Temporal and spatial associations of oestrogen receptor alpha and progesterone receptor in the endometrium of cyclic and early pregnant mares

L S Hartt, S J Carling, M M Joyce, G A Johnson, D K Vanderwall and T L Ott

Department of Animal and Veterinary Science, Center for Reproductive Biology, University of Idaho, Moscow, Idaho 83844-2330, USA and Department of Veterinary Integrative Biosciences, Texas A&M University, 4458 TAMU, College Station, Texas 77843-4458, USA

Correspondence should be addressed to T L Ott; Email: tott@uidaho.edu

Abstract

Uterine function is primarily controlled by the combined actions of oestrogen and progesterone working through their cognate nuclear receptors. The mechanism of establishment of pregnancy in the mare is of interest because it involves prolonged pre-attachment and conceptus migration phases, and both invasive and non-invasive placental cell types, and as such has been an important comparative model. This study characterised regulation of oestrogen (ER) and progesterone (PR) receptors in the endometrium of the mare during the oestrous cycle and early pregnancy. Endometrial tissues collected during the oestrous cycle and early pregnancy were analysed for steady-state levels of ER and PR mRNA and protein. Steady-state levels of ER and PR mRNA were highest on days 0, 17 and 20 in cyclic mares and lowest on days 11 and 14. A day-by-status interaction was detected, indicating that day 17 and day 20 pregnant mares exhibited low levels of ER and PR compared with the corresponding days of the oestrous cycle.

In situ hybridisation analyses showed receptor mRNA localisation primarily in the luminal epithelium (LE), glandular epithelium (GE) and stroma around oestrus. During dioestrus and early pregnancy, receptors were not detected in the LE, and were lower in the stroma and deeper GE. Changes in hybridisation intensity in these cell types were consistent with changes in mRNA levels detected by slot-blot hybridisation. ER and PR proteins were detected in the nuclei of LE, GE and stromal cells. Consistent with results from in situ hybridisation, levels of ER and PR immunoreactivity were higher around oestrus, declined to low levels during dioestrus and remained low during early pregnancy. Results described here for temporal and spatial changes in steroid receptor gene expression in mares show the greatest similarities with those described for cattle and sheep.

Reproduction (2005) 130 241–250

Introduction

Steroid hormones are important regulators of reproductive physiology in the mare. Both oestradiol and progesterone mediate dramatic changes in mare reproductive tissues during the oestrous cycle and early pregnancy (Ginther 1992). Mating and fertilisation require the presence of oestradiol, which allows the reproductive tract to become lubricated and flaccid, and drives reproductive behaviour (Andrews & McKenzie 1941), whereas progesterone influences uterine histotroph secretion, presumably aiding embryo survival to maintain pregnancy (Zavy et al. 1979, Spencer et al. 2004). The actions of these steroid hormones are mediated through receptors located in the nuclei of cells (DeFranco 1997). Steroid hormones bind receptors, and these ligand–receptor complexes serve as transcription factors that interact with DNA directly to regulate gene expression (Clark 1987). Understanding the temporal and spatial expression of oestrogen (ER) and progesterone (PR) receptors in the mare uterus is critical to understanding the physiological events occurring in the uterus during the transition from recurring oestrous cycles to establishment of pregnancy.

Studies in sheep (Ott et al. 1993, Spencer & Bazer 1995), pigs (Geisert et al. 1993, 1994) and mares (Tomanelli et al. 1991, Watson et al. 1992, McDowell et al. 1999) have all demonstrated relatively high endometrial levels of ER and PR mRNA and protein during oestrus, when peripheral levels of oestrogen are high, and relatively low levels during mid to late dioestrus, when circulating progesterone levels are high. In sheep, the species for which endometrial steroid receptor expression has been most comprehensively described, oestrogen generally up-regulates and progesterone generally...
down-regulates steroid receptor expression in endometrial epithelia (Spencer & Bazer 2002). However, in sheep endometrium, it is now clearly established that steroid receptor regulation of ER and PR is greatest in epithelial cells, both luminal and glandular, and is observed to a much lesser extent in the stromal cells. Therefore, temporal and spatial expression of ER and PR in various endometrial cell types must be examined in the horse.

For example, ER and PR mRNA levels in sheep endometrium are greatest at oestrus and decline fairly dramatically by days 6 to 11 of the cycle (Spencer & Bazer 1995). However, PR protein levels are actually greatest at day 6 in the luminal epithelium (LE) (Spencer & Bazer 1995). Progesterone, from the new corpus luteum (CL), then binds to endometrial PR to inhibit ER and PR expression first in the endometrial LE and later (and to a lesser degree) in the stroma (ST) and glandular epithelium (GE) (Spencer & Bazer 1995). In cyclic sheep, loss of PR is closely followed by increases in ER first in the ST and GE and later in the LE (Spencer & Bazer 1995). These increases are blocked in pregnant sheep through the actions of interferon-tau secreted by the conceptus (Spencer & Bazer 2002).

Similar to sheep, results from the horse suggest that endometrial expression of ER and PR is positively and negatively regulated by circulating levels of oestrogen and progesterone respectively. However, the temporal and spatial regulation of ER and PR mRNA and protein have not been thoroughly evaluated in the mare. In addition, due to the variable length of oestrus, the timing of ovulation, and regulation of luteolysis in the mare (Allen 2001), the temporal rise and fall in oestrogen and progesterone differ somewhat from ruminants (Meinecke et al. 1987, Townson et al. 1989). Furthermore, the equine embryo begins to produce oestrogens starting around day 10 of pregnancy (Allen 2001), much as occurs in pigs (Geisert & Yelich 1997), yet no role for conceptus oestrogens in CL maintenance has been shown in this species (see Allen 2001). There is no evidence that the equine conceptus produces a Type I interferon during the period of maternal recognition of pregnancy (Baker et al. 1991). As a consequence, the role of the conceptus in blocking the luteolytic mechanism remains an enigma in this species.

To date, the temporal and spatial regulation of ER and PR mRNA and protein have not been described in mare endometrium during the cycle and early pregnancy. This has created some differences in interpretation of the regulation of these receptors in the mare from studies that measured either receptor protein or receptor mRNA levels, but not both. For example, Watson et al. (1992) reported no change in ER or PR protein staining in the luminal and glandular epithelium between oestrus and mid-oestrus, whereas Aupperle et al. (2000) using similar techniques reported that ER and PR levels were lowest at mid-oestrus and highest at oestrus. Furthermore, McDowell et al. (1999) reported that ER mRNA levels were higher at oestrus than at days 5, 10 or 15, but detected no change in ER mRNA levels between the latter 3 days. In addition, there are no reports on the relative size of the transcripts for ER and PR in this species, nor has the spatial pattern of mRNA expression been described. Therefore, the objective of this study was to characterize the temporal and spatial expression of endometrial oestrogen receptor alpha (ERα) and progesterone receptor mRNA and protein during the oestrous cycle and early pregnancy in the mare.

**Materials and Methods**

**Tissue collection**

All animal procedures were reviewed and approved by the University of Idaho Animal Care and Use Committee (approval #2001-11). Mixed-breed mares, 3 to 12 years of age, weighing 300 to 500 kg were used in the study. The reproductive tracts of mares were examined using transrectal palpation and ultrasonography (Aloka 210; Corometrics, Wallingford, CT, USA). When mares developed an ovarian follicle of approximately 35 mm that was accompanied by prominent endometrial oedema they were: (1) treated with 2500 IU human chorionic gonadotrophin IV (Chorulon; Intervet, Millsboro, DE, USA) to induce ovulation, (2) randomly assigned to cyclic and pregnant groups, and (3) subsequently examined once daily until ovulation was detected (day 0). Mares assigned to the pregnant group were inseminated with at least 500 million progressively motile spermatozoa collected from one fertile stallion; insemination was performed on the day mares were assigned to the pregnant group and was repeated if ovulation had not occurred within 48 h. Transcervical endometrial biopsies were obtained from cyclic mares on days 0, 8, 11, 14, 17 and 20 (n = 3 mares/day) and from pregnant mares on days 11, 14, 17 and 20 (n = 3 mares/day). Immediately prior to endometrial sample collection, the reproductive tract of each mare was examined with transrectal ultrasonography to assess: (1) ovarian follicular activity, (2) presence or absence of a corpus luteum, (3) presence/degree of uterine oedema (evidence of oestrogen action on the uterus), and (4) presence of a conceptus in pregnant mares. Ovarian and uterine findings in the cyclic mares were consistent with their respective days of the cycle, and a conceptus was identified in each pregnant mare.

One sample of endometrium was fixed in 4% paraformaldehyde for subsequent use in histological and in situ hybridisation analyses and another sample was snap frozen in liquid nitrogen and then subjected to RNA extraction as described below.

**Northern blot analysis**

To purify polyadenylated-RNA (poly(A) RNA) for Northern blot analysis, endometrial samples were collected at
a slaughterhouse from two additional mares, one with a follicle greater than 45 mm and the second with a fresh corpus haemorrhagicum. Endometrial samples from mares with these ovarian structures would be expected to have been under the recent influence of high levels of oestrogen (Meinecke et al. 1987). After collection, the endometrium was stored in liquid nitrogen until RNA extraction. Total cellular RNA was extracted using TRIzol (Gibco-BRL, Grand Island, NY, USA), and purified using the Poly(A) Purist kit (Ambion Inc., Austin, TX, USA). Two-microgram samples of poly(A) RNA were separated on a 1% agarose formaldehyde gel and transferred to a nylon membrane (Nytran; Schleicher & Schuell, Keene, NH, USA). The nylon membranes were baked and the RNA cross-linked using ultraviolet illumination before hybridising overnight in 15 ml ULTRAhyb Reagent (Ambion) with 75 ng biotinylated ER or PR antisense probe (Yankey et al. 2001). Oestrogen receptor and progesterone receptor probes were prepared from oERa8 (pGEM4Z) and oPR7-7A (pCRII) (Spencer & Bazer 1995) plasmids using an in vitro transcription kit (Ambion). The nylon membranes were washed using buffers provided by the North2South Chemiluminescent Hybridisation and Detection kit (Pierce, Rockford, IL, USA) and bands were detected and quantified using a Fluor-S Multimager (Biorad, Hercules, CA, USA).

**RNA slot-blot analysis**

Slot-blot hybridisation was used to measure steady-state levels of ER and PR mRNA during the oestrous cycle and early pregnancy as described previously (Yankey et al. 2001). Four micrograms total cellular RNA for each mare (n = 3/status for each day) were loaded onto a nylon membrane by vacuum filtration (Nytran; Schleicher & Schuell). The membrane was prepared and hybridised with biotinylated ER and PR antisense probe and quantified as described for Northern blot analysis (see above). To adjust for loading, the membrane was stripped with 0.1% SDS at 90°C for 30 min and probed with a biotinylated 18S antisense RNA probe.

**In situ hybridisation**

Oestrogen and progesterone receptor mRNAs were localised in endometrial cross-sections as described previously (Johnson et al. 1999). Briefly, fixed tissues were embedded in Paraplast-Plus (Oxford Labware, St Louis, MO, USA), sectioned (5 µm) and transferred to SuperfrostPlus glass slides (Fisher Scientific, Pittsburg, PA, USA). In vitro transcription was used to incorporate α-35S-labelled UTP (Perkin Elmer Life Sciences, Boston, MA, USA) into antisense ER and PR probes and prepared from oERa8 (pGEM4Z) and oPR7-7A (pCRII) (Spencer & Bazer 1995). After the sections were deparaffinized through an ethanol gradient, rehydrated in 1 × PBS and digested with Proteinase K (20 µg/ml; at room temperature; 7.5 min) (Sigma, St Louis, MO, USA), the radiolabelled probe (5 × 106 c.p.m./slide) was hybridised with tissue sections (n = 3 animals/day/status) in a humidified chamber overnight. Slides were incubated for 30 min at 37°C with RNAlise A (10 µg/ml). Slides were then dehydrated with ethanol and placed on radiographic Kodak Biomax film (16 h) (Eastman Kodak, Rochester, NY, USA) to determine the length of incubation in liquid emulsion.

The sections were dipped in Kodak NTB-2 liquid emulsion (Eastman Kodak) and stored at 4°C for 21 days, after which the slides were developed in developer (Eastman Kodak), counterstained for 5 s in Harris’ modified haematoxylin (Fisher Scientific), and dehydrated through an ethanol gradient. Dehydrated sections were sealed under a coverslip using Permount (Fisher Scientific) and representative images were collected with a Nikon Eclipse E1000 microscope equipped with a Nikon digital DXM 1200 camera (Nikon, Melville, NY, USA) and Act-1 software (Nikon).

**Immunohistochemistry**

Oestrogen and progesterone receptor proteins were localised using a monoclonal rat anti-human ERα antibody (H222; provided by Dr Geoffrey Greene, The Ben May Institute for Cancer Research, The University of Chicago, IL, USA), a polyclonal mouse anti-human PR antibody (GR18; Oncogene Research Products, Boston, MA, USA) and the Rat/Mouse Biostain Super ABC kit (Biomeda, Foster City, CA, USA).

Tissues were prepared as previously described for in situ hybridisation. After rehydration, slides for ERα localisation were treated with Pronase E (2.5 mg/ml in 1 × PBS at 37°C) for 2 min. Antigen retrieval for PR was performed by treatment with 0.1 M boiling sodium citrate for 15 min. Endogenous peroxidase activity was quenched using 1% H2O2 in methanol, and slides were incubated with the protein blocking solution provided in the Biostain Super ABC kit (Biomeda). Primary antibodies were applied to each section (2.5 µg/ml). Rat (Pierce) and mouse (Sigma) IgG, at the same concentration as the primary antibody, were used as negative controls to establish background staining. Sections were incubated overnight in a humidified chamber containing 1 × PBS at 4°C.

Secondary antibody, provided by the Super ABC kit, was diluted and applied to slides. Slides were incubated with secondary antibody at 37°C for 30 min. Staining was detected by application of 3,3’-diaminobenzidine tetrachloride (DAB; Sigma) to slides and incubation in a humidified chamber for 30 min at 37°C. Tissues were counterstained with haematoxylin (Fisher Scientific), dehydrated and sealed under a coverslip.

Representative images were captured with a Nikon Eclipse E1000 microscope equipped with a Nikon digital
DXM 1200 camera (Nikon) and Act-1 software (Nikon). Photographic plates were assembled using Adobe Photoshop (version 6.0, Adobe Systems Incorporated).

**Statistical analysis**

Data from slot-blot analyses were analysed using GLM procedures of SAS (version 8.0; SAS Inst., Cary, NC, USA). Sources of variation were status, day, and their interaction. Regression analysis was utilised to characterise the effects of day on mRNA levels by calculating the highest order significant polynomial. Results from 18S hybridisation were included as a covariate in the model to adjust for differences in the amount of RNA loaded. There were no effects of status or day on the covariate. Data are presented as least square means ± pooled standard error (pooled s.e.). Probability values less than or equal to 0.05 were considered significant.

**Results**

At sampling, all mares exhibited ovarian and uterine ultrasonographic characteristics consistent with their assigned reproductive statuses. By examining ovarian structures and uterine oedema one can accurately assess the stage of the oestrous cycle in mares (Ginther 1995). Follicular growth and ovulation were confirmed in all mares as was the presence or absence of a conceptus and endometrial oedema (an indicator of oestrogen action on the uterus). All mares had prominent endometrial oedema prior to ovulation (day 0), and all cyclic mares exhibited oedema on day 20. As expected, oedema declined on the day of ovulation in all mares. None of the cyclic mares had oedema at days 8, 11 or 14 (expected time of the onset of luteolysis), and two of three mares showed oedema at day 17 consistent with the transition between the end of the luteal phase and the beginning of the follicular phase. None of the pregnant mares showed uterine oedema after ovulation.

**Northern and RNA slot-blot analysis**

Northern blot analysis of poly-adenylated RNA collected from oestrous mare endometrium was used to determine the message size of ER and PR mRNA. ER mRNA migrated at approximately 6.5 Kb (Fig. 1A), and PR demonstrated two distinct bands, one at approximately 3.6 Kb and a second at approximately 4.1 Kb in endometrial poly(A) samples (Fig. 1B).

RNA slot-blot analysis was used to determine steady-state levels of ER and PR in total cellular RNA from the endometrium of the mare. During the oestrous cycle, ER mRNA was high on day 0, decreased to low levels by day 14, and increased again to its highest levels between days 17 and 20 ($P = 0.01$; quartic, $y = 292.9 - 583.6 x + 208.1x^2 - 26.7x^3 + 1.4x^4$; $r = 0.85$; Fig. 2A). During early pregnancy, levels of ER mRNA did not change from day 11 to day 20. A day-by-status interaction ($P = 0.01$) revealed that levels of ER mRNA on days 11 and 14 of the oestrous cycle were similar to levels on the corresponding days of early pregnancy; however, ER mRNA levels were higher during the oestrous cycle on day 17 ($P < 0.05$) and
Progesterone receptor mRNA was also high on day 0 (ovulation) of the oestrous cycle, decreased to low levels on day 14, and increased to its highest levels on day 20 ($P = 0.01$; cubic, $y = 669.2 - 1000.6x + 316.2x^2 - 37.5x^3$; $r = 0.93$; Fig. 2B). Levels of PR mRNA did not change from day 11 to day 20 of pregnancy. A day-by-status interaction ($P \leq 0.01$) revealed that levels of PR mRNA on days 11 and 14 of the oestrous cycle were similar to levels on the corresponding days of early pregnancy; however, PR mRNA levels were higher during the oestrous cycle on day 17 ($P < 0.05$) and day 20 ($P < 0.05$), when mares were returning to oestrus, than during these same days of early pregnancy.

**In situ hybridisation**

Oestrogen and progesterone receptor mRNAs were localised in equine endometrial tissue using *in situ* hybridisation. ER mRNA hybridisation was strong in the luminal epithelium (LE) and glandular epithelium (GE), and moderate hybridisation was evident in the stroma (ST) on day 0 (day of ovulation) of the oestrous cycle (Fig. 3). Hybridisation of ER mRNA decreased across all cell types in the endometrium by day 14 and increased to high levels in the LE and GE and to moderate levels in the ST through day 20. Low levels of ER mRNA were detected in the ST, and minimal levels in the GE and LE on day 11 of pregnancy which was similar to patterns observed on day 11 of the oestrous cycle.
cycle. During early pregnancy (day 14 through day 20) levels of ER mRNA hybridisation were lower in all cell types than the corresponding days of the oestrous cycle.

During the oestrous cycle PR mRNA was high on day 0 in the LE, GE and ST (Fig. 4). PR mRNA hybridisation decreased to low levels on day 11 and day 14 in all cell types followed by an increase in hybridisation in LE, GE and ST by day 20 to levels equalling those observed at day 0. PR mRNA in the LE and GE on day 11 and day 14 of pregnancy were similar to those on the corresponding days of the oestrous cycle. However, progesterone receptor mRNA hybridisation remained low in all cell types through day 20 of early pregnancy in contrast to the day 17-20 rise during the oestrous cycle.

**Immunohistochemistry**

Immunohistochemistry was used to localise ER and PR protein in equine endometrial tissue during the oestrous cycle and early pregnancy. Consistent with in situ hybridisation analyses, ER and PR proteins were present in LE, GE and stromal cells (Figs 5 and 6), were elevated in all cell types on day 0 and day 20 and decreased to lower levels between day 11 and day 14 of the oestrous cycle (data not shown). ER and PR protein levels on day 20 of
Figure 5 Immunohistochemical localisation of ERα protein in representative endometrial cross sections from day 20 of the oestrous cycle and of pregnancy. On the left is immunostaining using rat anti-human ERα IgG, and on the right is immunostaining using an irrelevant rat IgG. D, day; C, oestrous cycle; P, pregnancy; GE, glandular epithelium; LE, luminal epithelium; ST, stroma.

Figure 6 Immunohistochemical localisation of PR protein in representative endometrial cross sections from day 20 of the oestrous cycle and of pregnancy. On the left is immunostaining using mouse anti-human PR IgG, and on the right is immunostaining using an irrelevant mouse IgG. D, day; C, oestrous cycle; P, pregnancy; GE, glandular epithelium; LE, luminal epithelium; ST, stroma.
Discussion

This study investigated the temporal and spatial localisation of ER and PR mRNA and protein in the endometrium of mares during the oestrous cycle and early pregnancy. These results show that ER and PR mRNA levels are high in LE, GE and stromal cells on day 0 and day 20 of the oestrous cycle. Levels for both receptors were low during late-dioestrus (day 11 through day 14) and on all days of early pregnancy through day 20. Significantly, although ER and PR mRNA levels were similar between pregnant and cyclic mares on day 11 through 14, patterns diverged after day 14. Levels for both transcripts increased to levels seen at ovulation by day 20 of the oestrous cycle, but this increase in expression was abrogated in pregnant mares where endometrial expression of ER and PR remained low through day 20. Furthermore, the changes in total endometrial mRNA levels and localisation of ER and PR protein reported in these studies extend and in some cases contrast with previous reports. The temporal and cell-specific expression of both mRNA and protein for oestrogen and progesterone receptors revealed a pattern largely consistent with that shown for domestic ruminants.

Our results also demonstrate message size for both ER and PR mRNA in the endometrium of the mare. The transcript size for ER is 6.5 Kb, and the two transcripts for PR are 3.6 Kb and 4.1 Kb. These sizes are similar to those observed in the ewe (Ott et al. 1993).

Expression of ER and PR appeared to be positively and negatively regulated by circulating levels of oestrogen and progesterone respectively. Previous studies in ewes (Ott et al. 1993, Spencer & Bazer 1995), sows (Geisert et al. 1993, 1994) and mares (Tomanelli et al. 1991, McDowell et al. 1999) demonstrated relatively high levels of oestrogen and progesterone receptor levels around oestrus, when peripheral levels of oestrogen are high, and low levels during mid- to late-dioestrus, when circulating progesterone levels are high. Therefore, we hypothesized that regulation of endometrial steroid receptors during the oestrous cycle of mares is similar to that described for sheep and involves both changes in circulating levels of oestrogen and progesterone as well as temporal and spatial changes in the expression of their respective receptors (Spencer & Bazer 2002). Consistent with this hypothesis, levels of ER and PR in the endometrium were highest around oestrus, their levels declined during the luteal phase and increased again in cyclic mares during the time when luteolysis normally occurs and mares return to oestrus. It is suggested that loss of PR removed the progesterone block to ER synthesis and resulted in increased endometrial ER expression by day 17, followed closely by ER-mediated increases in PR by day 20 of the oestrous cycle.

There is a discrepancy between the mRNA results described here and an earlier report. Although the pattern of PR mRNA expression observed in this study is consistent with a study conducted by McDowell et al. (1999), ER mRNA results are not. McDowell et al. (1999) observed no change in endometrial ER mRNA between days 5 and 15, whereas the present slot-blot and in situ hybridisation results clearly indicate a decrease in ER mRNA levels between days 8 and 14 of the oestrous cycle. Importantly, both studies do show that mRNA levels for both ER and PR are lower in endometrium collected after day 14 of pregnancy when compared with samples collected after day 14 of the oestrous cycle (McDowell et al. 1999). Taken together, these results suggest that days 14 through 17 represent a critical period of divergence in endometrial function between pregnant and cyclic mares that is likely the result of an endometrial response to an as yet unidentified pregnancy recognition signal secreted by the equine conceptus and/or the decline in plasma progesterone that occurs starting between days 14 and 15 in the non-pregnant mare (Meinecke et al. 1987).

Similar to the pig, it is known that the equine conceptus produces a significant amount of oestradiol as well as 17α-hydroxyprogesterone during the period of pregnancy recognition (Goff et al. 1993). There is no clear evidence that this oestrogen is responsible for blocking development of the luteolytic mechanism in mares (Allen 2001), and a role for 17α-hydroxyprogesterone has not been determined. Furthermore, in the pig, in contrast to ruminants, ER expression is maintained in the LE, but not in the GE and stroma around the time of pregnancy recognition signalling (Geisert et al. 1993). It has been suggested that this allows the luminal epithelium to respond to porcine conceptus-produced oestrogen to block luteolysis and stimulate endometrial secretory function (Geisert et al. 1993). The present results show that ER (and PR) disappear from the LE and are reduced in the GE and stroma in the mare, much like that shown for ruminants. Perhaps this explains the inability of exogenous oestrogen to lengthen luteal lifespan in mares as shown for pigs (see Bazer et al. 1986).

The endometrium is a complex tissue comprised of a number of different cell types. Therefore, understanding regulation of endometrial function by steroid hormones requires examining cell-specific patterns of expression. Previous studies characterised expression of oestrogen and progesterone receptor protein in LE, GE and stromal cells in the mare (Watson et al. 1992, Aupperle et al. 2000). Those studies demonstrated weak staining for oestrogen and progesterone receptors in LE and GE and stronger expression in the stroma during the oestrous cycle (Watson et al. 1992, Aupperle et al. 2000). In the present study, strong nuclear staining for ER and PR was evident in all cell types (LE, GE and stroma) on the days just prior to ovulation (days 17 and 20). In agreement with the previous studies, stromal expression of ER and PR proteins was high in the mare on the day of ovulation.
and decreased to low levels throughout dioestrus (Watson et al. 1992, Aupperle et al. 2000).

In a previous report, steroid receptor protein in the endometrial stroma on day 14 of pregnancy was similar to expression on day 14 of the oestrous cycle (Watson et al. 1992). Another study found that regardless of pregnancy status, oestrogen receptor and progesterone receptor protein levels were the same on day 15 (Tomanelli et al. 1991). Stomal expression of ER and PR protein was low when circulating progesterone levels were high and increased as peripheral levels of oestrogen increased (Watson et al. 1992, Aupperle et al. 2000). It is significant that results from the present study show ER and PR protein levels are higher in endometrium from cyclic compared with pregnant mares on day 20. ER and PR protein expression in the endometrium during early pregnancy is similar to levels seen on day 11 of the oestrous cycle and remained low through day 20. Therefore, similar to other domestic species, there exists a functional block to increased ER and PR gene expression in the endometrium of early pregnant mares. Whether this block is a direct result of the conceptus regulating oestrogen receptor levels as has been shown for sheep (Spencer & Bazer 2002), or whether conceptus-produced steroids (particularly 17α-hydroxyprogesterone) (Goff et al. 1993) locally suppress ER, or whether the effect is indirect via maintenance of circulating progesterone levels (McDowell et al. 1999) remains to be determined.

This study presents spatial and temporal patterns of expression of ER and PR mRNA and protein in mares that extend observations of previous reports, and are consistent with results from cattle and sheep. Levels of ER and PR mRNA and protein were up-regulated around oestrus, and were down-regulated during mid- to late-dioestrus, suggesting that ovarian oestrogen and progesterone, as well as their respective endometrial receptors act in concert to regulate ER and PR gene expression in the equine endometrium. During early pregnancy, levels of both ER and PR mRNA and protein remained low while levels on corresponding days of the oestrous cycle increased. These changes could result directly from the presence of the conceptus, or indirectly from the changing pattern of oestrogen and progesterone production that accompanies luteolysis, or both. To further delineate these mechanisms will require identification of the conceptus signals responsible for pregnancy recognition in the mare.

This study provides further characterisation of oestrogen and progesterone regulation in the endometrium of the mare during the oestrous cycle and early pregnancy. The results described here should advance our understanding of the physiology in the endometrium of the mare during these important reproductive periods. Combined with recent characterisation of the pattern of other reproductive hormones, such as oxytocin (Bae & Watson 2003) and prostaglandin F\textsubscript{2α} (Sharp et al. 1997, Handler et al. 2003), results presented here provide a better understanding of endometrial physiology during the oestrous cycle and early pregnancy in the mare.

Acknowledgements

This work was supported, in part, by NIH NCRR grant P20RR15587-01 to T L O. IAES manuscript #04A03. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

Bae SE & Watson ED 2003 A light microscopic and ultrastructural study on the presence and location of oxytocin in the equine endometrium. Theriogenology 60 909–921.
DeFranco DB 2002 Navigating steroid hormone receptors through the nuclear compartment. Molecular Endocrinology 16 1449–1455.
Goff AK, Leduc S, Poitras P & Vaillancourt D 1993 Steroid synthesis by equine conceptuses between days 7 and 14 and endometrial steroid metabolism. Domestic Animal Endocrinology 10 229–236.
McDowell KJ, Adams MH, Adam CY & Simpson KS 1999 Changes in equine endometrial oestrogen receptor and progesterone receptor mRNAs during the oestrous cycle, early pregnancy and after treat-


Received 7 December 2004
First decision 31 January 2005
Revised manuscript received 18 February 2005
Accepted 10 May 2005