Trophoblastin, an antiluteolytic protein present in early pregnancy in sheep

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Summary. Trophoblastin, an antiluteolytic component from the embryo, was identified in the ewe by the means of intrauterine injections of homogenates from trophoblasts at 14–16 days pregnancy. Homogenates from embryos and their membranes at 21–23 days pregnancy did not extend the life of the corpus luteum, suggesting that trophoblastin synthesis occurs for only a short period. The trophoblastin was thermolabile (80°C for 30 min) and inactivated by pronase. Treatment of ewes with oCS, hCG, and extracts of 120-day placentae did not affect the time of luteolysis. The protein appears to be insoluble at pH 7 or 8, but to dissolve readily at pH 9.6.

After injection of homogenates or extracts from 14–16-day-old trophoblasts, the initial CL were maintained for more than 1 month in most cyclic recipient ewes. Surgical removal of embryos at 21–23 days resulted in luteal maintenance for more than 1 month in over 50% of the operated animals. All the maintained CL were secretory although their average weight was about one-half of that CL of normal pregnancy, suggesting the existence of complementary luteotrophic placental factors. The uteri of most of these pseudopregnant ewes were distended with a clear, sterile fluid.

Introduction

Nidation of the embryo and its accessories requires a progesterone dominated environment in the ewe (Foote, Gooch, Pope & Casida, 1957; Moore & Rowson, 1959) as in all mammalian species. The inhibition of cyclic luteolysis, which is compulsory for the onset of pregnancy, results from an equilibrium between the luteolytic action of uterine prostaglandin (PG) F-2 α (McCracken, Barcikowski, Carlson, Grn & Samuelsson, 1972a; Goding, 1973) and the luteotrophic and antiluteolytic effects of a pituitary and trophoblastic hormonal complex (Denamur, Kann & Short, 1971; Martal & Lacroix, 1978a).

The luteotrophic role of LH and prolactin which is required to maintain the corpus luteum is well documented for the ewe (Denamur, Martinet & Short, 1973; Denamur, 1973). However, the nature of the substance(s) secreted by the conceptus is still unknown (Rowson & Moor, 1967; Denamur *et al.*, 1971).

Transfer of an embryo into a ligated uterine horn contralateral to the ovary bearing the cyclic corpus luteum in the ewe shows that the conceptus has no effect on the maintenance of luteal structures, although it inhibits their regression when introduced into the adjacent uterine horn (Moor & Rowson, 1966a). Inhibition of luteolysis by the conceptus is therefore essentially a local phenomenon.

In the present study we have tried (1) to specify the nature of the antiluteolytic component, for which the name trophoblastin is suggested, and (2) to examine the duration of its effect.

Materials and Methods

Animals

The studies were carried out on 247 primiparous and multiparous ewes of the Préalpes du Sud breed.

Control pregnant ewes. These were slaughtered on Days 15 or 60–90 of pregnancy and their CL were weighed.

Embryo donor ewes. These were divided into groups of 15 in which oestrous cycles were synchronized with progestagen-impregnated intravaginal sponges (30 mg 17 α -acetyoxy-9 α -fluoro-11 β -hydroxyprogesterone: Searle) (Roberts, 1966; Thimonier & Cognié, 1977). These sponges were removed after 14 days, and the ewes injected with 300 i.u. PMSG (Searle) on Day 14 of the synchronized oestrous cycle. The ewes were mated twice on the day of oestrus designated Day 0. On Days 14, 15 and 16 of pregnancy, embryos were obtained surgically by clamping the cervix and injecting sterile saline (0.9% (w/v) NaCl) into the tip of the uterine horn on the same side as the corpus luteum. Embryos were flushed out through a plastic cannula inserted into the opposite horn and frozen at -15°C until required.

Recipient ewes. These were divided into two groups such that some were operated upon during their natural sexual period (from July to February) and the others during the seasonal anoestrus. Oestrous cycles were induced in the anoestrous ewes by an artificial photoperiodic regime (Ortavant, 1977). After several normal cycles, the recipient ewes were anaesthetized with halothane (Fluothane: ICI), laparotomized along the linea alba and corpora lutea (CL) were marked with India ink. The uterine horn adjacent to the CL was cannulated with sterile polydimethylsiloxane tubing (i.d. 0.8 mm, o.d. 1.7 mm, length 80 cm: 'Silastic': Dow Corning). This tubing had two stops, in the median and terminal positions. The terminal stop was introduced into the tip of the uterine horn, tightened and uterus was sewn up again with catgut. The imperviousness of this system was checked. The other end of the cannula was sealed with linen thread, drawn out of the abdominal cavity and fixed to the wool.

After slaughter of the recipient ewes at various stages, the imperviousness of the tubing into the uterine horn was again checked. The India ink-marked CL were enucleated and weighed. The uterine contents were checked for bacteriological infection. Polyacrylamide gel electrophoresis of dialysed uterine fluid was performed according to the method of Davis (1964) using 7.5% polyacrylamide gels at pH 9.6.

Materials injected

Since the sheep embryonic disc is particularly small on Days 14–16 of pregnancy by comparison with the trophoblast, we have used the term trophoblast in a wide sense rather than refer to embryo. However, material from Days 21–23 of pregnancy is termed embryo and includes both the embryo proper and the trophoblast because the embryo itself is clearly visible to the naked eye at this stage of development. All materials were injected by the intrauterine route and always included 20 000 i.u. penicillin G/ml. The sexual behaviour of the recipient ewes was checked each morning and evening by use of vasectomized rams.

Treatment 0. Ewes were given an injection of 2 ml 0.9% (w/v) NaCl for 11 days.

Treatments 1 and 2. Homogenates of 14–16-day trophoblasts (Treatment 1) and 21–23-day embryos (Treatment 2) were prepared in sterile polypropylene syringes and saline (0.9% (w/v) NaCl), and were injected at the rate of 2 embryos/day per ewe.

Treatments 3 and 4. Homogenates of 14–16-day trophoblasts were heated at 80°C for 30 min (Treatment 3) or incubated at 37°C for 2 h with pronase (Sigma, non-specific protease from *Streptomyces griseus*, 3–4 units/mg, sterilized through Millipore filters) (Treatment 4).

Treatments 5, 6 and 7. Homogenates of 14–16-day trophoblasts were extracted for 4 h at 4°C at pH 7 in saline (0.9% (w/v) NaCl) (Treatment 5), or at pH 8 (Treatment 6) or pH 9.6

(Treatment 7) in 0.05 M-ammonium bicarbonate buffer with 0.1 M-KCl and subsequently centrifuged at 3000 g for 30 min.

Treatment 8. Homogenates of 14–16-day trophoblasts, extracted with 0.05 M-ammonium bicarbonate + 0.1 M-KCl at pH 9.6, were centrifuged at 105 000 g. The supernatant was applied to an Ultrogel AcA 5-4 (LKB) column (4.5 × 140 cm) equilibrated with the same buffer at pH 9.6. The effluent was dialysed, freeze-dried, and then injected for 10 days (3 trophoblast equivalents/day/animal).

Treatment 9. Doses of 15 or 1500 i.u. hCG (Organon) were given to ewes from Days 12 to 17 of the cycle.

Treatment 10. Ovine chorionic somatomammotrophin, oCS (Martal & Djiane, 1977a), was purified by the method of Martal & Djiane (1975) with small modifications (Martal, 1978). The activity of the oCS was about 20 i.u./mg when expressed in ovine prolactin equivalents (NIH-P-S7, 24 i.u./mg) and 0.8 i.u./mg in bovine growth hormone equivalents (NIH-B-GH, 1 i.u./mg). A daily dose of 50 µg oCS in saline was injected until oestrous behaviour occurred.

Treatment 11. Placental cotyledons from 120-day pregnant sheep were extracted in 5 volumes of 0.05 M-ammonium bicarbonate buffer, pH 9.6, with 0.3 M-KCl for 4 h at 4°C, then centrifuged at 15 000 g for 30 min. Recipient ewes were injected daily with 2 ml placental extract.

Serum progesterone concentrations

To identify possible 'silent heats' and to determine the functional life of the CL, jugular venous samples were collected daily in tubes (Becton-Dickinson) without anticoagulant. Progesterone concentrations were determined by a double-antibody radioimmunoassay. The progesterone antiserum, kindly supplied by Dr J. Fèvre and Dr P. Rombauts, was obtained by immunization of rabbits against progesterone-11 α -hemi-succinate-bovine serum albumin. The antiserum exhibited the greatest cross-reactivity (7.5%) with 5 α -pregnane-3,20-dione and with desoxycorticosterone; the relative activities of all other steroids tested were less than 2%. The serum samples were extracted with hexane and the rabbit progesterone antiserum was precipitated by sheep γ -globulin antiserum. Control serum from ovariectomized ewes was regularly introduced into each standard curve. The limit of sensitivity for the assay was about 0.2 ng/ml and the intra- and inter-assay coefficients of variation were respectively about 10 and 15%.

Results

Inhibition of cyclic luteolysis by total homogenates of trophoblasts

When homogenates of 14–16-day-old trophoblasts (Treatment 1) were injected for 11 days, the sexual behaviour was inhibited in 4 of 5 ewes until Day 23 when the animals were slaughtered (Table 1). The CL identified were well vascularized and their average weight (525 ± 32 mg, $n = 4$) was comparable to that of CL at Day 15 of pregnancy (596 ± 28 mg, $n = 34$) (Table 2). The serum progesterone values confirmed that the CL were functional.

When Treatment 1 was given for 4 days only, cyclic luteolysis was inhibited in only 4 of 7 animals (Table 1). When these 4 ewes were slaughtered at Day 58 of pregnancy, the CL were comparable in weight (454 ± 30 mg, $n = 4$) and activity to those of animals treated for 11 days.

Neither the presence of the catheter nor the injection of 0.9% (w/v) NaCl (Treatment 0) resulted in any extension of the oestrous cycle (Table 1). However, if penicillin was omitted the onset of infection inhibited luteolysis, as observed by Coudert & Short (1966).

Nature of the antiluteolytic substance

Cyclic luteolysis was not blocked by Treatments 3 or 4 (Table 3) suggesting that the trophoblastin is likely to be a protein, or to include protein material.

Table 1. Effect of daily intrauterine injections of homogenates of embryos or trophoblasts (from Day 12) on luteolysis in cyclic sheep

Nature	Treatment		No. of recipient ewes	Days to next oestrus (no. of ewes)	Progesterone conc.† (ng/ml)
	Duration (days)	Total no. of injected trophoblasts or embryos			
Saline (Treatment 0)	11	—	8	16.9 ± 0.3 (8)	0.6 ± 0.1
14–16-day trophoblasts (Treatment 1)	11	110	5	>23* (4)	2.7 ± 0.5
	4	56	7	16.7 ± 0.3 (3) >58* (4)	0.5 ± 0.1 1.9 ± 0.4
21–23-day embryos (Treatment 2)	6	72	6	16.9 ± 0.3 (5)	0.2 ± 0.1
				>53* (1)	2.5 ± 0.2

Values are mean ± s.e.m.

* Slaughter at Days 23, 58, 53 of pregnancy respectively.

† At day of oestrus or of slaughter (3 samples daily/animal).

Intrauterine injections of hCG (Treatment 9) had no effect on cyclic regression of the CL (Table 3) which had to be very carefully identified by the ink marks because new CL may be formed under the influence of hCG. Similarly, treatment with oCS (Treatment 10) at levels presumed to be up to 40 times higher than the physiological concentrations in early pregnancy (Martal & Djiane, 1977b), did not maintain the luteal structures in the recipient ewes (Table 3). Hence, trophoblastin appears to be a protein different from human chorionic gonadotrophin or ovine somatomammotrophin.

When crude extracts from placenta at 120 days pregnancy, containing large amounts of oCS, were injected (Treatment 11), the same negative results were obtained, suggesting that trophoblastin had disappeared from the placenta at these late periods of pregnancy (Table 3).

Table 2. Comparative aspects of the maintenance of CL in sheep

	Recipient ewes					Donor ewes after removal of embryos at Day 21–23
	Normal pregnant ewes	Treatment 0 (saline)	Treatment 1 (14–16-day homogenates)	Treatments 7 and 8 (pH 9.6 supernatant)		
Time of slaughter (days after mating or oestrus)	15	60–90	17	23	50–60	70–110
No. of ewes	14	16	8	4	4	6
No. of CL (total)	34	19	0	4	9	11
Weight of CL (mg)	596 ± 28	620 ± 31	—	525 ± 32	409 ± 36	306 ± 38
Progesterone conc. (ng/ml)	4.7 ± 0.6	3.5 ± 0.7	0.6 ± 0.1	2.7 ± 0.5	4.9 ± 0.7	2.8 ± 0.9

Values are mean ± s.e.m.

Table 3. The effects of various treatments (Days 12–17) on inhibition of luteolysis in ewes to show the nature of trophoblastin

Treatment	Daily amount/ewe	No. of ewes	Days to next oestrus* (no. of ewes)
1 (14–16-day homogenates)	2 trophoblasts	12	$\begin{cases} >22 (8)^\dagger \\ 16.9 \pm 0.4 (4) \end{cases}$
3 (14–16-day homogenates heated)	2 trophoblasts	3	$17.2 \pm 0.2 (3)$
4 (pronase alone)	2 mg	4	$16.7 \pm 0.3 (4)$
4 (pronase + homogenates)	2 mg + 2 trophoblasts	3	$17.3 \pm 0.3 (3)$
9 (hCG)	15 i.u.	4	$16.7 \pm 0.4 (4)$
	1500 i.u.	4	$16.2 \pm 0.3 (4)$
10 (oCS)	Prolactin equiv.	5	$17.2 \pm 0.2 (5)$
11 (120-day placenta)	2 ml	3	$16.5 \pm 0.1 (3)$

Values are mean $\pm$ s.e.m.

* Progesterone concentration in peripheral serum at moment of oestrus was 0.4 ± 0.1 ng/ml (N = 30).

† Slaughter at Day 23 or 58 of pregnancy.

Treatments 5 and 6 had no inhibitory action on cyclic luteolysis, but Treatments 7 and 8 blocked the regression of luteal structures in some animals (Table 4). The presence of activity in filtrates from the Ultrogel columns demonstrates that intact trophoblast cells are not required to inhibit CL regression, and that the factor is soluble.

Timing of trophoblastin synthesis

Injection of homogenates of 21–23-day-old embryos (Treatment 2) did not impair luteal regression in 5 out of 6 animals (Table 1, Text-fig. 1). Trophoblastin therefore seems to be absent, or nearly so, at this stage of pregnancy.

Table 4. The effect of various extraction procedures for trophoblastin on the inhibition of luteolysis in sheep

Treatment (Days 12–22)				No. of ewes returning to oestrus/ no. treated	Interval to next oestrus (days)
No.	pH of extraction	Fraction	No. of 14–16-day trophoblasts injected		
5	7	Pellet	30	1/1	19
		Supernatant	30	3/3	16.0 ± 0.3
6	8	Pellet	20	1/1	21
		Supernatant	20	1/1	18
7	9.6	Pellet	60	3/3	16.8 ± 0.2
		Supernatant	60	2/5	16.2 >30*
8	9.6	Ultrogel effluent	90	1/3	17 >30*

Values are mean $\pm$ s.e.m.

* Slaughter at Day 37 (1 ewe), 54 (3 ewes) or 58 (1 ewe).

Text-fig. 1. Progesterone concentrations (mean $\pm$ s.e.m.) in the peripheral serum of recipient ewes after the intrauterine injections of homogenates and extracts of trophoblasts. ●—●, Injections (†) to 4 ewes over 4 days of homogenates of 14–16-day trophoblasts; ○---○, injections to 5 ewes over 6 days of homogenates of 21–23-day embryos; △---△, injections to 3 ewes over 6 days of extracts at pH 9.6 of 14–16-day trophoblasts.

Persistence of trophoblastin action on cyclic luteolysis

After intrauterine injections of 14–16-day-old trophoblasts for 4 days, the CL were maintained for almost 2 months in 4 ewes out of 7 (Table 1, Text-fig. 1). Progesterone levels remained constant at about 2 ng/ml. The uterus appeared distended with about 100 ml of a clear, yellowish, sterile fluid of as yet unidentified composition. The uterine mucosa appeared sound and the fluid bore no comparison with pyometrial liquid.

When ewes were injected with trophoblast homogenate extracted at pH 9.6 (Treatments 7 and 8) progesterone was secreted at levels greater than 4 ng/ml for more than 1 month (Text-fig. 1), although the CL weighed less than those of ewes in Treatment 1 (Table 2). No luteinized follicles or newly formed CL were observed. The unusually high concentration of progesterone in this experiment may be ascribed to the high frequency of double corpora lutea of these same ewes. The uteri again contained abundant yellowish fluid, the bacteriological analysis of which did not reveal any pathogenic aerobic or anaerobic germs.

Removal of 21–23-day-old embryos from 26 donor ewes, the CL of which were inked and the progesterone levels measured, resulted in a marked delay of resumption of the oestrous cycle in some animals: oestrus occurred by 10–20 days after surgery in 12 ewes, 30–60 in 7 and 70–110 in 7 ewes. Removal of the embryos at 14–16 days did not prevent resumption of oestrus in the next cycle although it was slightly extended as observed by Moor & Rowson (1966b) and Edey (1967). The progesterone concentrations in animals deprived of their embryos at 21–23 days of pregnancy are shown in Text-fig. 2. The average value in 6 ewes up to 69 days after surgery was 4–6 ng progesterone/ml. The concentrations in individual sheep are also shown, e.g. in Ewe 4396 the CL regressed rather rapidly (23 days after surgery) while luteolysis occurred

Text-fig. 2. Progesterone concentrations in the peripheral serum of ewes after the removal of embryos on Days 21-23 of pregnancy. ●, Mean $\pm$ s.e.m. values for 6 ewes between Days 24 and 59 and for 3 ewes between Days 60 and 90; ○---○, Ewe 4396, showing early luteolysis; △---△, Ewe 5454, showing extended inhibition of luteolysis.

very late, 112 days after surgery in Ewe 5454. The sexual behaviour of the animals matched these data very closely. At slaughter between 70 and 110 days after surgery, the CL were still quite large and progesterone levels were reasonably high (Table 2). The uterus of most, but not all, animals was distended with fluid.

When 21–23-day-old embryo homogenates were injected (Treatment 2) into recipients, only 1 out of 6 ewes exhibited a well vascularized CL weighing 545 mg at slaughter (Table 1). The progesterone value exceeded 2 ng/ml and confirmed the functional state of the CL. The remaining 5 ewes showed normal oestrous behaviour and progesterone concentrations (Text-fig. 1).

A condition of 'natural pseudopregnancy' was observed at 14 days pregnancy in one of the embryo donor ewes: the uterus of this animal was devoid of an embryo, but distended with a clear, sterile fluid containing membranous debris, which suggested that a fetal resorption might have taken place. Progesterone levels exceeded 2 ng/ml until the resumption of oestrous behaviour at Day 82.

Discussion

At 14–16 days pregnancy, the embryonic disc of the sheep is only a minute cellular cluster of a few hundreds μm diameter, whereas the trophoblast is about a hundred times more developed (Wintenberger-Torrès, 1967; Bindon, 1973; Fléchon, Guillomot & Wintenberger-Torrès, 1978). In addition, the thread-like morphology of the trophoblast seems suited for a local antiluteolytic action. Hence it seems reasonable to assume that the antiluteolysin examined in this paper is secreted by the trophoblast proper, and can be called trophoblastin.

The protein nature of trophoblastin seems well established, since it is thermolabile and protease sensitive. Rowson & Moor (1967) had also emphasized the heat lability of the antiluteolytic activity of 14-day sheep embryos. It also seems clear that trophoblastin is distinct from hitherto known placental hormones, since neither ovine chorionic somatomammotrophin nor human chorionic gonadotrophin are able to simulate the same effects.

The period during which trophoblastin is secreted appears to be rather restricted, since its presence seems critical from Day 12 of pregnancy solely (first day of intrauterine injections) and it is not detectable after Days 21–23.

Prostaglandin F-2 α is secreted mainly during the second half of the cycle, simultaneously with the rise in oestrogens preceding and accompanying oestrus (Cox, Thorburn, Currie, Restall & Schneider, 1972). Oestrogens themselves are luteolytic at this period in the ewe (Denamur & Kann, 1973; Bolt & Hawk, 1975). Conversely plasma peaks of PGF-2 α are less numerous at the onset of pregnancy (Thorburn, Cox, Currie, Restall & Schneider, 1973; Barcikowski, Carlson, Wilson & McCracken, 1974; Nett *et al.*, 1976), although its concentration in the endometrium proper is higher than that in cyclic ewes (Wilson, Butcher & Inskeep, 1972; Lewis *et al.*, 1977). In addition, luteolysis, which is invariably observed when oestradiol-17 β is injected into cyclic ewes, is inhibited in about 30% of pregnant animals (Kittok & Britt, 1977). Hence the conceptus appears to secrete a substance which inhibits the luteolytic action of oestradiol.

Although the mechanism of action of trophoblastin is unknown, it could either be antiluteolytic by inhibiting the release or synthesis of prostaglandin F-2 α or it could be luteotrophic through the action of prostaglandin E-2, for instance. It would be unreasonable to assume that trophoblastin, which appears to be a protein, may act directly on the ovary, like PGF-2 α which has been shown to diffuse by a counter current process from the uterus (McCracken *et al.*, 1972b); this interpretation is strongly borne out by the experiments demonstrating that embryos transplanted in the isolated uterine horns opposite the CL do not prevent luteolysis (Moor & Rowson, 1966a). This appears to confirm that trophoblastin action is essentially a local one. Nevertheless it has been recently suggested (Inskeep, Smutny, Butcher & Pexton, 1975) that the

conceptus may still exert a direct action on the ovary, since PGF-2 α injections into this organ are active in the cyclic, but not in the pregnant ewe. Hence the occurrence of a diffusible antiluteolytic component counteracting the effect of PGF-2 α on the ovary remains a possibility. Prostaglandin E-2 might be such a component, since Pratt, Butcher & Inskeep (1977) showed that intrauterine injections of this compound, which obviously might diffuse through the same route as PGF-2 α , delays cyclic luteolysis for short periods (2 days). Simultaneous infusion of prostaglandin E-2 antagonizes the luteolytic action of prostaglandin F-2 α *in vivo* (Henderson, Scaramuzzi & Baird, 1977). In addition PGF-2 α is a vasoconstrictor at the CL level (Niswender, Reimers, Diekman & Nett, 1976) whereas PGE-2 is vasodilatory (Strong & Bohr, 1967). Although the evidence may suggest that PGE-2 synthesis may participate in the action of the conceptus on luteolysis, it has not yet been demonstrated that it can, like trophoblastin, extend the life-span of a corpus luteum for long periods.

A situation akin to pseudopregnancy is observed both in the cyclic ewes which are recipients for intrauterine trophoblast injections and in the donor ewes, provided that removal of the embryos has taken place at Days 21–23 after mating. This pseudopregnancy appears obvious from the morphological state of the uterus and from the functional state of the CL. In most pseudopregnant ewes, the uterus appears distended with fluid as in pregnancy, but mechanical distension of the organ is insufficient for extending the life-span of CL (Moor, 1968). However, some of the ewes pseudopregnant after removal of their embryos at 21–23 days show no such distension when slaughtered. A single case of natural pseudopregnancy is described, analogous to the condition mentioned by Amoroso, Harrison, Heap & Poyser (1973), who observed a ewe with a distended uterus which was filled with an abundant, viscous, odourless, sterile and PGF-2 α -rich fluid after a daily intramuscular injection of progesterone. Similar results were obtained in 5 pregnant ewes after isolation of the empty uterine horn and in non-pregnant ewes after autografting of the ovary (Harrison, Heap, Horton & Poyser, 1972). In all these instances, progesterone seems to play the paramount role in promoting the accumulation of fluid in the uterine lumen. The mechanism of the antiluteolytic action of trophoblastin could perhaps consist of a reversal of the direction of release of PGF-2 α from an endocrine to an exocrine polarity into the uterine lumen, as suggested by Bazer & Thatcher (1977) to explain the luteotrophic action of oestrogens in the sow.

The CL is well maintained during pseudopregnancy and secretes progesterone. The average weight of CL at slaughter (70 days pseudopregnancy), 352 ± 28 mg ($N = 20$), is less than that of CL from normal pregnant ewes (703 ± 24 mg, $N = 34$, up to 130 days pregnancy: Martal & Djiane, 1977b), but it is comparable to that of CL from hysterectomized ewes (Denamur *et al.*, 1973). It is remarkable that trophoblastin seems to act for such an extended period, in about half of the animals after removal of their embryos at 21–23 days pregnancy and in more than half of the ewes which were injected with homogenates or extracts from 14–16-day-old trophoblasts.

However, trophoblastin alone does not seem sufficient to prevent at least partial regression of CL, suggesting that complementary actions of placental luteotrophins may be operating in the pregnant ewe. Hypophysectomy (Denamur *et al.*, 1971) or incomplete prolactin suppression (Martal & Lacroix, 1978b) bear out this hypothesis. We have postulated that oCS (Martal & Djiane, 1977b) and an hCG-like factor (Martal & Lacroix, 1978a; Wintenberger, 1978) might account for the placental luteotrophic complex which is essential for maintaining a normal CL during pregnancy in sheep. Although the detection of these hormones is possible at early periods, their action does not appear to be prominent from the very beginning of pregnancy, when compared to the antiluteolytic effect of trophoblastin, since intrauterine injections of oCS, hCG or placental extracts do not inhibit luteal regression under our experimental conditions.

There seems to be a distinct difference between sheep and primates. In primates, which are devoid of a strong cyclic uterine luteolytic activity, hysterectomy does not extend the sexual cycle (Neill, Johansson & Knobil, 1969; Beling, Stewart & Markham, 1970) and chorionic gonadotrophin is frequently considered to be the hormone responsible for maintaining CL from the

onset of pregnancy (Jaffe, Lee & Midgley, 1969; Niswender, Menon & Jaffe, 1972; Neill & Knobil, 1972; Catt, Dufau & Vaitukaitis, 1975), informing the maternal organism of its pregnant state. Conversely, in the ewe the antiluteolytic action of trophoblastin suggests that it is this factor which acts as a signal from the embryo and enables the CL and the maternal organism to recognize the presence of a conceptus in the uterus.

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