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Ovarian steroids, oxytocin, and tumor necrosis factor modulate equine oviduct function

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ABSTRACT

The oviduct plays important roles in the early reproductive process. The aim of this study was to evaluate gene transcription and protein expression of progesterone receptor (PGR), estrogen receptors 1 (ESR1) and 2 (ESR2); oxytocin receptor (OXTR); prostaglandin F2α synthase (AKR1C3), and prostaglandin E2 synthase (PTGES) in mare oviduct in different estrous cycle stages. Estradiol (E_2), progesterone (P_4), oxytocin (OXT), and tumor necrosis factor α (TNF) effect on in vitro PGE_2 and prostaglandin F2α ($PGF_{2\alpha}$) secretion by equine oviduct explants or by oviduct epithelial cells (OECs) were also assessed. During the breeding season, oviduct tissue was obtained post mortem from cyclic mares. Protein of ESR1, ESR2, PGR, AKR1C3, and PTGES was present in OECs, whereas OXTR was shown in oviduct stroma. In follicular phase, protein expression of ESR1, ESR2, PGR, and OXTR increased in oviduct explants ($P < 0.05$), whereas no estrous cycle effect was noted for AKR1C3 or PTGES. In follicular phase, mRNA transcription was upregulated for *Pgr* but downregulated for *Oxtr, Ptges*, and *Akr1c3* ($P < 0.05$). Nevertheless, *Esr1* and *Esr2* mRNA levels did not change with the estrous cycle. In the ampulla, *Esr1, Esr2*, and *Oxtr* mRNA transcription increased, but not for *Pgr* or *Ptges*. In contrast, *Akr1c3* mRNA level was upregulated in the infundibulum ($P < 0.05$). In follicular phase, E_2, P_4, and OXT downregulated PGE_2 production by OEC ($P < 0.05$), but no difference was observed in mid-luteal phase. Explants production of PGE_2 rose when treated with OXT in follicular phase; with TNF or OXT in early luteal phase; or with TNF, OXT, or P_4 in mid-luteal phase. $PGF_{2\alpha}$ production by OEC was downregulated by all treatments in follicular phase but upregulated in mid-luteal phase ($P < 0.05$). Oviduct explants $PGF_{2\alpha}$ production was stimulated by TNF or OXT in all estrous cycle phases. In conclusion, this work has shown that ESR1, ESR2, OXTR, PTGES, and AKRLC3 gene transcription and/or translation is estrous cycle dependent and varies with oviduct portion (infundibulum vs ampulla) and cell type. Ovarian steroid hormones, OXT and TNF stimulation of $PGF_{2\alpha}$ and/or PGE_2 production is also estrous cycle dependent and varies in the different portions of mare oviduct. Differential transcription level and protein localization in various portions of the oviduct throughout the estrous cycle, as well as PG production, suggest coordinated physiologic actions and mechanisms of steroid hormones, OXT, and TNF in the equine oviduct.

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1. Introduction

The oviduct is an active organ that provides the necessary conditions for oocyte maturation, sperm capacitation, fertilization, and early embryonic development and transport. Since the equine embryo spends its first 6 d of life in the oviduct, near the ampulla-isthmus junction [1], the physiologic importance of the oviduct should be better investigated in this species. Once in the oviduct, the equine embryo affects the maternal secretion pattern of proteins involved in pregnancy-related pathways [2].

The regulation of oviduct muscular and secretory activity for optimal gametes and embryo transport is influenced by ovarian steroids [3–6], adrenergic nerves [7,8], nitric oxide [9–12], oxytocin (OXT) [3,4,13] prostaglandins (PGs) [3,4,14], and cytokine TNFα (TNF) [12], among others. Ovarian steroids also conduct a series of changes through proteomic and nongenomic pathways in the oviduct epithelium, affecting gene expression, proteome, and secretion of oviduct fluid [15]. Recent studies have shown that the equine oviduct is an organ highly responsive to local changes in progesterone (P_4) and 17β-estradiol (E_2) concentrations affecting oviduct steroidogenic capacities and P_4 receptor expression [5,6]. Both E_2 and P_4 exert their actions on the oviduct through their specific receptors. As master regulators of oviduct functions, estradiol receptors and P_4 receptors (PGRs) rule the expression of downstream target genes [16]. As tubal motility is decreased by the P_4-induced reduction in both beat frequency of cilia and frequency of contractions, this steroid hormone may have an inhibitory action on human tubal activity [4,17,18]. It has been shown that in vivo P_4 concentrations can be very high in mare oviductal tissue and fluid ipsilateral to the ovulation side [6]. Besides, P_4 and E_2 are able to modulate mare oviduct ciliary activity, cell ultrastructure, transcription of embryotrophic genes, as well as oviduct fluid composition, shown by changes in glucose consumption and lactate production [5].

Ovarian steroids themselves could also be involved in prostaglandin F2α ($PGF_{2\alpha}$) and E_2 (PGE_2) production in the rat oviduct [15]. Estradiol has been shown to upregulate the expression and activity of prostaglandin synthase 2 (PTGS2), an enzyme involved in PG synthesis. This stimulatory effect may be receptor mediated [15]. In the mare, oviduct treatment with PGE_2 hastens the transport of equine embryos throughout this organ, which suggests a role for embryonic PGE_2 in the initiation of selective oviduct transport [19,20]. In the presence of sperm cells, bovine oviduct epithelial cells produce $PGF_{2\alpha}$ that might stimulate spermatozoa transport [21]. The importance of PGE_2 and $PGF_{2\alpha}$ in oviduct, either present in seminal plasma or produced by oviduct cells themselves, may contribute for gametes and embryo transport and also for signaling between the embryo and the oviduct, which might be crucial for embryo development [21,22].

Oxytocin, whose action is mediated by specific OXT receptors (OXTR), also play a role in oviduct contraction or relaxation, in cyclic and/or pregnant females and in PG synthesis in several species such as cow, woman, or bitch [4,23–25]. In addition, a study of ours on mare endometrium indicates that TNF production is closely related to ovarian steroid actions and the interaction between TNF and PG regulates endometrium physiologic processes [33]. Thus, we hypothesized that ovarian steroids, OXT and TNF differentially modulate equine oviductal secretions. Therefore, tissue explants and cell cultures were used to test this hypothesis. Gene expression and the presence of ESR1, ESR2, PGR, OXTR, as well as AKR1C3 and PTGES in mare oviduct portions were investigated throughout the estrous cycle. Furthermore, the effect of E_2, P_4, OXT, and TNF on in vitro PG secretion by the equine oviduct was assessed.

2. Materials and methods

2.1. Collection of mare oviducts

Blood samples and internal genitalia were collected post mortem at the abattoir, as by-products, from randomly designated 30 cyclic mares (3 to 9 years old) from early April to late September. After stunning, mares were euthanized, according to Portuguese legislation (DL 98/96, Art. 1°) and European Legislation concerning welfare aspects of animal stunning and euthanasia methods (EFSA, AHAW/04-027) and approved by the Faculty of Veterinary Medicine Ethics Committee, University of Lisbon, Lisbon, Portugal.

Because the reproductive status of the mares was unknown, blood samples were collected at the time of exsanguination into heparinized tubes (Monovettes; Ref. 02.265, Sarstedt, Numbrecht, Germany) and the estrous phase was further confirmed by plasma P_4 concentrations, as described [27,28]. Follicular, early and mid-luteal stages of the estrous cycle were identified based on follicle size and morphological appearance of the *corpus luteum* (CL). Briefly, mid-luteal phase (MLP) was considered in the presence of a mature CL, associated with follicles 15 to 20 mm in diameter and $P_4 > 6$ ng/mL; early luteal phase (ELP) was considered in the presence of *corpus hemorragicum* (CH), absence of major follicles and $P_4 > 1$ ng/mL; follicular phase (FP) was considered in the presence of a preovulatory follicle 35–40 mm in diameter, absence of CL, visible edema of endometrium, and $P_4 < 1$ ng/mL. Oviducts from mares with apparent reproductive problems, such as endometritis, were discarded from the study. Thus, oviducts from the ipsilateral side to the predominant ovarian structure (ie, CH, CL, follicle) from healthy mares were used in this study and based on the criteria aforementioned and grouped as follows: follicular phase ($n = 10$), early luteal phase ($n = 10$) or mid-luteal phase ($n = 10$). Immediately after collection of mares internal genitalia, the oviducts from half of the mares ($n = 15$; 5/phase) were assigned for oviduct epithelial cells (OECs) culture, whereas the other half ($n = 15$; 5/phase) for explant culture, gene, and protein expression quantification and immunohistochemistry (IHC) studies. Thus, oviducts for explants study were gently separated into its 3 portions (infundibulum, ampulla, and isthmus) and immersed in specific solutions: (i) RNA later (AM7020, Ambion, Applied Biosystems, CA, USA) for gene and protein expression quantification; (ii) sterile transport medium Hank's balanced salt solution (HBSS; 55021C; Sigma) with 0.1% bovine serum albumin (BSA), 20-μg/mL gentamicin (G1397; Sigma), and 250-g/mL amphotericin