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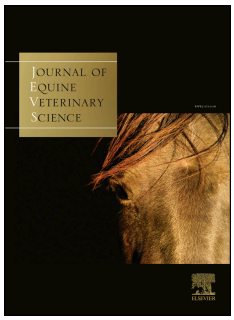

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Uterine glands agenesia in the mare

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Abstract

All mammalian uteri contain endometrial glands that synthesize and secrete a substance termed histotroph, which is essential for early pregnancy nutrition. Because of that function, endometrial glands play a crucial role as regulators of survival and development of conceptus. In all mammalian females as well as in woman, endometrial glands undergo extensive hyperplasia and hypertrophy during pregnancy as a response to increasing demands of the developing conceptus for uterine histotroph. The process of endometrial glands development is called adenogenesis. Inhibition of the development of these glands through gene mutation or epigenetic strategies results in infertility.

Two cases of total intrauterine glands developmental agenesis are presented, which were diagnosed on the base of histopathological examination of endometrial biopsy specimens. Despite the fact that endometrial glands agenesis in mare is a rare condition, it is worth while to emphasize that the endometrial biopsy is currently the best diagnostic tool to recognize the above developmental disturbance. Clinicians should be aware, that the mare with normal reproductive organs during macroscopic clinical investigation, can present with uterine glands agenesis.

Key words: uterine glands, agenesia, mares,

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All mammalian uteri contain endometrial glands that synthesize and secrete a substance termed histotroph, which is essential for early pregnancy nutrition. Because of that function, endometrial glands play a crucial role as regulators of survival and development of conceptus. In all mammalian females as well as in woman, endometrial glands undergo extensive hyperplasia and hypertrophy during pregnancy as a response to increasing demands of the developing conceptus for uterine histotroph. The process of endometrial glands development is called adenogenesis. Inhibition of the development of these glands through gene mutation or epigenetic strategies results in infertility.

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1. Introduction

Uterine glands found in all mammal species are responsible for synthesizing and secreting a mixture of substances termed histotroph, which provides nutrients to the conceptus during early pregnancy [1], [2], [3], [4]. Due to its nutritional role, uterine secretions act as primary regulators of survival and development of the conceptus, onset of pregnancy recognition signals as well as implantation [3], [4], [5], [6], [7], [8], [9], [10], [11], [12], [13]. Extensive hyperplasia and hypertrophy of uterine glands during pregnancy, which have been reported for all mammalian females, are likely to occur as a response to surging demand of the developing conceptus for histotroph [5], [14], [15].

The process of developing endometrial glands is called adenogenesis. Inhibition of the development of these glands through gene mutation or epigenetic strategies results in infertility. Uterine secretions are thought to be particularly important for conceptus survival and development in animals with superficial attachment and placentation preceded by a prolonged period of preimplantation conceptus development, such as sheep, cattle, pigs and horses [16].

This paper discusses two cases of total intrauterine gland agenesis in mares diagnosed on the basis of histopathological evaluation of endometrial biopsy specimens.

2. Materials and methods

Biopsy specimens were obtained from 343 mares – patients evaluated for their reproduction status. Including criteria were: unsuccessful multiply breading, repeated early embryonic deaths or abortions. Regarding this anamnesis, special attention was paid on endometrosis, subclinical endometritis and other uterine lesions difficult to be diagnosed during basic clinical examination. Biopsy specimens were taken during routine examination, as a principle in diestrus (estrus cycle phase was assessed on the basis of ultrasound examination and confirmed by measuring progesterone level in blood plasma) (Delfia Fluorometr 1232 with Progesterone

Kit, Walloc Oy, Finland) or in anoestrus when obtained out of breeding season. The specimens were collected from the uterine body close to junction with the horn, stained with hematoxylin and eosin and assessed according to Kenney classification to determine endometrial status [17]. Among all examined patients, two warmblood mares, 5 and 7 years old respectively, were subjected to the procedure of breeding soundness examination because of history of subfertility. In both cases anamnesis stated that mares showed a regular pattern of signs of estrus during the breeding season and the normal ovarian activity with ovulation, confirmed by ultrasound examination.. The first mare was repeatedly inseminated with fresh and frozen semen of different stallions for 2 consecutive seasons without effect. The second mare was mated naturally for one season and than inseminated for the next season with fresh semen of two different stallions. In both mares the early pregnancy diagnosis was carried out between 14- 20 days after each breeding, every time with negative results. Besides basic clinical examination, uterine biopsy specimens obtained from this two mares underwent histological evaluation. Apart from routine microscopic evaluation, the uterine specimens were investigated for estradiol receptors (ER). Immunohistochemical stainings for estrogen receptors (without differentiation on α and β receptor) were performed on paraffin embedded sections, previously fixed with 10% Neutral Buffered Formalin. Sections were deparaffinised in xylene, rehydrated through graded alcohols and treated in a 0.01 M citrate buffer for 20 min (pH 7) in a microwave oven. To block endogenous peroxidase activity and non-specific antibody staining, incubation with 0.03% hydrogen peroxide (15 min. at room temperature) and incubation with normal goat serum (20 min. at room temperature) was carried out. After overnight incubation in 4°C with anti-ER antibody (NCL-ER-LH2, clone CC 4-5, Novocastra Lab. Ltd. UK), secondary antibody (biotinylated goat anti-mouse immunoglobulin G) was used as a linker. Streptavidin-px complex was applied as detection antibody and stained with diaminobenzidine. The sections were lightly counterstained with hematoxylin. Specimen of the human mammary gland tumor

was used as the positive control. The negative control was estimated using the mare's endometrial sections not covered with primary antibodies during staining procedure.

3. Results

No abnormalities were observed during basic clinical evaluation of the reproductive tract (consisting of vaginoscopy, rectal palpation and ultrasound examination) of two mentioned infertile mares. Ultrasound examination revealed that biopsy in both cases was carried out during diestrus (corpus luteum on one of the ovary of each mare). Analysis of plasma progesterone profiles confirmed the above-mentioned diagnosis (P4 = 5 ng/ml and 7 ng/ml respectively).

The histopathological evaluation of endometrial specimens from infertile mares revealed total agenesis of intrauterine glands (Fig. 3,4). Moreover, in both cases animals lack nuclear estradiol receptors as evidenced by immunohistochemical reactions (Fig. 5).

4. Discussion

In humans adenogenesis is initiated in the fetus and completed postnatally during puberty. However, development of uterine glands in other animals – such as sheep, pigs and rodents – was found to occur postnatally [16]. Typically, the process of adenogenesis involves differentiation and budding of glandular epithelium from luminal epithelium. Subsequent stages include invagination and extensive tubular coiling and branching morphogenesis throughout the uterine stroma to the myometrium. The process of uterine gland formation is

regulated by prolactin, estradiol-17b, progesterone and their receptors. What is more, it has been determined that several genes, including forkhead box A2, beta-catenin and members of the Wnt and Hox gene families, are implicated in uterine gland development [18]. Unusually high rates of peri-implantation embryonic loss in humans and livestock may be prompted by defects in endometrial gland development caused by genetic errors, epigenetic effects of endocrine disruptors as well as pathological lesions [16]. Direct influence of hormones on adenogenesis was studied on many different species. Exposure of ewes to progestin during neonate life caused ablation of endometrial gland differentiation and produced adults that displayed a uterine gland knockout (UGKO) phenotype, which is characterized by the absence of endometrial glands [19], [20], [21], [22], [23], [24]. Partial to complete UGKO phenotypes were also obtained in adult cows exposed from birth to a combination of progesterone and estradiol benzoate (P+E) [19,25]. Studies of embryo development conducted on ovine UGKO model revealed that proper glandular structure of endometrium plays an essential role in peri-implantation conceptus survival and growth [21], [23], [24]. Similarly, decreased pregnancy rates were also reported in adult heifers with reduced number of endometrial glands that were exposed neonatally to P+E [25]. As far as the process of adenogenesis in horses is concerned, there is only limited data available. Allen and Gerstenberg [26] investigated adenogenesis in fillies from day 150 of gestation up to the first estrus. According to their findings, invagination of the luminal epithelium into the underlying stroma began at 250 day of gestation and the first cross-sections of very simple tubular gland structures were detected by day 300 of gestation. During the early postnatal period these tubular gland structures developed into clusters of rudimentary gland cross-sections. Then the process of gland development was arrested and the epithelial invaginations were restarted during the first estrus at the onset of puberty. However, final differentiation and maturation of the secretory compartments of the glands occurred only after the first ovulation.

Considering that findings, it become obvious that proper hormonal stimulation is essential for endometrial gland formation. Both hormonal disturbances and improper distribution of hormone receptors can result in incomplete adenogenesis or total uterine gland knockout. Total lack of estradiol receptors in both uterine specimens obtained from mares discussed in our work, fully confirmed that observation. It should be emphasized that this condition has probably a genetic background. It is well known that some chromosomal aberrations affect in many ways the process of genital system development in all species, including horses [27].

The fact that uterine glands undergo continuous changes connected with ovarian cycle with maximal development under estrogens influence and significant involution during anoestrus should be considered when microscopic evaluation of uterine biopsy samples is performed (Fig. 1,2). Endometrial atrophy during winter anestrus as well as in aged mares with diminished ovarian activity is a normal condition and should be differentiated from inherited uterine gland agenesis, which is the topic of our study. The ovarian cycle affects the level of estrogen receptors in the endometrium as well. Its higher appearance is observed during estrus [28], but unlike the endometrial specimens from the presented cases of the mares with uterine glands agenesis, there are detectable in every stage of the ovarian cycle (Fig. 3).

Despite the fact that a UGKO phenotype in the mare is a rare condition, it is worth emphasizing that mares with normal reproductive organs and ovarian activity can present with this developmental disorder. This disorder will not be recognized during basic breeding examination. At this time, endometrial biopsy is the best diagnostic tool to recognize the uterine agenesis in the mare.

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Description of figures

Fig. 1. Normal mare's endometrium in diestrus. Visible multiple uterine glands, scattered symmetrically in the endometrium. HE x 20

Fig. 2. Mares' uterine glands atrophy during seasonal anoestrus. HE x10

Fig. 3. Uterine glands agenesis. Mare no 1. HE x 10

Fig. 4. Uterine glands agenesis. Mare no 2. HE x20

Fig. 5. Negative expression of estrogen receptors in the endometrium of the mare with uterine glands agenesis. Mare No 1. IPOX×40

Fig. 6. Positive expression of estrogen receptors in the normal endometrium of the mare in diestrus. IPOX×40

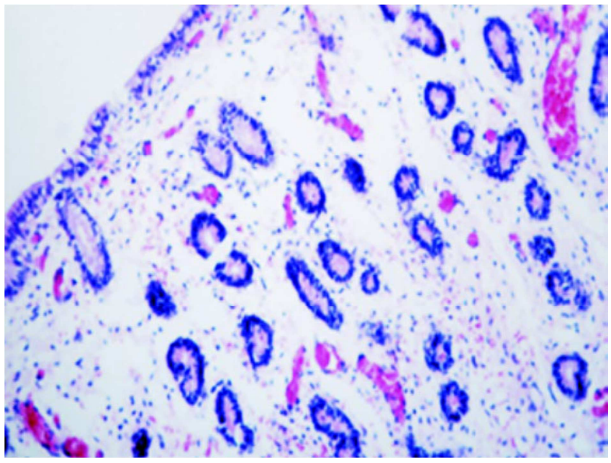

Fig. 1

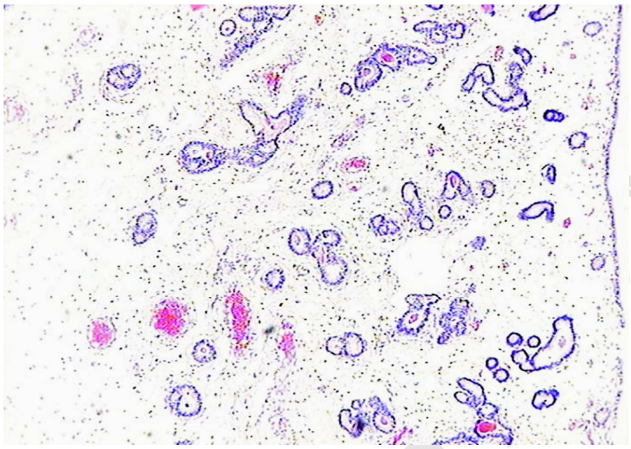

Fig. 2

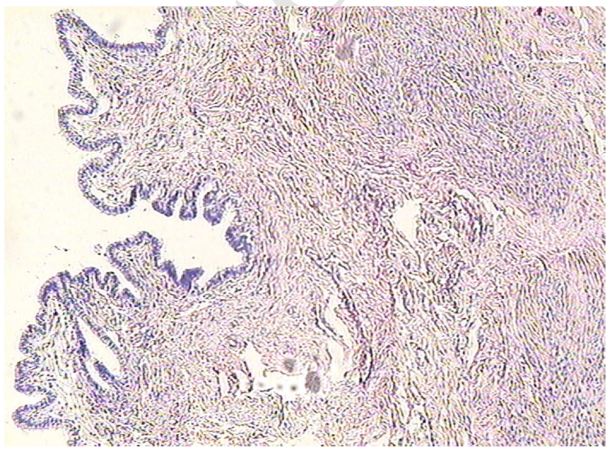

Fig. 3

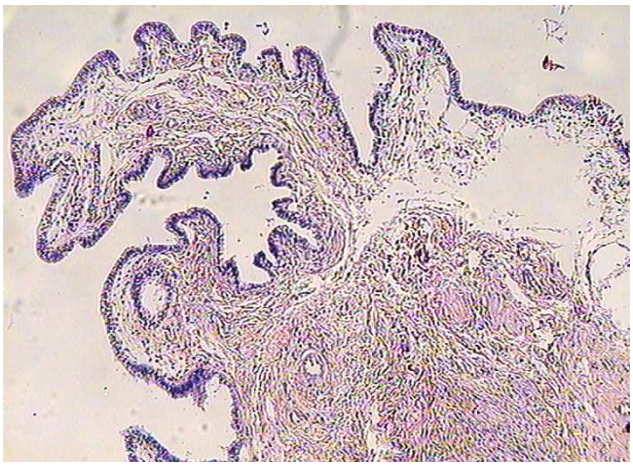

Fig. 4

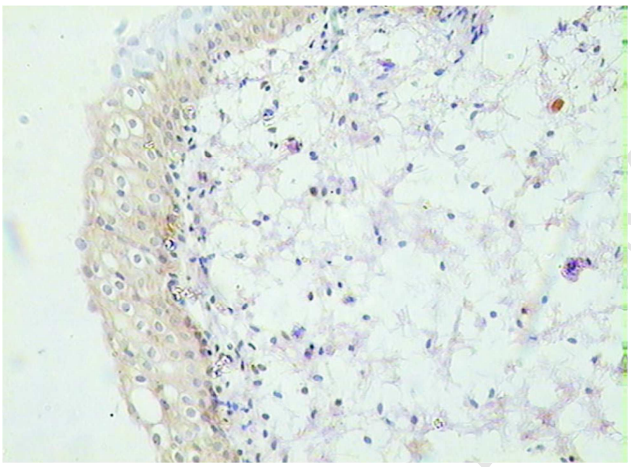

Fig. 5

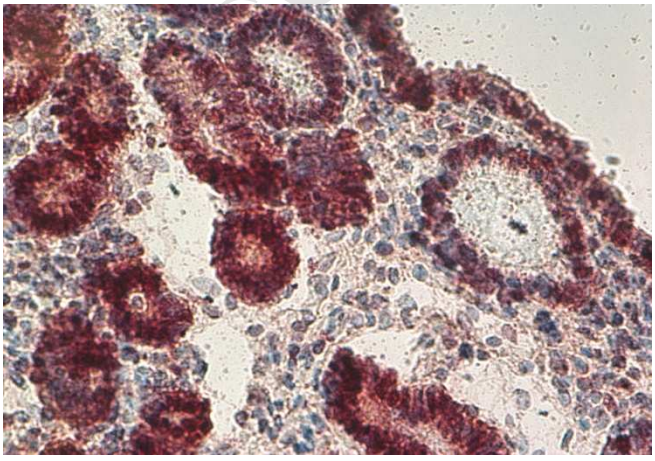

Fig. 6

*Over 300 uterine equine endometrial specimens underwent histological evaluation as a part of breeding soundness examination
*2 cases of endometrial glands agenesia were diagnosed
* Adenogenesis disturbances lead to infertility. Uterine biopsy is the procedure of choice to diagnose this developmental disorder