Animal Reproduction Science 151 (2014) 169–181

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Expression of receptors for ovarian steroids and prostaglandin E2 in the endometrium and myometrium of mares during estrus, diestrus and early pregnancy

E.S.M. Silva[b], K.E. Scoggin[a], I.F. Canisso[a], M.H.T. Troedsson[a], E.L. Squires[a], B.A. Ball[a,*]

[a] Reproduction Laboratory, Maxwell H. Gluck Equine Research, Department of Veterinary Science, University of Kentucky, Lexington KY 40546-0099, USA

[b] Faculdade de Medicina Veterinária e Zootecnia, UNESP–Universidade Estadual Paulista, Departamento de Reprodução Animal e Radiologia Veterinária, Botucatu, São Paulo, CEP: 18618-970, Brazil

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 31 October 2014
Accepted 3 November 2014
Available online 10 November 2014

Keywords:
Steroid receptor
Prostaglandin E2 receptor
Estrous cycle
Pregnancy
Uterus
Equine

ABSTRACT

The objective of this study was to compare expression of estrogen receptor alpha (ER-α), β (ER-β), progesterone receptor (PR), as well as prostaglandin E2 type 2 (EP2) and 4 (EP4) receptors in the equine myometrium and endometrium during estrus, diestrus and early pregnancy. Tissues were collected during estrus, diestrus, and early pregnancy. Transcripts for ER-α (ESR1), ER-β (ESR2), PR (PGR), EP2 (PTGER2) and EP4 (PTGER4) were quantified by qPCR. Immunohistochemistry was used to localize ER-α, ER-β, PR, EP2 and EP4. Differences in transcript in endometrium and myometrium were compared by the ΔΔCT method. Expression for ESR1 ($P<0.05$) tended to be higher during estrus than diestrus in the endometrium ($P=0.1$) and myometrium ($P=0.06$). In addition, ESR1 expression was greater during estrus than pregnancy ($P<0.05$) in the endometrium and tended to be higher in estrus compared to pregnancy in the myometrium ($P=0.1$). Expression for PGR was greater ($P<0.05$) in the endometrium during estrus and diestrus than during pregnancy. In the myometrium, PGR expression was greater in estrus than pregnancy ($P=0.05$) and tended to be higher during diestrus in relation to pregnancy ($P=0.07$). There were no differences among reproductive stages in ESR2, PTGER2 and PTGER4 mRNA expression ($P>0.05$). Immunolabeling in the endometrium appeared to be more intense for ER-α during estrus than diestrus and pregnancy. In addition, immunostaining for PR during pregnancy appeared to be more intense in the stroma and less intense in glands and epithelium compared to estrus and diestrus. EP2 immunoreactivity appeared to be more intense during early pregnancy in both endometrium and myometrium, whereas weak immunolabeling for EP4 was noted across reproductive stages. This study demonstrates differential regulation of estrogen receptor (ER) and PR in the myometrium and endometrium during the reproductive cycle and pregnancy as well as abundant protein expression of EP2 in the endometrium and myometrium during early pregnancy in mares.

1. Introduction

Changes in the mare's reproductive tract throughout the estrous cycle and early pregnancy are coordinated by

* Corresponding author.
E-mail address: b.a.ball@uky.edu (B.A. Ball).

http://dx.doi.org/10.1016/j.anireprosci.2014.11.001
0378-4320/

ovarian steroids (Ginther, 1992). Both estrogen and progesterone bind to nuclear receptors and act through induction of transcription for a variety of genes, thus regulating cell development and differentiation (DeFranco, 2002).

Estrogen mediates its effects through two types of receptors (i.e. α and β) encoded by different genes, ESR1 and ESR2 (Enmark et al., 1997). Estrogen receptor α (ER-α) is the predominant estrogen receptor (ER) in the uterus (Weihua et al., 2000). It plays a major role in the uterotrophic effects of estrogen, as evidenced by a loss of cellular proliferation and secretory protein production in the uterus of adult female ER-α knockout mice (Lubahn et al., 1993). Estrogen receptor β (ER-β) has a high degree of sequence homology with the classical ER-α (Hiroi et al., 1999) and has been described in the uterus of women (Matsuzaki et al., 1999), mice (Weihua et al., 2000), and more recently the horse (Honnens et al., 2011). Adult female ER-β knockout mice were infertile or had signs of subfertility, demonstrating that this receptor is important in female reproduction (Weihua et al., 2000). In addition, it has been suggested that ER-β modulates the uterotrophic effects of ER-α (Weihua et al., 2000).

Progesterone mediates its functions through two isoforms of nuclear receptors (PGR isoform A and PGR isoform B), which are encoded by the same gene (Mote et al., 2006). The isoform PGR-A plays a major role in mediating the actions of progesterone in the uterus and ovaries, while PGR-B is more important in the development of the mammary gland (Mulac-Jericevic et al., 2000, 2003). Studies with progesterone receptor (PR) in knockout mice demonstrated that PR is essential for secondary sexual development and function of progesterone targeted organs (Lydon et al., 1995).

In the horse, dynamics of ER and PR during the reproductive cycle and early pregnancy have been primarily described in the endometrium (Aupperle et al., 2000; Hartt et al., 2005; McDowell et al., 1999; Tomanelli et al., 1991; Watson et al., 1992). To date, there are no studies addressing the distribution of ER and PR in the equine myometrium during either the estrous cycle or early pregnancy. Thus, knowing the dynamics of these steroid receptors during the estrous cycle and early pregnancy will allow us to better understand normal mare physiology.

Prostaglandins, in addition to steroids, are potent mediators in the female reproductive tract (Sales and Jabbour, 2003). Prostaglandin E_2 (PGE_2) is probably the most versatile prostanoid modulator of reproductive events (e.g. ovulation, fertilization, implantation and parturition; Lim et al., 1997). Effects of PGE_2 are mediated by four receptor sub-types which are encoded by different genes: EP1, EP2, EP3 and EP4 (Narumiya et al., 1999). Isoforms EP2 and EP4 are known as relaxant receptors, which mediate an increase in cyclic AMP and induce smooth muscle relaxation (Narumiya et al., 1999; Narumiya and Fitzgerald, 2001). In contrast, EP2 is considered a contractile receptor, mediating intracellular Ca^{2+} mobilization and induction of smooth muscle contraction. The isoform EP3 is termed an inhibitory receptor, which mediates decreases in cyclic AMP and inhibits smooth muscle relaxation (Narumiya et al., 1999; Narumiya and Fitzgerald, 2001).

Of all the PGE_2 receptors, EP3 and EP4 are the most widely distributed throughout the body, with expression present in almost all murine tissues examined (Honda et al., 1993; Sugimoto et al., 1992). In the uterus, EP2 is the most abundant sub-type (Smock et al., 1999). Studies in knockout mice indicate that EP2, but not EP1, EP3 or EP4, is associated with impaired ovulation and reduction in litter size (Hizaki et al., 1999). Both isoforms, EP2 and EP4, are associated with uterine relaxation during pregnancy in different mammals, including rats (Papay and Kennedy, 2000), mice (Lim and Dey, 1997), sheep (Ma et al., 1999), cattle (Arosh et al., 2003), baboon (Smith et al., 2001) and human (Milne et al., 2001).

Expression and regulation of the EP2 and EP4 receptors in the uterus vary throughout the reproductive cycle and pregnancy in multiple species (Arosh et al., 2003; Blesson et al., 2012; Dong and Yallampalli, 2000; Milne et al., 2001). Prostanoid receptor EP2 is the major cyclic AMP-generating isoform expressed and regulated in bovine uterine tissues during the estrous cycle and early pregnancy (Arosh et al., 2003). No differences in EP2 expression were observed in the nonpregnant human uterus across the menstrual cycle; however, EP4 receptor mRNA expression was significantly higher during the late proliferative stage of the cycle (Milne et al., 2001). In ovariectomized steroid-treated mice, uterine PGE_2 receptors are regulated by estradiol and progesterone (Blesson et al., 2012). In the mare, EP2 transcripts were found to be increased during late diestrus and early pregnancy in the endometrium, while no variations throughout the reproductive stages were observed for EP4 (Atli et al., 2010). Nevertheless, distribution of PGE_2 receptors in the endometrium, as well as EP2 and EP4 expression and immunolocalization in the myometrium have not been reported throughout the estrous cycle or pregnancy in mares.

Therefore, the objectives of the present study were to compare transcripts and to describe protein expression of the steroid (ER-α, ER-β and PR) and PGE_2 (EP2 and EP4) receptors in the endometrium and myometrium during estrus, diestrus and early pregnancy in mares.

2. Materials and methods

2.1. Animals and tissue collection

All animal procedures were completed in accordance with the Institutional Animal Care and Use Committee of the University of Kentucky (Protocol #00843A2005). Sixteen clinically healthy mares of different light breeds, ranging from 4 to 18 y of age were used in this study. Uterine tissue samples were obtained postmortem from mares during estrus ($n = 7$), diestrus ($n = 6$) and early pregnancy ($n = 3$). Estrus was defined by the presence of at least one preovulatory follicle (≥35 mm), no visible corpus luteum and presence of endometrial edema observed via transrectal ultrasonography. Immediately before euthanasia, the mares were re-examined by transrectal ultrasound to confirm the presence of the preovulatory follicle. For diestrus tissues, ovulation was confirmed by daily transrectal palpation and ultrasonography, and mares were euthanized between 9 and 13 d post-ovulation. Pregnant mare tissues