Conceptus interferon in uterine flush, endometrial concentrations of oxytocin receptors and prostaglandin $F_{2\alpha}$ release *in vitro* after transfer of conceptuses to ewes induced to ovulate at 28 days postpartum

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We examined the key events underlying maternal recognition of pregnancy and the prevention of luteolysis in early postpartum ewes by synchronously transferring single expanded blastocysts recovered from control ewes on day 11 of pregnancy into the uterus of either postpartum recipients that had been induced to ovulate 28 days after lambing ($n = 12$) or control recipients ($n = 11$). Conceptus development, uterine flush interferon (oTP-1) concentrations, endometrial oxytocin receptor concentrations and endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) release *in vitro* were determined 5 days later (corresponding to day 16 of the ovarian cycle). By this stage, both conceptus mass and oTP-1 content of total uterine flush in the eight postpartum recipients that remained pregnant were significantly lower ($P < 0.01$) than in the eight pregnant control ewes (524 ± 116.6 versus 959 ± 80.6 mg and 968 ± 16.9 versus 1512 ± 106.2 ng oTP-1 for postpartum and control recipients, respectively). These effects were independent of ovulation rate and daily peripheral progesterone concentrations after blastocyst transfer, which were similar between groups. Endometrial oxytocin receptor density was variable in both groups when they were killed, and was generally higher in pregnant postpartum than in control recipients, and was significantly different ($P < 0.05$) when the values for the transfer but not the contralateral uterine horns were compared. Similarly, basal and oxytocin-stimulated endometrial $PGF_{2\alpha}$ release during a 4 h incubation were higher ($P < 0.01$) in pregnant postpartum versus control recipients. Irrespective of treatment group and when expressed per uterine horn, conceptus mass was highly negatively correlated with number of oxytocin receptors and $PGF_{2\alpha}$ release *in vitro*. The results of this study imply that suboptimal conceptus growth rates and secretion of oTP-1, resulting in an inability to regulate endometrial oxytocin receptor-mediated $PGF_{2\alpha}$ secretion, may be central to pregnancy failure in the early postpartum ewe.

Introduction

In ewes that have recently lambed, the use of exogenous hormones to induce ovulation followed by intrauterine insemination to ensure fertilization results in the production of viable oviductal-stage embryos at 3–5 weeks postpartum. These embryos develop to term if transferred on day 3 after oestrus to a normal uterine environment of recipient ewes whose interval from parturition is more than 150 days, suggesting that follicle recruitment and oocyte maturation are normal and embryos are inherently viable (Wallace *et al.*, 1989a). However, embryos rarely survive beyond the duration of a normal ovarian cycle if transferred or returned to a postpartum uterus (McKelvey *et al.*, 1989; Wallace *et al.*, 1989b). Similarly, in early postpartum beef cows, the oocyte shed at a weaning-induced oestrus 30–35 days postpartum and preceding either a short or normal luteal phase can be fertilized and undergo early embryo development up to day 3 or 6 after insemination (Breuel *et al.*, 1993; Schrick *et al.*, 1993) but further development in the

postpartum uterus must be compromised because, irrespective of luteal function, pregnancy rates are low.

The exact timing of embryonic mortality in both these species is unknown. However, when conceptus development was assessed at day 16 after insemination, only 33% of postpartum ewes remained pregnant compared with 87% of controls, and for pregnant postpartum ewes, conceptus mass per corpus luteum was significantly reduced (Wallace *et al.*, 1991a). This finding suggests that pregnancy failure occurs at or before maternal recognition of pregnancy. In ewes, pregnancy recognition begins at about day 12 after oestrus and depends on adequate secretion of the conceptus interferon tau (or oTP-1) into the uterine lumen (see review by Bazer *et al.*, 1994). The interferon acts locally via its receptor on the uterine epithelium to suppress expression of uterine oxytocin receptors. This in turn prevents establishment of a positive feedback loop between pulsatile secretion of oxytocin from the ovary and endometrial release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which would normally induce luteal regression at the end of the ovarian cycle.

Received 30 August 1994.

In this study we examined the key events underlying the maternal recognition of pregnancy in early postpartum ewes by synchronously transferring single expanded blastocysts recovered from control ewes on day 11 of pregnancy into the uterus of either postpartum recipients that had been induced to ovulate 28 days after lambing or into control recipients. Conceptus development, interferon concentrations of the uterine flush, endometrial oxytocin receptor content and endometrial PGF_{2α} release *in vitro* were determined 5 days later (corresponding to day 16 of the cycle).

Materials and Methods

Experimental design

Border Leicester × Scottish Blackface ewes, >150 days postpartum, were used to donate expanded blastocysts for transfer into recipients that had recently produced lambs. Ewes were individually penned under natural lighting conditions at the Rowett Research Institute (57°N, 2°W) and were offered either 1.4 kg (non-lactating) or 3.6 kg (lactating) of a complete diet supplying 9 MJ metabolizable energy kg⁻¹ every day. In all ewes, oestrus was synchronized by withdrawing a progesterone-impregnated controlled internal drug release device (CIDR; containing 0.3 g progesterone, Inter-Ag, Hamilton) 11 days after its insertion. At withdrawal of the CIDR device from the vagina, each ewe received an i.m. injection of 750 iu of pregnant mares' serum gonadotrophin (PMSG; Intervet Labs, Cambridge) and the subsequent onset of oestrus was assessed using a vasectomized ram at intervals of 12 h between 24 and 48 h after CIDR withdrawal. Donor ewes (*n* = 15) were inseminated into the uterus 52 h after CIDR withdrawal (day 0) as described by McKelvey *et al.* (1985). Fresh semen collected from a pool of rams was diluted 1:4 (v:v) with PBS and approximately 0.2 ml of diluted semen used per horn. On day 11 after insemination, blastocysts were recovered surgically from donor ewes via a midline laparotomy; their diameters were measured under a stereomicroscope and held at 37°C until transfer. A total of 23 blastocysts (mean diameter 860 µm) were synchronously transferred in singleton into the uterus of recipient ewes with the same breeding history as the donors (controls, *n* = 11) or to lactating ewes that had been induced to ovulate 28 days after parturition (postpartum *n* = 12). When two or more embryos were recovered from a single donor ewe, a blastocyst was transferred to each of the two recipient treatment groups. Blastocysts were transferred into the uterine horn (transfer horn) ipsilateral to the ovary with the greatest number of corpora lutea. Postpartum recipients had previously been induced to lamb synchronously during the mid-breeding season by injection of 15 mg betamethasone (Betsolan; Glaxovet plc, Greenford) on day 143 of pregnancy. Lambings occurred 36–72 h later and the average litter size was 1.5 ± 0.23.

Five days after blastocyst transfer (corresponding to day 16 of the ovarian cycle), the reproductive tract of recipient ewes was exposed at mid-ventral laparotomy, clamped at the cervix, removed under terminal anaesthesia (via inhalation of halothane (Halothane-M&B; Rhone-Mereux Ltd, Harlow) in a mixture of O₂ and nitrous oxide) and placed on ice. The uterine horns were carefully dissected along the external bifurcation

and individually irrigated with 20 ml PBS to recover conceptus tissue. The conceptus tissue was subjectively assessed and weighed. Any remaining tissue was removed from the uterine flush by centrifugation at 1000 g for 20 min at 4°C. The supernatant volume was recorded and the flush samples stored at -20°C for subsequent analysis. Within 10 min of removing the uterus, a representative mixture (about 5 g) of caruncular and intercaruncular endometrium from the length of each uterine horn was dissected and immediately placed in ice-cold Krebs-Ringer buffer (KRB) for measurement of PGF_{2α} release *in vitro*. A further 5 g of endometrium from both uterine horns was removed and stored in liquid N₂ (for up to 10 days) for subsequent membrane preparation for oxytocin-binding measurements. Ovulation rates were recorded and the corpora lutea dissected and weighed.

oTP-1 content of uterine flush

oTP-1 content of the uterine horn flush was measured by radioimmunoassay as described by Vallet *et al.* (1988) with the following minor modifications. Iodination was by the chloramine T method (Hunter and Greenwood, 1963) and during the assay procedure, bound oTP-1 was separated from free oTP-1 by second antibody precipitation using donkey anti-rabbit serum with normal immunized rabbit serum as carrier (Scottish Antibody Production Unit, Carlisle, Lanarkshire). Uterine flush samples were diluted 1:10 or 1:100 with assay buffer before analysis. The limit of detection was 0.5 ng per tube and the variation between duplicate pairs was 1.8 ± 0.12%.

Release of PGF_{2α} in vitro

To determine PGF_{2α} release *in vitro*, caruncular endometrial explants (100 mg) were incubated in polystyrene multiwell culture plates (Corning, New York) in 2 ml KRB (containing 10 mmol glucose l⁻¹ and 2 mmol CaCl₂ l⁻¹, pH 7.4). Explants were gently washed twice with ice-cold KRB and then pre-incubated at 37°C on a rocking platform (8 cycles min⁻¹) under an atmosphere of 95% O₂:5% CO₂. After 2 h, the KRB was removed, the explants were washed and fresh KRB containing 0 or 1 µmol oxytocin l⁻¹ was added (CRB Ltd, Harston, Cambridgeshire; triplicate determinations per uterine horn). The incubation medium was sampled after 4 h for determination of PGF_{2α}. Media samples were stored at -20°C until analysis. PGF_{2α} concentrations were measured in duplicate as described by Kelly *et al.* (1986). The sensitivity of the assay was 16 pg per tube and the inter- and intra-assay coefficients of variation were 13.5 and 12.3%, respectively.

Oxytocin receptor binding assay

Membrane fractions were prepared from endometrial tissue exactly as described by Wallace *et al.* (1991b). Protein concentrations of the resulting membrane fractions were determined using the method of Bradford (1976) with BSA as standard. The concentration of oxytocin-binding sites in all membrane fractions was measured using the iodinated oxytocin antagonist ¹²⁵I-labelled d(CH₂)₅[Tyr(Me)²Thr⁴Tyr-NH₂]-vasotocin (OTA; Peninsula Laboratories, St Helens, Merseyside) as described by Wallace *et al.* (1991b). The OTA was iodinated on

Table 1. Pregnancy rate, conceptus mass and oTP-1 content of uterine flush on day 16 of the ovarian cycle (oestrus = day 0) in control and postpartum ewes following single blastocyst transfer on day 11. Postpartum ewes were induced to ovulate 28 days after lambing.

Parameter	Control	Postpartum	Significance of differences [‡]
Number of recipient ewes	11	12	
Number pregnant at day 16	8	8	
Conceptus mass (mg) recovered/pregnant recipient			
Transfer horn	498 ± 40.5	431 ± 90.2	ns
Contralateral horn	461 ± 74.7	93 ± 36.6	<i>P</i> < 0.001
Combined total	959 ± 80.6	524 ± 116.6	<i>P</i> < 0.01
(Range)	(629–1385)	(188–1073)	
oTP-1 (ng) in uterine flush*			
Transfer horn	771 ± 55.9	505 ± 51.1	<i>P</i> < 0.001
Contralateral horn	739 ± 62.4	463 ± 80.1	<i>P</i> < 0.05
Combined total	1512 ± 106.2	968 ± 116.9	<i>P</i> < 0.01
(Range)	(1021–2016)	(502–1382)	

Values are means ± SEM. ns: not significant.

*Adjusted to take into account volume of flush recovered.

[‡]Student's *t* test.

the tyrosyl residue at position 9 using an Iodogen method (Elands *et al.*, 1987). The relative concentration of oxytocin-binding sites in all membrane fractions were estimated on two occasions in triplicate by determining the specific binding of each sample (100 µg protein per tube) at a single saturating concentration of ¹²⁵I-labelled OTA (180 pmol per tube) incubated at 30°C for 45 min. Specific binding was measured as the difference between total and nonspecific binding, where non-specific binding and total binding represent the amount of ¹²⁵I-labelled OTA bound in the presence and absence of excess displacing oxytocin (10 µmol oxytocin l⁻¹). The measurement of number of receptors at a single concentration of ¹²⁵I-labelled OTA in ewes is closely correlated with numbers of oxytocin receptors obtained using full saturation analyses (Wallace *et al.*, 1991c).

Blood sampling and progesterone radioimmunoassay

Blood samples were collected via jugular venepuncture at 09:00 h daily, to determine peripheral progesterone concentrations and hence corpus luteum function during the induced cycle and following blastocyst transfer. Progesterone concentrations were measured in 100 µl aliquots of plasma following extraction and radioimmunoassay as described by Djahanbakhch *et al.* (1981). The inter- and intra-assay coefficients of variation were 9.6% and 12.4%, respectively; the detection limit was 0.3 ng ml⁻¹ and the recovery of progesterone from plasma was 62.9 ± 0.98% (SEM, *n* = 430). Values were adjusted to take into account extraction losses.

Statistical analyses

Results were analysed by Student's *t* test and correlation analysis was by Pearson's Product Moment test, where appropriate.

Results

After blastocyst transfer on day 11 of the induced ovarian cycle, eight of 12 postpartum and eight of 11 control recipients remained pregnant 120 h later (corresponding to day 16 of the cycle). Total conceptus mass recovered from the eight pregnant postpartum ewes was highly variable and significantly lower (*P* < 0.01) than in the eight corresponding control ewes. Of the eight conceptuses recovered from postpartum ewes, four were fragmented and appeared to be degenerating, while four were of similar appearance to those harvested from control ewes. Flushing the uterine horns separately allowed assessment of the degree to which the conceptus was occupying the uterine horn contralateral to the site of transfer (Table 1). Conceptus tissue was recovered from both transfer and contralateral horns of all control recipients, including two ewes that had only corpora lutea on the ovary ipsilateral to the transfer horn. In contrast, conceptus tissue was not recovered from the contralateral horns of three of eight postpartum recipients, and of these ewes, two had ovulated unilaterally. Overall, significantly more (*P* < 0.001) conceptus tissue was recovered from the contralateral horn in control than in postpartum recipients.

Total concentrations of the major conceptus interferon, oTP-1, were significantly lower (*P* < 0.01) in the uterine flushings of pregnant postpartum versus pregnant control ewes (Table 1). There were no differences in oTP-1 concentrations in the flushings recovered from the transfer versus contralateral horns in either the control or postpartum groups. Irrespective of treatment group, total conceptus mass recovered was positively correlated with oTP-1 secretion *in vivo* (*r* = 0.744, *n* = 16, *P* < 0.001). No oTP-1 was detected in the uterine flushings of nonpregnant recipients but, as it was not clear at which stage the pregnancies had failed, no further data from these animals will be presented.

Table 2. Ovulation rate, peripheral progesterone concentrations and corpus luteum mass in pregnant, control and postpartum ewes following single blastocyst transfer on day 11 of the cycle. Postpartum ewes were induced to ovulate 28 days after lambing.

Parameter	Control	Postpartum	Significance of differences [†]
Number of pregnant recipient ewes	8	8	
Ovulation rate	3.6 ± 0.56	2.8 ± 0.37	ns
Progesterone concentrations on days 11–16	6.3 ± 0.65	7.9 ± 1.06	ns
Total corpus luteum mass (g)	2.39 ± 0.274	1.92 ± 0.237	ns

Values are means ± SEM.

[†]Student's *t* test.

Fig. 1. Changes in peripheral plasma concentrations of progesterone throughout the oestrous cycle in control (●) and postpartum (○) ewes induced to ovulate 28 days after lambing. A single blastocyst was transferred on day 11 after oestrus. Values represent means ± SEM.

Mean ovulation rate, daily peripheral progesterone concentrations throughout the induced cycle and total corpus luteum mass on day 16 of the cycle were similar for pregnant postpartum and control recipient ewes (Table 2, Fig. 1). Irrespective of treatment group, peripheral progesterone concentrations in the sample collected immediately before tissue harvesting on day 16 of the cycle were independent of both the conceptus mass recovered and the oTP-1 content of the uterine flush.

Data for the endometrial oxytocin receptor density when animals were killed on day 16 of the cycle and basal and oxytocin-stimulated endometrial PGF_{2α} release during incubation *in vitro* for 4 h are presented in Table 3. Endometrial oxytocin receptor density was variable in both groups and generally higher in pregnant postpartum recipients than in controls. This difference reached significance ($P < 0.05$) when values for the transfer but not the contralateral horns were compared. Basal endometrial PGF_{2α} release in culture was significantly higher ($P < 0.05$) in both the transfer and contralateral horns of the pregnant postpartum versus control recipients. Furthermore, within the postpartum group, basal PGF_{2α} release was significantly higher ($P < 0.05$) in the contralateral than in the transfer horn. Exogenous oxytocin stimulated PGF_{2α} release *in vitro* to varying extents in all ewes and

the mean PGF_{2α} concentrations at the end of the incubation period remained significantly higher in both horns of pregnant postpartum versus control recipients. When expressed per uterine horn, conceptus mass was negatively correlated with (i) oxytocin receptor number ($r = -0.522$, $n = 32$, $P < 0.01$); (ii) basal PGF_{2α} release *in vitro* ($r = -0.622$, $n = 32$, $P < 0.001$); and (iii) oxytocin-stimulated PGF_{2α} release ($r = -0.630$, $n = 32$, $P < 0.001$).

Discussion

The single blastocyst transfer and conceptus recovery procedures used in the study reported here allowed some of the key components underlying the maternal recognition of pregnancy in ewes induced to ovulate early postpartum to be examined.

After transfer of developmentally normal blastocyst tissue on day 11 after oestrus, subsequent development as measured by conceptus mass, migration into the contralateral horn and interferon content of oTP-1 in uterine flush was significantly reduced after exposure for 120 h to a postpartum compared with a control uterine environment. The factors underlying the reduced growth rate of the conceptus and its suboptimal oTP-1 secretion in a postpartum uterus have yet to be identified but may involve inappropriate secretion of one or more uterine growth factors. Insulin-like growth factors (IGF-I and IGF-II) are present in ovine uterine flushings collected during the period of pregnancy recognition (Ko *et al.*, 1991) and the endometrium is an abundant source of mRNAs encoding IGF-I and IGF-II at this time (Stevenson *et al.*, 1992; Simmen *et al.*, 1993). Simultaneous addition of both IGF-I and IGF-II to conceptuses at day 13 of development significantly stimulated oTP-1 secretion (Ko *et al.*, 1991) and mRNA encoding IGF-I, but not IGF-II, has been localized in preimplantation embryonic tissues from the one-cell to the expanded blastocyst stage (Watson *et al.*, 1994). Similarly, cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to enhance conceptus oTP-1 production (protein and mRNA) *in vitro* and mRNA encoding GM-CSF is localized in the uterine endometrium (Imakawa *et al.*, 1993). Secretion of growth factor by the early postpartum uterus has not been examined. Attempts to detect qualitative or quantitative differences in endometrial protein secretion during explant culture *in vitro* have largely failed to reveal major differences between

Table 3. Endometrial oxytocin receptor density and basal and oxytocin-stimulated PGF_{2a} release during *in vitro* culture of endometrium harvested on day 16 of the cycle from pregnant control and postpartum recipients. Postpartum ewes were induced to ovulate 28 days after lambing.

Parameter	Control	Postpartum	Significance of differences [‡]
Number of recipient ewes	8	8	
Number of endometrial oxytocin receptors (fmol ¹²⁵ I-labelled OTA mg ⁻¹ protein)			
Transfer horn	6.9 ± 0.94	21.3 ± 5.17	P < 0.05
Contralateral horn	16.2 ± 3.34	38.0 ± 13.94	ns
Combined mean	11.6 ± 2.06	29.6 ± 8.79	P < 0.06
Basal PGF _{2a} release (ng g ⁻¹ per 4 h)			
Transfer horn	111.4 ± 27.68	332.5 ± 89.08	P < 0.05
Contralateral horn	191.1 ± 51.28	858.6 ± 200.06	P < 0.01
Combined mean	151.3 ± 36.62	595.6 ± 125.72	P < 0.01
Oxytocin-stimulated PGF _{2a} release (ng g ⁻¹ per 4 h)			
Transfer horn	235.6 ± 51.54	775.7 ± 230.44	P < 0.05
Contralateral horn	449.8 ± 95.50	1436.4 ± 356.02	P < 0.05
Combined mean	342.7 ± 67.04	1106.1 ± 221.90	P < 0.01

Values are means ± SEM.

OTA: d(CH₂)₅[Tyr(Me)²Thr⁴Tyr-NH₂⁹]-vasotocin.

[‡]Student's *t* test.

pregnant or nonpregnant postpartum versus pregnant control endometrium at day 10, 15 or 16 after oestrus. However, a limited range of polypeptides was examined in these studies (Wallace *et al.*, 1991a, 1992, 1993a).

The results of the study reported here indicate that blastocysts cultured in a postpartum uterus secrete insufficient oTP-1 to exceed the threshold required to inhibit luteolysis. Positive relationships have been observed in ewes in which the interval from previous parturition exceeds 150 days, among conceptus development, pregnancy recognition and the amount of oTP-1 recovered in uterine flushings (Nephew *et al.*, 1989; Ko *et al.*, 1991) and the present data confirm and extend these findings. In these ewes, supplementation of the endogenous conceptus interferon signal by administration of recombinant bovine interferon during the pregnancy recognition period enhances both pregnancy rate and the number of lambs born (Nephew *et al.*, 1990; Martinod *et al.*, 1991; Schalue-Francis *et al.*, 1991). It has not been established whether similar interferon supplementation could enhance pregnancy rates in the early postpartum ewe model used in the present study. However, in dairy cows inseminated before 55 days postpartum, the intra-uterine transfer of trophoblastic vesicles on day 7 of the ovarian cycle (presumably providing a source of bovine conceptus interferon) significantly increases pregnancy rates (Ryan *et al.*, 1994).

The mechanism whereby the conceptus interferon, oTP-1, inhibits endometrial responsiveness to oxytocin, and hence prevents PGF_{2a} secretion and luteolysis, has not been completely elucidated but the results presented here strongly suggest that regulation of the uterine oxytocin receptor is a key component. In early pregnant ewes, the expression of endometrial oxytocin receptors (protein and mRNA) is suppressed on days 14–15 after oestrus (McCracken *et al.*, 1981;

Sheldrick and Flint 1985; Ayad *et al.*, 1993; Stevenson *et al.*, 1994). Furthermore, infusion of conceptus secretory proteins or recombinant ovine or bovine conceptus interferon into the uteri of nonpregnant ewes prevents oxytocin receptor formation and extends the duration of the cycle (Stewart *et al.*, 1989; Martal *et al.*, 1990; Vallet and Lamming, 1991; Parkinson *et al.*, 1992; Miranda *et al.*, 1993a). In the study reported here, endometrial oxytocin receptor concentrations were significantly higher in pregnant postpartum versus pregnant control ewes. The design of the study allowed the number of oxytocin receptors and basal and oxytocin stimulated PGF_{2a} release *in vitro* to be examined in relation to the relative degree of conceptus occupancy of the uterine horns 120 h after transfer. The negative correlations between conceptus mass per uterine horn and number of oxytocin receptors, basal PGF_{2a} and oxytocin-stimulated PGF_{2a} release *in vitro* are consistent with a direct paracrine action of oTP-1 on the uterine epithelium as first demonstrated by Lamming *et al.* (1991) using pre-mating uterine ligation to separate pregnant versus nonpregnant horns. It is not known whether the large number of oxytocin receptors in postpartum ewes is entirely due to suboptimal oTP-1 secretion by the developing conceptus or whether it reflects persistence of oxytocin receptors in postpartum uterine tissues *per se*. Autoradiography studies revealed that oxytocin receptors are present in the uterine tissues of untreated lactating ewes until at least day 35 postpartum (Wallace, 1994). These receptors are functional in that exogenous oxytocin stimulated the inositol phosphate-diacylglycerol second messenger system within the endometrium and PGF_{2a} release *in vitro*. Furthermore, functional oxytocin receptors have been detected throughout the ovarian cycle in ewes induced to ovulate at day 21 postpartum (Wallace *et al.*, 1993b). The number of oxytocin receptors in pregnant postpartum ewes at

day 16 after oestrus in the present study was low in relation to those measured previously in nonpregnant control ewes near the onset of luteolysis (Wallace *et al.*, 1991c). However, recent data in non-postpartum animals suggests that only a modest rise in the number of oxytocin receptors is required to mediate oxytocin-induced PGF_{2α} secretion and that the large increase in the number of oxytocin receptors late in the oestrous cycle occurs as a consequence rather than a cause of luteolysis (Mirando *et al.*, 1993b; Beard and Lamming, 1994).

In conclusion, the results of the present study suggest that an inability to suppress endometrial oxytocin receptor-mediated PGF_{2α} release completely, and hence prevent luteolysis, is central to pregnancy failure in ewes induced to ovulate early postpartum. While the factors underlying the reduced growth rate of the conceptus and its suboptimal oTP-1 secretion in the early postpartum uterus have yet to be identified, the early postpartum ewe could prove to be a useful model for investigating the role of uterine growth factors and cytokines in embryo growth and development during the period of pregnancy recognition. Indeed, preliminary evidence from a study in which embryos were transferred into diverse uterine environments at day 3 after oestrus and assessed on day 11 or day 16 suggests that the postpartum uterus can influence embryo survival and growth before the expanded blastocyst stage (J. M. Wallace, unpublished).

The authors thank I. Robertson for expert technical assistance, F. W. Bazer for oTP-1 antiserum and standard material and R. Kelly for PGF_{2α} antiserum. This research was financed by the Scottish Office, Agriculture and Fisheries Department.

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