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Short communication

Dual effect of hormones on mare reproductive physiology and dysfunction

Maria R. Rebordão[1,2], Ana Amaral[1], António Galvão[1,3], Anna Z. Szótek[3], Pedro Pinto-Bravo[2], Dariusz S., Skarzynski[3] and Graça Ferreira-Dias[1]

[1] CIISA, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2] Coimbra College of Agriculture, Coimbra, Portugal
[3] Institute of Animal Reproduction and Food Research, PAS, Olsztyn, Poland

Summary: In the last decades, classical and molecular biology approaches have greatly contributed for building up sound knowledge on the mechanisms regulating reproductive processes in the mare. Different perspectives on the role of hormones should be addressed and thus considered by both researchers and clinicians. While oxytocin is largely used as a post-insemination method to improve fertility or prevent endometritis, it may also be considered to prolong the luteal phase. Prostaglandin E2 (PGE2), known to sustain luteostasis, has also been related to inflammatory processes in sub-clinical endometritis in mares. In contrast, as other ecbolic agents, PGF2α besides being luteolytic and currently used for estrous cycle shortening and endometritis control, might also play a role in fibrogenesis in mare endometrium. Hormones, like oxytocin, ovarian steroids, prostaglandins (PG), as well as enzyme profiles involved in endocrine pathways may act in a dissimilar fashion, depending on the estrous cycle phase and on the physiologic or pathologic condition.

Keywords: mare / reproduction / oxytocin / prostaglandin / endometrium / fibrosis / ovarian steroids

Doppeleffekt von Hormonen auf die Reproduktionsphysiologie und -störung bei der Stute

In den letzten Jahrzehnten haben die klassischen und molekularbiologische Methoden in hohen Maße für die Entwicklung fundierten Wissens zu den Regelmechanismen reproduktiver Prozesse bei der Stute beigetragen. Es sollten die unterschiedliche Sichtweisen auf die Rolle der Hormone berücksichtigt werden, sowohl von Forschern als auch Klinikern. Während Oxytocin überwiegend als Verfahren nach der Insemination verwendet wird, um die Fruchtbarkeit zu verbessern oder eine Endometritis zu verhindern, kann es auch eingesetzt werden, um die Gelbkörperphase zu verlängern. Prostaglandin E2 (PGE2) ist bekannt, die Luteostase zu erhalten, wird aber auch mit entzündlichen Prozessen bei der equinen subklinische Endometritis in Verbindung gebracht. Im Gegensatz dazu könnte PGF2α, wie andere ekbolische Mittel, abgesehen davon, dass es luteolytisch wirkt und derzeit benutzt wird, den Ovarialzyklus zu verkürzen und eine Endometritis zu kontrollieren, auch eine Rolle bei der Fibrogenese im equinen Endometrium spielen. Hormone wie Oxytocin, Ovarialsteroide, Prostaglandine (PG), als auch Enzymprofile, die an endokrinen Regelkreisen beteiligt sind, können in einer unterschiedlichen Art und Weise wirken, abhängig von der Zyklusphase und dem physiologischen oder pathologischen Zustand.

Schlüsselwörter: Stute / Reproduktion / Oxytocin / Prostaglandin / Endometrium / Fibrose / ovarielle Steroide

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Correspondence: Prof. Graça Ferreira-Dias, Faculty of Veterinary Medicine, Av. Universidade Técnica, 1300-477 Lisboa, Portugal; E-mail: gmlfdias@fmv.ulisboa.pt

Introduction

The current use of both classical and molecular biology approaches to deepen equine endocrinology knowledge has enabled the use of hormones in horse reproduction, either in normal or in pathological conditions. Ovarian steroid hormones have long been known to play a crucial role on mare reproductive function. The main ovarian steroids that rule reproductive physiological events in the mare are estrogens (E2) synthetized by ovarian follicles in the follicular phase, and progesterone (P4) produced by the corpus luteum (CL) in the luteal phase. In fact, in the mare endometrium, during estrus, high levels of ovarian steroid receptors, such as ER , ER and PR mRNA and protein have been detected in luminal and glandular epithelia, as well as in stromal cells (*Watson* et al. 1992, *Gebhardt* et al. 2012, *Silva* et al. 2014). In addition, a positive correlation between mRNA expression of ER and ER has been reported in mare endometrium (*Honnens* et al. 2011). In the luteal phase, when circulating P4 levels are high, endometrium expression of ER and PR are inhibited. In mare endometrium, E2 up-regulates ER and ER nuclear receptors, and also P4 receptors (PR) (*Silva* et al. 2014). When ovarian steroids reach the equine endometrium, they modulate cell proliferation, angiogenesis and prostaglandin (PG) secretion (*Galvão* et al. 2013, *Szóstek* et al. 2014). Besides, they also up-regulate prostaglandin endoperoxide synthase 2 (PTGS2), prostaglandin E2 synthase (PGES), and prostaglandin F2α (PGF2α) synthase (PGFS) expression in mare endometrium (*Szóstek* et al. 2014). Therefore, ovarian steroid-stimulated PG synthesis could be an important mechanism of estrous cycle regulation and early pregnancy maintenance (*Szóstek* et al. 2014). Besides, the coordinated action of ovarian steroid hormones, cytokines and nitric oxide (NO) in mare endometrium may regulate endometrium cell proliferation, angiogenesis and secretory function, modulating PGE2 and PGF2α production (*Galvão* et al. 2013, *Roberto da Costa* et al. 2008).

Prostaglandin and Oxytocin in endometrium

Strong evidence supports a role for PG, enzymes involved in their synthesis, and prostaglandin receptor signaling pathways in a multitude of physiologic events in reproductive tissues including the promotion of ovulation, endometrial physiology, proliferation of endometrial glands, angiogenesis and vascular function (*Jabbour* et al. 2006). In each ovarian cycle, for ovarian activity resumption to occur in the absence of pregnancy, PGF2α produced by the endometrium, and the presence of oxytocin (OXT) and its receptors (OXTR) are necessary for luteolysis. Exogenous natural PGF2α and its analogs have also been used frequently as a luteolysin in reproductive management programs in horses (*Meyers* 1997). When PGF2α treatment is used on Days 4–6 of the luteal phase it has a luteolytic effect in almost all mares, while on Day 0 or 1 it has a retarding effect on P4 output, and on Day 2 or 3 a transient regressive effect with resurgence to control levels in most mares (*Gastal* et al. 2005).

In spite of OXT being produced in the hypothalamus, stored in posterior pituitary vesicles, and released into the peripheral circulation, its synthesis and secretion by endometrial cells into the uterine lumen was also shown. There, it plays an important role in the autocrine/paracrine control of uterine contractility and luteolysis (*Watson* et al. 2000). When mares are exposed to any breeding stimuli, such as visual contact with a stallion, active teasing, stallion call, genital tract and uterine distension or artificial insemination, there is OXT release and a rapid onset of myometrial contractions (*Madill* et al. 2000, *Nikolokopoulos* et al. 2000), but only rarely in PGF2α metabolite (*Nikolokopoulos* et al. 2000).

In mares at estrus, even though OXT-mRNA levels are positively correlated with serum E2 levels (*Watson* et al. 2000), OXTR in endometrium are low (Ruijter-Villani et al., 2014). In the late luteal phase (day 14–15), OXT endometrium gene expression and OXTR concentration increase and mediate PGF2α release (*Ruijter-Villani* et al. 2014), resulting in structural and functional luteolysis with plasma P4 decrease to values below 1 ng/mL (*Ginther* et al. 2011).

Even though during spontaneous luteolysis endogenous OXT production regulates endometrium PGF2α secretion, mare s PGF2α response to exogenous OXT depends on her physiological state (estrous cycle phase or pregnancy). In cyclic mares, this response is the highest at luteolysis (late luteal phase) and related to increased endometrial OXTR (*Ruijter-Villani* et al. 2014). Instead, OXT administration to mares in mid luteal phase (day 8 to 14) blocks luteolysis and extends the luteal phase (*Stout* et al. 1999, *Keith* et al. 2013). Nevertheless, the exact mechanism involved in this action needs to be determined. In our latest work, from Day 7 to Day 14 after ovulation, mares received OXT every 12 h, and endometrial expression of prostaglandin-endoperoxide synthase 2 (PTGS2), prostaglandin F2α synthase (PGFS), prostaglandin E2 synthase (PGES), prostacyclin I synthase (PGIS), OXTR, PR, ERα and ER was assessed. In 67% of OXT treated mares, extended luteal function was observed. In the endometrium of OXT treated mares with prolonged luteal function, PGES, PGIS, PR and OXTR, immunolabeling was increased. Those mares endometrium also presented overexpression of OXTR and under expression of ERα. These findings suggest that prolonged luteal function associated with chronic OXT treatment may involve desensitization rather than downregulation of OXTR. In addition, chronic OXT administration to mares may be related to non-genomic P4 actions that block OXT binding to OXTR with consequent inhibition of PGF2α luteolytic pulses.

Ecbolic hormones and disease

It has been long recognized in the field of reproduction that PGE2 has luteotrophic properties, while PGF2α is luteolytic. Nevertheless, besides mediating homeostatic functions, PG are lipid autacoids involved in some pathogenic mechanisms, such as inflammation. Ecbolic hormones such as PGF2α and OXT have been used to promote mare uterine contractility in mares in some pathologic conditions. In fact, post-mating uterine lavage and OXT treatment have been reported to improve fertility rate in mares (*Gores-Lindholm* et al. 2013), or as a treatment for post-breeding delayed uterine clearance in mares susceptible to endometritis (*LeBlanc* et al., 1994).

The ecbolic PGF2α, together with uterine neutrophil cell count, has lately been regarded as the most accurate marker of inflammation during mating-induced endometritis (*Nash* et al. 2010). In fact, mating-induced endometritis in pony mares using intrauterine frozen/thawed semen or frozen/thawed extender caused a rise in PGF2α concentrations in uterine fluid 16 h after treatments (*Nash* et al. 2010). This dramatic elevation of PGF2α in the beginning and middle of the luteal phase in mares with endometritis may be responsible for shortening of estrous cycle, which is frequently observed clinically (*Gajos* et al. 2015). But when, instead of an increase in PGF2α, a rapid decrease of this hormone occurs in late diestrus, prolongation of the estrous cycle and postponed luteolysis are often encountered clinically in mares exhibiting pyometra (*Gajos* et al. 2015). In inflamed mare uteri, besides changes in PGF2α in uterine fluid, increased secretion of PGE2 might be responsible for the presence of edema and enlarged endometrial folds depicted by ultrasound examination (*Gajos* et al. 2015).

In other fields besides reproductive physiopathology, pathways involving PG, which act locally through specific receptors, have been considered in pathological conditions such as fibrogenesis (*Oga* et al. 2009). In heart and lungs, PGF2α stimulates fibrogenesis, independent from fibrotic cytokines such as TGFβ-1, by increasing collagen I and III synthesis by fibroblasts via a PGF2α receptor (FP) (*Ding* et al. 2012, *Oga* et al. 2009, *Oga* et al. 2013). While PGE2 triggers several antifibrotic actions in some cells by binding to E prostanoid receptors (EP), transcriptional or epigenetic decreases in EP2 or EP4 expression can limit the inhibitor signaling of PGE2 in lung (*Bozyk* and *More* 2011) and renal (*Nakagawa* et al. 2012) fibroblasts. Thus, both PGF2α and PGE2 might act together towards fibrosis deposition (*Olman* 2009).

In woman endometrium, PG action through specific receptors and signaling pathways have been associated to benign pathologies, triggering vasoconstriction, increasing myometrial contractions and pain (*Jabbour* et al. 2006). Endometrosis is a chronic degenerative process characterized by paramount fibrosis development in the mare endometrium, which is responsible for embryo loss. In the course of endometrosis, PG production and mRNA transcription of prostaglandin (PG)

synthases differ from healthy mare endometrium (*Szóstek* et al. 2012). Thus, PG changes may influence estrous-cycle disorders, early embryo mortality and infertility.

As part of the innate immunity response to infectious stimuli, neutrophils (PMN) are able to cast out their DNA and form neutrophil extracellular traps (NETs) (*Brinkmann* 2004). We have shown that equine PMN are able to form NETs in vitro, when in presence of bacteria causing endometritis in the mare, and also in the uteri of mares with endometritis by E .coli or/and S. zooepidemicus (*Rebordão* et al. 2014). When we tested in vitro the role of NETs components on mare endometrium, a decrease in PGE2 production and a rise in mRNA type 1 collagen level were observed, which may lead to fibrosis establishment.

In conclusion, it appears that in persistent endometritis in the mare, modifications in PG production may not only cause early luteolysis, but may also be related to endometrial fibrosis establishment. Understanding the complexity of reproductive endocrinology, mainly the role of ecbolic hormones and PG-driven mechanisms is vital for identification of potential pharmacological targets for prevention and treatment of mare reproductive problems.

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