Embryonic steroids and the establishment of pregnancy in pigs

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Summary. In the pig, establishment of pregnancy begins about 11–12 days after the start of oestrus. The ability of pig conceptuses to synthesize and release oestrogens during this period, as well as the ability of exogenous oestrogens to induce pseudo-pregnancy when administered from Day 11–15 of the oestrous cycle, provide evidence for an involvement of oestrogen in the maternal recognition of pregnancy in the sow. Oestrogen derived from the conceptus or from administration to cyclic gilts stimulates uterine secretion of calcium and specific polypeptides on Day 11–12. The specific roles for the uterine secretory response to oestrogen in the maintenance of pregnancy are unknown. However, it has been proposed that oestrogen prevents luteolysis in the sow through reorientation of endometrial prostaglandin release, i.e. into the uterine lumen rather than into the uterine vasculature. Oestrogen may interact with prolactin and/or conceptus secretory proteins to shift the direction of prostaglandin movement.

Although conceptus oestrogen synthesis triggers a number of uterine secretory events on Day 11, a second sustained phase of oestrogen stimulation from Day 14 to 18 appears to be necessary for luteal maintenance beyond Day 25. Pig conceptuses synthe-size and release large amounts of oestrogens between Days 14 and 18 of pregnancy. Conceptus oestrogens are clearly involved with the establishment of pregnancy. How-ever, the conceptus also secretes a number of biologically active substances such as catechol oestrogens, prostaglandins and polypeptides which could interact with oestro-gen to prevent luteolysis. The roles of these factors in control of vascular permeability, blood flow, placental attachment and immunological protection certainly indicate that, in addition to oestrogens, other factors are involved in the establishment of pregnancy in pigs.

Keywords: oestrogen; embryo; pregnancy; prostaglandin; uterus; pig

Introduction

The mechanisms by which pregnancy is established and maintained in mammals have been the emphasis of research in a number of laboratories throughout the world. During the two decades since Short (1969) first described the mechanism by which pregnancy is established as the "maternal recognition of pregnancy", research and technological advances have provided insights into the biological factors which control the oestrous cycle and maintenance of pregnancy in a number of species (see Bazer *et al.*, 1986).

In the simplest interpretation, "maternal recognition of pregnancy" can be defined as the method by which the conceptus prolongs the functional lifespan of the corpora lutea (CL) established after ovulation. Strong evidence has indicated the involvement of conceptus oestrogen synthesis and release for maintenance of CL function in the pig (Bazer & Thatcher, 1977; Heap

et al., 1979). Although oestrogen is involved in preventing luteolysis in the gilt, there is considerable debate over the mechanism by which oestrogen affects luteal maintenance (Bazer *et al.*, 1982; Ford & Stice 1985; Akinlosotu *et al.*, 1986; Guthrie & Lewis, 1986; Krzymowski *et al.*, 1987). While there is some indication that oestrogen affects luteal maintenance directly at the ovary, evidence has also been presented which demonstrates that inhibition of endometrial prostaglandin (PG) F-2α synthesis and/or reorientation of endometrial PG release regulates CL maintenance. The available data suggest that other factors such as catechol oestrogens, prostaglandins and polypeptides interact with oestrogen to prevent luteolysis. The establishment and maintenance of pregnancy are not controlled by a single event, but by a series of complex biochemical and cellular interactions which are not mutually exclusive of one another. This review will attempt to integrate current information available on the establishment and maintenance of pregnancy in pigs.

Control of luteolysis

The role of endometrial PGF-2α synthesis and release in luteolysis and control of the oestrous cycle in the pig has been reviewed extensively (Bazer *et al.*, 1982, 1984). The following is a brief summary of the major contributions which demonstrate endometrial PGF-2α involvement in luteolysis. Uterine endometrium is the source of the luteolysin as destruction of the uterine endometrial epithelium or hysterectomy before Day 14 of the oestrous cycle extends CL function beyond 30 days (see Melampy & Anderson, 1968). Administration of PGF-2α 12 days after the start of oestrus in cyclic (Hallford *et al.*, 1974), hysterectomized (Moeljono *et al.*, 1976) or pregnant (Diehl & Day, 1974) gilts initiates CL regression. The corpora lutea of the pig appear to be refractory to PGF-2α before Day 11 of the cycle (see Bazer *et al.*, 1982). Increased CL sensitivity to PGF-2α after Day 11 is associated with an increase in numbers of PGF-2α receptors in the luteal cells (Gadsby *et al.*, 1988). This alteration in PGF-2α receptor number corresponds to the time when LH dissociates from its membrane receptor (Henderson & McNatty, 1975) and when maintenance of CL function requires continued pituitary LH support (du Mesnil du Buisson & Leglise, 1963; Anderson *et al.*, 1965). Endometrial synthesis and release of PGF-2α in-vitro (Watson & Patek, 1979; Guthrie & Lewis, 1986) and plasma concentration of PGF-2α in the utero-ovarian vein (Moeljono *et al.*, 1977) increase concomitantly with the decline in plasma progesterone values from Day 14 to 17 of the oestrous cycle. Collectively, these data strongly support the contention that endometrial PGF-2α is the endometrial luteolysin involved with demise of CL function in pigs. Release of PGF-2α from the non-gravid uterine horn not only affects CL function of the ipsilateral ovary (local effect), but can also invoke luteolysis in the contralateral ovary (du Mesnil du Buisson, 1961a; Anderson *et al.*, 1966). If a substantial portion of one uterine horn is unoccupied by embryos after Day 12, pregnancy is not maintained in a significant percentage of gilts (du Mesnil du Buisson, 1961b; Dhindsa & Dziuk, 1968). The systemic action of PGF-2α that occurs in the gilt could result from the lower rate of metabolism of PGF-2α by the lung compared to ruminants in which pregnancy is not affected by a non-gravid contralateral horn (Davis *et al.*, 1979). These results suggest that pig conceptuses are capable of preventing CL regression directly at the ovary when a small portion of the uterus is non-gravid. However, although the luteolytic effect may be delayed, the majority of animals cannot sustain luteal function when a large portion of the uterus is unoccupied.

Conceptus steroid production

The period at which pig conceptuses first exert an affect on extending CL function during pregnancy has been evaluated by flushing conceptuses from the uterine horns on various days after oestrus. Flushing embryos from uteri before Day 11 of pregnancy results in a 21-day interoestrous interval similar to that in cyclic gilts. However, flushing embryos on or after Day 12 will extend the

interoestrous interval by 4–10 days (Dhindsa & Dziuk, 1968; Ford *et al.*, 1982a; van der Meulen *et al.*, 1988). Pig conceptuses therefore initiate signals necessary for the maintenance of pregnancy on about Day 11–12.

Kidder *et al.* (1955) first indicated that oestrogen administration after Day 9 of the oestrous cycle would prolong the interoestrous interval of the gilt. Several studies have since shown that systemic or intrauterine administration of oestrogen prolongs CL function (Frank *et al.*, 1977; Ford *et al.*, 1982b; Saunders *et al.*, 1983; Geisert *et al.*, 1987; King & Rajamahendran, 1988). A physiological role for oestrogens in the maintenance of pregnancy was originally shown by Perry *et al.* (1973) in studies demonstrating oestrogen synthesis and release by conceptuses on Days 11–18 of pregnancy. These findings were subsequently confirmed and expanded by others (Gadsby *et al.*, 1980; Fischer *et al.*, 1985). Conceptuses are able to convert progesterone to oestrone and oestradiol-17β when they have developed to the 7-mm spherical stage (Fischer *et al.*, 1985). In addition to production of oestrone and oestradiol-17β, the pig conceptus appears to have a high capacity to convert oestradiol to an isomer of 16α,17β-oestriol (Fischer *et al.*, 1985; Chakraborty *et al.*, 1988). Oestrogen production increases as conceptuses develop from the spherical to tubular and filamentous forms on Day 11–12 of pregnancy. Oestrogens have been localized in the trophectoderm and endoderm of the conceptus on Day 12, with greatest intensity appearing in the yolk sac endoderm on Days 14–16 (King & Ackerley, 1985). Initiation of conceptus oestrogen synthesis may be controlled by mesoderm outgrowth from the embryonic disc, as oestrogens were present in the endoderm and trophectoderm separated by mesoderm on Day 12. The timing of trophectodermal oestrogen production with the rapid elongation of the trophoblastic membrane allows the conceptus to stimulate locally a large surface area of the endometrium. A requirement for the presence of at least 2 embryos in each uterine horn (Polge *et al.*, 1966), exemplifies the importance of a localized effect exerted on the endometrium by the expanding conceptus. Stimulation of only the endometrial tissue in contact with the blastocyst permits individual embryos to develop and expand in relation to their own developmental programme as long as adequate uterine space is available (Dziuk, 1985).

Since the capacity of the endometrium to synthesize oestrogens during early pregnancy is low (Fischer *et al.*, 1985), the content of oestrogens in the uterine lumen reflects conceptus synthesis and release. Conceptus oestrogen production, as measured in uterine flushings, is biphasic (Fig. 1). Uterine content of oestrogen increases during rapid conceptus elongation on Day 11–12 of pregnancy, declines on Day 13 and 14 followed by a second sustained increase after Day 14 (Zavy *et al.*, 1980; Geisert *et al.*, 1982a; Stone & Seamark, 1985). An increase in the uterine content of oestrogens does not occur until after Day 15 in cyclic gilts. Measurement of plasma oestrone sulphate concentration (Fig. 1), which reflects endometrial sulphation of conceptus oestrogens released into the maternal circulation (Pack & Brooks, 1974), reveals a similar biphasic pattern of oestrogen production in pregnant gilts (Robertson & King, 1974; Stoner *et al.*, 1981). Increases of oestradiol-17β have also been detected in the uterine vein (Ford *et al.*, 1982a) and lymphatic vessels of the uterus (Magness & Ford, 1982). The presence of oestrogens in the peripheral circulation suggests that conceptus oestrogen synthesis could have systemic, as well as local, endometrial effects throughout gestation. Sulphatase activity in the hypothalamus, pituitary and CL (Heap *et al.*, 1977) suggests that the sulphated oestrogens coming from the uterus during early pregnancy may play a regulatory role in control of luteal function.

Conceptus oestrogens and endometrial function

Biphasic synthesis and release of conceptus oestrogens have a biological role in the timing of endometrial secretion and control of PGF-2α movement. Figure 1 depicts the relationship(s) of steroid, protein, calcium and prostaglandin changes in the uterus and peripheral plasma during the oestrous cycle and pregnancy. The initial increase in conceptus oestrogens at the time of trophoblast
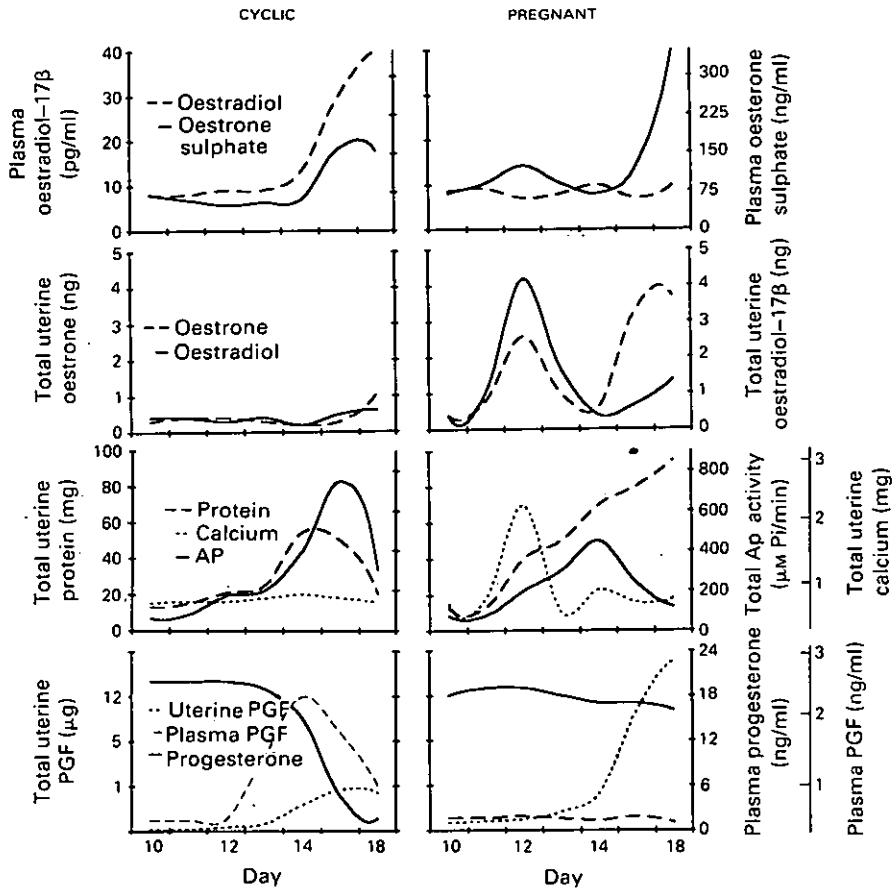
Fig. 1. Profiles of plasma and uterine luminal changes in steroids, prostaglandin F, calcium and protein from Day 10 to 18 of the oestrous cycle and pregnancy. (From Moeljono *et al.*, 1977; Zavy *et al.*, 1980; Stoner *et al.*, 1981; Geisert *et al.*, 1982a).

elongation stimulates a rapid release of calcium into the uterine lumen. The increase of uterine luminal calcium is associated with an earlier synchronized release of glandular secretory vesicles coincident with the increase in total uterine protein and the specific release of uteroferrin and plasmin inhibitors compared to cyclic animals (Geisert *et al.*, 1982a; Fazleabas *et al.*, 1983). Although increased, the synchronous surge release of uterine luminal calcium is absent in cyclic gilts on Day 12 and is not associated with protein and uteroferrin release. The total content of PGF-2α in uterine flushings of pregnant gilts increases on Day 12 in association with blastocyst elongation. The early increase in luminal prostaglandins on Day 12 appears to be of conceptus origin (Geisert *et al.*, 1982a, b). However, the endometrium does make a major contribution to the luminal content of PGF-2α after Day 14 as demonstrated in gilts treated with oestrogen to induce pseudopregnancy (Frank *et al.*, 1978). Administration of exogenous oestrogen on Day 11 of the oestrous cycle stimulates similar increases of calcium, protein and uteroferrin as measured in uterine flushings of pregnant gilts (Geisert *et al.*, 1982b).

It appears that uterine endometrial secretion of calcium and protein is not responsive to oestrogen until after the 10th day of the oestrous cycle, followed by a loss of sensitivity after Day 14 (Geisert *et al.*, 1987). Results also indicate that endometrial calcium release becomes refractory to continued oestrogen administration following the initial calcium surge. Failure of oestrogen to

stimulate endometrial secretion before Day 10 is consistent with the small amount of glandular secretory development at this time (Renegar, 1982).

Since exogenous administration of oestrogen stimulates uterine secretory events similar to those measured during pregnancy, as well as inducing CL maintenance, Geisert *et al.* (1987) conducted a study to establish the time and duration exogenous oestrogen administration needed to induce prolonged luteal function in the gilt (Table 1). The results indicated that a single injection of 5 mg oestradiol benzoate between Days 9·5 and 15·5 or on Days 11 and 14 of the oestrous cycle lengthened the interoestrous interval to about 28 days. Gilts treated with oestrogen on Day 15·5 exhibited either normal cycle lengths or interoestrous intervals of 28 days. These results suggest that oestrogen is not capable of rescuing CL function once luteolysis has been initiated. Plasma progesterone concentrations in gilts with interoestrous intervals of less than 31 days demonstrate that CL regression was initiated between Days 22 and 25 after oestrus compared with the sustained plasma progesterone concentrations of gilts treated with oestrogen on Days 11 through 15. Oestrogen administration on Day 9·5 of the oestrous cycle does not stimulate an increase in uterine content of calcium and uteroferrin 12 h after treatment as observed after treatment on Day 11; the ability of oestrogen treatment on Day 9·5 to extend the interoestrous interval is most probably due to the residual effects of the pharmacological levels of oestrogen still present on Day 11 (Geisert *et al.*, 1987). The ability of oestrogen treatment on Days 11 and 14–16 to produce a similar prolonged (> 60 days) extension of CL function, as reported by Frank *et al.* (1977), indicates that complete establishment of pregnancy in the gilt requires two phases of oestrogen stimulation.

Table 1. Interoestrous intervals in vehicle- and oestradiol benzoate-treated gilts

Treatment	Day of treatment	Days (mean ± s.e.m.)	Gilts with interval of > 35 days
Vehicle	11–15	19·3 ± 0·2	0/8
Oestradiol	9·5	28·0 ± 0·6	0/4
benzoate*	11·0	28·4 ± 1·3	0/8
	12·5	28·8 ± 1·1	0/4
	14·0	28·1 ± 2·9	0/8
	15·5	23·5 ± 3·2	0/4
	11 and 14	32·2 ± 1·0	0/4
	14–16	31·0 ± 10·2	1/4
	11 and 14–17	> 60	4/4
	11, 20–22	40·5 ± 11·3	2/4
	11–15	> 60	4/4

*5 mg oestradiol benzoate given i.m. in 250 μl vehicle.

Several lines of evidence support the two-phase theory for induction of extended luteal maintenance. First, the results in Table 1 indicate that oestradiol treatment on Day 11 or Days 14–16 alone failed to extend the interoestrous interval beyond 30 days, indicating that treatment on Days 11 and 14–16 is necessary for consistent luteal maintenance. Furthermore, failure of oestradiol-treatment on Days 11 and 14 to extend CL lifespan beyond 30 days suggests that a sustained, intensified oestrogen stimulation is required during the second phase of administration. Secondly interoestrous intervals of only 24–28 days have been achieved with intrauterine infusion of 100 μg oestradiol benzoate from Day 10 to 14 of the oestrous cycle (Saunders *et al.*, 1983) or placement of oestradiol-17β-impregnated Silastic beads into the uterine lumen on Day 10 (King & Rajamahendran, 1988). These findings suggest that the second increase in oestrogen after Day 14 is necessary for prolonging CL function beyond 30 days. Although the injection or infusion of pharmacological amounts of oestrogen may be questioned, a similar temporal change in blastocyst

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oestrogen secretion occurs during pregnancy (Zavy *et al.*, ·1980; Stone & Seamark, 1985). The secondary sustained increase in blastocyst oestrogen secretion appears to be involved with continued CL maintenance, since flushing embryos from the uterine horns before Day 18 is not as effective in extending CL maintenance beyond 30 days as flushing embryos on or after Day 18 of pregnancy (Dhindsa & Dziuk, 1968; Ford *et al.*, 1982a; van der Meulen *et al.*, 1988).

Since endometrial responsiveness to oestrogen could be associated with the concentration of oestrogen receptors, we measured endometrial receptor content during the oestrous cycle and pregnancy. Numbers of endometrial oestrogen receptor sites/cell from cyclic and pregnant gilts did not differ significantly from Days 0 to 18 (Fig. 2a), whereas total amount of oestrogen receptor/100 mg wet tissue (Fig. 2b) increased from Day 0 to 5. Although total receptor numbers remain elevated on Day 12 followed by a decline on Day 15 in cyclic and pregnant gilts, a second increase in oestrogen receptor occurs on Day 18 in pregnant but not cyclic gilts. Differences in receptor number on Day 18 are not due to an increase in receptor number per cell, but to an increase in the number of cells expressing the oestrogen receptor. On Day 18, the endometrium from cyclic gilts undergoes marked hydration with an increase in protein/cell, whereas in pregnant gilts the endometrium exhibits a proliferative response on Day 18 (data not shown). The increase in the number of endometrial cells responsive to oestrogen may allow sustained CL maintenance during the second phase of conceptus oestrogen stimulation.
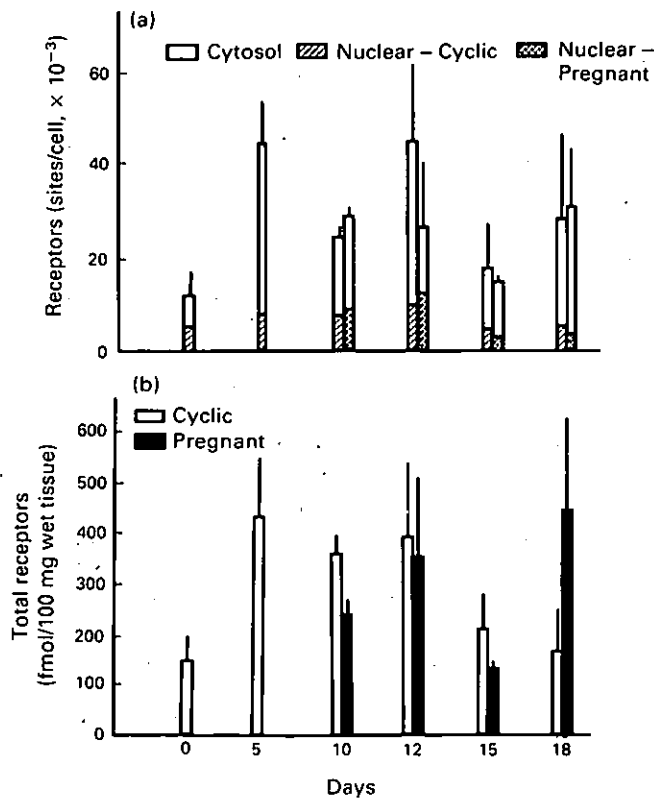

Fig. 2. Endometrial content (mean $\pm$ s.e.m.) of type I (a) oestrogen receptor sites/cell and (b) total oestrogen receptors/100 mg wet tissue weight during the oestrous cycle and pregnancy. Endometrium was not collected from pregnant gilts on Days 0 and 5.

Oestrogen control of endometrial prostaglandins

Several mechanisms by which conceptus oestrogens prevent luteal regression have been proposed. Oestrogen may produce a direct luteotrophic effect at the CL (Ball & Day, 1982; Conley *et al.*, 1989). It appears that oestrogen is capable of stimulating progesterone secretion by the CL possibly through an increase in luteal LH (Garverick *et al.*, 1982), prolactin receptors (Rolland *et al.*, 1976; Bramley & Menzies, 1987) and stimulation by catecholamines (Veldhuis & Klase, 1982). However, other data indicate that oestrogen assumes more of a supportive role in controlling luteal progesterone secretion rather than a protective function from PGF-2α-induced luteolysis. The inability of embryos to maintain pregnancy in the presence of a non-gravid uterine horn or when a large portion of the uterus is unoccupied (see Dziuk, 1985) does not support a systemic effect for conceptus oestrogens.

Secondly, it has been suggested that oestrogen prevents luteal regression by reducing the endometrial synthesis and release of PGF-2α (Guthrie & Rexroad, 1981). Although several studies have indicated that in-vitro endometrial PGF-2α production is reduced in pregnant or oestrogen-treated compared to cyclic gilts, the luminal content of PGF-2α is greater on Days 14–20 in oestrogen-treated (Frank *et al.*, 1977) than in cyclic gilts. Uterine content of PGF-2α is even greater in pregnant gilts (Zavy *et al.*, 1980), possibly reflecting contributions of prostaglandin synthesis by pig conceptuses (Guthrie & Lewis, 1986). The inverse relationship between plasma and uterine luminal content of PGF-2α (Fig. 1) provided support for the proposed theory that conceptus oestrogens alter transport of endometrial PGF-2α from an endocrine (towards uterine vasculature) to an exocrine (into uterine lumen) direction, preventing PGF-2α from reaching the CL (see Bazer *et al.*, 1984). Prostaglandins do not freely move across cell membranes and evidence from studies utilizing vaginal epithelium suggests that movement of prostaglandins is mediated through an active transport process (Bito *et al.*, 1976). Gross *et al.* (1988) utilized a bilateral perifusion device to measure in-vitro prostaglandin secretion from the luminal and myometrial surfaces of pig endometrium from cyclic, pregnant and oestrogen-treated gilts. Secretion of PGF-2α and PGE-2 were greater from the myometrial side (endocrine) for Day-10 pregnant and Day-14 cyclic gilts, whereas secretion was orientated to the luminal side (exocrine) for Day-12 and Day-14 pregnant gilts and for Day-14 oestrogen-treated gilts.

The complete mechanism by which oestrogens direct the secretion of prostaglandins into the uterine lumen during pregnancy is unknown. Oestrogen stimulation of endometrial calcium release would appear to be involved since treatment with calcium ionophore shifts orientation of PGF-2α secretion to the luminal side during in-vitro perifusion (Mirando *et al.*, 1988). Young *et al.* (1987) indicated that oestrogen administration did not affect endometrial cAMP content, but concentrations of cGMP increased rapidly. The role of calcium and cGMP as mediators in the shift in prostaglandin secretion and protein release requires further study.

Orientation of prostaglandin secretion into the uterine lumen involves an interaction between oestrogen and prolactin. Mirando *et al.* (1988) demonstrated that neither oestrogen nor prolactin alone alters PGF-2α secretion. However, perifusion of endometrium from an oestrogen-treated gilt with prolactin shifted PGF-2α secretion to an exocrine direction. Although plasma prolactin concentrations do not change during early pregnancy (Dusza & Krzymowska, 1981), the number of endometrial prolactin receptors increases between Days 14 and 30 (Dehoff *et al.*, 1984). Additionally, Young & Bazer (1988) have demonstrated a synergism between oestradiol and prolactin in enhancing uterine secretory activity. These data suggest that oestrogen-induced orientation of PGF-2α secretion towards the uterine lumen involves an interaction with prolactin or possibly conceptus secretory proteins. Although infusion of pig conceptus secretory proteins into cyclic gilts has no effect on the interoestrous interval (Harney, 1988), Dubois & Bazer (1988) indicate that conceptus secretory proteins enhance the effectiveness of oestrogen in redirecting PGF secretion towards the uterine lumen during in-vitro perifusion. Results strongly indicate an essential role for a local endometrial effect of conceptus oestrogens which may interact with prolactin or pig

conceptus secretory proteins through the induction of specific endometrial receptors. Although the first phase of conceptus oestrogen production is involved with switching prostaglandin movement, the role of the sustained secondary increase for long-term maintenance of CL function has not been established.

Other factors involved in maintenance of pregnancy

Establishment and maintenance of pregnancy not only entail the conceptus block to luteolysis, but also involve conceptus–endometrial interactions to control vascular permeability, blood flow, placental attachment and immunological protection. Uterine arterial blood flow increases coincident with increased synthesis of oestrogen by the pig conceptus (see Ford, 1989). Ford (1989) suggests that changes in uterine arterial vasodilatation during early pregnancy are mediated through conversion of conceptus oestrogens to catechol oestrogens. Pig conceptuses have the ability to metabolize oestradiol to 2- and 4-hydroxyoestradiol (Mondschein *et al.*, 1985). In fact, total catechol oestrogen synthesis by the conceptus tissue is highly correlated with increase in uterine blood flow and conceptus aromatase activity (Chakraborty *et al.*, 1989). Endometrial tissue can also convert oestrogens to 4-hydroxylated forms (Ford, 1989), possibly through peroxidase production of migrating eosinophils within the endometrium (Van Orden *et al.*, 1988). Since red blood cells contain catechol-*o*-methyl transferase, catechol oestrogens are quickly metabolized to inactive metabolites in the maternal circulation. Therefore, conceptus and/or endometrial catechol oestrogen synthesis may produce local effects on uterine blood flow, avoiding alterations in the systemic circulation. An increase in uterine vascular permeability, through increased fenestration of the subepithelial capillaries of the endometrium in close association with the attaching conceptus, has also been demonstrated (Keys *et al.*, 1986; Keys & King, 1988a). An involvement of oestradiol in altering vascular permeability, either directly or indirectly through conversion to catechol oestrogens and possibly by local stimulation of endometrial PGE production, would facilitate transcapillary transport of nutrients needed for conceptus development.

Conceptus oestrogen production appears to modulate the epitheliochorial placentation of the pig which occurs between Days 13 to 26 of gestation. An increase in endometrial folding and formation of a thick glycocalyx coating on the microvilli of the uterine surface epithelium is associated with oestrogen stimulation and conceptus attachment (Keys & King, 1988b). Treatment of pregnant gilts with oestrogen 2 days before conceptus oestrogen synthesis on Day 12 results in conceptus degeneration (Gries *et al.*, 1989). Although conceptus development is normal to Day 14 of pregnancy, considerable conceptus degeneration occurs between Days 16 and 18. Alteration in the secretion of specific uterine glycoproteins is associated with embryonic mortality. Our recent results indicated that administration of 5 mg oestradiol benzoate on Days 9 and 10 alters the glycocalyx covering the microvilli of the uterine epithelium (Fig. 3), a direct involvement of oestrogen in programming secretion and morphological changes in the uterine epithelium necessary for conceptus attachment and survival.

Pig conceptuses synthesize substantial amounts of PGE-2 and PGF-2α (Lewis & Waterman, 1983). Although there is little evidence to support the involvement of PGE-2 in luteal maintenance (Bazer *et al.*, 1984), PGE receptors are present in the uterine endometrium (Kennedy *et al.*, 1986). Inhibition of prostaglandin synthesis during conceptus attachment results in pregnancy failure (Kraeling *et al.*, 1985), indicating a role of prostaglandin synthesis in the establishment of pregnancy as previously reviewed by Bazer *et al.* (1984).

The developing pig conceptus secretes a number of unique proteins from Day 10 to 18 of pregnancy (Godkin *et al.*, 1982; Gries *et al.*, 1989). Although infusion of the conceptus proteins has no effect on the interoestrous interval in cyclic gilts (Harney, 1988), conceptus secretory proteins (CSP) increase endometrial PGE synthesis and interact with oestrogen to alter prostaglandin movement into the uterine lumen (Dubois & Bazer, 1988). Conceptus secretory proteins may serve a role similar to prolactin in establishing exocrine transport of endometrial prostaglandin
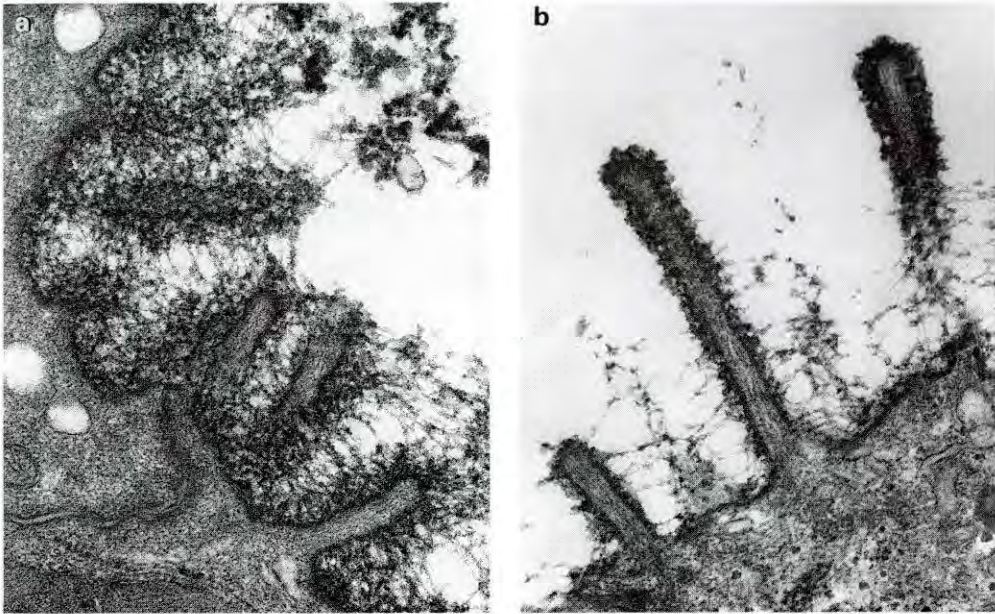
Fig. 3. TEM micrographs of the microvilli present on the uterine surface epithelium from (a) control, Day-16 pregnant gilt and (b) Day-16 pregnant gilt treated with oestrogen on Day 9 and 10 of pregnancy. Note the diminished appearance of the glycocalyx coating on the microvilli from the gilt treated with oestrogen. Tissue fixed in Karnovsky's containing ruthenium red, × 52 000.

production. Several conceptus proteins are synthesized at the time of the shift in prostaglandin movement and placental attachment. Baumbach *et al.* (1988) purified a major basic glycoprotein of M_r 43 000 from pig conceptuses at Days 14–17 of gestation. Immunocytochemical localization of the basic protein on the uterine surface epithelium opposite the trophectoderm suggests involvement with placental attachment. The pig conceptus also secretes a group of low molecular weight acidic proteins similar to conceptus secretion of the α interferon-like protein, oTP-1, present in sheep and other ruminant species (Imakawa *et al.*, 1987). Cross & Roberts (1988) demonstrated that the pig acidic proteins of M_r 22 000 cross-reacted with antiserum to human α interferon and possessed antiviral activity which is characteristic of interferons (Pestka & Langer, 1987). Measurement of antiviral activity in uterine flushings of pregnant gilts (R. E. Short, R. D. Geisert & M. T. Zavy, unpublished observations; M. A. Mirando, S. Beers & F. W. Bazer, personal communication) indicated that activity was low or undetectable on Day 12, but increased on Days 14–18 of pregnancy. The role of the low M_r acidic proteins in the gilt are unknown. Their synthesis from Day 12 through 18 suggests a role in the second phase of oestrogen stimulation of prolonged CL maintenance. The conceptus proteins may alter specific endometrial protein synthesis needed for conceptus development and control of prostaglandin synthesis as demonstrated in the ewe (Vallet *et al.*, 1988). Trophoblastic secretion of proteins, oestrogens and PGE at the site of placental attachment could provide a local mechanism for immune suppression of T cell activity (Croy *et al.*, 1987; King, 1988). Conceptus secretory proteins may be directly immunosuppressive or alternatively conceptus-derived oestrogen and/or secretory proteins may regulate the immune system through endometrial prostaglandin synthesis. Prostaglandins, especially PGE, can inhibit activation and function of immunocompetent cells (Webb *et al.*, 1985).

The present review was developed to integrate the mechanisms and factors which have been proposed to play a major role in the establishment and maintenance of pregnancy in the pig.
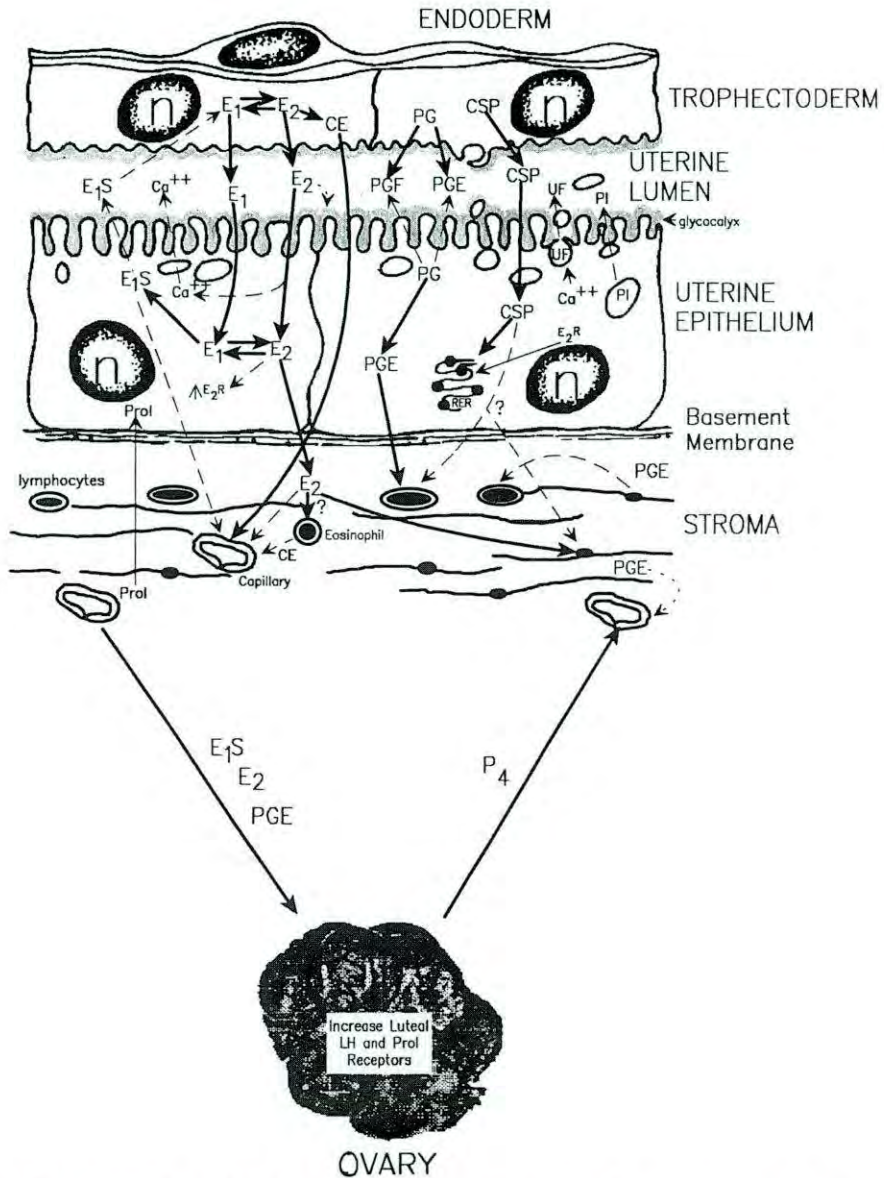
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Fig. 4. Diagrammatic model illustrating the interrelationships between conceptus, uterus and ovary in the establishment of pregnancy in pigs. E_1, Oestrone; E_2, oestradiol-17β; E_1S, oestrone sulphate; CE, catechol oestrogens; PG, prostaglandins; CSP, conceptus secretory proteins; UF, uteroferrin; PI, plasmin inhibitors; E_2R, oestrogen receptor; P_4, progesterone; Prol, prolactin.

Clearly this process involves a number of complex biochemical and cellular interactions which are not necessarily mutually exclusive. Conceptus oestrogen synthesis plays a critical role in the establishment of pregnancy, as was first illustrated in the summary figure of an earlier review by Perry *et al.* (1976). Based on the data available today, an expanded model illustrating the interrelationships between conceptus, uterus and ovary is provided in Fig. 4 and summarizes current thoughts on the "maternal recognition of pregnancy" in the pig.

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