Theriogenology 87 (2017) 193–204

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Endometrial prostaglandin synthases, ovarian steroids, and oxytocin receptors in mares with oxytocin-induced luteal maintenance

Maria R. Rebordão [a,b], António Galvão [a,c], Pedro Pinto-Bravo [a,b], Joana Pinheiro [b], Sandra Gamboa [b], Elisabete Silva [a], Luísa Mateus [a], Graça Ferreira-Dias [a,*]

[a] CIISA, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[b] Department of Animal Science, Coimbra College of Agriculture, Coimbra, Portugal
[c] Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research of PAS, Olsztyn, Poland

ARTICLE INFO

Article history:
Received 10 April 2016
Received in revised form 12 August 2016
Accepted 25 August 2016

Keywords:
Mare
Luteal phase
Oxytocin
Endometrium
Steroids receptors
Oxytocin receptor

ABSTRACT

Oxytocin (OXT) has been used to prolong the luteal phase in mares, but its mechanism of action is unknown. The aim of this study was to evaluate the effect of chronic exogenous OXT administration to mid-luteal phase mares on luteal maintenance. Also, endometrial expression of prostaglandin endoperoxide synthase 2 (PTGS2), prostaglandin $F_2\alpha$, E_2 and I_2 synthases (AKR1C3, PTGES, and PTGIS), oxytocin receptor (OXTR), progesterone receptor (PGR), and estrogen receptors 1 (ESR1) and 2 (ESR2) were assessed in mares experiencing luteal maintenance 2 weeks after chronic exogenous OXT administration. Control mares ($n = 5$; C group) received 6 mL of saline im, whereas OXT (60 units/mare) was administered im ($n = 6$; OXT group), every 12 hours, on days 7 to 14 postovulation. After endometrial biopsy in groups C (Day 10) and OXT (Day 24), luteolysis occurred within 3 or 6 days, respectively. Luteal maintenance took place in 4 of 6 (67%) of OXT-treated mares. Progesterone in C group was the highest on biopsy day ($P < 0.05$). In OXT mares, *PTGS2*, *ESR1* ($P < 0.05$), *PTGES*, *PTGIS*, *PGR*, and *ESR2* ($P < 0.01$) gene transcription decreased, whereas *OXTR* increased ($P < 0.05$) in comparison with the C group. In OXT-treated mares, endometrial ESR2 protein expression decreased ($P < 0.05$), but OXTR increased ($P < 0.05$) compared with control animals. In both experimental groups, PTGS2 was mainly immunolocalized in surface epithelium, whereas AKR1C3, PTGES, PTGIS, and PGR were in surface and glandular epithelia. ESR1 and ESR2 were found in glandular epithelium and OXTR in stromal cells. High immunolabeling for PTGES, PTGIS, PGR, and OXTR and low for ESR2 was detected in endometrium of OXT-group mares with extended diestrus. Prolonged luteal function associated with chronic OXT treatment may be related to different spatial expression of OXTR and PGR in the endometrium. The observed reduction of endometrial ESR2 may be responsible for the maintenance of PGR in luminal and glandular epithelium. Also, ESR2 may attenuate the transcriptional activity of *ESR1* in mare endometrium. This study offers new knowledge on the endometrial expression of ovarian steroids and OXT receptors in OXT pharmacologically induced luteal maintenance in the mare.

* Corresponding author. Tel./fax: +(351) 213 652889.
E-mail address: gmlfdias@fmv.ulisboa.pt (G. Ferreira-Dias).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.08.028

1. Introduction

Oxytocin (OXT) exerts a variety of actions and is involved in a large number of physiological processes, such as regulation of luteal function. Oxytocin is stored in posterior pituitary vesicles and released into the peripheral circulation in high-frequency bursts in late diestrus [1]. In the mare, this hormone is also synthesized by endometrial cells and secreted into the uterine lumen where it has an important role in the autocrine/paracrine control of uterine contractility and luteolysis [2]. Endometrial OXT concentrations are positively correlated with serum estradiol-17beta (E2) concentration [3,4]. Nevertheless, hormonal regulation of OXT receptors (OXTR) in equine endometrium appears to differ from ruminants [5] because in mares OXTR expression is low at estrus [6,7].

Luteolysis occurs on days 14 to 16 of the equine estrous cycle [8]. Although E2 is apparently not required for luteolysis in mares [9], in other mammals, E2 is considered a key regulator of *OXTR* gene expression [10]. In the mare, E2 plasma concentrations begin to increase approximately 2 days before the onset of luteolysis [9]. In late luteal phase (days 14 and 15), *OXT* gene expression [3] and OXTR concentration in mare endometrium increase and mediate prostaglandin (PG) release [6,11]. Afterward, OXTR activation stimulates mitogen-activated protein kinase, which regulates prostaglandin–endoperoxide synthase gene expression [12]. At Day 15, an increase in both mRNA and protein levels of prostaglandin endoperoxide synthase 2 (PTGS2) in luminal epithelium has been detected [13,14]. These enzymes convert arachidonic acid to PGH2, which can be converted to PGF2α by AKR1C3 in the uterus. OXT binding to its receptor is responsible for stimulating both frequency and amplitude of endometrial surges of PGF2α necessary for luteolysis [6,11]. In response to PGF2α luteolytic pulses over a 23-h period, the corpus luteum (CL) undergoes functional and structural regression (luteolysis) and progesterone (P4) progressively decreases to values <1 ng/mL [8].

Although endogenous OXT action is involved in PGF2α endometrial secretion during spontaneous luteolysis in mares [6,11,15], PGF2α response to exogenous OXT treatment depends on the phase of the estrous cycle [16,17]. Although PGF2α endometrial secretion is highest around the time of luteolysis (days 11–15 postovulation) and is associated with an increase in endometrial OXTR expression [6,11], when exogenous OXT is administered in mid-luteal phase (days 8–14), it blocks luteolysis and prolongs the luteal phase [15,18–21]. The exact mechanism involved in this action is unknown.

Because ovarian steroid hormones are essential mediators of utero-ovarian function acting through specific receptors and PG pathways, their relationship with OXT throughout the mare estrous cycle has to be considered. As in other mammalian species, in mare endometrium, E2 produced in the follicular phase upregulates its own nuclear receptors (estrogen receptors [ESR]) and P4 receptors (PGR) [22]. The effects of E2 are exerted in the endometrium *via* ESR1 and ESR2, which are the two main classical nuclear receptor isoforms [23]. Although there are two predominant isoforms of nuclear PGR (PGR-A and PGR-B), PGR-A is the major functional isoform in the uterus [24]. In the mare, during estrus, high endometrial levels of ESR1, ESR2, and PGR mRNA and protein have been detected in luminal, glandular epithelia, and stromal cells [22,25–28]. Moreover, the relative mRNA expression of *ESR1* and *ESR2* in mare endometrium seems to be positively correlated [26]. In the luteal phase, when circulating levels of P4 are high, endometrial expressions of ESR1, ESR2, and PGR are inhibited [22]. This expression decreases in stroma and deeper glandular epithelium and does not exist in luminal epithelium on days 11 and 14 of the mare estrous cycle [22]. Between days 17 and 20 of the estrous cycle, both ESR and PGR transcripts and protein increase in luminal and glandular epithelia and in stromal cells [22].

We hypothesized that chronic OXT administration to mares in mid-luteal phase would be able to prolong luteal function by modulating PG pathways, steroid hormones, and OXT receptors in the endometrium. Several studies have implicated those endometrial pathways in the beginning of OXT-induced luteal maintenance (Day 14 postovulation) [15,18,19,21]. Thus, the aim of the present study was to evaluate the endometrial expression of PTGS2, AKR1C3, PTGES, PTGIS, OXTR, PGR, ESR1, and ESR2 after the administration of exogenous OXT in mid-to-late luteal phase (days 7–14 after ovulation) to induce luteal maintenance.

2. Materials and methods

2.1. Animals

This study was carried out on Lusitano mares aged from 3 to 17 years, weighing between 450 and 500 kg. All had normal estrous cycles throughout the study period (between early June and early August). The animals were housed in boxes, fed grass hay, and commercial concentrate mix with free access to trace mineral salt blocks and water. All mares were routinely subjected to regular husbandry procedures, such as deworming and vaccinations. The protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

2.2. Experimental design

Throughout the entire experimental period, all mares were teased daily by a single stallion to detect estrous behavior. Each mare's genital tract was evaluated every-other-day by transrectal palpation and ultrasonography (Falco, 6 MHz linear transducer) for detection of ovulation (Day 0), CL size monitoring, and signs of estrus return (endometrial edema). A crossover design was used, so that the same animal received each of the 2 treatments sequentially. On Day 7 of the estrous cycle, mares were randomly assigned to 2 groups: control group ($n = 5$; C group) and oxytocin group ($n = 6$; OXT group). On days 7 to 14 after ovulation, OXT mares received 60 units (10 units/mL) of oxytocin, im, every 12 hours; control mares received 6 mL of saline solution im on the same schedule. Blood samples were obtained every-other-day *via* jugular venipuncture.