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Control of luteal function during early pregnancy in sheep

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Summary. Prolactin and LH are required for the maintenance of normal luteal structures during early pregnancy in the ewe, whilst luteolysis, induced by prostaglandin F-2α is inhibited by the presence of the conceptus. The trophoblast also secretes a local anti-luteolytic factor, trophoblastin, which is most probably a protein because it is thermolabile and inactivated by pronase. It is soluble at pH 9·6 and can be separated on Ultrogel. Trophoblastin is present for a short time (12th–22nd day of pregnancy) but its effect may last several months.

Introduction

Maintenance of the secretory activity of the corpus luteum is necessary during early pregnancy, although in some species, this action may subsequently be replaced by the placental secretion of progesterone. This is the case in sheep. Ovariectomy after Day 50 of gestation does not lead to abortion (Denamur & Martinet, 1955), but the secretion of progesterone from the ovary is required during early pregnancy because daily injections of this hormone to ovariectomized ewes commencing 3–4 days after mating prevents abortion (Foote, Gooch, Pope & Casida, 1957).

In most species in which the non-pregnant uterus has a luteolytic action (guinea-pig, rat, mouse, hamster, cow, ewe, goat, cow) (Anderson, Bland & Melampy, 1969; Mayer, Acker & Dulluc, 1973), the presence of the embryo prevents luteolysis. In these species, the conceptus inhibits the action of a uterine luteolytic factor. This signal has been studied in the ewe and an antiluteolytic protein, trophoblastin, has been isolated (Martal, Lacroix, Loudes, Saunier & Wintenberger-Torrès, 1979).

Luteolytic factors in sheep

Prostaglandin (PG) F-2α is the uterine luteolysin in the ewe, and is passed from the utero-ovarian vein to the ovarian artery by a mechanism of counter-current transfer (McCracken *et al.*, 1972a), thus preventing its degradation by the lungs (Piper, Vane & Wyllie, 1970). During oestrus, the drop in progesterone secretion and the involution of the corpora lutea is preceded by an increased secretion of oestradiol-17β (Cox, Thorburn, Currie, Restall & Schneider, 1972). Perfusion of oestradiol-17β into the uterine artery of the uterus, autotransplanted to the neck of a ewe, causes release of PGF-2α provided the infusion is given on Day 14 of the oestrous cycle (the onset of luteolysis approximately) (Barcikowski, Carlson, Wilson & McCracken, 1974). When the uterus is perfused on Day 6 or 10, oestradiol-17β has no effect. These findings are in agreement with those of Denamur & Kann (1973). The luteolytic action of oestrogens may be linked to the actions of progesterone. Brinsfield & Hawk (1973) showed that progesterone leads to an

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accumulation of lipid droplets in the endometrial cells. Such phospholipids may supply the arachidonic acid necessary for the synthesis of prostaglandins after the action of phospholipase A_2 (Kunze & Vogt, 1971). In thyroid cells the synthesis of the prostaglandins seems to involve the activation of phospholipase A_2 through a cyclic AMP-dependent mechanism (Haye, Champion & Jacquemin, 1973).

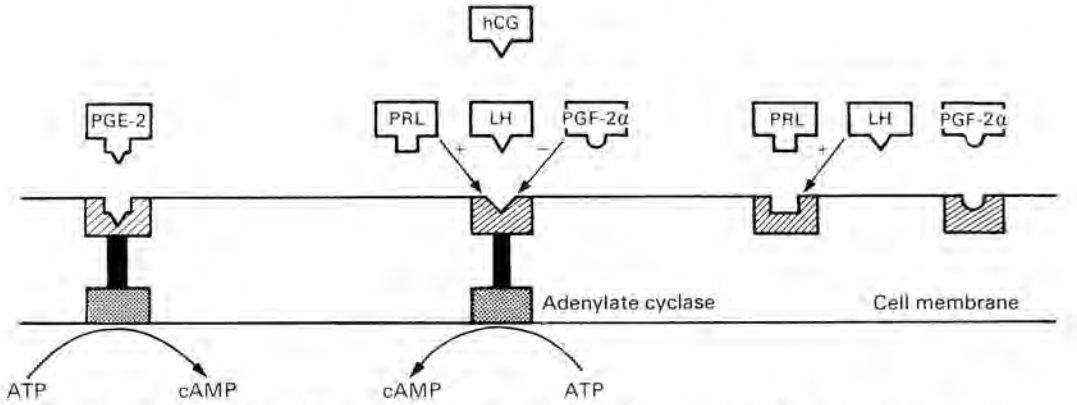
The luteolytic action of oestrogens probably brings about a de-novo synthesis of PGF-2α since a perfusion of indomethacin, which inhibits the prostaglandin synthetase system, reduces the effects of oestradiol-17β perfusion (Barcikowski *et al.*, 1974). Inhibitors of protein synthesis (cycloheximide) or mRNA synthesis (actinomycin D) do not change the uterine synthesis of PGF-2α stimulated by oestradiol-17β in the rat (Castracane & Jordan, 1976), and so oestrogens do not seem to induce the synthesis of enzymes but rather stimulate their activity.

In pregnant ewes, the peaks of PGF-2α in plasma are lower in number and in magnitude than in ewes displaying regular oestrous cycles (Thorburn, Cox, Currie, Restall & Schneider, 1973), although there are no significant differences in the mean plasma concentrations. The injection of oxytocin into the uterine artery *in vivo*, causes the release of PGF-2α into the blood of ewes on Day 16 of the oestrous cycle, but not in ewes on Day 16 of pregnancy (McCracken, 1980). In pregnant ewes, the number of oxytocin receptors in the endometrium (about 33 fmol/mg protein) is significantly lower than that found in ewes during the oestrous cycle (250 fmol/mg protein). When a transplanted uterus is subjected to progesterone pretreatment for 10 days starting on Day 2, 6 or 10 of the oestrous cycle, treatment for 6 h with oestradiol-17β is necessary to obtain an oxytocin-induced release of PGF-2α (McCracken, 1980). These results suggest that oestradiol-17β induces the synthesis of oxytocin receptors. During early pregnancy, the plasma levels of oestrogens are lower than at the end of the oestrous cycle, which may explain the lower sensitivity of the endometrium to oxytocin. Likewise, the abortive action of oestrogens in some species may also be explained by their ability to induce oxytocin receptors. In addition to its contractive effect on the myometrium, oxytocin may exert a specific luteolytic effect on the endometrial cell. In the cow, the administration of oxytocin reduces by half the duration of the oestrous cycle (Armstrong & Hansel, 1959). According to McCracken (1980), the rapid effect of oxytocin on the release of PGF-2α can be explained by the activation of cyclase and of phospholipase A_2. Thus, the peaks of PGF-2α release might result from stimulation by oxytocin. However, the presence of oxytocin does not seem to be essential for luteolysis which also takes place after total hypophysectomy (Denamur, Kann & Short, 1971), unless sufficient release of oxytocin occurs directly from the axons of the severed oxytocinergic neurones. The luteolytic action of PGF-2α may therefore be expressed in several ways.

The PGF-2α receptors of the ovine corpus luteum have been characterized (Powell, Hammarstrom & Samuelsson, 1974). The affinity of the receptors for PGF-2α is specific and they have little affinity for prostaglandins A, B, E or F-1. A molecule of PGF-2α is bound to its cell receptors through its three hydroxyl groups and its 5-6 cis double bond.

Prostaglandin F-2α considerably reduces the number of LH receptors in the corpus luteum of the rat and prolactin is able to inhibit this reduction (Grinwich, Hichens & Behrman, 1976) (Text-fig. 1). On the other hand, treatment with prolactin and LH does not hinder the lytic action of PGF-2α in hypophysectomized rats (Behrman, MacDonald & Greep, 1971). In the ewe, prolactin but not hCG or LH has been reported to protect the corpus luteum against the action of PGF-2α (McCracken, Barcikowski, Carlson, Green & Samuelsson, 1972b) although other experiments have failed to confirm this action (Chamley, Cerini & Goding, 1973). Prolactin could act in this context by preventing the degradation of progesterone by 20α-hydroxysteroid dehydrogenase (Behrman *et al.*, 1971).

The flow rate of the ovarian artery is not modified by injections of physiological doses of PGF-2α (McCracken *et al.*, 1972b), but the distribution of blood in the ovary seems to be altered and the luteolytic action of PGF-2α is characterized by marked vasoconstriction in the corpus luteum (Niswender, Reimers, Diekman & Nett, 1976).



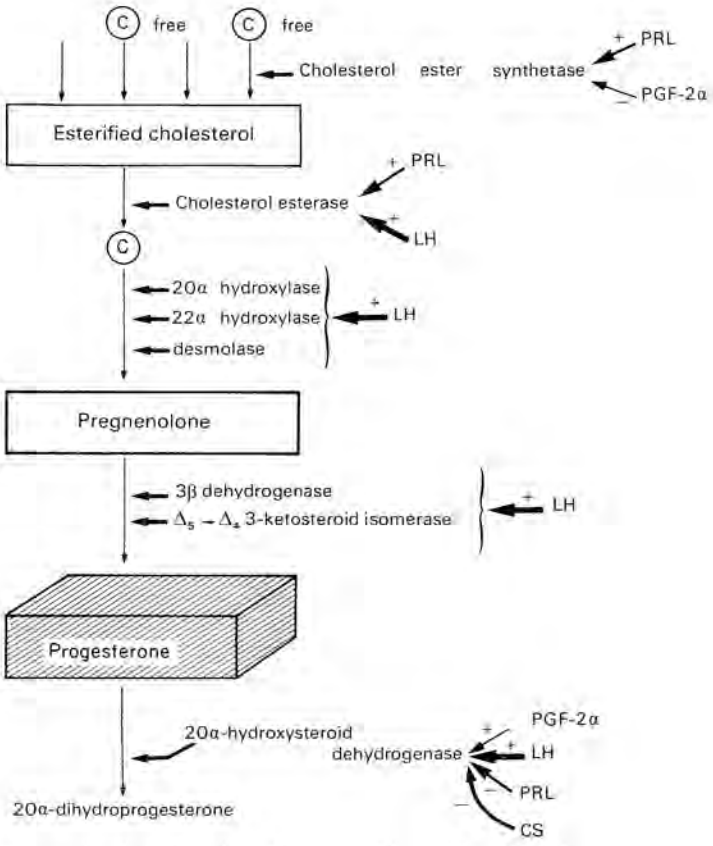
Text-fig. 1. Regulation of the number of LH and prolactin receptors in the corpus luteum of the rat. Prolactin (PRL) stimulates (+) the synthesis of LH receptors and LH stimulates (+) the synthesis of prolactin receptors. PGF-2α inhibits (−) the synthesis of LH receptors. PGE-2 and LH receptors are coupled with the adenylate cyclase system but prolactin and PGF-2α receptors are not.

Hypophysial luteotrophic factors

In the pregnant ewe hypophysectomy before Day 50 of gestation stops progesterone secretion, resulting in regression of the corpus luteum and abortion (Denamur & Martinet, 1961). When hypophysectomy is performed on Day 10 of gestation, injections of prolactin (250 i.u./day) or LH (0·5 mg/day) from Day 10 to 20 maintain gestation for a short time, whereas the combination of these two hormones (same doses) prevents abortion and leads to a normal development of the luteal structures (Denamur, 1973). In the intact pregnant ewe, the injection of antibodies to LH or prolactin from Day 10 to 20 allows pregnancy to continue for a while, whereas the combination of both antisera leads to immediate abortion (Kann & Denamur, 1974). The presence of both prolactin and LH therefore seems to be essential during early pregnancy in the ewe. However, the plasma concentrations of these hormones do not show any significant variations during early pregnancy (Kann & Denamur, 1974); thus it seems that very low levels are able to maintain the secretory activity of the corpora lutea.

The modes of action of prolactin and LH on steroidogenesis seem to be complementary (Text-fig. 2). In rats, prolactin stimulates the activity of cholesterol ester synthetase to increased storage of esterified cholesterol. Prolactin also stimulates cholesterol esterase activity which transforms esterified cholesterol into free cholesterol, used in the biosynthesis of progesterone, and in addition inhibits the degradation of progesterone into 20α-dihydroprogesterone *in vivo* (Wiest, Kidwell & Balogh, 1968) and *in vitro* (De la Llosa-Hermier, Leboulleux, Evrard & Hermier, 1979). However, prolactin has no effect *in vitro* on the synthesis of progesterone in corpora lutea (Kaltenbach, Cook, Niswender & Nalbandov, 1967; Armstrong, Knudsen & Miller, 1970). By contrast, LH activates cholesterol esterase (Behrman *et al.*, 1971) and stimulates the different steps in the biosynthesis of progesterone, *in vivo* and *in vitro* (Armstrong, O'Brien & Greep, 1964; Savard, Marsh & Rice, 1965). LH appears to be the more important hormone for steroid synthesis, but its in-vitro action on the corpora lutea from hypophysectomized rats only occurs if the animals have been injected with prolactin (Armstrong *et al.*, 1970). Thus, prolactin seems to be necessary for the storage of cholesterol by the luteal cell. In addition, prolactin is able to prolong and extend the action of LH on progesterone secretion by inhibiting the degradation of this steroid.

Both LH and hCG stimulate the activity of adenylate cyclase in luteal tissue (Marsh, 1970a) whereas prolactin does not (Mason, Schaffer & Toomey, 1973) and the cyclic AMP formed



Text-fig. 2. The mechanism of action of luteotrophic and luteolytic hormones on steroidogenesis in luteal tissue; +, luteotrophic activity; −, luteolytic activity; PRL, prolactin; PGF-2α, prostaglandin F-2α; LH, luteinizing hormone; CS, chorionic somatomammotrophin; C, cholesterol.

leads to activation of protein kinases (Menon, 1973). A cAMP-dependent protein kinase is involved in the cleavage of the side chain of cholesterol leading to formation of pregnenolone (Caron, Goldstein, Savard & Marsh, 1975). Another cAMP-dependent protein kinase (K II) is able to activate the phosphorylation of ribosomal proteins (Azhar & Menon, 1975a). Puromycin (Savard *et al.*, 1965), an inhibitor of translation, blocks any stimulation of progesterone synthesis by LH and cAMP. According to Hermier (1973), a regulatory protein which has a fast turn-over might control the penetration of free cholesterol into mitochondria. Indeed, the cleavage of the cholesterol side chain and 20α-hydroxylation leading to pregnenolone formation takes place at this site. Although the action of cAMP is similar to that of LH on the corpus luteum (Savard *et al.*, 1965), the stimulation of protein kinase K II might also be direct (without involvement of cAMP) (Azhar & Menon, 1975b). Azhar & Menon (1975b) even suggest that LH plays a specific role in transcription through the activated protein kinase.

Luteotrophic factors of trophoblastic origin

Hypophysectomy of ewes after 50–70 days of pregnancy leads to a 25% decrease in the weight of the corpus luteum (Denamur *et al.*, 1971), and hypoprolactinaemia, due to the administration of bromocriptine, is associated with only a 15% reduction (Martal & Lacroix, 1978b). As the

corpus luteum of pregnancy remains functional until Day 140 (Martal & Djiane, 1977), these data suggest the existence of luteotrophic hormones of placental origin, analogous to the hypophysial luteotrophic complex (LH + prolactin) (Denamur, 1973). Administration of anti-LH and anti-prolactin immune sera between Days 10 and 20 of pregnancy leads to luteolysis whereas this is not the case between Days 30 and 40 (Kann & Denamur, 1974). Lacroix & Martal (1979), using a radioreceptor assay, detected LH-like activity in the ewe placenta. Ovine chorionic gonadotrophin (oCG) has also been found in amniotic fluid, but because of low levels and interference by serum factors, this hormone has not been measured in blood: it does not exhibit any immunological reaction with ovine LH, human LH and hCG. From Day 15 of gestation, a level of 0·17 i.u. oCG (in hCG equivalents) has been measured per trophoblast. The maximum placental concentration, about 200 i.u. per placenta, is reached from Day 80 of pregnancy and remains constant until near term, when it decreases rapidly to approximately 50 i.u. per placenta.

Some LH-like activity was also observed by Wintenberger-Torrès (1978) in cultures of ovine trophoblasts from Day 10 to 13 of gestation; secretion was also very low, especially when compared with that of chorionic gonadotrophin in primates (Jaffe *et al.*, 1969; Neill & Knobil, 1972). In the rabbit (Haour & Saxena, 1974) and the rat (Haour, Tell & Sanchez, 1976) a low LH-like activity has also been found in placental extracts.

A prolactin-like hormone, initially named ovine placental lactogen, has been purified and characterized from the sheep placentâ. Because of its growth-promoting activity and following the international nomenclature, this hormone is now called ovine chorionic somatomammotrophin (oCS) (Martal & Djiane, 1977a). This hormone is present in the ewe trophoblast on Day 16 of gestation (Martal & Djiane, 1977b) and its activity corresponds to about 0·5 μg prolactin equivalent/trophoblast. This increases to about 10 μg/trophoblast within the next 15 days. At 70–75 days of pregnancy about 5 mg oCS/placenta (in prolactin equivalents) are present, and a maximum concentration occurs towards Days 110–120 of gestation (about 15 mg/placenta) (Martal & Djiane, 1977b).

Ovine chorionic somatomammotrophin is secreted by large binucleated cells, with a PAS-positive staining, in the unistratified epithelium of the chorionic villi (Martal, Djiane & Dubois, 1977). These cells have been observed from Day 16 of gestation onwards (Boshier, 1969). The chorionic somatomammotrophin binds to the prolactin receptors of ovine corpora lutea (Martal & Lacroix, 1978a; Chan, Robertson & Friesen, 1978) and might represent the prolactin factor of the luteotrophic complex necessary for maintaining the corpus luteum during pregnancy. In the rat, human chorionic somatomammotrophin (hCS) is luteotrophic and there may even be a synergy between the action of hCS and that of hCG in hypophysectomized pseudopregnant rats (Josimovich, 1968). Like prolactin, oCS and hCS inhibit the catabolism of progesterone into 20α-dihydroprogesterone (J. Martal & M. P. De la Llosa-Hermier, unpublished data). However, oCS secretion alone cannot explain the inhibition of luteolysis at the time of trophoblast implantation in the ewe. Indeed, the daily intrauterine administration of this hormone (40 μg prolactin equivalent) does not prevent the return to oestrus of mated ewes treated from Day 12 of the oestrous cycle. This dose is substantially higher than the amount of oCS measured in the trophoblast at implantation (Martal *et al.*, 1979).

In addition to oCS and oCG, prostaglandin (PG) E-2 is found in large quantities in the sheep trophoblast (M. C. Lacroix, personal communication), and this participates in the maintenance of the corpus luteum. The luteal cells contain PGE-1 and PGE-2 specific receptors with similar affinity but with very poor affinity for other prostaglandins (PGF, PGA and PGB) (Rao, 1974). These receptors are different from those for gonadotrophins but PGE stimulates adenylate cyclase activity and the synthesis of progesterone by the corpus luteum in the cow (Marsh, 1970b) (Text-fig. 1). Like PGF-2α, PGE-2 probably diffuses by counter-current into the ovarian artery. Pratt, Butcher & Inskeep (1977) showed that intrauterine injections of PGE-2 delayed luteolysis for 2 days. A simultaneous perfusion of PGE-2 may antagonize the luteolytic action of PGF-2α *in vivo* (Henderson, Scaramuzzi & Baird, 1977). Furthermore, PGF-2α is a

vasoconstrictor in the corpus luteum (Niswender *et al.*, 1976) whereas PGE-2 is a vasodilatator (Rankin & Phernetton, 1976). Although the synthesis of PGE-2 may participate in the action of the conceptus on luteolysis, it has not been established if this hormone has a long lasting effect on the survival of the corpus luteum.

Trophoblastin, an antiluteolytic factor

According to Moor & Rowson (1966) it is possible to transfer a 14–16-day old embryo from a pregnant to a non-pregnant ewe provided that the recipient ewe has not exceeded the 12th day of the oestrous cycle. Placed in the uterine horn adjacent to the corpus luteum or in the opposite horn, the trophoblast prevents luteolysis. On the other hand, the introduction of an embryo into the contralateral horn does not prevent the regression of the corpus luteum if that horn is isolated by a ligature. Such experiments show that the trophoblast inhibits the luteolytic action of PGF-2α by a local mechanism.

Daily injections into the uterine lumen of ewes from Day 12 of the oestrous cycle of homogenates from 14–16-day trophoblast leads to maintenance of the corpus luteum (Rowson & Moor, 1967) and progesterone secretion (Martal *et al.*, 1979) (Table 1). Four or five intrauterine injections were sufficient to maintain luteal function for several months in more than half of the recipient ewes (Martal *et al.*, 1979). The corpora lutea remained large and well vascularized, whilst the uterus often contained a large amount of a clear, yellowish and sterile fluid (about 100 ml at slaughter). However, mechanical distension of the uterus does not maintain the corpus luteum during gestation (Moor, 1968) and some animals with maintained corpora lutea do not possess this fluid. Injections of homogenates of 21–23-day embryos under the same conditions did not lead to maintenance of luteal structure in 4 out of 5 ewes (Table 1). The trophoblastic antiluteolysin or trophoblastin has therefore disappeared by Day 21 (Martal & Lacroix, 1978a). Rowson & Moor (1967) did not observe inhibition of luteolysis by injecting homogenates of 25-day embryos. Furthermore, the surgical removal of embryos after 21–23 days of gestation results in maintenance of the corpus luteum in more than half of the animals for a period ranging between 1 and 4 months (Martal *et al.*, 1979). Although present for a short time only, the effects of this embryonic signal may last much longer.

Nature of trophoblastin

At 14–16 days of gestation, the embryonic disc is a tiny mass of cells measuring some hundreds of microns in diameter, whereas the trophoblast is about 50 times this size (Flechon, Guillomot & Winterberger-Torrès, 1978). Because of its thread-like morphology, the trophoblast seems to be particularly well suited for the secretion of a local antiluteolytic factor. Hence, it seems reasonable to assume that trophoblastin is secreted by the trophoblast and not by the embryonic disc. Homogenates of 14–16-day-old trophoblasts heated for 30 min at 80°C or treated with pronase (a proteolytic enzyme) do not inhibit luteolysis in recipient ewes. Trophoblastin is therefore most probably a protein or a protein-containing substance (Table 1) (Martal *et al.*, 1979). According to Rowson & Moor (1967) the antiluteolytic activity of 14-day-old sheep embryos is thermolabile. Although insoluble at pH 7 or 8, trophoblastin may be extracted at pH 9·6. After gel filtration, trophoblastin is still able to maintain the luteal structures (Martal *et al.*, 1979).

As indicated previously oCS does not possess the local antiluteolytic activity of trophoblastin (Table 1). Likewise, ovine chorionic gonadotrophin (oCG), analogous to hCG, might be different from trophoblastin since an intrauterine injection of hCG does not inhibit luteolysis (Table 1). The injection of 120-day-old placental extracts, exhibiting high oCS and

oCG activities, does not prevent the regression of the corpus luteum of the oestrous cycle (Table 1) (Martal *et al.*, 1979). The latter results were confirmed by Ellinwood, Nett & Niswender (1979).

Table 1. Effects of various treatments on the maintenance of the corpus luteum of the ewe

Intrauterine injection	Daily amount per ewe (from Day 12 of oestrous cycle)	Treatment length (days)	Maintenance of corpus luteum (no. of ewes)
NaCl (0·9% w/v)	2 ml	11	0/8
Homogenates of 14–16-day trophoblasts	2 trophoblasts	11	4/5
	2 trophoblasts	4	4/7
Homogenates of 21–23-day trophoblasts	2 trophoblasts	6	1/6
Homogenates of 14–16-day trophoblasts (heated)	2 trophoblasts	6	0/3
Pronase (3–4 units/mg)	2 mg	6	0/3
Homogenates of 14–16-day trophoblasts + pronase	2 trophoblasts + 2 mg	6	0/3
hCG	15 i.u.	6	0/4
	1500 i.u.	5	0/4
oCS	40 μg (prolactin equivalent)	6	0/5
120-day placental extracts	2 ml	6	0/3
Supernatant of 14–16-day trophoblast extracts at pH 7 or 8	2–3 trophoblasts	6	0/4
Supernatant of 14–16-day trophoblast extracts at pH 9·6	2 trophoblasts	6	3/5
Ultrogel (LKB) effluent	3–4 trophoblasts	10	2/3

Mechanisms of action of trophoblastin

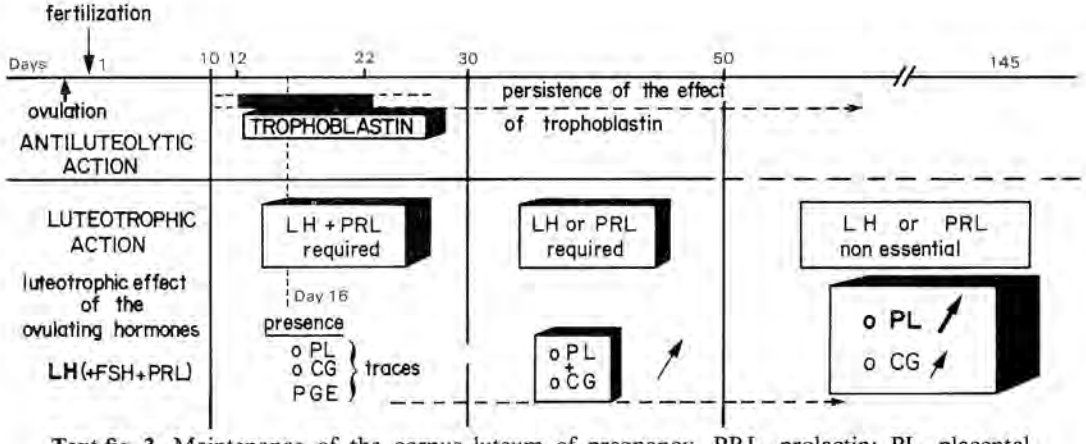
Although the mode of action of trophoblastin has not been established, several hypotheses may be put forward. Its antiluteolytic action may be expressed by a partial inhibition of PGF-2α secretion or synthesis, or by stimulation of the luteotrophic action of PGE-2. The role of thromboxanes and prostacyclins should be considered, but no data are available on the presence of these compounds during early pregnancy. Trophoblastin might reduce the synthesis of arachidonic acid from phospholipids, inhibit the conversion of arachidonic acid into prostaglandins or inhibit cyclo-oxygenase activity. An inhibitory mechanism of cyclo-oxygenase by destruction of its main activator has already been reported in other tissues (Cook & Lands, 1976).

Trophoblastin could influence the binding or synthesis of receptors to oestrogens or oxytocin, whose action on luteolysis has been considered (McCracken, 1980). This possibility is emphasized by the observation that the luteolytic action of oestradiol is reduced in pregnant ewes (Kittok & Britt, 1977).

Inskeep, Smutny, Butcher & Pexton (1975) have suggested that the conceptus might have a direct action on the ovary since PGF-2α injections into the ovary are luteolytic in ewes during the oestrous cycle, but not in pregnant ewes. The existence of an antiluteolytic compound diffusible by a counter-current mechanism and blocking the action of PGF-2α on the ovary is thus possible. PGE-2 could function in this way, but PGE-2, unlike trophoblastin, is not able to extend the life-span of the corpus luteum substantially. It is also likely that trophoblastin acts by favouring the synthesis of PGE-2 in the endometrium. The existence of enzymes such as PGE-2-9 ketoreductase and PGE-2α-9 hydroxydehydrogenase which convert PGE-2 into PGF-2α and *vice versa* has been reported for various tissues such as the kidney (Dunn & Hood, 1977) and the ovary (Watson, Shepherd & Dodson, 1979).

It may also be asked whether the essential mechanism of the antiluteolytic action of trophoblastin is a partial inversion in the direction of PGF-2α secretion. Instead of being secreted into the ovarian vein, PGF-2α could be excreted to a larger extent into the uterine lumen. Such an inversion has been suggested in the sow to explain the luteotrophic role of oestrogens (Bazer & Thatcher, 1977). In experiments carried out in ewes fitted with ovarian grafts under the neck skin, Harrison, Heap, Horton & Poyser (1972) observed that progesterone played a predominant role in the accumulation of uterine fluid rich in PGF-2α. Indeed, after injection of trophoblast homogenates at pH 9·6 or after removal of 21–23-day-old embryos, an abundant, clear and sterile fluid was found when the animals were slaughtered a long time after treatment. The presence of these fluids indicates a change in the direction of the diffusion of water, proteins and various substances. By inhibiting luteolysis, trophoblastin produces the same effect but probably in an indirect way through the action of progesterone. The amount of fluid in the uterine lumen at the moment of implantation is very small and an accumulation of fluid can be observed only in the presence of progesterone.

Text-figure 3 presents a summary of the roles played by the various luteotrophic and luteolytic factors in the maintenance of the ovine corpus luteum during gestation.



Text-fig. 3. Maintenance of the corpus luteum of pregnancy. PRL, prolactin; PL, placental lactogen; CG, chorionic gonadotrophin.

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