Theriogenology xxx (2015) 1–10

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Altered secretion of selected arachidonic acid metabolites during subclinical endometritis relative to estrous cycle stage and grade of fibrosis in mares

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

[a] EQUI-MEDICA Equine Practice, Chojnice, Poland
[b] Department and Clinic of Reproduction, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Science, Wrocław, Poland
[c] Department of Pathology, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Science, Wrocław, Poland
[d] Department of Reproductive Immunology and Pathology, Polish Academy of Sciences, Olsztyn, Poland

ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form 10 February 2015
Accepted 30 March 2015

Keywords:
Endometritis
Prostaglandin
Leukotriene
Mare

ABSTRACT

Mares that fail to become pregnant after repeated breeding, without showing typical signs of clinical endometritis, should be suspected of subclinical endometritis (SE). Contact with infectious agents results in altered synthesis and secretion of inflammatory mediators, including cytokines and arachidonic acid metabolites, and disturbs endometrial functional balance. To address the hypothesis that SE affects the immune endocrine status of the equine endometrium, spontaneous secretion of prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 6-keto-$PGF_{1\alpha}$ (a metabolite of prostacyclin I_2), leukotriene B_4 (LTB_4), and leukotriene C_4 (LTC_4) was examined. In addition, secretion of these factors was examined relative to the grade of inflammation, fibrosis, and estrous cycle stage. Eighty-two warmblood mares, of known breeding history, were enrolled in this study. On the basis of histopathologic assessment, mares were classified as suffering from first-grade SE, second-grade SE, or being healthy. The grade of fibrosis and the infiltration of endometrial tissue with polymorphonuclear leukocytes were examined by routine hematoxylin–eosin staining. In mares suffering from SE, the secretion profiles of PGE_2, 6-keto-$PGF_{1\alpha}$, LTB_4, and LTC_4 were changed compared to mares that did not suffer from endometritis. The secretion of PGE_2 and 6-keto-$PGF_{1\alpha}$ was increased, whereas that of LTB_4 and LTC_4 was decreased. Secretion of 6-keto-$PGF_{1\alpha}$ was increased in first- and second-grade SE ($P < 0.01$). The concentration of PGI_2 metabolite was increased only in inflamed endometrium, independently of the inflammation grade, but was not affected by fibrosis. Prostaglandin E_2 secretion was increased in second-grade SE ($P < 0.05$). The secretion of LTB_4 decreased in both first- and second-grade SE ($P < 0.05$), whereas secretion of LTC_4 was decreased only in second-grade SE ($P < 0.05$). Fibrosis did not change the secretion profile of PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ during the course of SE. However, the secretion profile of LTB_4 was affected during the course of fibrosis. Evident divergences between PGE_2 and $PGF_{2\alpha}$ profiles and in PGE_2:$PGF_{2\alpha}$ ratios in the control versus SE mares observed during the course of diestrus contribute to shortened or prolonged interestrous intervals observed clinically in SE mares.

* Corresponding author. Tel.: +48 89 539 31 28; fax: +48 89 539 31 30.
E-mail address: m.siemieniuch@pan.olsztyn.pl (M.J. Siemieniuch).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.03.038

1. Introduction

Endometritis is one of the main causes of subfertility or infertility in mares, and it seriously reduces reproductive efficiency. Endometrial infections are usually caused by *Streptococcus equi* or *Escherichia coli* and are not only responsible for lowering of conception rates but also lead to early embryo losses, abortion, placentitis, stillbirths, and delivering of intrauterine-infected foals [1]. A subclinical endometritis (SE) in mares is characterized neither by fluid accumulation in the uterine lumen nor by the presence of a vulvar discharge. This lack of obvious clinical signs of inflammation renders SE difficult to diagnose and treat [1].

Studies concerning the pathogenesis and treatment of postmating-induced endometritis have reported that older mares are more susceptible than younger ones and that the course of infection varies depending on animal age. Increased mare age (>13 years) is the most limiting factor in reproductive performance in warmblood mares [2–4]. Data show that advanced age in mares is also associated with a disturbed systemic inflammatory response characterized by increased production of inflammatory cytokines, including IFN-γ and TNF-α [5,6]. Furthermore, gene expression and the arachidonic acid metabolite secretion pattern in the equine endometrium are affected during the course of fibrosis [7]. Subclinical endometritis is elicited by pathogens, including pathogenic or opportunistic bacteria and fungi, and is sustained by an inadequate immunologic response in mares. In addition to β-hemolytic *Streptococcus* spp., which is commonly isolated from clinical cases, several gram-negative bacteria are overrepresented in subclinical inflammations, including *E coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus* spp., and various fungi [1,8].

At present, the method for effective diagnosis of SE, which is usually weakly visible using ultrasound techniques, is cytologic or histopathologic examination for influx of polymorphonuclear neutrophils (PMNs) into the endometrium, compared to microbiological examination [9–11]. Although the cytologic brush approach is useful and valuable for collecting material for microbiological examination [8,11,12], it is less effective for histopathologic examinations [9,11]. Using histopathologic examination of the endometrium, both assessment of the inflammatory process and evaluation of fibrosis grade are possible. In the endometrium of healthy mares, shortly before estrus, only a few PMN cells are present, in amounts not exceeding 2% [11,13].

Data collected so far regarding alterations in inflammatory responses and deterioration of reproductive performance in older thoroughbred mares correlate these changes with an increasing risk of fibrosis, supporting the suspicion that the secretion of arachidonic acid metabolites is disturbed in SE, while the grade of fibrosis is also consistent with these changes. The aim of the present study, therefore, was to evaluate the spontaneous endometrial secretion of arachidonic acid metabolites in SE within the context of estrous cycle stage and grade of fibrosis. For this purpose, the secretion profiles of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), 6-keto-$PGF_{1\alpha}$ (a metabolite of prostacyclin I_2), leukotriene B_4 (LTB_4), and leukotriene C_4 (LTC_4) were investigated in endometrial tissue culture using endometrial biopsies.

2. Materials and methods

2.1. Animals

2.1.1. Ethical approval for the 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: 18th April 2011.

The material was collected from 67 mares suspected of SE (aged 6–23 years) and from 15 maiden mares (young, aged 3–4 years, with no history of breeding), which served as a control group. Control maiden mares have never been mated, thus were not suffering from endometritis or endometriosis. Control mares in this study were not suspected of endometritis and were suspected to be fertile if mated. The material was collected between February and September 2012 at a number of stud farms in Poland.

Mares suspected of SE had been bred three or more times unsuccessfully in the same breeding season or had a history of 1 year of reproductive failure. Some of these mares had been treated with intrauterine antibiotic infusions in a previous breeding season and, despite the treatment, they had not become pregnant. All mares were examined by transrectal palpation and ultrasonography for genital health and determination of cycle stage [14,15]. None of the mares included in the study showed fluid in the uterus. Thirty-six mares were in estrus and had a dominant follicle, and 46 mares were in diestrus and had a CL.

The mares were classified into four groups: an estrus group, early diestrus (from ovulation to 5 days after ovulation), middiestrus (from 6 to 12 days after ovulation), and late diestrus (from 13 days after ovulation) on the basis of their history, ultrasonographic examination of the reproductive tract, and determination of progesterone (P_4) concentration in the blood [7,14–16]. Mares were considered to be in estrus when ultrasonographic examination of the reproductive tract showed endometrial edema, and a follicle with a diameter more than 3.5 cm was found. Additionally, P_4 concentrations were below 1 ng/mL. The early diestrus group was characterized by the presence on the ovary of a large CL (with or without a nonechogenic central area) that had a bright echogenicity within its echogenic portion, and P_4 concentrations ranged from 1 to 8 ng/mL. Middiestrus mares had a CL characterized by less echogenicity, there was no endometrial edema, and P_4 concentrations ranged from 4 to 14 ng/mL. Late diestrus mares had a small bright CL or a CL was not found, there was also no endometrial edema, and P_4 concentrations ranged from <1 to 3 ng/mL. Jugular vein blood samples were collected into heparinized tubes. Samples were kept refrigerated until centrifuged (1500 × *g* for 20 minutes) and decanted. Plasma was stored at −20 °C until assayed. Progesterone concentrations were determined using a commercial Progesterone ELISA kit (ADI-901-011; ENZO Life Sciences Inc., Farmingdale, NY, USA).

Endometrial biopsies were collected as already described [8]. Briefly, a sterilized biopsy punch was used (EQUIVET; Kruuse, Denmark). The instrument was passed through the vagina and cervix into the uterus with a sleeved and lubricated arm. After the forceps were placed