Regulation of oxytocin receptor in the placentome capsule throughout pregnancy in the ewe: the possible role of oestradiol receptor, progesterone receptor and aromatase

S T Leung, T S Reynolds and D C Wathes

Department of Veterinary Basic Sciences, The Royal Veterinary College, Boltons Park, Hawkshead Road, Potters Bar, Hertfordshire, EN6 1NB, UK
(Requests for offprints should be addressed to D C Wathes)

Abstract

The hormonal regulation of uterine oxytocin receptors (OTR) during the establishment of pregnancy and at parturition has been studied extensively, but little information is available during mid-pregnancy. This study investigated the localisation of OTR mRNA in the ovine placentome throughout gestation and related this to expression patterns for the putative regulatory agents aromatase, oestradiol receptor, progesterone receptor and oxytocin. Placentomes were collected at regular intervals throughout pregnancy for *in situ* hybridisation analysis and immunocytochemistry (oestradiol receptor only). Results were quantified by optical density measurements of autoradiographs. Progesterone receptor mRNA was localised to the caruncular tissues on day 30 but became undetectable by day 34. Aromatase mRNA appeared in the fetal villi at days 34–40, with concentrations peaking at days 52–55 and again at days 132–137. Oestradiol receptor mRNA was localised to the caruncular tissues from days 13 to 30 and found in the maternal villi and placentome

capsule from days 45 to 70. Oestradiol receptor protein was barely detectable in either tissue. OTR mRNA was localised to the placentome capsule at days 34–40, remaining high at day 45 and declining to basal levels by days 132–137. Oxytocin mRNA was not detected in the placentome. In conclusion: (1) progesterone acting via its receptor may suppress the expression of aromatase and OTR in early pregnancy; (2) the up-regulation of OTR expression in the capsule may not involve the oestradiol receptor; (3) there is a differential regulation between different regions of the uterus as the increase in the placentome capsule occurs at a time when concentrations in the rest of the endometrium and myometrium remain low; (4) oestradiol receptor expression in the placentome may be regulated at the translational level; and (5) there is no local production of oxytocin in the sheep placenta. The role of OTRs in the capsule during mid-pregnancy remains to be determined.

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Introduction

The development of endometrial oxytocin receptors (OTR) plays an important role in inducing the production of prostaglandin (PG) $F_{2\alpha}$ during luteolysis in cyclic ewes. Inhibition of these receptors is an essential step in the maternal recognition of pregnancy (for reviews see Stewart *et al.* 1992, Flint *et al.* 1994, Wathes & Lamming 1995). Oxytocin induced myometrial contractions of the reproductive tract, mediated by OTR, are also important to facilitate delivery of the fetus during parturition (Owiny *et al.* 1992, Word *et al.* 1992, Kimura *et al.* 1996). The regulation and role of OTR at these two time periods have, therefore, been investigated extensively. Fewer studies, however, have examined the period in between, that encompassing most of pregnancy.

We have reported previously that OTR mRNA is present in the uterine tissues and the capsule of the placentome during mid-pregnancy in sheep (Wathes *et al.*

1996a). The capsule is a layer of maternal connective tissues rich in collagen and fibroblast surrounding the placentome which becomes distinguishable at around day 35 of pregnancy. OTR expression, both mRNA and protein, increases significantly at around day 70 and again at parturition in the endometrium and myometrium (Fuchs *et al.* 1992, Wathes *et al.* 1996a, Wu *et al.* 1996). In contrast, the concentration of OTR mRNA in the capsule of the placentome elevates significantly at around day 40 when its expression in the endometrium and myometrium are low (Wathes *et al.* 1996a). Therefore, a differential regulation of the expression of OTR must exist between these different regions along the reproductive tract.

Aromatase plays an important role in converting progesterone to oestrogen in the placenta (Flint *et al.* 1975, Mason *et al.* 1989). The modulation of the concentration of these steroid hormones may have a profound effect on the development of their receptors since oestradiol stimulates

the expression of the oestradiol receptor (Fang *et al.* 1996, Ing *et al.* 1996, Katzenellenbogen 1996, Ing & Tornesi 1997). Although the increase in free oestradiol concentration is only significant at term, aromatase may be responsible for the gradual increase in oestradiol sulphate, which can be unconjugated to produce free oestrogen (Mitchell *et al.* 1984, Chibbar *et al.* 1986, Janszen *et al.* 1995). In order to understand the possible role of aromatase during pregnancy, it is necessary to examine its expression pattern and site of synthesis.

Oestradiol and progesterone may play a role in opposing and facilitating the establishment of pregnancy respectively. It is generally believed that oestradiol and progesterone induce and suppress the expression of OTR respectively (Maggi *et al.* 1991, Ostrowski *et al.* 1995, Larcher *et al.* 1995, Wathes *et al.* 1996b). Although the oestrogen and progesterone concentrations in sheep have been monitored throughout pregnancy (Bassett *et al.* 1969, Carnegie & Robertson 1978), little is known about the development of their receptors during mid-pregnancy. Therefore, the investigation of the sites of expression of oestradiol and progesterone receptors in the placenta of sheep may help us to understand the possible role of these steroids in achieving differential regulation of expression of OTR during pregnancy.

The aims of this experiment were to localise and correlate the expression patterns of aromatase, oestradiol receptor, progesterone receptor, oxytocin and OTR mRNA in the ovine placentome using *in situ* hybridisation throughout pregnancy. Immunocytochemistry was also carried out to investigate the translation of the oestradiol receptor message.

Materials and Methods

Animals

Clun Forest ewes were run with entire rams and raddle marks were checked to indicate mating (day 0). The gestation length of sheep is between 145 and 147 days. Ewes were slaughtered by an overdose of pentobarbitone i.v. (Euthatal, Tallaght, Dublin, Ireland). Tissues were collected within 15 min of slaughter at the following stages of pregnancy: days 13–15 ($n=4$), days 21–22 ($n=4$), days 29–30 ($n=4$), days 34–40 ($n=3$), days 45–46 ($n=3$), days 52–55 ($n=5$), days 65–70 ($n=7$), days 90–105 ($n=6$) and days 132–137 ($n=7$). Pieces of placentome were removed, wrapped in aluminum foil, frozen in isopentane immersed in liquid nitrogen and stored at -80°C . Samples were also obtained from non-pregnant ewes at oestrus ($n=4$) and on day 9 of the cycle ($n=3$).

Reagents

Chemicals were purchased from Sigma Chemical Co. (Poole, Dorset, UK) or Merck (Poole, Dorset, UK) unless otherwise specified.

Section preparation

Cross sections ($10\text{ }\mu\text{m}$) were cut and thaw mounted on poly-L-lysine (0.1 mg ml^{-1}) coated slides. The sections were fixed in 4% w/v paraformaldehyde (BDH, Poole, Dorset, UK) in $1\times$ phosphate-buffered saline (PBS, 0.13 M NaCl , $0.007\text{ M Na}_2\text{HPO}_4$) for 5 min, washed in $1\times$ PBS for 2 min ($3\times$) and dehydrated in 75% followed by 95% absolute ethanol for 5 min each. The sections were then stored in 95% absolute ethanol at 4°C until used.

In situ hybridisation

The *in situ* hybridisation procedures were applied as previously described (Wathes *et al.* 1996a). Briefly, the mRNA specific probes (antisense; 45 mer synthetic oligonucleotide), were end labelled with dATP- S^{35} (NEN Research Products, Stevenage, Herts, UK). The reaction mix containing the labelled probes in hybridisation buffer ($100\text{ K c.p.m. per }100\text{ }\mu\text{l}$ hybridisation buffer per slide) was added to the sections, covered with a parafilm coverslip, and incubated at 43°C overnight. After incubation, the sections were washed at room temperature for 30 min followed by 1 h at 55°C in $1\times$ sodium saline citrate (SSC; 15 mM sodium chloride, 15 mM sodium citrate, pH 7.0) containing 0.2% (w/v) sodium thiosulphate-5 hydrate. The slides were then dehydrated in a gradient of ethanol, air-dried and exposed to hyperfilm- βmax (Amersham International plc, Amersham, Bucks, UK) for the defined period (see below). The sense sequence of the respective probe was used as the negative control. The uterus of a ewe at oestrus was used as the positive control for the oestradiol receptor and OTR probes, a day 2 ovine corpus luteum as the positive control for the oxytocin peptide probe and a day 2 uterus as the positive control for the progesterone receptor probe.

Probes (antisense sequence)

The probe sequences used in this study were based on published cDNA sequences. Oligonucleotide 45 mers were synthesised (Babraham Institute, Cambridge, UK) after specific sequences were chosen.

Aromatase (exposure time 2 weeks; Hinshelwood *et al.* 1993, bases 127–1317):

5'-TCA CCG GGT AGC CAT CGA TGA CAT CAT CCT CTA AGG CTT TGC GCA-3'

Progesterone receptor (exposure time 2 weeks; Ing *et al.* 1996, bases 2148–2196):

5'-CCG AAA ACC TGG CAG TGA CTT AGA CCA CTT GAC CAC TGA GAG AAG-3'

Oestradiol receptor (exposure time 3 weeks; Ing *et al.* 1996, bases 564–608):

5'-TGG CCT GTA GTA GGC GGC AGG GCC GGC TTC GCG CAC CGC ATA GCC-3'

Oxytocin peptide (exposure time 2 weeks; Ivell *et al.* 1990, bases 770–814):

5'-AGC ACC GCA CGC TTG CCG CCC AGG
GGG CAG TTC TGA ATG TAG CTG-3'

OTR (exposure time 2 weeks; Stevenson *et al.* 1994, bases 887–923):

5'-TTC CTT GGG CGC ATC GGC ATC CCA
GAC ACT CCA CAT CTG CAG GAA-3'

Photographic emulsions

The procedures were similar to the instructions provided by the manufacturer (LM-1, Amersham International plc). Briefly, completely dried slides were dipped into the emulsion vertically for 5 s at 43 °C and allowed to dry horizontally at room temperature and then on a pre-cooled metal plate, with dry ice underneath, for 10 min each. The slides were then placed into a light-tight box with anhydrous silica gel in the base of the box, sealed, and incubated at 4 °C until developing. The incubation time depended on the probe used: (a) aromatase, (b) progesterone receptor, (c) oxytocin peptide, (d) OTR for 3 weeks, and (e) oestradiol receptor for 4 weeks. After incubation, the slides were dipped in developer (Phenisol; Ilford Limited; Ilford, UK) for 5 min, stop bath (0.5% acetic acid v/v) for 1 min, fixative (47% w/v, sodium thiosulphate pentahydrate) for 10 min and then distilled water for at least 10 min before counterstaining with Harris' haematoxylin (BDH) and eosin (Sigma) to identify the cell types.

Oestradiol receptor localisation

Immunocytochemistry was carried out on placentomes collected at days 21–22, 29–30, 40–46, 52–55, 70 and 105. The procedures were applied as described previously (Wathes & Hamon 1993). Cross sections (5 µm thick) were cut and thaw-mounted onto 3-aminopropyltriethoxysilane (2% v/v) coated slides and fixed in formaldehyde (3.7% v/v) for 10 min. The sections were then treated with acetone (100%) and methanol (100%) containing hydrogen peroxide (1% v/v; BDH) at room temperature for 5 s each followed by washing in 1 × PBS for 5 min (2 ×). All subsequent incubations were in a humidified box at room temperature unless otherwise specified. After washing, the sections were incubated with normal rabbit serum (NRS, 2.5%; Sigma) for 10 min. The NRS was then blotted off prior to the incubation with 100 µl mouse anti-oestradiol receptor antiserum (1:80 diluted in PBS; DAKO, Ely, Cambridge, UK) at 4 °C overnight. For control sections, mouse anti-IgG (1 µg ml⁻¹; Sigma) was added in place of anti-oestradiol receptor antiserum.

After overnight incubation, the slides were washed in 1 × PBS for 5 min (2 ×) before incubating with: (i) rabbit anti-mouse antiserum (1:100, Sigma) for 30 min; then

(ii) 100 µl peroxidase anti-peroxidase (1:50, Sigma) for 30 min; and (iii) 100 µl activated diaminobenzidine-tetrahydrochloride (DAB; 0.5 mg ml⁻¹; Sigma) for 10 min in the dark. After incubation, the excess DAB was washed away by distilled water and the slides were mounted after a serial dehydration in 75%, 100% ethanol, and xylene (100%) for 2 min each.

Data analysis

After exposure, the *in situ* hybridisation images on autoradiographs were quantified by measuring the optical absorbance of specific areas identified by Harris' haematoxylin and eosin staining using an image analysis system (Seescan plc, Cambridge, Cambs, UK). The results were expressed as optical density (OD) units on an arbitrary scale with a detection limit of 0.01. Three readings per section and four sections per sample were taken from the probed slides. The sense values were subtracted from the antisense values to produce a mean value of specific hybridisation in each region. The immunocytochemistry results were given a score of zero (no staining) to three (very strong staining). The final results were log transformed to ensure homogeneity of variance and analysed by ANOVA followed by pooled variance *t*-test. If the data were not homogeneous, a Kruskal–Wallis test (non-parametric ANOVA) was used followed by a Mann–Whitney test. Values are given as the mean ± s.e.m. Significance is defined as a *P* value < 0.05.

Results

Aromatase

The oligonucleotide probe used was specific for both the ovarian and placental isoforms of aromatase. No aromatase mRNA was detected in any region of the uterus at oestrus, nor during early pregnancy before day 30 (OD < 0.01; *n* = 12). The aromatase mRNA was first localised in the fetal villi of the cotyledon at days 34–40 (OD = 0.05 ± 0.022; *n* = 5; Fig. 1), increasing significantly at days 52–55 (0.08 ± 0.006; *n* = 5; *P* < 0.05) and returning to a similar concentration to that measured on days 34–46 at days 65–105 (*n* = 12; Fig. 2). Another and greater significant elevation of aromatase mRNA in the fetal villi was observed at days 132–137 (OD = 0.25 ± 0.071; *n* = 3; *P* < 0.05; Fig. 2). The aromatase mRNA was below the detection limit (OD < 0.01) in all other regions of the placentome.

Oestradiol receptor

The oestradiol receptor mRNA concentrations in the caruncle and luminal epithelium (LE) of the uterus at oestrus were 0.24 ± 0.049 (*n* = 4) and 0.12 ± 0.022 (*n* = 4)

Figure 1 The expression of aromatase mRNA was localised using *in situ* hybridisation. An ovine specific antisense probe was hybridised with a cross section of placentome on day 135 of pregnancy (a). The specific site of expression was examined by photographic emulsion (c). Aromatase mRNA was expressed in the fetal villi (FV). The sense sequence was used as the negative control (b and d). The scale bars represent 23 mm (a and b) and 10 µm (c and d) respectively.

Figure 2 The expression of aromatase (ARM) mRNA in the placentome throughout pregnancy was investigated using *in situ* hybridisation. Samples were obtained at regular time intervals throughout pregnancy (p; $n=3-7$ per stage). The concentration of the ARM mRNA in the fetal villi was measured on the autoradiographs and expressed as mean $\pm$ S.E.M. OD units. Aromatase expression was not detectable (ND) in either the fetal cotyledon or maternal caruncle on day 30 of pregnancy. A low concentration of ARM mRNA was expressed when the placentome was first recognised at around days 34–40 of pregnancy. A significant increase in ARM concentration was observed at days 52–55 (which returned to a lower level by day 65 until a second peak was observed at around day 135 of pregnancy. (a>b, $P<0.05$; b>c, $P<0.05$; a>c, $P<0.01$).

OD units respectively. During early pregnancy, from day 13 to day 30, a low concentration of oestradiol receptor mRNA was localised in the caruncular tissues. As pregnancy progressed and the placentome became bigger, the

Figure 3 The expression of oestradiol receptor mRNA in the placentome was investigated using *in situ* hybridisation. A cross section of the placentome (day 45 of pregnancy) was hybridised with an antisense ovine specific probe. The results showed that oestradiol receptor was expressed in the maternal villi (MV; a and b) and in the placental capsule (Cap; c and d). The sense sequence was used as the negative control (b and d). The scale bars represent 35 mm.

results showed that the mRNA signal originated in the maternal villi (Fig. 3). The concentration of oestradiol receptor mRNA in the maternal villi was highest at days 34–46 (0.07 ± 0.01) but became undetectable by day 90 of pregnancy (Fig. 4). It was also localised in the placental capsule, first appearing on day 45 ($P<0.001$; Fig. 3), then progressively decreasing from days 52 to 70 and disappearing by day 90 of pregnancy (Fig. 5). A low level of oestradiol receptor mRNA was observed in early pregnancy in the LE on day 13 (0.03 ± 0.008), reaching basal levels by days 21 to 30. It then became undetectable throughout the rest of the study period (data not shown).

The concentration of oestradiol receptor protein in the uterus from a ewe at oestrus (used as a positive control) scored two and three in the endometrial LE and myometrium respectively (data not shown). These values are comparable with those reported previously (Wathes & Hamon 1993). No oestradiol receptor protein was detected in either the LE nor the glandular epithelium at any stage of pregnancy examined (days 21–105). In contrast, a low concentration of oestradiol receptor protein was detected in the maternal villi (scored 0.41 ± 0.148 , $n=11$) from days 40 to 105 of pregnancy. This subsequently disappeared. Oestradiol receptor protein was also found in the capsule at a similar low concentration (scored 0.67 ± 0.235 , $n=11$) from days 40 to 105 of pregnancy.

Progesterone receptor

A very low concentration of progesterone receptor mRNA (0.02 ± 0.006 , $n=3$) was localised in the caruncular tissues

Figure 4 The expression of oestradiol receptor (E2R) mRNA in the maternal caruncles or villi was localised by *in situ* hybridisation. A uterus from an oestrous sheep was used as the positive control to illustrate the relative degree of expression between cyclic and pregnant ewes. The results were expressed as mean $\pm$ S.E.M. OD units measured from autoradiographs. Samples were collected at regular time intervals throughout pregnancy (p, $n=3-7$ per stage) or during the oestrous cycle (NP, $n=3-4$ per stage). Low E2R mRNA expression was found in the caruncular tissues on day 13 of pregnancy until day 34, when the placentome could be recognised histologically. No significant variation was observed in the maternal villi until day 70 of pregnancy. However, E2R mRNA was below detection (ND) from day 90 until day 137 of pregnancy.

Figure 5 The expression of oestradiol receptor (E2R) mRNA in the placentome capsule was studied throughout pregnancy at regular time intervals (p; $n=3-7$ per stage). The results were expressed as mean $\pm$ S.E.M. OD units. No E2R mRNA was detected in the placentome capsule on days 34-40 of pregnancy (ND). However, a significant increase in E2R mRNA was observed on day 45 ($P<0.001$), with concentration remaining high until days 65-70 and becoming undetectable (ND) by day 90.

on day 30 of pregnancy (data not shown). It was undetectable by day 34 and throughout the rest of the period studied. In contrast, progesterone receptor mRNA was highly expressed in the deep and superficial uterine glands of a ewe on day 2 of the oestrous cycle (control tissue, $OD=0.25 \pm 0.083$ and 0.37 ± 0.112 respectively) but was not expressed in the myometrium, LE nor caruncle at this stage.

Figure 6 The expression of OTR mRNA in the placentome was investigated using *in situ* hybridisation. An antisense ovine specific probe was hybridised with a cross section of the placentome at day 45 of pregnancy (a). The site of expression was localised using photographic emulsion (c). The results showed that OTR was strongly expressed in the capsule (Cap) of the placentome. The sense sequence was used as the negative control (b and d). The scale bars represent 45 mm (a and b) and 10 μ m (c and d) respectively.

Oxytocin receptor and oxytocin

There was a significant variation of OTR mRNA concentrations in the capsule layer of the placentome throughout pregnancy (ANOVA; $P<0.001$). OTR mRNA first appeared in the capsule on day 35 of pregnancy ($P<0.0001$) at around the time when the capsule layer could be distinguished histologically (Fig. 6). The concentration remained high at days 45-46 ($P<0.0001$) and then progressively decreased from days 52 to 55, reaching basal levels by day 132 (Fig. 7). The peak OTR mRNA concentration in the capsule in mid-pregnancy was equivalent to that measured in the LE and myometrium during labour (Wathes *et al.* 1996a). No oxytocin mRNA was detectable in any region of the placentome throughout pregnancy ($OD<0.01$) whereas the corpus luteum from a ewe on day 2 of the oestrous cycle (positive control) showed a high concentration of oxytocin mRNA ($OD=1.31 \pm 0.179$).

Discussion

Progesterone is an essential hormone required to maintain pregnancy. Oestrogen becomes the dominant hormone at parturition in the ewe, when it induces cervical ripening (Ellwood 1980, Winn *et al.* 1994, Cheah *et al.* 1995, Silva *et al.* 1995) and the formation of myometrial gap junctions (Garfield *et al.* 1980, Ciray *et al.* 1996). Although the

Figure 7 OTR mRNA expression in the placentome was investigated throughout pregnancy at regular intervals (p; $n=3-7$ per stage) using *in situ* hybridisation. The results were expressed as OD units $\pm$ S.E.M.. No OTR mRNA expression was detected before day 34 of pregnancy (ND). A significant up-regulation of OTR mRNA in the placentome capsule on days 34-45 of pregnancy was observed, which dropped to a lower level by day 52 and then progressively decreased to basal levels by day 132 of pregnancy. (a>c, $P<0.0001$; b>c, $P<0.05$; a>b, $P<0.05$). The concentration of OTR mRNA in the uterine LE and myometrium (Myo) in labour is included to illustrate the relative degree of OTR expression in the placentome capsule throughout pregnancy.

concentrations of oestrogen and progesterone have been measured throughout pregnancy (Bassett *et al.* 1969, Carnegie & Robertson 1978), little is known about the development of their receptors in the placenta during most of gestation. Both oxytocin and its receptor are believed to be inhibited and stimulated by progesterone and oestrogen respectively, presumably through binding to their corresponding receptors. This study provides the first description of the relative expression pattern of aromatase, progesterone receptor, oestradiol receptor, oxytocin and OTR in the ovine placentome throughout pregnancy and, therefore, clarifies the possible roles of progesterone receptor and oestradiol receptor on the expression of OTR during pregnancy.

Aromatase catalyses the last step in the conversion of progesterone to oestrogen. Aromatase activity is thus critical to the biosynthesis of oestrogen. Previous studies in ovine placental tissues show that the catalytic activity of aromatase is increased significantly near term (Mason *et al.* 1989), which is consistent with the findings of this study. However, our results differ from those of Tsumagari *et al.* (1993) in bovine cotyledon who observed that the catalytic activity of aromatase peaked in mid-gestation around the fifth month and immediately after, but not before, parturition. This discrepancy may be due to a species difference. Another possibility is that the aromatase catalytic activity may not be proportional to its mRNA concentration in the cow, although the aromatase mRNA in this study peaked in parallel to its reported catalytic activity in sheep (Mason *et al.* 1989).

The constant expression of aromatase in early pregnancy (after day 34) and the significant up-regulation in late pregnancy in the fetal villi may explain the gradual increase in oestradiol sulphate in both the fetal and maternal system, which also show a significant increase preceding the onset of parturition (Carnegie & Robertson 1978). Since the aromatase mRNA is of fetal origin, it suggests that the maternal oestrogen level may be modulated by the fetus as gestation progresses. Cortisol has been implicated as the transcriptional stimulator of aromatase at term (Flint *et al.* 1975, Ricketts *et al.* 1980, Mason *et al.* 1989). Our results support this hypothesis but cortisol may not be the sole transcriptional stimulator since aromatase mRNA was expressed throughout most of the gestation period. It has been reported that a non-steroidal aromatase inhibitor is produced and secreted by the corpus luteum and placenta in sheep during the last third of gestation (Al-Gubory *et al.* 1994, 1995). Withdrawal of such an inhibitor would allow a quick increase in oestradiol synthesis. Therefore, it seems likely that the activity of aromatase is strictly regulated at both the transcriptional and post-translational levels.

The results from this study suggest that the expression of oestradiol receptor was unlikely to be regulated solely by oestradiol. The expression of oestradiol receptor in the placentome follows a tissue and time specific pattern during gestation. The up-regulation of oestradiol receptor mRNA in the capsule of the placentome between days 45 and 65 corresponds to the appearance of oestradiol sulphate found in the fetal and maternal systems, although the concentration of free oestradiol is low at this stage. In addition, oestradiol receptor mRNA concentration in this tissue dropped below the detection limit by day 90 of pregnancy until term although the oestradiol sulphate and free oestradiol continue to increase until parturition. This suggests that an unknown stimulus may be produced by the placental system to regulate the transcriptional expression of oestradiol receptor during pregnancy. More importantly, only a limited amount of oestradiol receptor protein was localised in the placentome capsule from days 40 to 105 whereas its mRNA was high up to day 65 and dropped below the detection limit by day 90 of pregnancy. This suggests that the expression of oestradiol receptor may also be regulated at the translational level and that a very low concentration of oestradiol receptor protein may be sufficient to serve its purpose throughout pregnancy.

The inhibition of the expression of OTR by interferon γ in early pregnancy is essential to prevent luteolysis (for reviews see Stewart *et al.* 1992, Flint *et al.* 1994). This may in part be via down-regulating the concentration of oestradiol receptor (Mirando *et al.* 1993, Spencer *et al.* 1995a,b). This study showed that a low level of oestradiol receptor mRNA is present on days 13-15 of pregnancy in the caruncle and the LE, suggesting that a basal

activity of oestradiol receptor may still be required during implantation.

After implantation, the trophoblast stops producing interferon and the expression of uterine OTR resumes. Its concentration remained at a low but detectable level in the endometrial LE throughout gestation, with a small increase at mid-pregnancy and a significant up-regulation during parturition (Wathes *et al.* 1996a). In contrast, the concentration of oestradiol receptor in the LE was below the detection limit during most of the gestation period and only a low concentration was measured at parturition (Leung *et al.* 1997). This suggests that the expression of OTR in the LE may occur in a constitutive and inducible manner such that the oestradiol receptor is only required to facilitate the significant up-regulation of OTR expression. Furthermore, the expression of OTR mRNA preceded that of the oestradiol receptor in the placentome capsule in mid-pregnancy, confirming that the oestradiol receptor is not the main stimulus for the induction of OTR expression. However, the oestradiol receptor may be required to sustain OTR expression. It has been demonstrated that oestradiol can both stimulate and suppress OTR expression in the uterus of cyclic sheep (Wathes & Lamming 1995), supporting the hypothesis that oestradiol has a differential regulatory effect on OTR expression. We have previously reported that OTR follows a similar expression pattern to that of oestradiol receptor in the caruncular stroma, myometrium and cervix of cyclic sheep whereas OTR expression in the LE is not correlated with oestradiol receptor expression (Wathes & Hamon 1993, Leung *et al.* 1995). In addition, OTR expression in the uterus and cervix are highly coordinated in cyclic ewes with maximal expression in both tissues at oestrus but such coordination is absent during parturition when the increase only occurs in the uterus (Leung *et al.* 1995, Wathes *et al.* 1996a). This suggests that tissue specific factor(s) are required to regulate OTR expression. If the presence of such local regulatory factor(s) is confirmed, the steroidal effect on OTR expression in some tissues may be mediated through modifying the activity of these regulatory agent(s). This may provide an explanation of the differential expression of OTR in a tissue and time specific manner.

Oxytocin, mediated by OTR, is able to induce the production of PGF_{2α} and PGE₂ in the placental tissues (Moore *et al.* 1988, Meier *et al.* 1995, Fuchs *et al.* 1996). PGE₂ can stimulate vascular dilation (Armstrong *et al.* 1995) whereas PGF_{2α} may promote placental growth since PGF_{2α} treatment increases the placentome weights at days 90–100 of pregnancy (Weems *et al.* 1994). The significant increase in OTR expression, possibly supported by oestradiol receptors, in the placentome capsule coincides with the time of the fastest placentome growth rate at around day 40 (Amoroso 1952). In addition, the peak of OTR and oestradiol receptor expression in the placentome capsule coincided with the sudden onset of regular mus-

cular contractility in the pregnant horn of the uterus from days 49 to 66. No uterine activity was detectable before day 41 and only steady uterine activity was detected after day 66 until parturition (Garcia-Villar *et al.* 1984). This suggests that OTR may be responsible for the induction of uterine activity during pregnancy but the function of this activity is unclear.

The lack of oxytocin expression in the placental tissues confirms our previous results (Wathes *et al.* 1996a) and suggests that an oxytocin paracrine/autocrine system within the uterus may be absent in sheep. Nevertheless, the pituitary may provide the source of oxytocin since a basal level of oxytocin was measurable in human serum throughout pregnancy (Leake *et al.* 1981) and a low pulsatile secretion of oxytocin was also found in pregnant cows (Fuchs *et al.* 1986).

A high progesterone concentration plays an essential role in maintaining pregnancy, including stimulating uterine secretions (Stephenson *et al.* 1989), acting as an immunosuppressant (Stites & Siteri 1983) and inhibiting myometrial contractions. Previous workers have shown that immunoreactive progesterone receptor was present in early pregnancy up to day 21 in the stroma but not in the LE (Wathes & Hamon 1993, Zheng *et al.* 1996). In this study, progesterone receptor mRNA was present in the caruncular tissues in early pregnancy on day 30 but became undetectable during the rest of gestation. It is possible, however, that progesterone may act at another site external to the placenta. Another possibility is that the technique used in this study was not sensitive enough to detect very low expression of progesterone receptor mRNA. Nevertheless, the drop in progesterone receptor mRNA by day 35 preceded the up-regulation of aromatase and OTR mRNA at days 35–40, strongly suggesting that progesterone receptor may have an inhibitory effect on the expression of OTR and aromatase in the placentome during early pregnancy.

In conclusion, the expression of aromatase, which parallels the concentration of oestrogenic derivatives during pregnancy, and OTR may be inhibited by progesterone receptor during early pregnancy. The expression of oestradiol receptor may be regulated at both the transcriptional and translational levels in a tissue and time specific manner, independent of the concentration of free oestradiol in the circulation and may be unaffected by progesterone receptor. Finally, the results from this study show that the up-regulation of OTR in the placentome capsule precedes the appearance of the oestradiol receptor, and is therefore unlikely to be regulated directly by oestradiol.

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