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Original Research

Uterine Glands Agenesia in the Mare

Maciej Witkowski [a,*], Maria Katkiewicz [b], Joanna Kochan [c], Duccio Panzani [d]

[a] University Center of Veterinary Medicine UJ-UR, University of Agriculture in Krakow, Krakow, Poland
[b] Department of Clinical Science, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland
[c] Department of Veterinary, Animal Reproduction and Welfare, Institute of Veterinary Science, University of Agriculture in Krakow, Krakow, Poland
[d] Department of Veterinary Sciences, University of Pisa, Pisa, Italy

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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.

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 [1–4]. 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 [3–13]. 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

Animal welfare/ethical statement: All methods and procedures used in the study: "Uterine glands agenesia in the mare", were in the compliance with the guidelines of the Polish law and EU directive (2010/63/EU) on the protection of animals used for scientific purposes.

Conflict of interest statement: The authors declare that they don't have any conflict of interest.

* Corresponding author at: Maciej Witkowski, University Center of Veterinary Medicine UJ-UR, University of Agriculture in Krakow, al Mickiewicza 24/28, Krakow 30-059, Poland.

E-mail address: mawitkow@gmail.com (M. Witkowski).

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criteria were as follows: unsuccessful multiple breeding, repeated early embryonic deaths or abortions. Regarding this anamnesis, special attention was paid on endometriosis, 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 anestrus 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, 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 two consecutive seasons without effect. The second mare was mated naturally for one season and then inseminated for the next season with fresh semen of two different stallions. In both mares, the early pregnancy diagnosis was carried out between 14 and 20 days after each breeding, every time with negative results. Besides basic clinical examination, uterine biopsy specimens obtained from these two mares underwent histological evaluation. Apart from routine microscopic evaluation, the uterine specimens were investigated for estradiol receptors (ERs). 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 deparaffinized in xylene, rehydrated through graded alcohols, and treated in a 0.01-M citrate buffer for 20 minutes (pH 7) in a microwave oven. To block endogenous peroxidase activity and nonspecific antibody staining, incubation with 0.03% hydrogen peroxide (15 minutes at room temperature) and incubation with normal goat serum (20 minutes at room temperature) were 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 antimouse 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 aforementioned diagnosis (P4 = 5 ng/mL and 7 ng/mL, respectively).

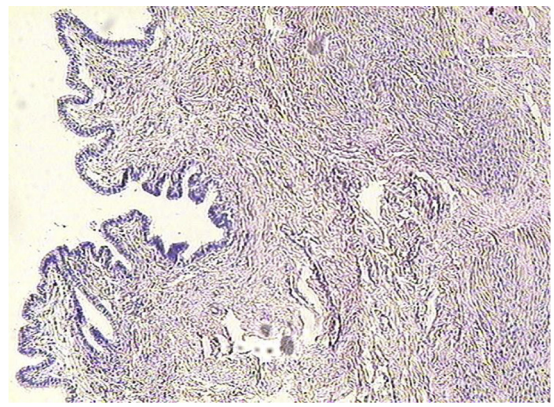

Fig. 1. Uterine glands agenesis. Mare no 1. Hematoxylin eosin staining ×10.

The histopathological evaluation of endometrial specimens from infertile mares revealed total agenesis of intrauterine glands (Figs. 1 and 2). Moreover, in both cases, animals lack nuclear ERs as evidenced by immunohistochemical reactions (Fig. 3).

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 periimplantation 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 [19–24]. 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