD, Gimenez T, Hoang-Vu C, Munster E. Effect of seminal oestrogens on prostaglandin F2alpha release from the uterus of the sow. Anim Reprod Sci 1990;23:145–56.
[13] Campbell MLH, England GCW. Effect of teasing, mechanical stimulation and the intrauterine infusion of saline on uterine contractions in mares. Vet Rec 2004;155:103–10.
[14] Campbell MLH, England GCW. Effect coitus and the artificial insemination of different volume of fresh semen on uterine contractions in the mare. Vet Rec 2006;159:843–9.
[15] Hino T, Yanagimachi R. Active peristaltic movement and fluid production on the mouse oviduct: their role in fluid and sperm transport and fertilization. Biol Reprod 2019;101:40–9.
[16] Smith TT, Yanagimachi R. Attachment and release of spermatozoa from the caudal isthmus of the hamster oviduct. J Reprod Fertil 1991;91:567–73.
[17] Suarez SS. Formation of a reservoir of sperm in the oviduct. Reprod Domest Anim 2002;37:140–3.
[18] Cohen J. Immunological aspects of sperm collection and transport. In: Crighton DB, editor. Immunological aspects of reproduction in mammals. Kent: Butterworths; 1984. p. 77–89.
[19] Troedsson MTH. Uterine clearance and resistance to persistent endometritis in the mare. Theriogenology 1999;52:461–71.
[20] England GCW, Russo M, Freeman SL. The bitch uterine response to semen deposition and its modification by male accessory gland secretions. Vet J 2013;195:179–84.
[21] Alghamdi AS, Foster DN, Troedsson MTH. Equine seminal plasma reduces sperm binding to polymorphonuclear neutrophil (PMNs) and improves the fertility of fresh semen inseminated into inflamed uteri. Reprod127 2004: 593–600.
[22] Brook D. Cytological and bacteriological examination of the mare's endometrium. Eq Vet Sci 1985;5:16–22.
[23] Hughes JP, Loy RG. Investigations on the effect of intrauterine inoculation of Streptococcus zooepidemicus in the mare. Proc Am Assoc Eq Pract 1969;15: 289–92.
[24] Evans EI. The transport of spermatozoa in the dog. Am J Physiol 1933;105: 287–93.
[25] Holst PA, Phemister RD. The prenatal development of the dog: preimplantation events. Biol Reprod 1971;5:194–206.
[26] Concannon PW, McCann JP, Temple M. Biology and endocrinology of ovulation, pregnancy and parturition in the dog. J Reprod Fertil Suppl 1989;39: 3–25.
[27] Doak RL, Hall A, Dale HE. Longevity of spermatozoa in the reproductive tract of the bitch. J Reprod Fertil 1967;13:51–8.
[28] England GCW, Allen WE, Middleton DJ. An investigation into the origin of the first fraction of the canine ejaculate. Res Vet Sci 1990;49:66–70.
[29] Linde C. Transport of radiopaque fluid into the uterus after vaginal deposition in the oestrous bitch. Acta Vet Scand 1978;19:463–5.
[30] Silva LDM, Onclin K, Verstegen JP. Cervical opening in relation to progesterone and oestradiol during heat in beagle bitches. J Reprod Fertil 1995;104:85–90.
[31] Allen WE, France C. A contrast radiographic study of the vagina and uterus of the normal bitch. J Small Anim Pract 1985;26:153–66.
[32] Tsutsui T, Kawakami E, Murao I, Ogossa A. Transport of spermatozoa in the reproductive tract of the bitch: observations through uterine fistula. Jpn J Vet Sci 1989;3:560–5.
[33] Wheaton LG, Pijanowski GJ, Weston PG, Burke TJ. Uterine motility during the estrous cycle; studies in healthy bitches. Am J Vet Res 1988;49:82–6.
[34] England GCW, Moxon R, Freeman SL. Stimulation of mating-induced uterine contractions in the bitch and their modification and enhancement of fertility by prostatic fluid. Reprod Domest Anim 2012;6:1–5.
[35] Fukushima FB, Malm C, Henry M, Gheller VA, Serakidis R, Neves MM, Macedo SP, Figueiredo MS, Andrade MEJ, Chaves MS, Silva MX, Rezende CMF, Melo EG. Site of intra-uterine artificial insemination in the bitch does not affect sperm distribution within the uterus. Reprod Domest Anim 2010;45: 1059–64.
[36] Rijsselaere T, Van Soom A, Van Cruchten S, Coryn M, Görtz K, Maes D, de Kruif A. Sperm distribution in the genital tract of the bitch following artificial insemination in relation to the time of ovulation. Reproduction 2004;128: 801–11.
[37] Rijsselaere T. New techniques for canine semen assessment and characterization of the sperm reservoir in the bitch. PhD thesis. Faculty of Veterinary Medicine, Ghent University; 2004. p. 189–212.
[38] Rijsselaere T, Van Soom A, Van Cruchten S, Coryn M, Görtz K, Maes D, de Kruif A. Effect of the timing of artificial insemination on the number of spermatozoa discovered in the uterine crypts of the bitch. Reprod Fertil Dev 2005;17:152 [abstract)].
[39] Rijsselaere T, England GCW, Freeman SL, Maes D, Van Soom A. Current knowledge on the transport and fate of spermatozoa in the reproductive tract of the bitch. Reprod Domest Anim 2014;49:2–7.
[40] Pacey AA, Freeman SL, England GCW. Contact of dog spermatozoa with homologous uterine tube epithelium prolongs flagellar activity in relation to the stage of the estrus cycle. Theriogenology 2000;54:109–18.
[41] England GCW, Allen WE, Blythe SA. Variability of the time of calculated luteinising hormone release in 218 canine pregnancies. Vet Rec 1989;125: 624–5.
[42] England GCW, Burgess CM, Clutterbuck AL, Freeman SL. Epithelial surface changes and spermatozoa storage in the reproductive tract of the bitch. Vet J 2013;195:185–91.
[43] Karre I, Meyer-Lindenberg A, Urhausen C, Beineke A, Meinecke B, Piechotta M, Beyerbach M, Günzel-Apel AR. Distribution and viability of spermatozoa in the canine female genital tract during post-ovulatory oocyte maturation. Acta Vet Scand 2012;54. 49.
[44] Freeman SL, England GCW. Storage and release of spermatozoa from the preuterine tube reservoir. PLoS One 2013;8(2):e57006. https://doi.org/10.1371/journal.pone.0057006.
[45] Iguer-ouada M. Oestrus cycle stage dependent effects of plasma and vaginal fluid on dog semen motility parameters. Medically assisted procreation in canine species: analysis and 4°C preservation of semen, PhD-thesis, Faculty of Veterinary Medicine. Liège University; 2000pp187–202.
[46] England GCW, Moxon R, Freeman SL. Delayed uterine fluid clearance and reduced uterine perfusion in bitches with endometrial hyperplasia and clinical management with post-mating antibiotic. Theriogenology 2012;78: 1611–7.
[47] Hurtgen JP. Pathogenesis and treatment of endometritis in the mare: a review. Theriogenology 2006;66:560–6.
[48] Schlafer DH, Gifford AT. Cystic endometrial hyperplasia, pseudoplacentational endometrial hyperplasia, and other cystic conditions of the canine and feline uterus. Theriogenology 2008;70:349–58.
[49] Hadley JC. The development of cystic endometrial hyperplasia in the bitch following serial uterine biopsies. J Small Anim Pract 1975;16:249–57.
[50] Dow C. The cystic hyperplasia-pyometra complex in the bitch. J comp Path 1959;69:237–50.
[51] Bigliardi E, Parmigiani E, Cavirani S, Luppi A, Bonati L, Corradi A. Ultrasonography and cystic hyperplasia-pyometra complex in the bitch. Reprod Domest Anim 2004;39:136–40.
[52] England GCW, Burgess CM, Freeman SL. Perturbed sperm-epithelial interaction in bitches with mating-induced endometritis. Vet J 2012;194:314–8.
[53] Fontaine E, Levy X, Grellet A, Luc A, Bernex F, Boulouis HJ, Fontbonne A. Diagnosis of endometritis in the bitch: a new approach. Reprod Domest Anim 2009;44:196–9.
[54] Mir F, Fontaine E, Albaric O, Greer M, Vannier F, Schlafer DH, Fontbonne A. Findings in uterine biopsies obtained by laparotomy from bitches with unexplained infertility or pregnancy loss: an observational study. Theriogenology 2013;79:312–22.
[55] Gifford AT, Scarlett JM, Schlafer DH. Histopathologic findings in uterine biopsy samples from subfertile bitches: 399 cases (1990-2005). J Am Vet Med Assoc 2014;244:180–6.
[56] García Mitacek MC, Praderio RG, Stornelli MC, de la Sota RL, Stornelli MA. Prostaglandin synthesis enzymes' gene transcription in bitches with endometritis. Reprod Domest Anim 2017;52:298–302.
[57] Moxon R, Whiteside H, England GCW. Prevalence of ultrasound-determined cystic endometrial hyperplasia and the relationship with age in dogs. Theriogenology 2016;86:976–80.
[58] Freeman SL, Russo M, England GCW. Uterine artery blood flow characteristics during oestrus in pregnant and non-pregnant bitches. Vet J 2013;197:205–10.
[59] Freeman SL, Green MJ, England GCW. Uterine fluid from bitches with mating-induced endometritis reduces the attachment of spermatozoa to the uterine epithelium. Vet J 2013;198:76–80.
[60] Pycock JF, Newcombe JR. Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. Vet Rec 1996;138: 320–3.
[61] Pycock JF, Newcombe JR. The relationship between intraluminal uterine fluid, endometritis and pregnancy rate in the mare. Equine Pract 1996;18:19–22.
[62] Praderio RG, García Mitacek MC, Núñez Favre R, Rearte R, de la Sota RL, Stornelli MA. Uterine endometrial cytology, biopsy, bacteriology, and serum C-reactive protein in clinically healthy diestrus bitches. Theriogenology 2019;131:153–61.
[63] Lyman CC, Hornberger KT, Hallman RM, Holyoak GR. Theriogenology question of the month. J Am Vet Med Assoc 2018;253:1267–70.
[64] Freeman SL1, Green MJ, England GCW. Prevalence and effect of uterine luminal free fluid on pregnancy and litter size in bitches. Theriogenology 2013;80:73–6.
[65] Dhaliwal GK, England GC, Noakes DE. The effects of endometrial scarification on uterine steroid receptors, bacterial flora and histological structure in the bitch. Anim Reprod Sci 2002;69:239–49.