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Equine endometrial vascular pattern changes during the estrous cycle examined by Narrow Band Imaging hysteroscopy

Henning Otzen[a], Harald Sieme[b,*], Harriëtte Oldenhof[b], Ana Kassens[b], Franziska Ertmer[b], Kristina Rode[c], Kristin Müller[d], Kristin Klose[d], Karl Rohn[e], Heinz-Adolf Schoon[d], Burkhard Meinecke[a]

[a] Institute for Reproductive Biology, University of Veterinary Medicine Hannover, Bünteweg 2, 30559 Hannover, Germany
[b] Clinic for Horses-Unit for Reproductive Medicine, University of Veterinary Medicine Hannover, Bünteweg 15, 30559 Hannover, Germany
[c] Institute for Anatomy, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany
[d] Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 33, 04103 Leipzig, Germany
[e] Institute for Biometry, Epidemiology and Information Processing, University of Veterinary Medicine Hannover, Bünteweg 2, 30559 Hannover, Germany

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ABSTRACT

The aim of this study was to evaluate the uterine blood supply and endometrial vessel architecture, during the equine estrous cycle. Narrow Band Imaging (NBI) hysteroscopy was used for evaluating changes in the endometrial vasculature during the estrous cycle [six mares, d 0 (representing the day of ovulation), d 6 and 11 in four locations]. In addition, endometrial biopsy samples were used for immunodetection of markers for angiogenesis (Vascular Endothelial Growth Factor A, its receptor 2, as well as angiopoietin-2 and its receptor-tyrosine-kinase Tie2) during the estrous cycle (three mares, d 0, 5 and 10; one biopsy per mare). Detailed analysis of hysteroscopic images revealed an increase in the vascular density from estrus towards diestrus. In contrast, microscopic specimens prepared from biopsies revealed no evidence for changes in the endometrial vessel number during the estrous cycle. Studies on expression of angiogenesis markers indicated that cyclic changes in the endometrial vascular density observed by NBI-hysteroscopy were not due to formation of new vessels. It is concluded that vessels are involved in blood supply of a smaller area during diestrus, facilitating better distribution of nutrients during this phase.

1. Introduction

The endometrium is supplied with nutrients via its vascular system, which is especially critical for the developing embryo (Allen and Wilsher 2009). Using non-invasive Doppler sonography, it has been determined that the uterine blood supply in mares not only changes during gestation, but also exhibits differences between estrus and diestrus as well as during diestrus (Bollwein et al., 1998; Ousey et al., 2012; Klewitz et al., 2015).

* Corresponding author. Fax: +49 511 953 82 8530.
E-mail addresses: Henning.Otzen@tiho-hannover.de (H. Otzen), Harald.Sieme@tiho-hannover.de (H. Sieme), Harriette.Oldenhof@tiho-hannover.de (H. Oldenhof), Ana.Kassens@tiho-hannover.de (A. Kassens), Franziska.Ertmer@tiho-hannover.de (F. Ertmer), Kristina.Rode@tiho-hannover.de (K. Rode), kristin.mueller@vetmed.uni-leipzig.de (K. Müller), kristin.klose@vetmed.uni-leipzig.de (K. Klose), Karl.Rohn@tiho-hannover.de (K. Rohn), schoon@vetmed.uni-leipzig.de (H.-A. Schoon), Burkhard.Meinecke@tiho-hannover.de (B. Meinecke).

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For primates, including human, it has been described that the endometrium is prepared for a pregnancy by growth of the stratum functionalis and angiogenesis (Reynolds et al., 1992). In case of an absence of implantation, the endometrial lining will be shed. For maresgrowth and ablation of the endometrium in response to presence or absence of an embryo have not been found and there is no evidence for endometrial angiogenesis in healthy mares exhibiting a normal estrous cycle (Merkl et al., 2010).

Investigations on formation of blood vessels in the endometrium have made use of vasculature measurements as well as immunohistochemistry of specific markers for angiogenesis in endometrial biopsy samples, including Vascular Endothelial Growth Factor A (VEGF) and its Receptor 2 (VEGF-R2). In case of VEGF, different expression patterns have been reported. Allen et al. (2007) found an increased expression in endometrial glands and epithelium during diestrus compared to estrus, whereas in capillary endothelium the expression was increased during estrus. In contrast, Silva et al. (2011) did not detect any changes in VEGF expression. Its receptor, VEGF-R2, has been detected in both endometrial glands and vessel endothelium though at different phases of the equine estrous cycle (Allen et al., 2007; Silva et al., 2011). Allen et al. (2007) reported detection in the epithelium during estrus while Silva et al. (2011) reported detection only in diestrus. Assessments on vascular densities of the endometrium revealed no differences between estrus and diestrus (Ferreira-Diaz et al., 2001, da Costa et al., 2007; Silva et al., 2011); whereas, there are no reports on expression of angiopoietin 2 and Tie2 in the equine endometrium, angiogenic processes have been described for ovarian remodeling (Müller et al., 2009).

In contrast to assessments on endometrial features (i.e. vasculature) from microscopic specimens, hysteroscopic approaches allow for in vivo evaluation of a large area of the endometrium. For examination of the intrauterine environment in mares, the development and use of flexible endoscopes have proven particularly helpful. Improvements in the resolution of obtained images in turn allow for visualization of greater details in the mucosa, whereas application of specific filters as done in Narrow Band Imaging (NBI), results in improved visualization and contrast of mucosal vessels (Yoshida et al., 2004; Wen et al., 2012). Hysteroscopic evaluations using NBI have recently gained increased attention, especially for discriminating different vascular patterns and forms of endometriosis in women as well as for detecting other gynecological problems (Kuroda et al., 2009; Kisu et al., 2012).

The aim of this study was to evaluate the endometrial vessel architecture during the equine estrous cycle, using NBI hysteroscopy. In addition, assessments on serum progesterone content and uterine blood flow via Doppler sonography were performed. NBI images were analyzed to reveal possible changes in the vascular density, vessel sizes (i.e. diameter), and blood supply (i.e. Voronoi cell area; Yoshii and Sugiyama, 1988) during the estrous cycle. Furthermore, expression levels and localization of markers for angiogenesis (VEGF, VEGF-R2, Angiopoietin-2 [Ang2], and its receptor receptor-tyrosine-kinase Tie2 [Tie2]) were studied using specific antibodies on microscopic specimens prepared from endometrial biopsies.

2. Material and methods

2.1. Animals

Experiments were performed in agreement with German animal welfare legislation and approved by the Lower Saxony State Office for Consumer Protection and Food Safety, Hannover (reference number: 33.4-42502-05-12A262).

Hysteroscopic and sonographic examinations were performed on a total of six mares [8–20 years; warmblood ($n=4$) and trotter breed ($n=2$), nulliparous ($n=3$) and multiparous ($n=3$)], during the breeding season (June to mid-September 2013), at day (d) 0 (representing the day of ovulation), d 6 and d 11 of their estrous cycle. To avoid artefacts from previous hysteroscopies, a new estrous cycle was used for each hysteroscopy. Horses were kept on pasture with access to water ad libitum and shelter, at the Clinic for Horses of the University of Veterinary Medicine Hannover, Germany. Estrous cycle monitoring was carried out by teasing with a stallion and transrectal ultrasound examinations. For histological analysis of the endometrium biopsies collected during the estrous cycle (at d 0, d 5 and d 10) from three different mares (4–10 years) which were held at/present in the collection of the Institute for Veterinary Pathology, University of Leipzig.

2.2. Narrow Band Imaging hysteroscopic evaluation of the equine endometrium

Mares were sedated via intravenous application of a combination of detomidine and butorphanol (0.01 mg/kg, Cepesedan and 0.01 mg/kg, Alvegesic; both CP-Pharma, Burgdorf, Germany). Furthermore, the tail was covered using tape and the perineal region was cleaned with iodine soap and water. For NBI hysteroscopic evaluations, an Evis Exera II system was used (Olympus, Hamburg, Germany). The system was composed of a video processor (CV-180) and light source (CLV-180) and a capped (D-201-12704) colonoscope (PCF-H180-AL). This setup allowed for NBI examinations, by making use of only two wavelength peaks (415 nm and 540 nm) for illumination. For later correction and calibration of images, with each hysteroscopy session, an image was taken from a grid composed of multiple squares of defined sizes (RE 435; Roth Elektronik, Hamburg, Germany). All hysteroscopies were performed by the same operators, one for introducing the endoscope transvaginally and another for directing the endoscope. The uterus was expanded with filtered air, and NBI hysteroscopic images were collected from the mucosa of the left and right horn. A pressure of 15 to 20 mm Hg was used, similar to that previously described (Bartmann and Schiemann 2003). Hysteroscopy sessions took on average 54 ± 12.3 min (54 ± 13.4, 58 ± 14.7 and 51 ± 6.0 min for d0, d6 and d11, respectively). In order not to touch the mucosa before taking images, first images of the dorsal and ventral corpus were collected followed by images of the horns