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Identification of vessel degeneration and endometrosis in the equine endometrium, using narrow-band imaging hysteroscopy

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ABSTRACT

In this study, endometrosis and angiosclerosis in mares were studied. Endometrosis is a severe, progressive, and irreversible fibrotic condition that affects the endometrium, whereas angiosclerosis refers to thickening of vessel walls due to degenerative changes leading to reduced elasticity of the walls and lower perfusion. Histologic evaluations were performed on biopsies and compared with vascular features of the endometrial surface obtained via narrow-band imaging (NBI) hysteroscopy. First, it was determined if hysteroscopic evaluation of the endometrium using NBI resulted in a better visualization of the vascular pattern (i.e., vessel-versus-background contrast was increased) compared with using white light. This was found to be the case for examinations *in vivo* (n = 10), but not when using abattoir uteri (n = 3). In the second part of this study, it was determined if vascular densities and sizes as derived from NBI images could be used as indicators for the degree of degenerative changes of the equine endometrium and its vessels. Narrow-band imaging hysteroscopic evaluations were performed (n = 10), and endometrial biopsies (n = 32) were collected. Histologic specimens were evaluated for degree of endometrosis and angiosclerosis, and they were classified in Kenney categories. Narrow-band imaging images were analyzed for vascular pattern. Samples classified to Kenney category I, or without signs of vessel degeneration, had significantly higher vascular densities than samples from Kenney category IIa or with angiosclerosis. In conclusion, narrow-band imaging facilitates enhanced visualization of the vasculature of the equine endometrium during hysteroscopies, which has applications in detection of endometrosis and angiosclerosis.

1. Introduction

Broodmares are subjected to breeding soundness examinations for many reasons. Commonly performed uterine examinations include transrectal palpation and ultrasonography, as well as collection of uterine swabs for

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microbiological and cytologic evaluations, and/or endometrial biopsies [1–4]. In addition, hysteroscopic examinations can be performed to examine the intraluminal surface of the uterus for revealing the presence of adhesions [5], endometrial cysts [6], uterine neoplasia, and/or uterine infections [7].

When endometrial biopsies are taken, these can be used for preparing specimens for histologic evaluation of the appearance of, e.g., glands, stroma, and vessels. It allows for identifying inflammatory and degenerative processes of the endometrium (endometrosis), as well as confirmation of the hormonal status [8]. Endometrial biopsies are especially valuable for evaluation of vascular changes like vasculitis and angiosclerosis [9]. Typically one endometrial biopsy sample is taken when examining a mare with the assumption that it is representative of the whole endometrium [10,11].

A mare is diagnosed with endometrosis when signs of active or inactive periglandular and/or stromal endometrial fibrosis are present in an endometrial biopsy. Endometrosis is an irreversible and progressive condition, for which there is no therapy and/or treatment [4,12–15]. Endometritis results in activation of fibrotic stromal cells, which have a role in degeneration of the endometrium [13,14]. Endometrosis negatively affects fertility. The presence of a higher degree of endometrosis correlates with a decreased chance of giving birth to a foal [4]. Also, the severity of endometrosis typically increases with increasing age [15].

In case of angiosis, the walls of endometrial vessels show signs of degeneration. This occurs more heavily with increasing parity and results in a reduced endometrial perfusion and lymphatic drainage [16,17]. An increased uterine artery resistance index has been correlated with an increase in endometrosis and/or angiosis [18,19].

An approach which allows for the collection of biopsies at the site of hysteroscopic examination has recently been developed [20]. In the present study, we have used this approach in combination with narrow-band imaging (NBI). Narrow-band imaging uses two wavelength peaks, which are absorbed by hemoglobin and is suggested to result in improved visualization of mucosal and vascular structures [21]. The aim of this study was to determine if hysteroscopic evaluation of the equine endometrium using NBI resulted in a better visualization of the vascular pattern (i.e., increased contrast of vessels versus background) compared to using white light. An additional goal was to determine if vascular features as derived from NBI images could be used as indicators for the degree of degenerative changes of the endometrium and its vessels.

2. Materials and methods

2.1. Animals

This study was approved by the Lower Saxony State Office for Consumer Protection and Food Safety Hannover (reference number 33.4-42502-05-12A262). Furthermore, procedures and maintenance of animals were according to German animal welfare legislation.

Hysteroscopic examinations were performed on a total of 10 mares (age: 3–22). Eight of the 10 mares were of the Warmblood breed, one mare was a Thoroughbred, and one mare was a Trotter. It was assumed that the incidence of endometrosis or angiosclerosis did not exhibit breed specific differences. The studies included mares which were held at the Clinic for Horses of the University of Veterinary Medicine Hannover ($n = 2$) as well as patients who underwent breeding soundness examinations ($n = 8$). Using an ultrasound scanner (Logiq P5 US with probe I739, 6–10 MHz; both GE Ultraschall, Solingen, Germany), mares were examined transrectal for uterine health and estrous cycle stage. Only mares which did not exhibit estrous behavior nor showed follicles greater than 3.5 cm on ultrasound images were included in the studies; as it has been shown in our laboratory that mares during diestrus (Days 6 and 11 after ovulation) have no significant differences in endometrial vascular density [22].

2.2. Abattoir material

Before performing hysteroscopies on mares *in vivo*, hysteroscopic examination was performed on abattoir-acquired mare reproductive tracts. This allowed for easy comparison of similar locations with different illuminations. Uteri from three mares (2, 9, and 14-years old; warmblood breed) were collected directly after slaughter, stored in saline solution at 4 °C, and used for hysteroscopic evaluations within 8 hours.

2.3. Hysteroscopic examinations and retrieval of biopsies of the equine endometrium

Hysteroscopic procedures were performed as described by Bartmann et al. [6]. Briefly, mares were placed in stocks, and their perineum was washed with iodine soap, followed by clean tap water, and dried. Sedation was done by giving a bolus (0.05-mg/kg romifidin and 0.02-mg/kg butorphanol) followed by a drip infusion (13 droplets/10 seconds) of 500-mL saline supplemented with romifidin (0.15 mg/kg; Boehringer Ingelheim, Ingelheim, Germany) and butorphanol (0.05 mg/kg; CP-Pharma, Burgdorf, Germany).

For hysteroscopic evaluations an Evis Exera II system was used (Olympus, Hamburg, Germany), equipped with a light source (CLV 180), video processor (CV 180), suction pump (KV-5), and colonoscope (PCF-H180AL). A soft disposable distal attachment (D-201-12704) was attached to the tip of the endoscope. This setup allowed for use of white light and narrow-band imaging (NBI). For correcting the barrel distortion effect and calibrating images during later analysis, at the beginning of each hysteroscopy session, an image was taken from a grid (RE 435; Roth Elektronik, Hamburg, Germany). The same operators performed each hysteroscopy, one for introducing the endoscope transvaginally, one for directing the endoscope and another for handling the endoscopic instruments.

During hysteroscopic sessions, endometrial biopsies were recovered for histologic evaluation by the cap-assisted endoscopic mucosal resection technique using a high frequency surgical device (Erbotom T 400B, Erbe, Tuebingen, Germany), retrieval basket (137-949-30, Pauldrach medical, Garbsen, Germany), asymmetric electrosurgical snare (SD-221U-25, Olympus, Hamburg, Germany), forceps (Lot G4303244,