J. Reprod. Fert., Suppl. **30** (1981), 171–182 *Printed in Great Britain*

Blastocyst–endometrial interactions in early pregnancy in the sheep

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Summary. This review analyses the endocrine recognition of pregnancy in the sheep. Particular emphasis is placed on the effect of a blastocyst on prostaglandin and protein metabolism in the endometrium. The preimplantation period is associated with increased prostaglandin content in the endometrium and uterine lumen, increased leucine incorporation into endometrial protein and increased blood flow to the uterus. It is concluded that there are substantial alterations in endometrial function during the protracted preimplantation period and that these alterations constitute an endocrine form of maternal recognition of pregnancy. However, it is not yet possible to attribute these alterations to specific embryonic signals.

Introduction

The mammalian conceptus can develop independently of the uterine environment until blastulation. During the remainder of the preimplantation period, the blastocyst depends increasingly on the uterine environment for survival and development, necessitating a close interaction between the blastocyst and the maternal endometrium. This is shown by experiments involving embryo transfer between animals (and to ectopic sites within animals) and by a study of the phenomenon of delayed implantation (Heap, Flint, Gadsby & Rice, 1979). The nature of the interaction between the blastocyst and the maternal system is not well understood. More is known about signals of embryonic origin which influence the maternal system than *vice versa*. This is probably because the effects of a blastocyst on the maternal environment are more readily discernible.

In all eutherian mammals studied, continued progesterone secretion by the corpus luteum is essential for the establishment and maintenance of early gestation (Amoroso & Perry, 1977). In a number of species, including ruminants, the signal prolonging the life-span of the corpus luteum precedes attachment and implantation and is one of the earliest indications of recognition of pregnancy by the maternal endocrine system (Short, 1969) (Table 1). There may be other forms of maternal recognition of pregnancy during the preimplantation period, for example, involving the immune system (Noonan, Halliday, Morton & Clunie, 1979; Nancarrow, Wallace & Grewal, 1981).

This review will consider the endocrine recognition of pregnancy in sheep during the preimplantation period. Attention will be focussed on factors originating from the blastocyst and their effects on prostaglandin (PG) and protein synthesis in the attachment (caruncular) and inter-attachment (intercaruncular) regions of the endometrium.

0449-3087/81/020171-12$02.00/0

Table 1. A comparison of the time (days *post coitum*) of recognition of pregnancy, attachment and parturition in the ewe, cow, sow and mare

Species	Endocrine recognition of pregnancy*	Definitive attachment of embryonic and uterine tissues	Parturition
Ewe	12–13	16	145
Cow	16–17	18–22	282
Sow	12	18	114
Mare	14–16	36–38	340

* Defined as the day when a blastocyst must be present in the uterus to prevent regression of the corpus luteum.

The oestrous cycle of the ewe

Hormones in the oestrous cycle

Changes in blood concentrations (Yuthasastrakosol, Palmer & Howland, 1975; Pant, Hopkinson & Fitzpatrick, 1977) and secretion rates of hormones (Baird, Land, Scaramuzzi & Wheeler, 1976; Baird & Scaramuzzi, 1976) during the ovine oestrous cycle have been extensively described. The concentration of progesterone, the major gestagen produced by the corpus luteum, increases from Days 3–4 (Day 0 = day of oestrus) to a maximum (4–6 ng/ml) by Days 7–8. The progesterone levels remain high until Days 14–15 when luteolysis occurs; progesterone levels then decline to very low levels (<0·2 ng/ml) 1–2 days before the next oestrus. The concentration of free oestrogen (mainly oestrone and oestradiol-17β) in peripheral blood is relatively low (up to 5 pg/ml) and apart from an increase during the preovulatory period, there is no consistent pattern. Following the preovulatory surge at oestrus, luteinizing hormone (LH) levels fall to a minimum by the mid-luteal phase (Hauger, Karsch & Foster, 1977); a small but significant increase in plasma LH levels occurs about the time of luteolysis when progesterone levels are falling. The levels of prolactin remain relatively constant during the cycle except for an increase about oestrus (Cumming, Brown, Goding, Bryant & Greenwood, 1972). Prostaglandin (PG) F levels (in uterine blood) increase from Day 12 onwards reaching maximum concentrations 1–2 days before oestrus. An increase in the concentration of the PGF-2α metabolite 13,14-dihydro-15-keto-PGF-2α in peripheral blood (Peterson, Tervit, Fairclough, Havik & Smith, 1976) parallels the increase in PGF in the uterine vein.

Control of the corpus luteum in non-pregnant ewes

Pituitary luteotrophic complex. The formation of the corpus luteum and maintenance of its function requires pituitary support in most mammals. Denamur, Kann & Short (1973) reviewed the evidence for LH and prolactin forming a luteotrophic complex to support the corpus luteum in sheep. There is no evidence that the fall in LH levels by mid-cycle initiates luteolysis.

The uterine luteolysin. The relationship between the uterus and corpus luteum in all ruminants is of particular importance in the establishment of pregnancy. The uterus has a local, luteolytic action on the ipsilateral corpus luteum of non-pregnant ewes to bring about a decrease in progesterone secretion towards the end of the oestrous cycle (Moor, 1968). Evidence to date supports the notion that the uterine luteolysin in the sheep is PGF-2α (Goding, 1974), produced by the endometrium under the influence of the ovarian steroids, progesterone and oestradiol-17β (Baird, 1978), and possibly oxytocin (Sheldrick, Mitchell & Flint, 1980). PGF-2α, first detected in the uterine vein after Day 12, reaches the ovarian artery by a 'countercurrent' mechanism, with transfer in the region of the junction of the ovarian and uterine veins where there is a close

apposition of the venous vessels with the convoluted ovarian artery. The transfer of PGF-2α is down a concentration gradient; a log relationship has been described between the concentrations of PGF in the uterine vein and ovarian artery (Land, Baird & Scaramuzzi, 1976).

The mechanism by which PGF-2α exerts its luteolytic action on the corpus luteum is not known (Horton & Poyser, 1976). There is evidence of a vascular component as a fall in capillary blood flow to the corpus luteum is associated with but does not precede luteolysis (Niswender, Moore, Akbar, Nett & Diekman, 1975). Although receptors for PGF-2α have been demonstrated on luteal cell membranes from sheep (Powell, Hammarstrom & Samuelsson, 1974), binding of PGF-2α to these receptors has not been directly linked with luteolysis. PGF-2α can affect the biochemistry of the luteal cell. A decline in the activity of 3β-hydroxysteroid oxoreductase–Δ^4 isomerase activity converting pregnenolone to progesterone has been measured during natural luteolysis (Deane, Hay, Moor, Rowson & Short, 1966) and within 5 h of PGF-2α treatment (Hoppen, Williams & Findlay, 1976). This is probably not the primary event because injection of PGF-2α directly into the corpus luteum does not reduce progesterone secretion (Chamley & O'Shea, 1976). Henderson & McNatty (1975) suggested that PGF-2α initiates luteolysis by uncoupling LH and the adenyl cyclase system. Diekman, O'Callaghan, Nett & Niswender (1978) demonstrated a loss of LH receptors from lutein cells following PGF treatment of ewes. However, luteal progesterone levels declined well before a decrease in the content of LH receptors was detected. This suggests that the principal mechanism by which PGF-2α induces luteolysis is not via loss of LH receptors.

It seems likely that the luteolytic action of PGF-2α in the ewe is at the ovarian, rather than the pituitary, level and that it probably involves a vascular component as well as a direct action on the lutein cell. The question remains as to the sequence in which these effects of PGF-2α occur.

Prostaglandins in early pregnancy

Antiluteolytic role of the conceptus

In pregnancy, production of an antiluteolysin by the blastocyst is essential to neutralize the lytic action of the uterus (Moor, 1968; Martal, Lacroix, Loudes, Saunier & Wintenberger-Torres, 1979), at least until Day 50 when sufficient progesterone is produced by the placenta (Denamur *et al.*, 1973; Amoroso & Perry, 1977). Moor & Rowson (1966a) have shown a local action of the uterine luteolysin in bringing about regression of the corpus luteum in the non-pregnant ewe. Likewise, a viable conceptus, confined to one uterine horn, has a local action and will only maintain the ipsilateral corpus luteum (Moor & Rowson, 1966b). The blastocyst is only capable of preventing the demise of the corpus luteum if there is support for the corpus luteum by the luteotrophic complex of pituitary LH and prolactin. Although placental lactogen in sheep is first detected in the cotyledons on Days 16–17 (Martal & Djiane, 1977), sheep trophoblast does not produce sufficient luteotrophin (like chorionic gonadotrophin in primates) to support the corpus luteum of pregnancy during the preimplantation period.

The events of early pregnancy in the ewe are summarized in Table 2. Short (1969) divided the first 50 days of pregnancy in the ewe into three periods related to the endocrine recognition of pregnancy: the indifferent period up to Day 11, when removal of the blastocyst does not affect the subsequent return to oestrus; the antiluteolytic period from Day 12 to 25, when the presence of the embryo negates the lytic influence of the uterus; and a luteotrophic period from Day 40 onwards, when the embryo is able to support the corpus luteum in the absence of the pituitary gland. Of particular interest is the rapid expansion and elongation of the blastocyst at about the beginning of the antiluteolytic period. The rapid growth of trophectoderm has been related to expression of the antiluteolysin and depends on the development of the progestational endometrium (Lawson & Findlay, 1977). By the time definitive attachment takes place between Days 16 and 18, the length of the chorionic sac exceeds 10 cm.

Table 2. Events associated with endocrine recognition of pregnancy in the ewe (after Short, 1969)

Period	Day of pregnancy	Event
Indifferent	0	Oestrus and ovulation
	2–4	Entry of zygote to uterus
	6–7	Hatching of blastocyst
Antiluteolytic	11	Blastocyst 1 cm length
	12	Removal of blastocyst before Day 12 does not alter cycle length
	12–15	Elongation of blastodermic vesicle > 10 cm. Mesoderm outgrowth and formation of chorionic sac
	16	First signs of attachment. Maintenance of luteal progesterone levels
	17	Missed oestrus. Formation of chorio-allantoic placenta
Luteotrophic	40	Luteotrophic influence of embryo
	50	Corpus luteum can be removed without causing abortion; progesterone production by placenta adequate to maintain gestation

Nature of the antiluteolysin, trophoblastin

Moor (1968) described the antiluteolysin as a water-soluble, heat-labile, species-specific substance, present in sheep trophoblast on Days 14–15. The capacity of conceptus extracts to extend the life-span of the corpus luteum when infused into the uterine lumen (but not when infused systemically) has been confirmed and extended (Lawson & Findlay, 1977; Martal *et al.*, 1979; Martal, 1981; Ellinwood, Nett & Niswender, 1979a). In more recent studies, however, we have noted that distension of the uterus after intrauterine infusion of lamb serum as a control was associated in a significant number of ewes with maintenance of the corpus luteum (L. D. Staples, R. A. S. Lawson & J. K. Findlay, unpublished observations), reinforcing the need for appropriate controls in such experiments. Martal *et al.* (1979) called the antiluteolysin 'trophoblastin' and describe it as an acidic protein of molecular weight 70 000. It seems probable that trophoblastin is distinct from placental lactogen or a chorionic gonadotrophin because neither of these hormones (ovine placental lactogen and hCG) prolonged luteal function when infused in a similar manner. A report of 'hCG-like' activity in the ovine conceptus before attachment (Wintenberger-Torres, 1978) has not been confirmed (Ellinwood *et al.*, 1979a). Placental lactogen has not been detected in trophoblast until around the time of attachment (Martal & Djiane, 1977), after the onset of the antiluteolytic influence on Day 12.

Mechanism of action of trophoblastin

Assuming that PGF-2α is the luteolysin in the ewe, trophoblastin could exert its antiluteolytic action to maintain corpus luteum function in 3 ways: by changing the uterine synthesis, metabolism and/or release of PGF-2α so that less PGF-2α would be available for transfer to the ovary; by interfering with the local transfer of PGF-2α from uterus to ovary via the 'countercurrent' system; or by antagonizing the action of PGF-2α at the ovarian level. This review will focus on the first possibility, since information on the other two is meagre. There is evidence for a direct luteotrophic influence of the gravid uterine horn on the ovary (Inskeep, Smutny, Butcher & Pexton, 1975; Mapletoft, Del Campo & Ginther, 1975; Mapletoft, Lapin & Ginther, 1976), but it is not known if this involves trophoblastin or a different factor.

There have been conflicting reports on whether or not PGF production by the sheep uterus is altered during the preimplantation period (Findlay, Cerini, Staples & Cumming, 1978). Because PGF is secreted in a pulsatile fashion (Thorburn, Cox, Currie, Restall & Schneider, 1973) and is rapidly cleared by the lung (Davis, Fleet, Harrison & Maule Walker, 1980), it is necessary to take samples at frequent intervals (5–10 min) to assess the levels in blood. When such precautions are taken, there is evidence that the concentration and frequency of release of PGF

into uterine venous blood is reduced in early pregnancy compared to the non-pregnant cycle (Thorburn *et al.*, 1973; Barcikowski, Carlson, Wilson & McCracken, 1974; Nett *et al.*, 1976). Until secretion rates are measured, doubt must remain about the absolute level of PGF production *in vivo* because there is increased blood flow to the uterus in early pregnancy (Greiss & Anderson, 1970) which could have a diluting effect. However, the conclusion that PGF levels decrease in uterine venous blood in early pregnancy is supported by the failure to detect increased concentrations of the metabolite 13,14-dihydro-15-keto-PGF-2α, found in peripheral blood, during the later stages of the oestrous cycle (Peterson *et al.*, 1976).

Despite evidence for a decrease in PGF in the uterine venous blood, there are now a number of reports that the capacity of the endometrium to produce PGF *in vitro* is not decreased in early pregnancy (Ellinwood *et al.*, 1979b; Findlay *et al.*, 1981). Furthermore, PGF and PGE-2 content and concentration in the endometrium increase in the preimplantation period, particularly by Day 15 (Lewis *et al.*, 1977; Lewis, Jenkins, Fogwell & Inskeep, 1978; Ellinwood *et al.*, 1979b).

These findings support the model, based on studies in the pig (Bazer & Thatcher, 1977), that PG synthesis by the endometrium remains unaltered or may even increase in early pregnancy and that there is a re-distribution of PG towards the uterine lumen away from the venous drainage. Studies using immunofluorescent localization of PGF in the intercaruncular endometrium have shown that the distribution of PGF is predominantly in the lamina propria of pregnant ewes after Day 12 whereas in non-pregnant ewes it is mainly in the epithelial cells lining the uterine lumen and uterine glands (Cerini, Cerini, Findlay & Lawson, 1979). The idea that there is a re-distribution of PG in the gravid uterus is further supported by the work of Ellinwood *et al.* (1979b) who reported increased concentrations of PGF and PGE-2 (and protein) in uterine flushings of pregnant ewes during Days 13–17. The relative increase in PGE-2 to PGF is notable in view of the demonstration that PGE-2 can antagonize the luteolytic action of PGF-2α in sheep (Henderson, Scaramuzzi & Baird, 1977).

If this concept on the re-distribution of uterine PG in early pregnancy of the ewe is correct, it raises a number of questions. Is the change in PG distribution confined to the caruncular region of the endometrium? How is the PG content of the uterine tissues and lumen of pregnant animals maintained above that of non-pregnant animals? Are these changes in PG content and distribution due to the action of trophoblastin?

The relative contribution of the caruncular and intercaruncular regions of the endometrium to PGF production is not clear. Current evidence suggests that both regions are equally capable of the synthesis of PGF in pregnant and non-pregnant ewes when provided with excess arachidonate substrate (Findlay *et al.*, 1981), and that the differences in earlier studies (Louis, Parry, Robinson, Thorburn & Challis, 1977) were due to the incubation conditions and/or failure to separate myometrium from intercaruncular endometrium. However, PGF content of caruncles from pregnant ewes on Day 15 (1 day before attachment) was much higher than that of intercaruncular tissue, and of caruncular and intercaruncular tissue of non-pregnant ewes (Table 3). Furthermore, after incubation for 4 h in the presence of indomethacin, caruncular endometrium from pregnant ewes still retained a significant amount of PGF compared to all other tissues which released their PGF content into the medium (Table 3). Since the absolute capacity of the tissues to synthesize PGF did not differ, the higher tissue content of PGF in caruncular endometrium of pregnant ewes suggests that a PG binding moiety may be present.

It seems unlikely that all the increased protein and PG content of the uterine lumen is entirely of embryonic origin. There are pregnancy-associated proteins in the uterine lumen during the preimplantation period (Roberts, Parker & Symonds, 1976; Staples, Lawson & Findlay, 1978), but their capacity to bind PGs has not been studied. The ovine (J. Hyland, personal communication) and the bovine (Shemesh, Milaguir, Ayalon & Hansel, 1979) conceptus releases significant quantities of PGF and PGE-2 *in vitro*. We do not know if trophoblastin is involved in these changes in uterine PG content. A proposed mode of action of trophoblastin would be that

Table 3. Mean (± s.e.m.) tissue content of prostaglandin F (PGF) in caruncular and intercaruncular endometrium of pregnant and non-pregnant ewes on Day 15 (from Findlay *et al.*, 1981)

Endometrial tissue	Pregnancy status	No. of ewes	PGF tissue content* (ng/g) Before indomethacin	PGF tissue content* (ng/g) After indomethacin
Caruncular	Pregnant	4	213 ± 24^a	34 ± 9^c
	Non-pregnant	3	48 ± 18^b	9 ± 3^d
Intercaruncular	Pregnant	4	63 ± 23^b	$<2^d$
	Non-pregnant	3	47 ± 18^b	$<2^d$

* Measured before and after incubation in the presence of indomethacin (1·5 μg/ml) at 37°C for 90 min.

Values in columns with different superscripts are different at the 5% level using Duncan's F-test.

it acts on the endometrium to produce a substance (? protein) capable of sequestering prostaglandins in higher concentrations in the caruncle and uterine lumen. Trophoblastin may also act on the synthetic pathway of PG production to increase the relative abundance of PGE-2 over PGF-2α, perhaps by increasing the activity of 9-keto-reductase converting PGF-2α to PGE-2 (Watson, Shepherd & Dodson, 1979).

The discussion so far has centred on the mechanism by which the conceptus can exert its antiluteolytic effect on the uterus to ensure continued progesterone secretion by the corpus luteum. If this is achieved by sequestering PG in the uterine caruncles and lumen, and by increasing the amount of PGE-2 relative to PGF-2α, the possibility that these changes in uterine PG and protein are directly beneficial to the preimplantation blastocyst should not be overlooked.

Protein synthesis

Effect of ovarian steroids

Endometrial proteins contribute to the secretions of the uterine glands to support the preimplantation blastocyst. The endometrium also synthesizes enzymes for intermediary metabolism and steroid hormone metabolism. It is well established that the ovine endometrium is under the control of ovarian steroids (Miller & Moore, 1976; Miller, Murphy & Stone, 1977b; Stone, Murphy & Miller, 1978). The rate of protein synthesis *in vitro*, the activation of many endometrial enzymes and the tissue content of energy substrates such as glycogen are steroid-dependent, showing changes related to oestrogen and progesterone concentrations in the circulation (Findlay, Maule Walker & Heap, 1980). There are differences in enzyme activities between regions of the endometrium. The lysosomal enzymes, acid phosphatase and β-glucuronidase, have a greater activity in caruncular than in intercaruncular tissue, and reach their maximum activities during the luteal phase (Murdoch & White, 1968; Murdoch, 1970; Findlay *et al.*, 1981). Murdoch (1970) suggested that these enzymes in the caruncles might be important for attachment and implantation, although there is no evidence of enhanced activity just before attachment.

Effect of pregnancy

The protein content of the lumen of the uterus increases between Days 13 and 17 of pregnancy (Ellinwood *et al.*, 1979b); glycosidase activity in uterine flushings of ewes also

increases prior to attachment (Roberts *et al.*, 1976). It is possible that some of these proteins originate from the blastocyst. Electrophoretic studies on uterine flushings collected on Day 15 of pregnancy revealed 10 uterine specific bands of which 5 were characteristic of pregnant animals (Roberts *et al.*, 1976). It is also likely that much of the protein in the lumen is of endometrial origin so a local action of the embryo on endometrial protein synthesis needs to be considered as well as the effects of ovarian steroids.

Until recently, there were no reports comparing protein synthesis in the endometrium of pregnant and non-pregnant ewes during the preimplantation period. Miller, Moore, Murphy & Stone (1977a) studied protein synthesis on Days 4 and 10 in the endometrium of ovariectomized ewes made pregnant by embryo transfer and treated with oestrogen and progesterone. However, no comparison was made with non-pregnant animals. We found a significant increase on Day 15 of pregnancy in the incorporation of radiolabelled leucine into acid-insoluble protein *in vitro* for caruncular and intercaruncular endometrium (Table 4) (Findlay *et al.*, 1981). An increase in protein synthesis was observed in ovariectomized ewes treated with oestradiol-17β (implant for 15 days), but not with progesterone (implant for 12 days); when the steroid treatments were combined, progesterone did not significantly reduce the oestradiol-induced increase in protein synthesis. Electrophoretic studies (one dimension, double label) of the newly synthesized protein from both regions of the endometrium did not reveal any bands specifically associated with pregnancy or steroid treatment (Findlay *et al.*, 1981). The electrophoretic method may have been too insensitive to detect small increases in the synthesis of specific proteins.

Table 4. Mean (± s.e.m.) incorporation of radiolabelled leucine into trichloracetic acid-insoluble protein after quadruplicate incubations with 50 mg endometrium from pregnant or non-pregnant ewes on Day 15 (from Findlay *et al.*, 1981)

Endometrial tissue	Pregnancy status	No. of ewes	Leucine incorporation (nmol/mg protein)
Caruncular	Pregnant	4	$14{\cdot}7 \pm 0{\cdot}9^{a}$
	Non-pregnant	5	$10{\cdot}3 \pm 0{\cdot}9^{bc}$
Intercaruncular	Pregnant	4	$13{\cdot}1 \pm 1{\cdot}9^{ac}$
	Non-pregnant	5	$8{\cdot}9 \pm 0{\cdot}5^{b}$

Different superscripts are different at the 5% level of Duncan's F-test.

Factors increasing endometrial protein synthesis in pregnancy

The available evidence suggests an increase in the rate of protein synthesis by the ovine endometrium during Days 13–17 of the preimplantation period. This may be due to an interaction between the blastocyst and the uterus to increase uterine blood flow and/or to enhance the responsiveness of the endometrium to stimulation by steroids.

Uterine blood flow. There is an increase in blood flow to the gravid uterine horn, and to the endometrium in particular, beginning on Day 12–13 (Greiss & Anderson, 1970; Greiss & Miller, 1971). On Day 16, about the time of attachment, there is hyperaemia in the caruncles (Boshier, 1970) probably associated with a higher overall metabolic activity in the endometrium and therefore increased protein synthesis. It has been suggested that the increased uterine blood flow results from a local influence of the blastocyst on the uterine vascular bed, whereby neurotransmitter effects on arterial smooth muscle cells are reduced, resulting in vasodilation (Ford, Weber & Stormshak, 1977; Pope & Stormshak, 1979). The vasodilatory influence of the blastocyst on uterine blood flow is probably not mediated by PGE-2 (Pope & Stormshak, 1979) present in increased concentration in pregnant endometrium (Lewis *et al.*, 1978; Ellinwood *et al.*, 1979b). Increased blood flow to the gravid horn could result from a local action of oestrogen,

known to increase uterine blood flow in the sheep (Huckabee, Crenshaw, Curet, Mann & Barron, 1970; Anderson, Hackshaw, Still & Greiss, 1977).

Local oestrogen action. Because oestrogen treatment can mimic the effects of pregnancy on endometrial protein synthesis and uterine blood flow, it is tempting to suggest a key role for this steroid in blastocyst–endometrial interactions. A role for oestradiol-17β as an embryonic signal concerned with maternal recognition of pregnancy need not conflict with its luteolytic action in sheep. Oestradiol is luteolytic when given systemically at midcycle in large doses (Stormshak, Kelly & Hawk, 1969), presumably acting on the uterus to increase secretion of PGF-2α (Barcikowski *et al.*, 1974). If oestradiol-17β is involved in interactions between the blastocyst and the endometrium, the production and action of the steroid are probably local. This view is supported by the fact that pregnancy can continue in ovariectomized, adrenalectomized ewes, provided they are treated with progesterone and mineralocorticoid (Cumming, Baxter & Lawson, 1974). However, there is no evidence that the sheep conceptus has the capability to produce oestrogens (Gadsby, Heap & Burton, 1980), unlike pig trophectoderm (Perry, Heap & Amoroso, 1973). Willis, Fields, Wise, Dueben & Bazer (1979) have reported aromatase activity in endometrium from ewes on Day 20 of pregnancy; we found no evidence for conversion of androstenedione to oestradiol or oestrone by endometrium of ovariectomized ewes treated with oestradiol and progesterone (Findlay *et al.*, 1981). It seems likely, therefore, that production of oestrogen by blastocyst or endometrium before attachment is negligible; after attachment the cotyledonary placenta acquires the capacity to synthesize oestrogen and oestrone sulphate in particular (Carnegie & Robertson, 1978).

Steroid receptors. If oestradiol-17β is not acting as an embryonic signal in blastocyst–endometrial relations, the stimulatory effect of ovarian oestradiol on endometrial protein synthesis might be enhanced by the presence of a blastocyst, via a blastocyst action on uterine receptors for oestradiol. Receptors for oestrogen and progesterone are present in cytosol and nuclear fractions of the sheep uterus (Shutt & Cox, 1972: Miller *et al.*, 1977b; Koligian & Stormshak, 1977a; Stone *et al.*, 1978; Stone, Wild & Miller, 1979). In the non-pregnant ewe, the ratio of RNA : DNA, rates of protein synthesis and cytoplasmic concentrations of oestrogen and progesterone receptor all decline from a maximum about oestrus to a minimum during the last 4 days of the luteal phase of the oestrous cycle (Miller *et al.*, 1977b). The decline in cytoplasmic oestrogen receptor during the luteal phase was associated with a decline in nuclear uptake of this receptor (Koligian & Stormshak, 1977a). Evidence suggests that the decline in cytoplasmic oestrogen receptor was due to high levels of progesterone preventing its replacement (Koligian & Stormshak, 1977b; Stone *et al.*, 1979; Miller, Wild & Stone, 1979). However, progesterone treatment was without effect on oestradiol-induced increases in protein synthesis, RNA : DNA ratio, alkaline phosphatase or peroxidase, despite the decrease in levels of both oestrogen and progesterone receptor in the cytoplasm (Stone *et al.*, 1979; Miller *et al.*, 1979; Findlay *et al.*, 1981).

If the biosynthetic response of the endometrium is regulated primarily by the concentration of oestrogen receptors, then the antagonistic effect of progesterone on oestradiol receptor availablity would have to be overcome in pregnancy. On the other hand, Miller *et al.* (1979) concluded that an effect of progesterone on the replacement of oestrogen receptor is not necessarily associated with other anti-oestrogenic actions of progesterone and suggest that, in the sheep, the initial anti-oestrogenic actions of progesterone are not mediated via a specific effect on such replenishment. The uterotrophic and anti-oestrogenic effects of progesterone on ovine endometrium require further investigation to resolve the actions of steroids in implantation. Steroid receptor concentrations and affinity in the cytoplasm and nucleus of caruncular and intercaruncular endometrium during the preimplantation period need to be measured and compared to values in non-pregnant animals. We have preliminary evidence that the number of oestrogen receptors in the caruncular and intercaruncular endometrial cell cytoplasm are reduced in pregnancy by Day 9 and remain lower than in non-pregnant animals until Day 15.

There is no change in the affinity with time or pregnancy status (J. K. Findlay, J. Swaney, N. Colvin, B. Doughton & I. J. Clarke, unpublished observations).

In summary, the mechanism by which the presence of the blastocyst brings about increased protein synthesis in the endometrium is not clear. The failure to detect a local increase in oestrogen production by blastocyst or endometrium suggests that, in the sheep, increased blood flow to the gravid horn and/or increased responsiveness of the endometrium to ovarian steroids may bring about the increased protein synthesis. However, both of these possibilities beg the question of how the blastocyst interact with the endometrium to change blood flow and responsiveness to steroids.

Conclusions

The available evidence suggests that the blastocyst exerts a direct action on the endometrium during the preimplantation period, although it is not yet possible to attribute the changes in endometrial function to specific embryonic signals, except perhaps for the action of trophoblastin as an antiluteolysin. The protracted period of implantation in the sheep provides a valuable model for resolving the nature and relative importance of short-range (direct) signals of embryonic origin that may be obligatory for attachment and implantation.

I am indebted to Dr Brian Heap and his colleagues at the A.R.C. Animal Research Institute, Babraham, U.K., for collaborating on experiments reported here, to my colleagues at the Animal Research Institute, Werribee, for continued support and collaboration and to the Australian Wool Research Trust Funds, the National Health and Medical Research Council of Australia and the Ford Foundation for financial support. My thanks to Noelene Colvin for editorial assistance and Jill Volfsbergs and Leigh Hudson for typing.

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