Theriogenology 103 (2017) 191–196

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Advanced age in mares affects endometrial secretion of arachidonic acid metabolites during equine subclinical *endometritis*

Marta J. Siemieniuch [a, b, *], Katarzyna Gajos [c], Roland Kozdrowski [d], Marcin Nowak [e]

[a] Dep. of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Tuwima 10-St, 10-748 Olsztyn, Poland
[b] Research Station of Institute of Animal Reproduction and Food Research of PAS, Popielno 25, 12-220 Ruciane-Nida, Poland
[c] EQUI-MEDICA Equine Practice, Strzelecka 25-St, 89-600 Chojnice, Poland
[d] Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Science, Dep. of Reproduction and Clinic of Farm Animals, Pl. Grunwaldzki 55, 50-366 Wrocław, Poland
[e] Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Science, Dep. of Pathology, Norwida 31-St, 50-375 Wrocław, Poland

ARTICLE INFO

Article history:
Received 26 February 2017
Received in revised form
20 July 2017
Accepted 27 July 2017
Available online 29 July 2017

Keywords:
Subclinical *endometritis*
Arachidonic acid metabolites
Aging
Mare

ABSTRACT

Even if mares continue to breed up to an advanced age, in aging mares reproductive failure is quite common. Subclinical *endometritis*, which occurs more often in aging mares than in younger counterparts, may cause prolongation or shortening of the inter-estrus period or the corpus luteum lifespan. We hypothesized that during subclinical *endometritis* the secretion of selected arachidonic acid metabolites may differ in aging mares compared to younger females. To verify this thesis, *ex vivo* organ cultures of endometrium were established with subsequent measurements of concentrations of prostaglandin E_2 (PGE_2), 6-keto-$PGF_{1\alpha}$ and both leukotrienes (LTs), LTB_4 and LTC_4 in the culture supernatants. The endometrial biopsies were obtained from 82 mares of known breeding history. This study revealed that the concentrations of the selected arachidonic acid metabolites, which act both as immunological mediators and endocrine modulators in the reproductive organs, depends on the mares' ages. Spontaneous endometrial secretion of PGE_2, 6-keto-$PGF_{1\alpha}$ and LTC_4 was increased in mares aged 16–23 years that suffered from subclinical *endometritis*, compared with control counterparts. Moreover, secretion of these metabolites was higher in *endometritis*-positive mares aged 16–23 years than in younger females. We conclude that advanced age in mares further disturbs the immuno-endocrine balance in *endometritis*-positive mares.

1. Introduction

Mares continue to breed until they reach an advanced age. The persistence of ovulatory cycles is independent of senescence in mares, however in aging mares the inter-estrus intervals and follicular phases may be prolonged as a result of extended maturation of the dominant follicle or the simultaneous presence of Cushing's syndrome [1,2]. Interestingly, aged mares present the tendency to ovulate smaller follicles [1]. Foals dropped by aging mares may be undersized or weaker than counterparts developed by younger mares, because of endometrial abnormalities such as chronic fibrosis of the endometrium, disturbances of placental vascularisation or placental undernutrition [2,3].

Infertility or subfertility in horses is one of the most important health problems in equine management, seriously reducing the benefits from equine production. It is assumed that 30% of the cases of infertility are caused by chronic fibrotic degeneration of the endometrium (*endometrosis*) [4]. Another 25–60% cases of infertility result from various kinds of inflammatory processes of the endometrium (*endometritis*) [5,6]. The majority of inflammatory processes in the equine endometrium is caused by infectious agents, such as bacteria. As a consequence, *endometritis* may lead to early embryonic losses, abortion, *placentitis* and delivery of stillborn or intrauterine-infected foals [7]. It is generally accepted that equine *endometritis* results from a compromised mechanism of uterine

* Corresponding author. Dep. of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Tuwima 10-St, 10-748 Olsztyn, Poland.
E-mail addresses: m.siemieniuch@pan.olsztyn.pl (M.J. Siemieniuch), gajka19@interia.pl (K. Gajos), roland.kozdrowski@upwr.edu.pl (R. Kozdrowski), marcin.nowak@up.wroc.pl (M. Nowak).

http://dx.doi.org/10.1016/j.theriogenology.2017.07.043
0093-691X/

clearance of fluid that contains spermatozoa, bacteria and cellular debris after mating or post-partum lochia disposal [8-10]. Based on the severity of inflammation, which is related to the influx of different cell types into the uterine wall and then the presence of cells in the discharge, clinical *endometritis* should be separated from its subclinical form, which is much more difficult to diagnose and cure. Subclinical *endometritis* is described as a form of endometrial inflammation without accumulation of fluid in the uterine lumen and vaginal discharge. In this hidden form of endometrial inflammation, clinical signs are not usually present, which often renders this condition undiagnosed. The clinical signs are unclear and equivocal, and awkward to interpret, depend on the type of infecting microorganisms, or a diffuse or focal type of infection [7]. Contact with infectious agents activates innate immunological mechanisms localized in the endometrium and stimulates production and accumulation of mediators of inflammation like interleukins and arachidonic acid metabolites in the endometrium, leading to immuno-endocrine disturbances in the uterine environment.

In a previous study we showed that specific immune mechanisms are activated less intensively in the less severe form of subclinical *endometritis*, i.e., chronic *endometritis*, than in subacute suppurative *endometritis* [11]. Besides an observation that the severity of inflammation affects the immune response in the endometrium [11], we also noted that the immune response of the equine endometrium correlates with the stage of the estrous cycle as well as the grade of endometrial fibrosis [12,13]. Studies on the course of clinical, post-breeding induction of *endometritis* (PBIE) as well as on post-partum inflammations showed that aging mares are more susceptible to chronic, hard to cure infections of the endometrium, in contrast to younger females in which acute *endometritis* is present more often than the chronic form, and besides this they recover much faster [14,15]. Based on these observations, we hypothesized that in the course of subclinical *endometritis* the secretion of selected arachidonic acid metabolites differ in aging mares compared with younger females, and potentiate the clinically observed abnormalities such as a prolonged estrous cycle caused by delayed luteolysis.

2. Materials and methods

2.1. Ethical approval for use of animals

This study was approved by the II Local Ethics Committee in Wrocław (Wrocław University of Environmental and Life Sciences, Poland). Reference number of approval: 43/2011, date: 18 April 2011.

2.2. Animals and endometrial biopsy sampling

The material was collected from 82 warmblood mares (aged 2–23 years) either suspected of subclinical *endometritis* (SE) and not suspected of *endometritis*, between February and September 2012 at a number of stud farms in the lower Silesia region of Poland (south-west Poland). Uterine biopsies and blood samples were collected with the informed consent of animals' owners.

Criteria for mares to be enrolled in the SE group were that they had been bred three or more times unsuccessfully during the breeding season, or had a history of $\geq$ one year of reproductive failure. None of the mares was in foaling heat, additionally none of the mares included in the study showed fluid in the uterus and involution of the uterus was complete. None of the mares had dystocia, retained fetal membranes or had problems during the puerperium. A blood sample was collected from the jugular vein of each mare. All mares were examined by transrectal palpation and USG (Honda HS-1500V) for genital tract evaluation and determination of estrous cycle stage and by measurement of serum progesterone (P_4) level [16,17], as described in previous studies [11,13,18]. None of the mares included in the study showed fluid in the uterus, so that no cases of clinical *endometritis* were enrolled in this study. Thirty-six mares were in estrus and had a dominant follicle, and 46 mares were in diestrus and had a corpus luteum (CL). Blood samples were kept refrigerated until centrifuged ($1500 \times g$ for 20 min) and pipetted to collect serum. Serum was stored at −20 °C until assayed. Progesterone concentrations were determined using a commercial Progesterone ELISA kit (ENZO Life Sciences Inc., Farmingdale, NY, USA; ADI-901-011).

After USG examination, the tail was bandaged, and then the vulva and perineum were cleaned with iodopovidone (Betadine, EGIS, Warsaw, Poland), rinsed three times with water, and dried with a paper towel. We collected endometrial biopsies (EB) by means of a sterilized biopsy punch (Equi-Vet, Kruuse; Denmark), as already described [11,13,18]. The instrument was passed through the vagina and cervix into the uterus with a sleeved and lubricated arm. After the forceps were placed in the uterine lumen, the arm was withdrawn from the vagina and inserted into the rectum to guide the forceps to the desired location. A uterine biopsy was obtained from a randomly selected base of the uterine horn. Samples of EB were immediately smeared onto culture media for microbiological examination as described in a previous report [11,13,18]. Afterwards, each EB was divided with sterile scissors and forceps into two pieces. One piece was fixed in 4% formaldehyde for histopathological examination, while the second piece was plunged into Dulbecco's Modified Eagle's Medium (DMEM) without phenol red (Sigma Aldrich, St. Louis, MI, USA; D6429) and transported to the laboratory at 4 °C, all within 4 h after sampling.

2.3. Histology

Endometrial biopsies were fixed in 4% formaldehyde and stained routinely with hematoxylin and eosin. The cross-sections were evaluated by light microscopy according to Kenney and Doig [19] for classification of fibrosis grade *(endometrosis,* or chronic degenerative *endometritis*). The cytological determination of SE was based on counting both mononuclear and polymorphonuclear neutrophil (PMN) cell infiltration of the endometrial luminal epithelium and *stratum compactum*. This examination was based on the criteria proposed by Ricketts [20], Ricketts and Alonso [21] and Nielsen [22]. A total of 45 (54.8%) of the mares were positive for *endometritis* and 67 (81.7%) for periglandular fibrosis. Judging by age, a total of 82 mares was assigned into 4 groups. Data concerned number of individuals in each stage of the estrous cycle, genitally normal or suffered from SE, are presented in Table 1. Of 17 mares aged 2–5 years, two had fibrosis grade 2a in histological examination.

In 23 mares aged 6–10 years, six exhibited no fibrosis, eleven exhibited fibrosis grade 2a and another six mares exhibited fibrosis

Table 1
Number of individuals in each stage of the estrous cycle, genitally normal or suffered from SE.

Stage of the estrous cycle	Age			
	Age of mares			
	2–5	6–10	11–15	16–23
	Ctr/SE	Ctr/SE	Ctr/SE	Ctr/SE
Estrus	6/2	3/6	5/4	3/6
Early diestrus	2/1	2/3	1/3	1/4
Mid diestrus	2/1	3/3	3/3	1/2
Late diestrus	2/1	2/1	1/2	1/2