MINIREVIEW

Mediators of Maternal Recognition of Pregnancy in Mammals[1] (43371A)

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Maternal recognition of pregnancy results from signaling between the conceptus (embryo and its associated membranes) and the maternal system (1). These signals ensure maintenance of structural and functional integrity of the corpus luteum (CL), which would otherwise regress at the end of the estrous of menstrual cycle. The CL produces progesterone, the hormone of pregnancy, which is required to stimulate and maintain endometrial functions that are permissive to early embryonic development, implantation, placentation, and successful fetal and placental development. The terms luteotrophic, luteal protective, antiluteolytic, and luteolytic will be defined before mechanisms for maternal recognition of pregnancy are discussed. A luteotrophic signal, e.g., chorionic gonadotrophin (CG), is produced by primate conceptuses, and appears to act directly on the CL, via receptors for luteinizing hormone, to ensure maintenance of its structural and functional integrity. It is important to keep in mind that the ovarian cycle of primates is uterine independent, i.e., luteolytic events responsible for regression of the CL and cessation of progesterone secretion at the end of a menstrual cycle result from intraovarian effects of prostaglandins, oxytocin, or other, as yet undefined, luteolytic agents. A luteolytic agent causes structural and functional demise of the CL or luteolysis. Prostaglandin (PG) $F_{2\alpha}$ is the luteolytic signal common to most, if not all, mammals. There may also be luteal protective signals, e.g., PGE_2 (PGE), that antagonize potential luteolytic effects of PGF.

The ovarian cycle of subprimate mammals is uterine dependent and hysterectomy extends CL maintenance for a period characteristic of the species' gestational period. The uterine endometrium, primarily surface epithelium and perhaps superficial glandular epithelium, produces PGF, which is responsible for luteolysis. Signals from conceptuses of subprimate mammals are termed antiluteolytic because they either inhibit endometrial release of luteolytic amounts of PGF or alter the pattern of endometrial secretion of PGF to abrogate its luteolytic effects. In subprimate mammals, conceptus signals responsible for maternal recognition of pregnancy appear to act in a paracrine manner to interrupt endometrial production of luteolytic PGF, but do not act directly on the CL. Antiluteolytic signals include estrogen and lactogenic hormones (pig) and Type I trophoblast interferons (ruminants).

Endocrine Requirements for Luteolysis

Primates. The menstrual cycle in humans is from onset of menses in one cycle to the onset of menses in the subsequent cycle and averages 28 days. Ovulation occurs in response to a surge of luteinizing hormone released on about Day 14, and CL regression, a prelude to onset of menses, results from intraovarian effects of PGF (2), oxytocin (3), or other unidentified hormones acting independently or in concert. However, the luteolytic mechanism in primates remains poorly understood.

Luteolysis in primates is uterine independent; however, changes in numbers of endometrial steroid receptors and their localization are affected by day of the menstrual cycle and pregnancy (4–6). Endometrial steroid receptors will be discussed briefly for primates even though they do not influence luteolytic events, because

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this information is relevant when one compares endometrial functions of cyclic and pregnant mammals. In general, concentrations of estrogen receptors are greatest in surface and glandular epithelium and stromal cells on Days 10–14 and then decline to their lowest levels on Days 22–28 just prior to onset of menses. Progesterone receptors (PR) are also highest in endometrial epithelium on Days 10–14 and then decline to their lowest levels on Days 15–21. However, PR in stromal cells do not change appreciably between Days 10 and 21, but do decrease thereafter. During pregnancy, high levels of PR are present in endometrial decidual tissue (5) to ensure that a progesterone-responsive endometrium, permissive to the establishment and maintenance of pregnancy, is maintained. Estrogen receptors (ER) are absent from epithelial and stromal cells of the endometrium of pregnant baboons as early as Day 18 after ovulation (7).

Production of CG by trophoblast of primate conceptuses begins around the time of implantation and CG acts directly on the CL to ensure continued secretion of progesterone (8). The β-subunit of CG is detectable as early as the 6–8 cell stage of development of human embryos, but secretion of CG is associated with later events of implantation and trophoblast outgrowth. In humans, for example, implantation begins on Days 7–9 and CG is detectable in peripheral plasma between Days 9 and 12; a similar sequence of events occurs in the chimpanzee, rhesus, and baboon (8). Structural and functional demise of the CL, followed by termination of pregnancy, results from either passive (within 6 days) or active immunization (within 4 weeks) against CG of marmoset monkeys at 3–5 weeks of pregnancy. Although CG is the primary luteotrophic signal from the primate conceptus during early pregnancy, other hormones and growth factors may also participate (8).

Maternal recognition of pregnancy in primates appears to involve independent interactions between the conceptus and uterus, as well as between the conceptus and ovary. Interactions between the conceptus and uterus result in maintenance of high levels of PR, but not ER, in the endometrium. Progesterone, through interaction with its receptor in stromal cells, maintains an endometrium that is permissive to conceptus development, implantation, decidualization, placentation, and fetal and placental development to term. The significance of the absence of ER in endometrial epithelium and stroma of primates (baboons) during early pregnancy is not known. Finally, CG, produced by trophoblast, appears to be the mediator of maternal recognition of pregnancy in primates. Production of CG begins during the peri-implantation period and it exerts its luteotrophic effect directly on the CL; however, growth factors, prostaglandins other than PGF, or other hormones, e.g., relaxin or lactogenic hormones, may influence luteal function directly or indirectly during pregnancy.

Ruminants. Sheep, cattle, and goats have estrous cycles of about 17, 21, and 20 days, respectively. An ovulatory surge of luteinizing hormone occurs near the time of onset of estrus, when the female first accepts the male for mating. Luteinizing hormone initiates events that culminate in ovulation about 30 hr later. With maturation of the CL, concentrations of progesterone in peripheral blood peak around middiestrus, and, in cyclic females, luteolysis is induced by pulsatile release of PGF from endometrial epithelium during late diestrus. The antiluteolytic signals for maternal recognition of pregnancy in ruminants are called Type I trophoblast interferons (9, 10) and they share high amino acid and nucleotide sequence homology across species. The Type I trophoblast interferons exert a paracrine, antiluteolytic effect on the endometrium to inhibit endometrial production of luteolytic pulses of PGF. Other conceptus and uterine products secreted during pregnancy, e.g., PGE and platelet activating factor, may also exert luteal protective effects.

Three ovarian hormones are known to influence endometrial production of luteolytic pulses of PGF; progesteone, estrogen, and oxytocin. A number of mechanisms associated with endocrine regulation of luteolysis are understood in sheep and appear to be applicable to most ruminant species. Endometrium of ewes is stimulated by progesterone to increase phospholipid stores (arachidonic acid source) and cyclooxygenase enzymatic activity necessary for conversion of arachidonic acid to PGF (11).

Oxytocin secreted by the CL and posterior pituitary stimulates pulsatile endometrial secretion of PGF required for luteolysis. High levels of mRNA for oxytocin and its associated neurophysin are present in granulosa cells of the preovulatory follicle and corpus hemorrhagicum of ewes, cows, and goats between Days 0 and 7 after onset of estrus (12–16). Oxytocin and its associated neurophysin are synthesized and stored in secretory granules of large luteal cells until their release into the ovarian vasculature beginning about Day 5 of either the estrous cycle or pregnancy (17, 18). Oxytocin, primarily from the CL, but also from the posterior pituitary, acts through receptors on endometrial epithelium to stimulate the inositol phospholipid second messenger system, which, in turn, releases luteolytic pulses of PGF (19). In sheep (19) and cows (20–23), endometrial receptors for oxytocin are present between estrus and about Day 4 of the cycle, are low or undetectable between Days 5 and 13, and the increase rapidly between Days 14 and 16 (sheep) or Days 17 and 20 (cows). Recent evidence indicates that the oxytocin receptors are localized to the endometrial surface epithelium during the luteolytic period (24).

McCracken *et al.* (25) proposed that progesterone,

binding to its receptor, initiates events that inhibit synthesis of oxytocin receptors by endometrial epithelium for 10 to 12 days, a period referred to as the "progesteone block." Afterward, endometrial receptors for oxytocin increase. In cows, progesterone down-regulates its own receptors after about Day 12 of the cycle to end the progesterone block, and the decrease in endometrial PR is followed by an increase in endometrial receptors for oxytocin that appears to be enhanced by estrogen (20). The progesterone block to synthesis of oxytocin receptors is also absent during metestrus. During metestrus, PR numbers are high; however, circulating levels of progesterone are inadequate to occupy those receptors and initiate the progesterone block to oxytocin-receptor synthesis. During late diestrus, the opposite situation develops, i.e., circulating levels of progesterone are high, but loss of endometrial PR terminates the progesterone block. This allows up-regulation of oxytocin receptors essential for the initiation of endometrial production of luteolytic pulses of PGF.

Results of studies with ovariectomized, steroid-treated ewes indicate that treatment with progesterone alone for at least 12 days results in up-regulation of oxytocin receptors, as well as the presence of adequate cyclooxygenase enzymatic activity and phospholipid stores for oxytocin induction of high levels of endometrial production of PGF (26–28). Estrogen, acting on an endometrium exposed to progesterone for 10 to 12 days or during late diestrus, enhances up-regulation of endometrial oxytocin receptors (29) or the ability of oxytocin to stimulate endometrial secretion of PGF (26, 27, 30, 31). Endometrial receptors for estrogen (32) and oxytocin (24) are localized to surface and superficial glandular epithelium during the luteolytic period of the estrous cycle. Localization of the endometrial PR during the estrous cycle has not been determined for ruminants.

During the estrous cycle of cattle, endometrial ER and PR are highest during the first 10–12 days after onset of estrus and then decline to their lowest levels on about Day 13 (20). The ER then increase between Days 14 and 21, with oxytocin receptors increasing between Days 17 and 21, when PR are also increasing very slowly. In cyclic ewes, endometrial PR and PR mRNA decrease from Day 10 to Day 14 and then increase up to Day 16. This coincides with increasing ER, ER mRNA, and oxytocin receptors between Days 14 and estrus (33; T. L. Ott, T. P. Ogle, and F. W. Bazer, unpublished results). In contrast, endometrial PR are stable between Days 10 and 16 of pregnancy, despite a gradual decrease in PR mRNA and a steady decline in ER and ER mRNA (T. L. Ott, T. P. Ogle, and F. W. Bazer, unpublished results). Similarly, in sheep and goats, endometrial receptors for oxytocin increase rapidly 48–72 hr prior to estrus. In ewes and cows, therefore, the endometrium during the luteolytic period is characterized by low PR, increasing ER, and increasing numbers of oxytocin receptors.

During the luteolytic period, about 98% of the PGF pulses are associated with a pulse of oxytocin. However, only about 50% of the oxytocin pulses result in a pulse of PGF (34), suggesting that oxytocin is responsible for coordinating luteolytic events. The PGF pulse frequency may be less than that for oxytocin because of the time required for replenishment of pools of phospholipids from which arachidonic acid can be mobilized for synthesis of PGF (35). Treatment of ewes (36) or goats (37) with an oxytocin antagonist, passive or active immunization of ewes against oxytocin (38, 39), or continuous infusion of oxytocin to down-regulate oxytocin receptors (40) prevents or significantly delays luteolysis. These results indicate a central role for oxytocin in the luteolytic mechanism, which is dependent upon pulsatile release of PGF (41). Uterine release of about five pulses of PGF per 25 hr is required to initiate luteolysis (25). Low amplitude pulses of PGF from the uterus act on large luteal cells to cause trafficking of secretory granules containing oxytocin to the cell surface and exocytosis of oxytocin, which then induces a pulse of uterine PGF (19). This mechanism is repeated at 4- to 5-hr intervals between Days 14 and 17 or until the CL is depleted of its finite stores of oxytocin. Other mechanisms to explain the episodic release of oxytocin and PGF have been proposed, because release of oxytocin from the CL, even when there is one CL on each ovary, and the posterior pituitary is synchronous in sheep (34). Control of oxytocin release by hormones other than PGF or PGE is possible, but such a factor has not been defined.

In long-term ovariectomized ewes, oxytocin receptors are high in the absence of ovarian hormone replacement, suggesting that their presence is due to the lack of an inhibitory hormone, e.g., progesterone. However, due to the lack of progesterone effects to increase arachidonic acid pools and cyclooxygenase enzyme activity, oxytocin is unable to stimulate PGF production by the uterus of long-term ovariectomized ewes (26). The role of estrogen in the luteolytic process is not established. Using the long-term ovariectomized ewes and steroid replacement therapy, estrogen alone does not further stimulate synthesis of oxytocin receptors, arachidonic acid pools, or cyclooxygenase enzymatic activity of the endometrium. However, when these ewes are treated with progesterone and estrogen, the frequency of PGF pulses is increased. Estrogen may enhance oxytocin-receptor synthesis and postreceptor events mediated by oxytocin to increase the frequency of PGF pulses from sheep endometrium during luteolysis (30). The use of x-irradiation of ovaries to destroy follicles or immunization of ewes against estrogen delays luteolysis (42). Estrogen from follicles may ensure a PGF

pulse frequency that is adequate to induce luteolysis by influencing the oxytocin pulse frequency or an undefined estrogen-sensitive PGF pulse generator (42), and protracted interpulse intervals for PGF result in failure of luteolysis (43). Similarly, administration of a gonadotrophin-releasing hormone agonist every 3 days to prevent development of steroidogenically competent ovarian follicles in cows prevents luteolysis for at least 45 days (44).

Pregnancy Recognition in Sheep. The presence of the conceptus in the uterus prevents luteolysis because an antiluteolytic signal(s), produced by the conceptus, prevents uterine production of luteolytic pulses of PGF. Type I trophoblast interferons are the antiluteolytic signals produced by conceptuses of ruminants. Potential mechanisms of action include: (i) stabilization or up-regulation of endometrial PR to extend the progesterone block and prevent endometrial synthesis of oxytocin receptors or up-regulation of ER; (ii) direct inhibition of ER to attenuate episodic release of PGF required for luteolysis; (iii) direct inhibition of synthesis of endometrial oxytocin receptors; or (iv) inhibition of postreceptor mechanisms that prevent oxytocin-induced pulsatile release of PGF. Available results indicate that endometrial epithelium of pregnant ewes, cows, and goats have few or no receptors for either oxytocin (see 45) or estrogen (32).

Pregnant ewes fail to experience luteolysis in response to doses of exogenous oxytocin (31) and estradiol (31, 46, 47) that cause luteolysis in cyclic ewes and cows (48). Release of oxytocin and oxytocin neurophysin has been reported to be reduced (49), increased (18), or not different (34) in pregnant compared with cyclic ewes between Days 13 and 16 after estrus. However, a consistent finding has been that oxytocin receptor numbers are very low or absent in pregnant ewes (19, 25). Basal secretion of PGF by sheep endometrium is substantially higher for pregnant ewes than for cyclic ewes (31, 47); however, the pulsatile release of PGF required for luteolysis is abolished during pregnancy (25, 34, 50).

Homogenates of sheep conceptuses extend the interestrous interval in ewes when infused into the uterine lumen, but not the utero-ovarian venous drainage (51–53). Sheep conceptus homogenates do not contain either chorionic gonadotrophin-like or prolactin-like activity (54). Through *in vitro* culture of sheep conceptuses and analysis of radiolabeled proteins released into the culture medium, the first major protein secreted by mononuclear cells of ovine trophectoderm was identified as ovine trophoblast protein-1 (oTP-1; see 45).

oTP-1 is secreted between Days 10 and 21 of pregnancy, has a mol wt of 19,000, and binds to endometrial receptors (see 45). There is a second period of secretion of immunoreactive and bioactive oTP-1 by chorion between Days 25 and 45 of pregnancy (55). oTP-1 has high amino acid sequence homology with α_{II}-interferons (56–58) and potent antiviral activity (59). Infusion of highly purified oTP-1 (60) or recombinant oTP-1 (61, 62) into the uterine lumen from Days 12 to 14 extends the interestrous interval and CL lifespan; therefore, oTP-1 alone is assumed to be the antiluteolytic factor produced by sheep conceptuses. oTP-1 is thought to exert a paracrine antiluteolytic effect on the endometrium, since there is no evidence that it is transported from the uterus to directly affect the CL (63).

By using endometrium taken on Day 15 of the estrous cycle (oxytocin receptor present), it was determined that oTP-1 does not compete with oxytocin for its receptor, inhibit oxytocin stimulation of endometrial inositol phospholipid metabolism, or inhibit oxytocin stimulation of endometrial secretion of PGF (64). The antiluteolytic effect of oTP-1 must, therefore, prevent development of the luteolytic mechanism. Secretion of oTP-1 (ng/uterine flushing) begins on about Day 10 (65) and increases as conceptuses change morphologically from spherical (312 ng) to tubular (1380 ng) to filamentous (4455 ng) forms on Days 12–13 (66). Successful transfer of embryos to cyclic ewes can be accomplished only as late as Day 12, i.e., 48–72 hr prior to the luteolytic period. This suggests that oTP-1 is secreted prior to the luteolytic period to directly or indirectly inhibit endometrial synthesis of oxytocin receptors and uterine release of luteolytic pulses of PGF.

Both inositol phospholipid metabolism (67, 68) and PGF secretion (60, 67) in response to oxytocin are reduced significantly when endometrium of cyclic ewes is exposed to oTP-1 on Days 12 through 14. These results indicate the absence of functional endometrial oxytocin receptors in ewes treated with oTP-1. Functional endometrial receptors for oxytocin are present in low numbers in pregnant ewes when measured directly (19, 25) or indirectly (67, 68). Intrauterine infusion of oTP-1 between Days 11 and 15 of the estrous cycle reduces the number of oxytocin receptors, the affinity of the oxytocin receptor for oxytocin, and endometrial ER protein; however, effects of oTP-1 on endometrial PR were not detected (69).

Oxytocin-receptor affinity decreases in the absence of estrogenic stimulation of rat myometrium (70) and oxytocin-receptor affinities tend to be lower for endometrium from pregnant cows (1.5 ± 0.5 vs 0.9 ± 0.1 n*M*; 22). oTP-1 may inhibit synthesis of oxytocin receptors and reduce their affinity for oxytocin, perhaps by down-regulating the endometrial ER or stabilizing endometrial PR. Rapid enhancement of endometrial ER in ewes follows withdrawal of progesterone (29), which suggests that failure of endometrial ER to increase during pregnancy is associated with either stabilization of PR by oTP-1 or direct inhibition of ER synthesis by oTP-1. During pregnancy, endometrial ER

and ER mRNA are significantly lower for pregnant than cyclic ewes on Day 16 (71; T. L. Ott, T. P. Ogle, and F. W. Bazer, unpublished results) and for cyclic ewes receiving intrauterine infusions of oTP-1 on Days 11–15 and hysterectomized on Day 16 (M. A. Mirando, T. P. Ogle, J. P. Harney, T. L. Ott, and F. W. Bazer, unpublished results). In addition, immunocytochemical studies indicate the absence of ER in endometrial surface and superficial glandular epithelium of Day 15 pregnant ewes (32). Interferons can inhibit synthesis, turnover, or movement of receptors within membranes (72, 73), and treatment of patients having steroid-dependent adenocarcinoma with β-interferon increased PR and decreased ER in tumor cells (74).

Temporal changes in endometrial receptors for progesterone during the estrous cycle and early pregnancy of sheep have not been reported previously. However, unpublished results (T. L. Ott, T. P. Ogle, and F. W. Bazer) indicate that: (i) endometrial PR is lower on Days 12 and 14 than on Days 10 and 16 of the estrous cycle; (ii) endometrial PR did not change significantly between Days 10 and 16 of pregnancy, indicative of stabilization of PR, despite a gradual decrease in PR mRNA; (iii) changes in PR mRNA differed between cyclic and pregnant ewes, tending to increase between Days 12 and 16 of the estrous cycle and decrease during the same period for pregnant ewes; and (iv) the ratios for PR to ER and PR mRNA to ER mRNA were higher for pregnant ewes. Intrauterine infusion of ovine conceptus secretory proteins increased endometrial PR about 40% when endometrium was taken before concentrations of progesterone in plasma declined and estrogen up-regulation of PR occurred (M. A. Mirando, R. J. Moffatt, T. L. Ott, and F. W. Bazer, unpublished results), but oCSP had no detectable effect in a second experiment, when endometrium was taken after plasma progesterone had declined and up-regulation of ER had begun (M. A. Mirando, T. L. Ott, T. P. Ogle, and F. W. Bazer). *In vivo*, the antiluteolytic effects of oTP-1 are dependent upon the presence of progesterone (75). In the absence of progesterone, oTP-1 actually stimulates *in vivo* PGF response to exogenous oxytocin, while inhibiting this response in the presence of progesterone. Altering relative concentrations of endometrial PR and ER or the PR to ER ratios may influence conceptus-mediated antiluteolytic mechanisms. At present, consistent inhibitory effects of oTP-1 on ER and ER mRNA have been demonstrated, but effects of oTP-1 on PR and PR mRNA are equivocal.

High-affinity, low-capacity binding sites for oTP-1 are present in endometrial membranes (63) and human α-interferon will displace oTP-1 from those receptors (57). Unoccupied oTP-1 receptors are similar for cyclic and pregnant ewes on Days 8 and 12, but decrease thereafter for pregnant ewes (76). Sheep endometrium may have high- and low-affinity receptors for oTP-1, but only high-affinity receptors for recombinant bovine IFN-α_1I (rbIFN-α; 77). Antiluteolytic effects of oTP-1 may require that it bind to both types of receptor, and this may explain why oTP-1 has greater antiluteolytic activity than rbIFN-α. Daily intrauterine infusion of rbIFN-α extended interestrous intervals of ewes to greater than 19 days when 2000 μg, but not 200 μg, were infused over each 24-hr period from Days 9 through 19 (78). Intrauterine infusion of oTP-1 (100 μg/day) is considerably more effective than rbIFN-α (78) and human IFN-α_2I (79) in extending interestrous intervals of sheep, suggesting that antiluteolytic properties of oTP-1 are not shared equally with rbIFN-α and recombinant human IFN-α.

Concentrations of PGE in utero-ovarian vein plasma of pregnant ewes increase on Days 13 and 14 and PGE has been suggested to play a luteal-protective role (80); however, this conclusion is not supported by the results of others (18). Since PGE stimulates the release of oxytocin from luteal cells, it may initiate or accelerate depletion of luteal oxytocin before endometrial receptors for oxytocin increase, which would reduce stimulation of oxytocin receptors that may be synthesized during early pregnancy (30). This may explain why mean concentrations of oxytocin are higher in blood from the inferior vena cava of pregnant ewes between Days 4 and 14 after onset of estrus (18), while pulses of oxytocin are of lower amplitude in pregnant ewes during the period of expected luteolysis (81). The presence of a factor(s) in uterine venous blood that delays luteolysis in ewes has been suggested (82), and that factor may be PGE (83).

oTP-1, the mediator of maternal recognition of pregnancy in sheep, acts as an antiluteolytic signal to prevent uterine secretion of luteolytic pulses of PGF. Our current working hypothesis is that oTP-1 stabilizes endometrial PR and down-regulates endometrial ER to prevent up-regulation of endometrial receptors for oxytocin. Failure of pregnant ewes to respond to the potential luteolytic effects of estradiol and oxytocin could be explained by the absence of receptors for each of these hormones. In addition, the resistance of CL of pregnant ewes to luteolytic effects of PGF may be due to antagonistic effects mediated by PGE or other secretions of the conceptus that protect the structural and functional integrity of the CL.

Pregnancy Recognition in Cows. The corpus luteum lifespan in recipient cows and ewes is extended following interspecies reciprocal transfer of trophoblastic vesicles (84), indicating that antiluteolytic signals from conceptuses of sheep and cows are similar. Bovine conceptuses produce bovine trophoblast protein-1 (bTP-1), which cross-reacts immunologically with oTP-1 (85), has high amino acid sequence homology with both oTP-1 and α-interferon (86), and possesses potent antiviral activity (87). Secretion of bTP-1 is maximal

around Days 16–19 of pregnancy (88); however, mRNA for bTP-1 can be detected as early as Day 12 (89). Secretion of bTP-1 increases during elongation of the conceptus (90), and chorion may secrete bTP-1 until at least Day 38 of pregnancy (88, 91).

When infused into the uterine lumen of cyclic cows between Days 14 and 17, bTP-1 extends the lifespan of CL and decreases concentrations of PGF in the posterior vena cava (92). Antiluteolytic effects of bTP-1 may result, in part, from inhibition of PGF secretion by inducing an intracellular inhibitor of PGF synthesis (93), an effect not detected in sheep. This factor, isolated from the cytosolic fraction of endometrium from pregnant cows (94, 95), may inhibit cyclooxygenase and block conversion of arachidonic acid to both PGF and PGE (95). The inhibitor is noncompetitive with respect to arachidonic acid substrate, is protease sensitive, is precipitable with 20% ammonium sulfate, and has M_r forms of 25,000–35,000 and 70,000–75,000 (95).

A problem with assigning a functional role to the inhibitor is the fact that it can inhibit both PGF and PGE synthesis. However, bTP-1 acts on endometrial explants to decrease PGF secretion and increase PGE secretion (93). The inhibitor may be compartmentalized within the endometrium. During the estrous cycle, the major source of endometrial PGF is epithelial cells, while the major source of PGE is stromal cells (96). Perhaps bTP-1 induces the inhibitor in epithelial cells, but not stromal cells.

During maternal recognition of pregnancy, ovarian follicular populations are altered (97) and follicular waves on the ovary bearing the CL, but not the contralateral ovary, are suppressed in cattle (98). These effects may be supportive of the antiluteolytic mechanism whereby local suppression of follicular development reduces secretion of estradiol that could otherwise enhance uterine secretion of luteolytic pulses of PGF (44). Endometrial receptors for oxytocin are significantly reduced in pregnant compared with cyclic cattle during the luteolytic period (22, 23), e.g., 563 ± 117 vs 18 ± 5 fmol/mg protein for Day 18 cyclic and pregnant cows, respectively (23). As with sheep, bTP-1 may directly or indirectly inhibit synthesis of endometrial receptors for oxytocin to abrogate uterine production of luteolytic pulses of PGF.

Pregnancy Recognition in Goats. Goat conceptuses exert an antiluteolytic effect similar to that of sheep conceptuses. Goat conceptuses survive and extend luteal function when transferred to ewes (99) and goat conceptuses secrete proteins with biochemical characteristics similar to those of oTP-1 (100). The uterine luteolysin in goats is PGF and the conceptus interferes with oxytocin-induced pulsatile release of PGF (101). The removal of goat conceptuses from the uterine lumen between Days 13 and 15 does not affect the interestrous interval, but their removal on Day 17 extends luteal lifespan by 7–10 days (100). This suggests that maternal recognition of pregnancy in goats occurs around Day 17. Goat conceptuses secrete caprine trophoblast protein-1 between Days 16 and 21 that can be immunoprecipitated with antiserum to oTP-1; therefore, caprine trophoblast protein-1 may be the antiluteolytic protein (100). Pulsatile release of oxytocin and PGF is suppressed in pregnant compared with cyclic goats between Days 10–12 and estrus or Day 20 of pregnancy (101), suggesting that antiluteolytic mechanisms in the goat are similar to those for sheep and cows.

Pigs and Horses

Luteolytic Events. Endocrine requirements for luteolysis in pigs and horses have not been clearly deliniated. However, it is known that luteolysis occurs during late diestrus, i.e., following stimulation of the uterine endometrium by progesterone for 10 to 12 days. Luteolysis occurs when pulsatile release of uterine PGF into the uterine venous drainage begins on about Day 15 or 16 of the estrous cycle (102). The CL of pigs contains very low levels of oxytocin and vasopressin (103, 104) and undetectable levels of oxytocin mRNA (105); but the potential role of these neuropeptides of ovarian or posterior pituitary origin in luteolysis in pigs has not been established. The endometrium of pigs must contain receptors for oxytocin (106) because it responds *in vitro* to oxytocin with increased secretion of PGF (114) and inositol phospholipid turnover (107).

The uterine endometrium of mares releases PGF which results in luteolysis, but neither the pattern of release of PGF required for luteolysis nor endocrine regulation of uterine production of luteolytic PGF is established. It is known that cervical stimulation of oxytocin release via the Ferguson reflex stimulates uterine secretion of PGF (108) and that administration of exogenous oxytocin stimulates uterine release of PDF in mares (109).

The CL of pigs is refractory to luteolytic effects of PGF until about Day 13 of the estrous cycle, because luteal receptors for PGF are insufficient to allow PGF to exert a luteolytic effect until Days 12–14 of the estrous cycle (110). The CL of mares, however, is very responsive to luteolytic effects of PGF after about Day 5 after ovulation, as is the case for sheep, cattle, and goats.

Pregnancy Recognition in Pigs. The theory of maternal recognition of pregnancy in pigs has been reviewed extensively (102, 111). The major assumptions are that uterine endometrium secretes the luteolysin PGF and that the conceptuses secrete estrogens that are antiluteolytic. The present theory is that PGF is secreted in an endocrine direction, toward the uterine vasculature, in cyclic gilts and transported to the CL to exert its luteolytic effect. However, in pregnant pigs,

the direction of secretion of PGF is exocrine, into the uterine lumen, where it is sequestered to exert its biological effects *in utero* or be metabolized to prevent luteolysis.

Mean concentrations, peak frequency, and peak amplitude of PGF in utero-ovarian vein plasma are lower in pregnant and estrogen-induced pseudopregnant gilts than in cyclic gilts (111, 112). On the other hand, uterine flushings of pseudopregnant and pregnant gilts have significantly higher amounts of PGF than do those from cyclic gilts (111). These results indicate that PGF is released primarily into the uterine venous drainage (endocrine) in cyclic gilts, but into the uterine lumen (exocrine) in pregnant and pseudopregnant pigs, and that secretion of PGF is not inhibited during pregnancy or pseudopregnancy.

A perifusion device, which allows one to discriminate between release of PGF from the luminal and myometrial sides of the endometrium (113), was used to demonstrate (114) that endometrium from cyclic pigs secretes PGF primarily from the myometrial side (endocrine) and that pregnant gilts secrete PGF primarily from the luminal side (exocrine). The transition from endocrine to exocrine secretion occurs between Days 10 and 12 of pregnancy which is temporally associated with initiation of estrogen secretion by elongating pig conceptuses. Estrogens, secreted by the conceptus or injected, induce a transient release of calcium into the uterine lumen within 12 hr. Re-uptake of that calcium by endometrial or conceptus tissues occurs about 12 hr after concentrations of calcium in uterine secretions reach maximum values. The switch in direction of endometrial secretion of PGF from an endocrine to an exocrine orientation is closely associated with this period of release and re-uptake of calcium by the endometrium in pregnant and pseudopregnant gilts. When the endometrium from Day 14 cyclic gilts was treated with the calcium ionophore A23187 (an inducer of calcium flux across epithelial membranes), secretion of PGF changed from an endocrine toward an exocrine direction. These results suggest that induction of calcium cycling across endometrial epithelium is associated with redirection of secretion of PGF (115). Recent evidence suggests that estrogen induces endometrial receptors for prolactin in pigs (116), which allow prolactin to act on the endometrium to induce calcium cycling across the epithelium (115). Exogenous estradiol must be administered to gilts on Day 11 and Days 14–16 to consistently obtain interestrous intervals of greater than 60 days (117). The requirement for two phases of estradiol, similar to that produced by conceptuses on Days 11–13 and Days 15–30, for prolonged secretion of PGF in an exocrine direction may be necessary for initial induction of receptors for prolactin and then replenishment of those receptors.

Pig conceptus secretory proteins (pCSP) recovered from culture medium of Day 15 conceptuses (118) have antiviral activity (119–121) due to secretion of both α- (25%) and γ- (75%) interferons between Days 15 and 21 of gestation (122). Intrauterine infusion of pCSP on Days 12–15 of the estrous cycle has no effect on interestrous interval or temporal changes in concentrations of progesterone in plasma (123). The pCSP do stimulate endometrial production of PGF and PGE which may be beneficial to the establishment and maintenance of pregnancy (123, 124). Inhibition of secretion of pros-

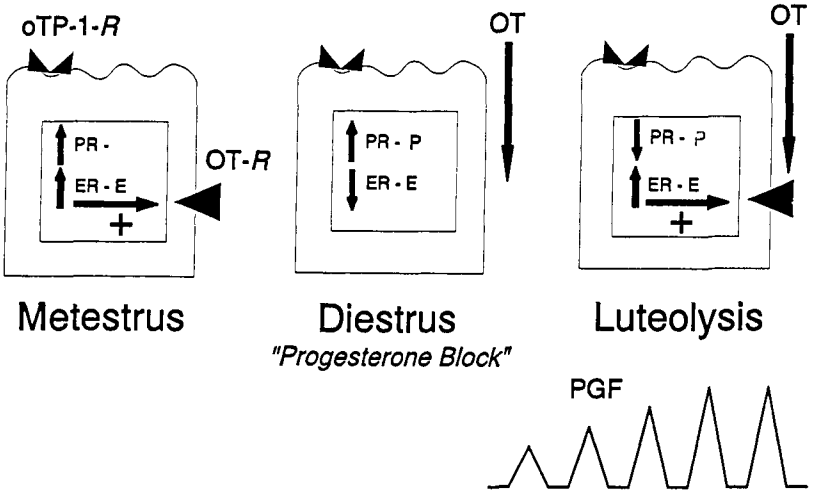

Figure 1. The current model for cyclic ewes assumes that oxytocin receptors are present on uterine epithelium during metestrus because occupied ER are present. Endometrial PR are also present, but low circulating levels of progesterone result in insufficient numbers of occupied PR to suppress synthesis of oxytocin receptors. During diestrus, endometrial ER and estradiol in plasma are low and occupied PR initiate and maintain the progesterone block to synthesis of ER and oxytocin receptors for 10 to 12 days. During late diestrus, progesterone down-regulates PR to allow up-regulation of ER and oxytocin receptors, an event that is facilitated by increasing rates of secretion of estradiol by ovarian follicles. The pulsatile release of oxytocin from CL and posterior pituitary induces release of luteolytic pulses of PGF from the endometrium to destroy the CL.

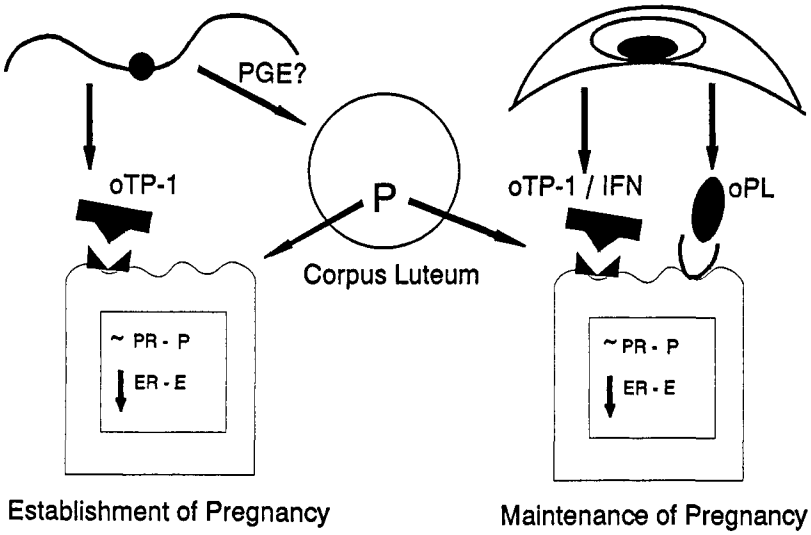

Figure 2. The conceptus of ruminants may secrete a luteal protective hormone such as PGE to increase the resistance of the CL of pregnancy to potential luteolytic effects of PGF. However, the model currently being tested assumes that Type I trophoblast interferons, e.g., oTP-1, are primary antiluteolytic paracrine hormones that act on endometrial epithelium to stabilize PR and possibly PR mRNA and inhibit up-regulation of ER and ER mRNA. Consequently, synthesis of endometrial oxytocin receptors is inhibited. For maintenance of pregnancy beyond Days 25–30, there is a secondary period of secretion of immunoreactive and bioactive oTP-1 that may be reinforced by effects of placental lactogen to stabilize endometrial PR and PR mRNA, as well as inhibit up-regulation of ER and ER mRNA. The paracrine effects of oTP-1 and placental lactogen prevent uterine secretion of luteolytic PGF that might otherwise cause luteolysis and termination of pregnancy. Endometrial sensitivity to oxytocin-induced and estrogen-induced secretion of PGF does occur between Days 25 and 30 of pregnancy in ewes.

taglandins between Days 12 and 20 after mating results in pregnancy failure in pigs (125). Available results indicate that estrogens of blastocyst origin are essential for maternal recognition of pregnancy in pigs and that pCSP, including interferons, play other roles during early pregnancy in pigs.

Endometrial concentrations of progesterone decrease from about 20 pmol/mg DNA on Day 14 to less than 2 pmol/mg DNA on Day 20, while endometrial ER decrease from about 1.5 pmol/mg DNA on Day 12 to less than 0.5 pmol/mg DNA on Day 20 of the estrous cycle (126). However, endometrial concentrations of progesterone remain stable (10–15 pmol/mg DNA) between Days 14 and 20 of pregnancy and ER decrease to less than 0.25 pmol/mg DNA during that period. Data are not available for changes in PR, PR mRNA, or ER mRNA in cyclic and pregnant pigs during early pregnancy, and one can only speculate that the changes in endometrial concentrations of progesterone are related to endometrial PR. Estrogen and prolactin can interact to increase PR (127) and endometrial secretion of progesterone-induced proteins (116, 127). It is possible that combined effects of prolactin and estrogen which stabilize endometrial PR and initiate down-regulation of the ER may be influenced by γ- and α-interferons secreted by trophoblast (122).

Pregnancy Recognition in Mares. The uterine luteolytic substance in mares is PGF and the conceptus appears to inhibit production of PGF by the uterine endometrium (108). In cycling mares, PGF concentrations in uterine venous plasma and uterine flushings increase on Days 14–16, when luteolysis occurs and plasma progesterone levels decline. The amount of PGF bound by luteal receptors is maximal on Day 14 of the estrous cycle and Day 18 of pregnancy. Since the CL of mares can respond to circulating PGF during pregnancy, the conceptus must evoke an antiluteolytic mechanism. Pregnant mares have little PGF in uterine fluids; PGF in uterine venous plasma is reduced, and PGFM in peripheral plasma shows no episodic pattern of release (108). In the presence of the conceptus, endometrial production of PGF is markedly reduced in response to cervical stimulation (108) and exogenous oxytocin (109), which indicates the absence of endometrial receptors for oxytocin in mares during early pregnancy.

The equine conceptus migrates from one uterine horn to the other 12–14 times per day on Days 12–18 of pregnancy (128) to inhibit endometrial PGF production and protect the CL. Thus, the equine conceptus does suppress PGF production by the endometrium, but the agent has not been identified (108). The equine conceptus also produces increasing amounts of estradiol between Days 8 and 20 of gestation. A similar trend, but of greater magnitude, was found for estrone. Attempts to prolong CL lifespan in mares by injection of estrogens have provided conflicting results (see 108).

Horse conceptuses secrete three major proteins between Days 12 and 14 of pregnancy with mol wt of greater than 400,000, 50,000 and 65,000. However, the role(s) of these proteins is not known (108). Estrogens or conceptus secretory proteins may provide the mater-

nal recognition of pregnancy signal in the mare by directly or indirectly inhibiting endometrial PGF production of luteolytic pulses of PGF.

Future Research and Working Hypotheses

Primates. The direct luteotrophic effect of CG on CL function appears central to establishment of pregnancy in primates. Current research is addressing the question of the intraovarian luteolytic mechanisms and whether intraovarian luteal protective factors, e.g., growth factors, may be important to the establishment and maintenance of pregnancy.

Ruminants. The working hypothesis being tested in our laboratory is summarized in Figures 1 and 2. The principal feature of this working hypothesis is that, during the estrous cycle, progesterone down-regulates its own receptor. Subsequently, endometrial ER increase and allow estrogens to enhance up-regulation of oxytocin receptors and allow oxytocin from the CL or posterior pituitary to stimulate uterine secretion of luteolytic pulses of PGF (Fig. 1). During pregnancy, we propose that oTP-1 (Type I trophoblast interferons) stabilizes the PR and, directly or indirectly, prevents up-regulation of ER and oxytocin receptors. Consequently, the uterine endometrium does not become competent to secrete luteolytic pulses of PGF (Fig. 2). Secondary antiluteolytic signals from the conceptus may include lactogenic hormones, e.g., placental lactogen, that further stabilizes or up-regulates endometrial PR to ensure maintenance of pregnancy.

Pigs. The theory of endocrine versus exocrine secretion of PGF by uterine endometrium has been supported by results of numerous studies; however, the mechanism by which this partitioning of PGF is accomplished is not known. Endocrine regulation of this process and the mechanisms responsible must be established. As proposed for ruminants, stabilization of endometrial PR and failure of up-regulation of ER appear to be associated with exocrine secretion of PGF. In pigs, this may result from effects of estrogens secreted by the conceptus to up-regulate endometrial receptors for prolactin. Consequently, endometrial epithelium is affected, perhaps through mechanisms induced by calcium, to secrete PGF in an exocrine direction to prevent luteolysis. Our understanding of endocrine regulation of luteolysis in pigs remains limited. In order to understand antiluteolytic mechanisms in this species, we must first understand the luteolytic mechanism, and this is the subject of considerable research at present.

Mare. Our understanding of endocrine regulation of luteolysis in mares is deficient and much work is required in this area. During early pregnancy, it seems clear that endometrial production of PGF is reduced in response to the presence of the conceptus; however, the conceptus secretory product responsible and its mechanism of action are not known.

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