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Repeated pregnancy in a bitch, with a diagnosis of extensive cystic endometrial hyperplasia, after the exact determination of the optimal time for breeding

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Abstract: A 6-year-old bitch was presented to the clinical examination with the aim of determining the optimal time for intrauterine insemination by frozen-thawed semen. The bitch had already had two litters in the past. Extensive cystic endometrial hyperplasia was diagnosed by an ultrasound examination in the bitch. The timing of the endoscopic transcervical insemination by a combination of ultrasonographic examination of the ovaries and determining the progesterone concentrations contributed to the successful conception in the bitch. The ultrasonographic examination of the ovaries represents a non-invasive method which allowed us to view the changes on the ovaries in real time and, thus, is a very suitable method for the specification of the ovulation time in bitches. Cystic endometrial hyperplasia is considered to be a possible cause of infertility. Nevertheless, the bitch repeatedly conceived and had larger litter sizes. Moreover, a considerable reduction in the number of endometrial cysts was observed after the third pregnancy of the bitch. In the described clinical case, the influence of the cystic endometrial hyperplasia on the conception was not demonstrated.

Keywords: cystic endometrial hyperplasia; dog; pregnancy; ultrasonography

Cystic endometrial hyperplasia (CEH) is considered to be an abnormal response of the uterine epithelium to the stimulation of the uterus by the ovarian hormones – oestrogen and progesterone, during the course of the reproductive cycle. The development of CEH occurs over multiple reproductive cycles. The incidence of CEH in bitches increases with the age (Moxon et al. 2016). CEH is considered to be a possible cause of infertility in bitches (England et al. 2013).

This article describes a clinical case of a breeding bitch which, despite the finding of extensive CEH,

had repeatedly conceived and had larger litter sizes. In this clinical case, we further discuss the breeding management based on a combination of the ultrasonographic (USG) examination of the ovaries and measuring the concentration of serum progesterone.

Case description

A 6-year-old bitch of the Weimaraner breed was presented to the department of reproduction with a request to find the optimal time for insemination

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and perform endoscopic transcervical insemination (TCI) with frozen-thawed semen. Previously, the bitch had two litters. During this clinical case, the bitch underwent a total of five clinical examinations at the department of reproduction. The USG examination (ultrasound system MyLab 40 Vet, microconvex 8.0 MHz probe; Esaote, Genova, Italy) focusing on the assessment of the uterus and ovaries was performed and peripheral blood samples were taken to determine the progesterone levels [measured by an immunoassay analyser (IMMULITE 1000 immunoassay system; Siemens, Munich, Germany)]. The findings from the performed examinations are described in Table 1. Extensive cystic endometrial hyperplasia was detected in the bitch during all of the subsequent examinations (Figure 1).

A repeated USG examination of the ovaries showed follicular growth, followed by ovulation

and formation of the *corpora lutea*, which was confirmed by the corresponding progesterone values. Mason demonstrated that artificial insemination with frozen-thawed semen should be performed when the serum progesterone concentrations exceed 10 ng/ml (Mason 2017). In this case, the TCI was performed on the day of the last clinical examination, when the progesterone concentration reached 16.8 ng/ml and the USG examination showed the presence of *corpora lutea* on the ovaries, therefore, the presence of fertilisable oocytes in the female reproductive tract was presumed. The TCI was performed according to the method described by Hollinshead and Hanlon (2017). A rigid endoscope (Uretero-Renoscope, distal tip 8fr; Karl Storz, Tuttlingen, Germany) and a special insemination catheter (TCI Canine catheter: CH 5 single port, 70 cm long; Minitube GmbH, Tiefenbach, Germany) were used for the insemination. The

Table 1. USG findings on the uterus and ovaries and detected concentrations of progesterone in the peripheral blood performed on the examined bitch in the context of the timing of the intrauterine insemination

Parameters	The day of the presence of a typical oestrus discharge				
	10	13	15	17	18
Uterus (mm)	cervix 27 × 17, uterine body and horns 8–9	cervix 34 × 17, uterine body and horns 8–9	cervix 35 × 19, uterine body and horns 8–9	cervix 35 × 19, uterine body and horns 8–9	cervix 36 × 19, uterine body and horns 8–9
Right ovary (mm)	17 × 13	19 × 14	19 × 14	22 × 20	22 × 19
Left ovary (mm)	15 × 10	16 × 12	16 × 14	16 × 15	16 × 15
Description of uterus	a large number of endometrial cysts of 3–4 mm in size were found throughout both the uterine horns and uterine body; an anechoic content 2 mm in width was shown in the lumen of the uterine body and horns				
Description of right ovary	oval shape, 5 anechoic spherical structures with a diameter of 4–5 mm	oval shape, 5 anechoic spherical structures with a diameter of 5–6 mm	oval shape, 5 anechoic spherical structures with a diameter of 7 mm, free fluid in the ovarian bursa	unequal shape, bumpy surface, 5 irregular spherical anechoic structures with a size of 10–11 mm	unequal shape, bumpy surface, 5 irregular spherical isoechoic structures with a size of 8–10 mm
Description of left ovary	oval shape, 3 anechoic spherical structures with a diameter of 4 mm	oval shape, 4 anechoic spherical structures with a diameter of 5 mm	oval shape, 4 anechoic spherical structures with a diameter of 6–7 mm, free fluid in the ovarian bursa	unequal shape, bumpy surface, 3 irregular spherical anechoic structures with a size of 10–12 mm	unequal shape, bumpy surface, 3 irregular spherical isoechoic structures with a size of 8–10 mm
Progesterone (ng/ml)	0.45	2.2	4.3	10.0	16.8

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Figure 1. Ultrasonographic view of extensive CEH before the intrauterine insemination
The uterus is marked by arrows
CEH = cystic endometrial hyperplasia

insemination catheter was inserted into the uterine body to a depth of 4 cm and 1.5 ml of ejaculate was deposited into the uterus. The ejaculate was collected, processed and examined by a specialised laboratory. Re-insemination was performed in the same way one day later. The bitch was confirmed to be pregnant on day 31 after the insemination and she gave birth to 6 puppies.

One year later, the owners came with this bitch for an examination with a request to determine the optimal time for natural breeding. The USG examination showed a significantly lower number of endometrial cysts in the uterine body and uterine horns compared to the previous USG examination of the uterus (Figure 2). The size of the left ovary was 18 × 16 mm, with the finding of 2 anechoic spherical structures with a diameter of 5–6 mm. The size of the right ovary was 22 × 25 mm with the finding of 6 anechoic spherical structures with a diameter of 10 mm. Free fluid was shown in both the left and right ovarian bursa. The progesterone concentration was 7.27 ng/ml. Due to the fact that the USG examination of the ovaries and progesterone value confirmed the ovulation, the owners were recommended to initiate natural breeding. As reported by the owners, the bitch conceived and consequently had 11 puppies.

Whereas CEH is often associated with an accumulation of fluid in the uterus and, thus, becomes a predisposition to the subsequent development of bacterial inflammation (De Bosschere et al. 2001), the bitch underwent a preventive ovariohysterectomy several months after the last parturition.

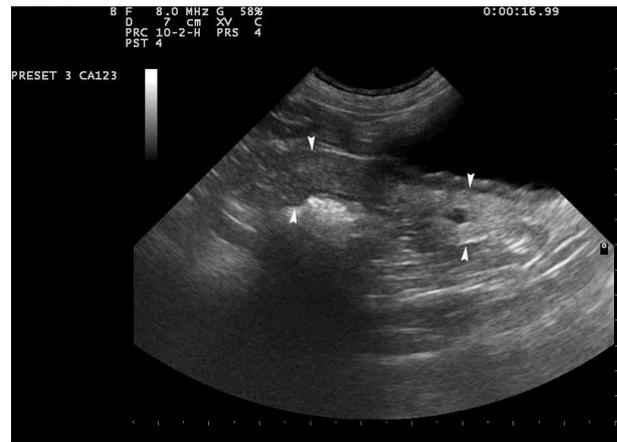


Figure 2. Ultrasonographic view of a reduced number of endometrial cysts after the third pregnancy of the bitch
The uterus is marked by arrows

DISCUSSION

In the clinical case described above, extensive cystic endometrial hyperplasia was detected by a USG examination in the bitch. The USG examination is a reliable method to detect CEH (Bigliardi et al. 2004). In the aforementioned study, the findings from the USG examination were confirmed by a histological examination.

The occurrence of CEH in bitches could significantly affect their fertility (England et al. 2013). In bitches, the development of a certain inflammatory uterine response after mating is described and includes an increase in the uterine contractility, influx of polymorphonuclear neutrophils into the uterine lumen and vasodilatation of the uterine arteries (England et al. 2013). The inflammatory response is particularly aimed at removing the excess and dead sperm, bacteria and other contaminants from the uterine lumen (England et al. 2013). In bitches with CEH, a decreased uterine contractility, an increased number of polymorphonuclear neutrophils and a reduced vasodilator vascular response after mating have been observed (England et al. 2012). Lower conception rates as well as smaller litter sizes were found in these bitches (England et al. 2012).

In this clinical case, a reduced function of the above-mentioned mechanism and a predisposition to infertility associated with that disorder could be assumed. Nevertheless, the bitch has repeatedly conceived and had large litters.

As the occurrence of CEH is more often described in animals that have never been pregnant,

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the protective effect of pregnancy on the development of CEH should be considered (Moxon et al. 2016). In our case, it is noteworthy that the number of endometrial cysts demonstrated by the USG examination before the last pregnancy of the bitch was considerably reduced in comparison with the USG examination performed before the TCI at our workplace (Figures 1 and 2). According to the authors of this manuscript, the improvement in the CEH status could be caused by changes in the endometrium associated with the placentation and subsequent regeneration of the endometrium at the sites of placental attachments in the puerperium.

Determining the appropriate time to initiate natural breeding or artificial insemination is considered as an essential point in breeding management. The suitable timing is especially based on the detection of the luteinizing hormone (LH) surge and subsequent ovulation. Currently, the most common method for detecting these parts of the reproductive cycle is to determine the concentration of the progesterone during oestrus (Hollinshead and Hanlon 2019). Nevertheless, it is appropriate to combine the method of determining the progesterone concentrations with another method, which will allow the specification of the ovulation time. A USG examination of the ovaries allowed us to view changes on the ovaries in real time and, thus, it is a very suitable method for the specification of the breeding date.

The size, shape and echogenicity of the ovaries fluctuate during the reproductive cycle, which is due to the gradual development and regression of ovarian follicles and *corpora lutea*. The growing follicles reach a size of 3–5 mm approximately 2–3 days before the LH surge (Hase et al. 2000). The largest follicle size of 5–8 mm is achieved 1–2 days after the LH peak which corresponds to the preovulatory period (Hase et al. 2000). Preovulatory follicles are described as anechoic spherical structures with a thin wall (Eker and Salmanoglu 2006; Levy and Fontbonne 2007). Because the follicles in the bitch luteinize before ovulation and these structures do not usually collapse after ovulation, it is difficult to distinguish preovulatory follicles from young *corpora lutea* (Concannon 2009). Only 30% to 40% of bitches show a typical course of ovulation with post-ovulation follicle collapse (Levy and Fontbonne 2007). Some studies also reported the presence of free fluid in the ovarian bursa at the time of ovulation (Eker and Salmanoglu 2006).

The newly formed *corpora lutea* have a thin wall and a fluid-filled cavity (Barr and Gaschen 2011). From the above-stated information, it is evident that the interpretation of ovarian findings during the period of ovulation is among the more complex clinical examinations. England and Yeager (1993) demonstrated that a USG examination for a reliable detection of ovulation would need to be performed at least twice a day. However, Fontbonne (2008) described that a once daily performed USG examination of the ovaries is sufficient not to miss the day of ovulation. Although, in this clinical case, it was not possible to perform a daily USG examination (at the request of the bitch owner), the approximate time of ovulation and subsequent formation of *corpora lutea* on the ovaries were detected.

This clinical case report presents the repeated successful conception in a bitch, which, due to the finding of CEH, had a predisposition to a low level of conception and, in case of conception, to a small litter size. Nevertheless, the influence of CEH on the conception was not demonstrated. Specification of the period of ovulation through a USG examination of the ovaries contributed to the successful conception in the bitch.

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Conflict of interest

The authors declare no conflict of interest.

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