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Role of conceptus-induced vasodilation in controlling utero-ovarian function of ewes, cows and sows

by

Lawrence Paul Reynolds

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Steroids

The length of the estrous cycle is about 16 days for the ewe and 21 days for the cow and sow (Cole and Cupps, 1977). Uterine blood flow (UBF) varies regularly throughout the estrous cycle in the ewe (Greiss and Anderson, 1969), cow (Ford et al., 1979) and sow (Ford and Christenson, 1979), and also in the guinea pig (Markee, 1932) and rat (Harvey and Owens, 1976). Uterine blood flow is highest at or just before estrus and lowest during the luteal phase of the cycle. For ewes, cows and sows, these regular patterns of UBF are temporally associated with the ratio of estrogen to progesterone ($E:P_4$) in systemic blood (Yuthasastrakosol et al., 1975; Ford et al., 1979; Henricks et al., 1972). The greater the $E:P_4$ ratio, the greater the quantity of blood flowing through the uterine vascular bed.

Administration of estrogen stimulates increased UBF in the ovariectomized ewe (Killam et al., 1973), cow (Ford and Reynolds, 1983a) and sow (Van Orden et al., 1983b), as well as in the ovariectomized guinea pig (Bacsich and Wyburn, 1940) and rat (Kalman, 1958). This increase in UBF in response to estrogen is equally distributed between the myometrial, endometrial and caruncular vascular beds of the nonpregnant ovine uterus (Rosenfeld et al., 1973; Anderson and Hackshaw, 1974). In those species studied to-date, UBF does not begin to increase until

approximately 30 min after estrogen administration and reaches maximal levels by 1-4 hr after estrogen (Spaziani and Suddick, 1967; Killam et al., 1973; Ford and Reynolds, 1983a; Van Orden et al., 1983b). Although all estrogens studied thus far cause a similar pattern of increase in UBF, their potencies differ. In the ovariectomized ewe, the order of potency for increasing UBF is: estradiol-17β ($E_2\beta$) $\geq$ estriol (E_3) > diethylstilbestrol > estrone (E_1); which is the same as the order of potency for causing uterine fluid imbibition (Resnik et al., 1974). Although the ability of $E_2\beta$ and E_3 to induce an increase in UBF and uterine water imbibition is similar, $E_2\beta$ is much more potent than E_3 in stimulating uterine growth (Anderson et al., 1975). This difference may be related to the observation of Anderson et al. (1975) that $E_2\beta$ is retained in the nucleus of uterine cells for greater than 24 hr, whereas E_3 is retained for only 3 hr. It has, therefore, been suggested that the increase in UBF elicited by the various estrogens does not require nuclear retention of estrogen, but instead may be mediated by events occurring in the cytosol of the uterine cells (Resnik et al., 1974). In support of this proposal, the vasodilatory response of the uterus to estrogen can be blocked by inhibitors of protein synthesis in ovariectomized rats (Spaziani and Suddick, 1967) and ewes (Killam et al., 1973), but not by actinomycin D, a transcription inhibitor (Resnik et al., 1975).

A decrease in the vasodilatory response of the uterine vascular bed to repeated challenge with estrogen after ovariectomy has been reported for ewes (Greiss and Anderson, 1970a), cows (Ford and Reynolds, 1983a) and sows (Van Orden et al., 1983b). This phenomenon may involve depletion

of vascular estrogen receptors because a continuous infusion of $E_2\beta$ into the uterine artery of ovariectomized ewes resulted in tachyphylaxis of the uterine vasodilatory response to $E_2\beta$ (Clewell et al., 1980). The response to estrogen can be restored by treatment with and subsequent withdrawal of progesterone (P_4; Greiss and Anderson, 1970a), and a local increase in P_4 concentrations was associated with increased binding sites for $E_2\beta$ in uterine arterial tissue of ewes (Koligian and Stormshak, 1977). Thus, a period of exposure to and subsequent withdrawal from P_4 may be required before uterine arteries become responsive to estrogen.

Administration of P_4 alone has little direct effect on UBF (Dickson et al., 1969; Greiss and Anderson, 1970a; Garris and Mitchell, 1979). However, P_4 has been shown to inhibit estrogen-induced increases in UBF of ewes and guinea pigs when both steroids were administered concomitantly (Greiss and Anderson, 1970a; Resnik et al., 1977; Garris and Mitchell, 1979), and the magnitude of this antagonism appears to be related to the $E:P_4$ ratio (Caton et al., 1974). Thus, reduced UBF during the luteal phase of the estrous cycle may be due to the inhibitory effect of P_4 on UBF, and (or) due to the low concentrations of estrogen present. Increased UBF at estrus appears to result from estrogen-induced vasodilation, and may be dependent upon a previous exposure to and withdrawal from P_4.

Ovarian blood flow (OBF) also varies regularly throughout the estrous cycle of ewes (Niswender et al., 1975), cows (Ford and Chenault, 1981) and sows (Magness et al., 1983). However, OBF follows a pattern opposite to that of UBF, being highest during the luteal phase of the estrous cycle and lowest at estrus. Ovarian blood flow is positively correlated

with the concentrations of P_4 and negatively correlated with the $E:P_4$ ratio in systemic blood of ewes, cows and sows (Niswender et al., 1975; Ford and Chenault, 1981; Magness et al., 1983). However, these correlations do not necessarily imply that P_4 is a vasodilator and estrogens are vasoconstrictors of the ovarian vascular bed. In fact, administration of estrogen has been shown to induce ovarian vasodilation in ewes (Rosenfeld et al., 1976; Rosenfeld, 1980). The increase in OBF during the luteal phase of the estrous cycle may simply result from the presence of functional corpora lutea (CL), which receive approximately 90% of total OBF in these species (Niswender et al., 1976; Bruce and Moor, 1976; Ford et al., 1982c). Decreased OBF at estrus is probably due to the regression of the CL, rather than a vasoconstrictor effect of estrogen. In support of this hypothesis, a preferential decrease in blood flow to the luteal vascular bed has been observed during natural and prostaglandin (PG) $F_2\alpha$-induced luteal regression (Niswender et al., 1976; Nett and Niswender, 1981). Therefore, the effect of estrogens to stimulate increased OBF may be masked by the tremendous decrease in the size of the ovarian vascular bed during the follicular phase of the estrous cycle.

Prostaglandins

Infusion of PGE_1 or PGE_2 into the uterine artery causes dilation, whereas $PGF_2\alpha$ causes constriction of the uterine vascular bed of ewes (Resnik and Brink, 1978; Still and Greiss, 1978). Thus, prostaglandins can have a direct effect to alter UBF. Prostaglandins might also be involved in mediating estrogen-induced increases in UBF. As mentioned

previously, UBF does not begin to increase until approximately 30 min after administration of estrogen in ewes (Killam et al., 1973), cows (Ford and Reynolds, 1983a) and sows (Van Orden et al., 1983b). This observation, plus the observation that estrogen-stimulated increases in UBF can be blocked by inhibition of protein synthesis but not by inhibition of transcription (Spaziani and Suddick, 1967; Killam et al., 1973; Resnik et al., 1975), have led to the proposal that estrogen may elicit uterine vasodilation by stimulating local production of another vasoactive substance. Meclofenamate and indomethacin (inhibitors of prostaglandin synthesis) were able to partially suppress estrogen-induced uterine prostaglandin production and vasodilation in rats (Ryan et al., 1974) and ewes (Clark et al., 1976). Clark et al. (1980), however, observed an effect of indomethacin on estrogen-induced uterine vasodilation only when toxic levels of indomethacin were administered to ewes. Prostaglandins may therefore play only a limited role in mediating the ability of estrogens to increase UBF.

Elevated concentrations of PGF have been observed in utero-ovarian venous blood of ewes (Thorburn, et al., 1972), cows (Shemesh and Hansel, 1975) and sows (Gleeson et al., 1974) in association with regression of the CL and decreasing P_4 concentrations in systemic blood. The period of luteal regression is also associated with a decrease in OBF of ewes (Niswender et al., 1976), cows (Ford and Chenault, 1981) and sows (Magness et al., 1983). Administration of exogenous $PGF_{2\alpha}$ induces luteolysis in these species (McCracken et al., 1970; Rowson et al., 1972; Moeljono et al., 1976), and reduces OBF of ewes (Baird, 1974; Niswender et al.,

1976). It has therefore been suggested that natural, or $PGF_{2\alpha}$-induced, luteolysis may result from a reduction in blood flow to the CL (Pharriss, 1970; Nett and Niswender, 1981). In agreement with this proposal, Nett and Niswender (1981) observed a 90% reduction in luteal blood flow within 2 hr of an intrauterine infusion of $PGF_{2\alpha}$, even though total OBF and systemic concentrations of P_4 did not decrease for another 2 to 4 hr. A reduction in luteal blood flow with no change in total OBF can be explained by the observation that $PGF_{2\alpha}$ causes dilation of the ovarian stromal vascular bed (Novy and Cook, 1973; Janson et al., 1975; Varga and Folly, 1977). Thus, the luteolytic activity of $PGF_{2\alpha}$ may be mediated, in part, by its vasoconstrictor effect on the luteal vascular bed.

Periarterial sympathetic nerves-interactions with steroids and prostaglandins

It has been known for many years that blood flow to the abdominal viscera, including the reproductive tract, is under the influence of sympathetic (adrenergic) vasoconstrictor nerves (Langley and Anderson, 1895, 1896; Bayliss, 1923). Most of the information on the distribution of sympathetic innervation within blood vessels has been obtained using the formaldehyde fluorescence method for catecholamines (Falck et al., 1962). Using this technique, a dense sympathetic perivascular plexus has been demonstrated for the small arteries and arterioles within the uterus and ovaries (Unsicker, 1974; Stefenson et al., 1981; Sorger et al., 1983). This sympathetic plexus is comprised of nonmyelinated, postganglionic fibers derived from the periaortic plexus (Shabanah et al., 1964). These sympathetic fibers become incorporated into the uterine

and ovarian arterial walls at their points of origin and therefore traverse the entire length of the arteries, forming their main sympathetic perivascular plexus. Vasoconstrictor nerves supplying the uterine and ovarian vasculature therefore bypass the hypogastric nerves, which supply the uterine myometrial smooth muscle (Shabanah et al., 1964). The uterine artery receives additional sympathetic innervation from the pelvic (hypogastric) plexus (Shabanah et al., 1964).

The walls of the distributing arteries and arterioles, including those supplying the uterus and ovaries, are composed of 3 layers (Rhodin, 1980). The innermost layer is the tunica intima and consists of a single continuous layer of squamous endothelial cells and a basal lamina. In the middle layer, or tunica media, the only cells present are spindle-shaped smooth muscle cells arranged in concentric layers, which number from 25-35 layers for large muscular arteries to 1-2 layers for arterioles (Rhodin, 1980). All of the smooth muscle cells of the tunica media are surrounded by a thin lamina, consisting of collagenous and elastic fibrils, which is interrupted at points of contact (nexuses) between the cells. The outermost layer of the arterial wall is the tunica adventitia, which is composed of dense fibroelastic tissue, probably secreted by the numerous fibroblasts (Rhodin, 1980). The adventitial layer also contains the vascular supply of the arterial wall, which includes arterioles, venules, capillaries and lymphatic vessels, collectively referred to as the vasa vasorum. In addition, an internal elastic lamina, between the media and intima, and an external elastic lamina, between the media and adventitia, invest the tunica media.

These elastic laminae, as well as the collagen and elastin fibrils surrounding each smooth muscle cell of the tunica media, are thought to be secreted and maintained by the smooth muscle cells themselves (Pease, 1962; Rhodin, 1980). The internal elastic lamina is highly fenestrated, which probably facilitates diffusion of substances between the intima and media (Rhodin, 1980).

The adrenergic innervation of blood vessels is found characteristically in the inner adventitia and at the adventitial-medial junction, as a continuous meshwork (plexus) surrounding the smooth muscle of the tunica media (Bevan, 1979). This sympathetic perivascular plexus has been described as "an irregular, highly branched, multiaxonal sheath which surrounds the tunica media of the vessels" (Bevan et al., 1980). Each axon exhibits periodic swellings, or varicosities, of 1.5 - 2 μm in diameter at intervals of 3 - 10 μm. These varicosities contain a large number of granular or "dense core" vesicles (35-60 nm diameter) that represent the storage sites for norepinephrine (NE), which is the primary neurotransmitter for perivascular adrenergic synapses (Bevan et al., 1980). Upon nerve stimulation, NE is released by exocytosis into the synaptic cleft (Bevan et al., 1980). For smaller blood vessels, which usually have synaptic clefts of less than 100 nm, released NE affects only a few subjacent smooth muscle cells, and the spread of the resultant contraction to other areas appears to occur primarily by myogenic propogation (Bevan and Ljung, 1974; Bevan, 1979). This myogenic propogation is thought to occur by means of the previously mentioned nexuses between the smooth muscle cells, many of which may

represent gap junctions (Ljung, 1976). In larger vessels, with wider synaptic clefts, NE diffuses throughout the surrounding tunica media (Bevan, 1979). Thus, in small arteries and arterioles, where neurotransmitter is restricted to the area of close neuromuscular association, a relatively rapid vasoconstrictor response, associated with myogenic propogation, is observed. In larger arteries, a comparatively slow development of neurogenic tone is seen (Bevan et al., 1980). For vessels of intermediate size, the vasoconstrictor response is a combination of the fast, propogated contraction and the slow, nonpropogated contraction which depends upon diffusion of NE throughout the tunica media (Dolozel et al., 1975; Bevan et al., 1980).

Norepinephrine induces contraction of vascular smooth muscle cells by interacting with membrane-bound α-adrenergic receptors, whereas interaction with β-adrenergic receptors causes relaxation and subsequent vasodilation (Bevan et al., 1980). The response of a particular vessel to NE depends on the relative amounts of the two receptor types. Alpha-adrenergic receptor-mediated vasoconstriction is thought to predominate in blood vessels of the abdominal viscera, including those of the uterus and ovaries (Innes and Nickerson, 1970). Electrical stimulation of uterine periarterial sympathetic nerves (Greiss and Gobble, 1967), or exposure to exogenous NE (Ladner et al., 1970; Barton et al., 1974), was able to reduce UBF of ovariectomized, nonpregnant and pregnant ewes nearly to zero. This constrictor response of the uterine vascular bed to sympathetic nerve stimulation or NE was inhibited by the α-adrenergic antagonist phenoxybenzamine (Greiss and Gobble, 1967; Ladner et al., 1970).

In addition, phentolamine, a competitive α-adrenergic receptor blocking drug, caused an immediate increase in UBF of ovariectomized cows when infused directly into the uterine artery (Ford and Reynolds, 1983a). Beta-adrenergic receptor activity, resulting in a relatively modest vasodilation, has been demonstrated for the uterine vascular beds of ovariectomized, nonpregnant and pregnant ewes (Ladner et al., 1970; Greiss, 1971; Barton et al., 1974) and ovariectomized cows (Ford and Reynolds, 1983a). Cholinergic vasodilator activity could not be demonstrated in the uterine vascular bed of pregnant ewes (Greiss and Gobble, 1967; Greiss et al., 1967). However, Greiss et al. (1967) did observe a transient and modest uterine vasodilatory response to acetylcholine in nonpregnant ewes. In support of these observations, Ford et al. (1977b) demonstrated that phentolamine inhibited a substantial portion of the vasoconstrictor response of ovine uterine arteries to sympathetic nerve stimulation *in vitro*, whereas propranolol (a competitive β-adrenergic receptor blocker) or atropine (a competitive cholinergic antagonist) had no effect on the vasoconstrictor response to nerve stimulation. It appears, therefore, that α-adrenergic receptor-mediated vasoconstriction does predominate in the uterine vascular bed, with only a minor role for β-adrenergic or cholinergic vasodilation. The ovarian vascular bed also appears to be influenced primarily by α-adrenergic vasoconstriction. Varga et al. (1979) observed a potent vasoconstrictor effect of NE on the vascular bed of human ovaries during *in vitro* perfusion, but only limited β-adrenergic receptor-mediated vasodilation. Thus,

one possibility is that blood flow to the uterus and ovaries is controlled by the presence or absence of α-adrenergic vasoconstrictor tone.

The ovarian steroids, estrogen and P_4, may alter uterine and ovarian blood flow by regulating the function of the periarterial sympathetic vasoconstrictor nerves within these vascular beds. McKercher et al. (1973) observed a reduction in NE content of uterine periarterial sympathetic nerves coincident with elevated UBF in estrogen-treated rats. In addition, the uterine vascular bed of estrogen-treated ovariectomized ewes was approximately tenfold less sensitive to the vasoconstrictor effects of NE than that of control ovariectomized ewes (Barton et al., 1974). During in vitro perfusion, uterine arterial segments from ovariectomized estrogen-treated ewes exhibited reduced vasoconstriction to sympathetic nerve stimulation or exogenous NE compared with uterine arterial segments from P_4-treated ovariectomized ewes (Ford et al., 1977c). When ovariectomized ewes were treated with both estrogen and P_4, the vasoconstrictor response was similar to that of vehicle-treated controls (Ford et al., 1977c). An association between levels of estrogen and P_4 in systemic blood and uterine arterial contractility has also been observed for intact ewes. Uterine arterial contractility to periarterial nerve stimulation or exogenous NE was reduced for estrus ewes compared with luteal-phase ewes (Ford et al., 1977b). In addition, in unilaterally ovulating ewes and cows, uterine arterial segments removed ipsilateral to the CL-bearing ovary exhibited a greater vasoconstrictor response to sympathetic nerve stimulation than arteries from the contralateral side (Ford et al., 1976). Pope et al. (1982) has

demonstrated that bovine uterine arterial tissue obtained ipsilateral to a CL-bearing ovary contains greater concentrations of P_4 than uterine arterial tissue from the contralateral side. The ovarian steroids appear to have effects on the ovarian periarterial sympathetic nerves which are similar to their effects on uterine periarterial sympathetic nerves. The contractility of ovine and bovine ovarian arterial segments to nerve stimulation and to exogenous NE was greater during the luteal phase of the estrous cycle than at estrus, and was also greater for arterial segments ipsilateral than for segments contralateral to the CL-bearing ovary (Kuhl et al., 1974; Ford et al., 1977a).

Although the studies cited above indicate that estrogen and P_4 act in an antagonistic manner to regulate the function of uterine and ovarian periarterial vasoconstrictor nerves, the mechanism by which this regulation is accomplished is not known. Progesterone increased contractility of aortic smooth muscle strips to NE, in association with a reduction in the activity of catechol-o-methyl transferase (COMT; Kalsner, 1969). Because COMT is the primary extra-neuronal enzyme responsible for conversion of NE to an inactive metabolite (in this case, normetanephrine), inhibition of COMT by P_4 would lead to a potentiation of the effects of NE (Bevan et al., 1980). Treatment of ovariectomized rats with estrogen reduced the release of ^{3}H-NE during contraction of portal vein strips *in vitro* (Bengtsson, 1978), and also reduced the content of NE in uterine periarterial sympathetic neurons (McKercher et al., 1973). Thus, both estrogen and P_4 are capable of affecting NE turnover in vascular smooth muscle.

Recently, Ford and Reynolds (1983a) observed that the competitive α-adrenergic receptor blocker, phentolamine, caused an immediate increase in UBF of ovariectomized cows when infused directly into the uterine artery. When $E_2\beta$ was administered, the typical 30 min delay was observed before UBF increased, suggesting the production of a second messenger. The increase in UBF was not additive when equimolar amounts of phentolamine and $E_2\beta$ were administered during the same treatment period, suggesting that both $E_2\beta$ and phentolamine may be interacting with α-adrenergic receptors. The possible effects of P_4 on vascular α-adrenergic receptors have not been evaluated. However, an interaction of ovarian steroids with vascular α-adrenergic receptors is reasonable, because estrogen and P_4 have been shown to alter the numbers of myometrial α-adrenergic receptors in an antagonistic manner (Williams and Lefkowitz, 1977).

Prostaglandins also appear to modulate the activity of uterine and ovarian perivascular sympathetic neurons. As mentioned previously, PGE causes vasodilation and $PGF_2\alpha$ vasoconstriction of the uterine vascular bed. Clark et al. (1977) demonstrated that infusion of meclofenamate into the canine uterine artery reduced PGE and had no effect on $PGF_2\alpha$ secretion by the uterus. In association with this decrease in PGE secretion, the vasoconstrictor response of the uterus to sympathetic nerve stimulation or exogenous NE was increased twofold. Thus, PGE may exert a tonic, inhibitory effect on perivascular sympathetic neurotransmission. In support of this concept, the studies of Hedquist (1970) and Kadowitz et al. (1971) demonstrated that PGE interferes with release

of NE from perivascular sympathetic nerve terminals. In contrast, Pope and Stormshak (1979) were unable to demonstrate an effect of PGE_2 on vasoconstriction of ovine uterine arteries to sympathetic nerve stimulation *in vitro*.

Kadowitz et al. (1972) demonstrated that $PGF_{2}\alpha$ induces vasoconstriction by augmenting the release of NE from perivascular sympathetic nerve terminals. If, as discussed previously, P_4 increases and estrogen decreases the levels of uterine and ovarian arterial NE and (or) α-adrenergic receptors, then exposure of the utero-ovarian vasculature to the ovarian steroids should affect the vasoconstrictor effects of $PGF_{2}\alpha$. This concept agrees with the data of Ford et al. (1977b,c), who observed that $PGF_{2}\alpha$ augmented contractility for uterine arteries from P_4-dominated intact (day 10 postestrus) and P_4-treated ovariectomized ewes, but not for uterine arteries from estrogen-dominated intact (estrus or Day 3 postestrus) or estrogen-treated ovariectomized ewes. In addition, $PGF_{2}\alpha$ increased the vasoconstrictor response to sympathetic nerve stimulation for ovine and bovine uterine arterial segments obtained ipsilateral, but not contralateral, to the CL-bearing ovary (Ford et al., 1976). A stimulatory effect of $PGF_{2}\alpha$ on the contractility of ovarian arteries obtained ipsilateral to the ovary that contained the corpus luteum has also been observed in cows (Ford et al., 1977a).

From the literature discussed thus far, it can be concluded that ovarian steroids and prostaglandins can affect periarterial sympathetic nerve function to cause changes in uterine and ovarian blood flow throughout the estrous cycle. Recent evidence suggests that a mechanism exists by which the local concentrations of estrogens and P_4 are selectively

increased, thereby amplifying their effects on the uterine and ovarian vascular beds. As mentioned above, Pope et al. (1982) found a greater concentration of P_4 in uterine arterial tissue ipsilateral to a CL-bearing ovary than in uterine arterial tissue from the contralateral side in cows. It has recently been demonstrated that the concentration of estrogens (E_1 and $E_2\beta$)in uterine arterial tissue of sows is greater during estrus than during the luteal phase of the estrous cycle (S. P. Ford and L. P. Reynolds, Iowa State Univ., unpublished observation). Uterine and ovarian lymph contain high levels of estrogens and P_4 (Lindner et al., 1964; Magness and Ford, 1982), and the rate of uterine lymph flow is much lower than that of UBF (Staples et al., 1982). The uterine and ovarian lymphatics are closely associated with the perivascular sympathetic neurons of the uterine and ovarian blood vessels (Hoggan and Hoggan, 1882; Rhodin, 1980), and free exchange of molecules of low molecular weight (< 600 Daltons) between the uterine lymph and blood has been shown to occur (McRae and Kennedy, 1979). Thus, it is possible that uterine and ovarian periarterial nerves are exposed to high concentrations of steroids during the slow passage of lymph along the lymphatic channels. It has also been suggested that prostaglandins may be transported in uterine lymph (Kotwica, 1980), in a manner similar to that proposed for steroids, thereby increasing the exposure of periarterial sympathetic neurons to prostaglandins.

Changes in Blood Flow to the Uterus and Ovaries During Early Pregnancy

Steroids

When embryos are flushed from the uterus before Day 12 postmating in ewes and sows (Moor and Rowson, 1966; Ford et al., 1982a) or Day 16 postmating in cows (Northey and French, 1980), regression of the CL, and subsequent estrus, occur at the normal time for each species. If, however, preimplantation embryos are not flushed until after Day 12 (ewes and sows) or Day 16 (cows), luteal lifespan is extended for 5 to 6 days beyond normal. These observations have led to the concept that the early conceptus, in some way, protects the CL from luteolysis, and this phenomenon has been termed "maternal recognition of pregnancy". The demonstration that intrauterine infusion of conceptus homogenates, obtained around the time of maternal recognition of pregnancy, was able to extend luteal function in ewes (Rowson and Moor, 1967), sows (Ball and Day, 1982) and cows (Northey and French, 1980), provides further evidence that the conceptuses are responsible for maintaining luteal function during early pregnancy.

A transient two to threefold increase in UBF has been observed for ewes on Days 13 to 15 (Greiss and Anderson, 1970b), sows on Days 12 and 13 (Ford and Christenson, 1979) and cows on Days 15 to 17 (Ford et al., 1979) postmating. Implantation does not occur until after pregnancy recognition in the ewe (Amoroso, 1952), sow (Crombie, 1970) and cow (Melton et al., 1951), and is associated with a secondary, prolonged increase in UBF in all 3 species (Greiss and Anderson, 1970b; Ford and

Christenson, 1979; Ford et al., 1979). That the conceptus is responsible for the early, transient increase in UBF, which is associated with maternal recognition of pregnancy, is suggested by the observation that blood flow only increased to the uterine horn containing the conceptus(es) in cows (Ford et al., 1979) and unilaterally pregnant sows (Ford and Christenson, 1979). This observation also emphasizes the local influence of the conceptus on the uterine blood supply. During early pregnancy, UBF of sows and cows is similar to that observed during the estrous cycle and is correlated with the $E:P_4$ ratio in systemic blood until Day 11 and Day 14, respectively (Ford and Christenson, 1979; Ford et al., 1979). On Days 12 and 13 postmating in sows and Days 15 to 17 postmating in cows, UBF was no longer related to the $E:P_4$ ratio in systemic blood, again suggesting the local nature of conceptus-induced vasodilation (Ford and Christenson, 1979; Ford et al., 1979). In association with increased UBF, a transient increase in OBF has also been observed at the time of pregnancy recognition in the sow (Magness et al., 1983) and cow (Ford and Chenault, 1981). In sows, this transient increase in OBF was observed only for the luteal vascular bed and not for the rest of the ovary (Ford et al., 1982c).

The significance of these transient elevations in UBF and OBF during early pregnancy is not understood. However, the luteotropic effect of the ovine (Mapletoft et al., 1976) and bovine (Del Campo et al., 1980) conceptus is exerted through a local vascular pathway from the uterus to the ovaries. Thus, the early increase in UBF may function to enhance transport of a conceptus-induced luteotropin to the CL-bearing

ovary. As mentioned previously, a primary mechanism for natural and induced luteolysis may be a reduction in blood flow to the CL. The transient increase in luteal blood flow of pregnant sows observed by Ford et al. (1982c) occurs when regression of the CL would normally begin in nonpregnant animals, and may therefore enhance luteal function during this critical period. This proposal is supported by the observation that luteal P_4 secretion is highly correlated with OBF in ewes (Niswender et al., 1976), sows (Magness et al., 1983) and cows (Ford and Chenault, 1981), and a transient increase in systemic P_4 levels has been observed coincident with increased OBF during early pregnancy in sows (Magness et al., 1983) and cows (Ford et al., 1979).

The factor(s) responsible for the conceptus-induced increases in UBF and OBF are not known. In vitro production of estrogens by conceptuses can be demonstrated by Day 12 postmating in the sow (Perry et al., 1976) and Day 13 in the cow (Shemesh et al., 1979; Chenault, 1980). In addition, estrogens are elevated in uterine flushings and venous blood of sows and cows at the time of pregnancy recognition for each species (Ford et al., 1981). Infusion of near physiological levels of $E_2\beta$ into an isolated uterine horn in nonpregnant sows resulted in an eight to tenfold increase in blood flow to both uterine horns (Ford et al., 1982b). These researchers also observed bilateral maintenance of luteal function, but with a preferential stimulation of P_4 secretion from the ipsilateral ovary. As mentioned earlier, administration of estrogen also stimulates increased OBF. Thus estrogens, of embryonic origin, may act locally to stimulate increased UBF and OBF during early pregnancy in ewes, sows and cows.

Prostaglandins

Prostaglandins may also be involved in the vascular changes that occur during early pregnancy. Production of PGE_2 can be demonstrated for cultured ovine blastocysts by Day 14 (Lacroix and Kann, 1982; Hyland et al., 1982) and bovine blastocysts by Day 13 (Shemesh et al., 1979; Lewis et al., 1982) postmating. Uterine luminal and venous levels of PGE_2 have been shown to be elevated by Day 15 in pregnant compared with nonpregnant ewes (Ellinwood et al., 1979). In addition, intrauterine infusion of PGE_2 in nonpregnant ewes results in an extension of luteal P_4 secretion (Magness et al., 1981). As previously discussed, PGE_2 will induce uterine vasodilation, but is much less potent in this regard than estrogens (Resnik and Brink, 1978). The vasodilatory effects of PGE_2 on the ovarian vascular bed have not been evaluated. However, PGE_2 will block $PGF_2\alpha$-induced luteolysis when both are infused simultaneously into the ovarian artery (Henderson et al., 1977) or ovarian vascular pedicle (Reynolds et al., 1981). It is conceivable that this antiluteolytic effect of PGE_2 is due to dilation of the ovarian vascular bed, which would counteract the vasoconstrictor effects of $PGF_{2\alpha}$.

Periarterial sympathetic nerves-interactions with steroids and prostaglandins

During early pregnancy, the conceptus may stimulate increased UBF by altering the function of periarterial sympathetic nerves. It has been previously mentioned that the vasoconstrictor response to sympathetic nerve stimulation was greater for uterine arterial segments obtained ipsilateral compared with those obtained contralateral to the CL-bearing

ovary of nonpregnant ewes and cows (Ford et al., 1976), even though blood flow was similar for either uterine horn of nonpregnant cows (Ford et al., 1979). In addition, it was observed that $PGF_2\alpha$ only augmented vasoconstriction to nerve stimulation for uterine arteries obtained ipsilateral to the luteal-bearing ovary (Ford et al., 1976), possibly due to greater concentrations of NE and (or) α-adrenergic receptors in P_4-dominated arteries. When uterine arterial segments were obtained from ewes on Day 15 or cows on Day 17 postmating, the ability of $PGF_2\alpha$ to augment contractility to nerve stimulation, for uterine arteries ipsilateral to the CL-bearing ovary, was no longer observed (Ford et al., 1976). These data suggest an effect of the conceptus on reducing uterine arterial NE and (or) α-adrenergic receptor concentrations. A similar inhibition of $PGF_2\alpha$-induced vasoconstriction was observed when a conceptus brei was perfused through uterine arterial segments of nonpregnant ewes (Ford et al., 1976) and cows (Ford, 1978). This decrease in responsiveness of uterine arteries from nonpregnant animals to $PGF_2\alpha$ and sympathetic nerve stimulation is similar to that observed for estrogen-dominated nonpregnant ewes (Ford et al., 1977b,c). As discussed for arteries of estrous animals, exposure of uterine and ovarian arteries to estrogens of conceptus origin could induce depletion of arterial NE and (or) α-adrenergic receptors, thereby inhibiting the vasoconstrictor effects of $PGF_2\alpha$ and causing vasodilation. Similarly, PGE_2 of conceptus origin may inhibit the release of uterine and ovarian arterial NE induced by $PGF_2\alpha$ and nerve stimulation, as discussed previously (Kadowitz et al., 1971).

Exposure of the sympathetic neurons of the uterine and ovarian vascular bed to the conceptus-induced vasodilatory factor may be via the lymphatics. Magness and Ford (1982) found greater concentrations of estrogens in uterine lymph on Days 11, 13 and 15 of pregnancy than on the same days of the estrous cycle in gilts, and as previously discussed, there is a close association between the lymphatics and the periarterial sympathetic neurons. Transport of prostaglandins in uterine and ovarian lymph has been suggested (Kotwica, 1980), but has not been evaluated during early pregnancy.

STATEMENT OF PROBLEM

During the estrous cycle of the ewe, cow and sow, regular variations in uterine blood flow (UBF) and ovarian blood flow (OBF) are observed. Uterine blood flow is highest at estrus and remains low throughout the luteal phase of the estrous cycle. Ovarian blood flow follows a pattern opposite to that of UBF, being least at estrus and greatest during the luteal phase. The increase in UBF at estrus is highly correlated with the estrogen: progesterone ratio ($E:P_4$) in systemic blood. The negative correlation between OBF and the $E:P_4$ ratio in systemic blood may simply be due to the decreased size of the ovarian vascular bed at estrus, and, in fact, administration of estrogen stimulates increased UBF and OBF in ewes. The vasodilatory effect of estrogen may be mediated, in part, by prostaglandins of the E series (PGE), which also have a direct vasodilatory effect on the uterine vascular bed. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induces uterine and ovarian vasoconstriction, and this may be involved in luteal regression at the end of the estrous cycle in nonpregnant animals. The vasoconstrictive or vasodilatory effects of the ovarian steroids and prostaglandins have been shown to be due to an interaction between these compounds in altering the function of perivascular sympathetic vasoconstrictor neurons. These alterations may include effects on neurotransmitter levels, neurotransmitter release, concentrations of neurotransmitter at neuroeffector junctions and effects on α-adrenergic receptors.

During early pregnancy, transient uterine and luteal vasodilation has been observed coincident with the time that conceptuses must be present in

the uterus to maintain luteal progesterone (P_4) secretion. These increases in UBF and OBF are mediated by the conceptus, and may be necessary for the transport of a luteotropic substance from the gravid uterus to the ovary bearing the corpus luteum (CL). Additionally, they may act directly to counteract the luteal vasoconstriction that results in regression of the CL in the absence of conceptuses. Thus, the transient increases in UBF and OBF at the time of pregnancy recognition may be important for the maintenance of luteal P_4 secretion, which is essential for the successful establishment of pregnancy. The conceptuses of farm animal species can produce estrogens and PGE_2, and these compounds are secreted from the gravid uterus during maternal recognition of pregnancy. Similar to uterine arteries of nonpregnant animals at estrus, the sympathetic vasoconstrictor activity is reduced for uterine arteries during early pregnancy. The vasoconstrictor effect of $PGF_2\alpha$ is also absent during early pregnancy. Estrogens and (or) PGE of embryonic origin may, therefore, act on the perivascular sympathetic neurons in a local fashion to induce transient uterine and ovarian vasodilation, which may be essential for the establishment of pregnancy.

Experiments were therefore designed to investigate the mechanisms by which the effects of the conceptus on the uterine and ovarian perivascular sympathetic neurons are accomplished. Additional studies were designed to further investigate the local effects of estrogen and PGE_2 in stimulating UBF and maintaining luteal P_4 secretion during early pregnancy.

EXPERIMENT I. ROLE OF PERIARTERIAL SYMPATHETIC NERVES IN ALTERING UTERINE BLOOD FLOW DURING THE ESTROUS CYCLE AND EARLY PREGNANCY IN GILTS

Introduction

During the estrous cycle of ewes (Greiss and Anderson, 1969) and sows (Ford and Christenson, 1979), regular variations in uterine blood flow (UBF) are observed. These variations in UBF are temporally associated with the daily estrogen to progesterone ratio ($E:P_4$) in systemic blood of each species (Yuthasastrakosol et al., 1975; Henricks et al., 1972), with UBF being greatest at estrus and least during the luteal phase of the estrous cycle. Administration of estrogen stimulates increased UBF in the ewe (Killam et al., 1973) and sow (Dickson et al., 1969). Progesterone (P_4) antagonizes the uterine vasodilatory effect of estrogen (Caton et al., 1974; Resnik et al., 1977) and the magnitude of this inhibition is related to the ratio of the two steroids (Caton et al., 1974). Blood flow to the uterus and ovaries appears to be controlled primarily by periarterial sympathetic vasoconstrictor nerves (PSVN; Ford, 1982). Exposure to P_4 increases, whereas estrogen decreases, the function of uterine PSVN in nonpregnant ewes and rats, possibly through changes in norepinephrine (NE) or α-adrenergic receptor levels (McKercher et al., 1973; Barton et al., 1974; Ford et al., 1976, 1977b,c).

A transient two to threefold increase in UBF is observed coincident with maternal recognition of pregnancy in ewes (Moor

and Rowson, 1966; Greiss and Anderson, 1970b) and sows (Ford et al., 1982a). Although systemic estrogen concentrations remain low in sows during early pregnancy (Magness et al., 1983), the transient increase in UBF may result from a local increase in estrogen levels. Early porcine blastocysts are capable of estrogen production in vitro (Perry et al., 1976), and estrogens are elevated in uterine luminal fluid, uterine venous blood and uterine lymph of sows at the time of pregnancy recognition (Ford et al., 1981; Magness and Ford, 1982). In addition, the presence of the conceptus is associated with reduced function of uterine PSVN during early pregnancy in ewes (Ford et al., 1976).

This study was therefore conducted to determine the association between uterine arterial contractility, NE concentrations and α-adrenergic receptor numbers, and the concentrations of estrone (E_1), estradiol-17β ($E_2\beta$) and P_4 in systemic blood during the estrous cycle and early pregnancy in gilts.

Materials and Methods

Experimental procedures

Yorkshire gilts of similar age and weight (10-12 mo, 140-160 kg) that had exhibited estrous cycles of normal duration (18-22 days) were assigned randomly to be sacrificed during the follicular phase (FP, Days 19 to 21; n = 8) or luteal phase (LP, Day 13; n = 7) of the estrous cycle, or on Day 13 of pregnancy (P; n = 7). Gilts in the P group were mated to an intact boar on the first day

of estrus (first day of estrus or mating = Day 0). Immediately before sacrifice, a sample of blood was obtained from the anterior vena cava of each gilt and plasma frozen at -20°C until assayed for P_4, E_1 and $E_2\beta$ by radioimmunoassay. Pregnancy was verified by flushing each uterine horn of P gilts with 0.9% saline to recover conceptus tissue.

At sacrifice, a 3.0 cm segment of the middle uterine artery supplying one uterine horn was excised immediately before its first bifurcation in the mesometrium, and placed into a container of oxygenated (95% O_2, 5% CO_2) Krebs-Ringer (KR) solution (22°C; millimolar composition = NaCl, 118.1; $NaHCO_3$, 25.0; KCl, 4.7; $CaCl_2.6H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 1.2; glucose, 11.1). The remainder of the uterine arterial tissue within the mesometrium of both uterine horns was excised, weighed, placed in a vial containing 0.9% saline and immediately frozen in an acetone: ethanol (1:1, v/v) bath on dry ice. In addition, branches of the cranial mesenteric artery of each gilt, that were similar in size to uterine arteries, were collected, weighed and frozen to serve as control tissue. The arterial tissue was then stored at -80°C until assayed for α-adrenergic receptor binding and NE content.

At the laboratory, each segment of the middle uterine artery that had been placed in KR solution was prepared for perfusion as previously described for ovine and bovine uterine arteries (Ford et al., 1976). Breifly, each arterial segment was cannulated

with polyethylene tubing, and mounted in a perfusion chamber within 60 min after sacrifice. Arterial segments were perfused both intraluminally and extraluminally with continuously oxygenated KR solution (37°C). The extraluminal flow rate was 10 ml/min. The intraluminal perfusion rate (20 ml/min) was held constant throughout the perfusion of each artery. Uterine arterial segments were allowed a 30-min equilibration period, by which time all arteries had established a baseline perfusion pressure (BPP), which remained constant for the duration of perfusion. After equilibration, each arterial segment received 3 sequential 10-min perfusion periods with 0.9% saline, NE and saline, respectively. At the end of each 10-min perfusion period, PSVN were subjected to electrical stimulation (30 sec, 30 HZ, 70 V; Ford et al., 1976). The change in perfusion pressure above BPP in response to electrical stimulation was defined as uterine arterial contractility. The final concentration of NE, administered into the intraluminal perfusate, was 500 ng/ml. The first saline perfusion was given to determine the endogenous response of uterine arterial segments to electrical stimulation of PSVN. Norepinephrine was administered to provide the PSVN with a maximal amount of releasable NE, without altering the BPP. The second saline perfusion was used to verify the continued viability of the preparation. Preliminary studies demonstrated that a major portion of the vasoconstrictor response of gilt uterine arterial segments to electrical stimulation was mediated by α-adrenergic receptors because 10-min perfusion of

phentolamine (100 ng/ml; a competitive α-adrenergic antagonist) reduced the response to electrical stimulation by 72 ± 9% (SEM; n = 8), compared with saline control perfusions.

Alpha-adrenergic receptor binding was determined in the laboratory of Dr. D. E. Van Orden, Univ. of Iowa, for uterine and mesenteric arterial tissue using the α_1-selective adrenergic antagonist ^{3}H-WB-4101 (Amersham, Arlington Heights, IL), as described by Colucci et al. (1981). To facilitate homogenization, each vial of arterial tissue was thawed on ice and cut into 1-cm sections. These sections of arterial tissue were then wrapped in aluminum foil, frozen in liquid nitrogen and pulverized. One-half to 2 g of this tissue powder was homogenized in 0.25 M sucrose using a Polytron (Brinkmann Instruments, Inc., Westbury, NY). The resulting homogenate was centrifuged at 1500 x g for 15 min at 4°C. The supernatant was poured through a gauze filter and further centrifuged at 100,000 x g for 1 hr at 4°C. The membrane-rich pellet obtained after the final centrifugation was re-dissolved in Tris-HCl buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, pH 7.6) to yield a final protein concentration of 2-3 mg/ml. Protein concentrations were determined by the method of Lowry et al. (1951) using BSA as the standard. Receptor binding studies were conducted at 22°C, and all solutions of adrenergic agonists and antagonists were prepared immediately before use. Saturation curves for ^{3}H-WB-4101 were obtained by adding 25 μl of ^{3}H-WB-4101 solution (0.10-3.00 nM) and 25 μl of distilled H_2O or 6.00 mM (-)-epinephrine

bitartrate (Calbiochem, San Diego, CA) to 100 µl of the arterial membrane preparation from each animal. This mixture was allowed to incubate for 20 min, and was then diluted with 4.5 ml Tris-HCl buffer (22°C) and immediately filtered through a glass fiber filter (Whatman GF/C). The glass fiber filter was washed with two additional 4.5 ml aliquots of Tris-HCl, placed in a scintillation vial and radioactivity determined by liquid scintillation spectrometry. Specific binding was defined as the difference between the amount of ^{3}H-WB-4101 bound in the presence and absence of epinephrine. Specific binding averaged ∿70% of total binding at a ^{3}H-WB-4101 concentration of 0.40 nM (approximately the equilibrium dissociation constant, Kd, for ^{3}H-WB-4101). Saturation curves for binding of ^{3}H-WB-4101 by membrane preparations from each gilt were analyzed by the method of Scatchard (1949) for determination of Kd and the amount of radioligand bound at saturation (BMAX). The specific binding was saturable, rapid (reaching equilibrium in about 4 min) and remained stable for 20 min. Figures 1 and 2 show representative saturation curves obtained from membrane preparations of uterine and mesenteric arteries. Scatchard analysis demonstrated that binding of ^{3}H-WB-4101 by all membrane preparations was linear ($r \simeq 0.97$), indicating a single class of binding sites. To determine specificity of ^{3}H-WB-4101 binding for uterine arteries, increasing concentrations (10^{-10} to 10^{-3} M) of adrenergic agonists [(-)-epinephrine bitartrate and (-)-norepinephrine bitartrate, Calbiochem, San Diego, CA; (-)-isoproterenol -HCl, Sigma, St. Louis,

Figure 1. Representative saturation curve for binding of ^{3}H-WB-4101 by membrane preparations from uterine arteries of gilts. Inset: Scatchard analysis of saturation curve

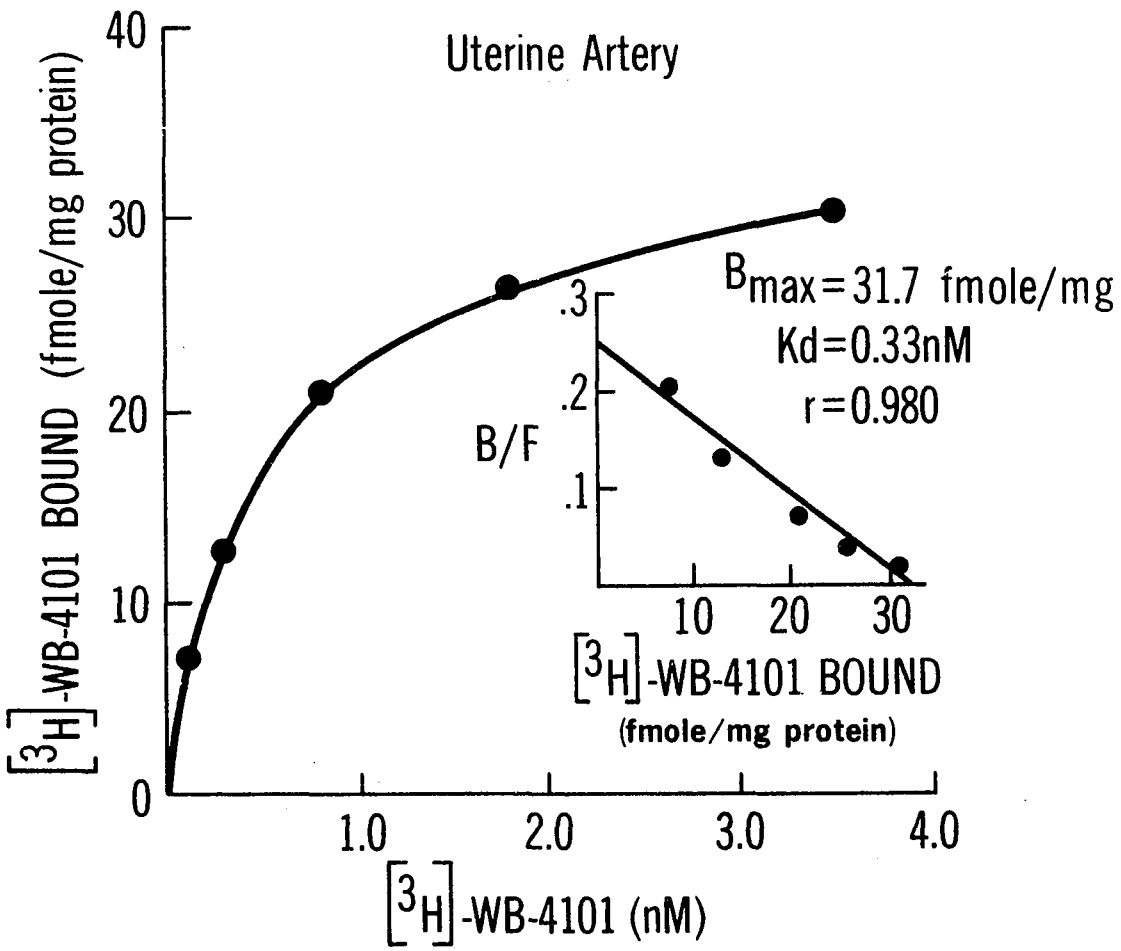
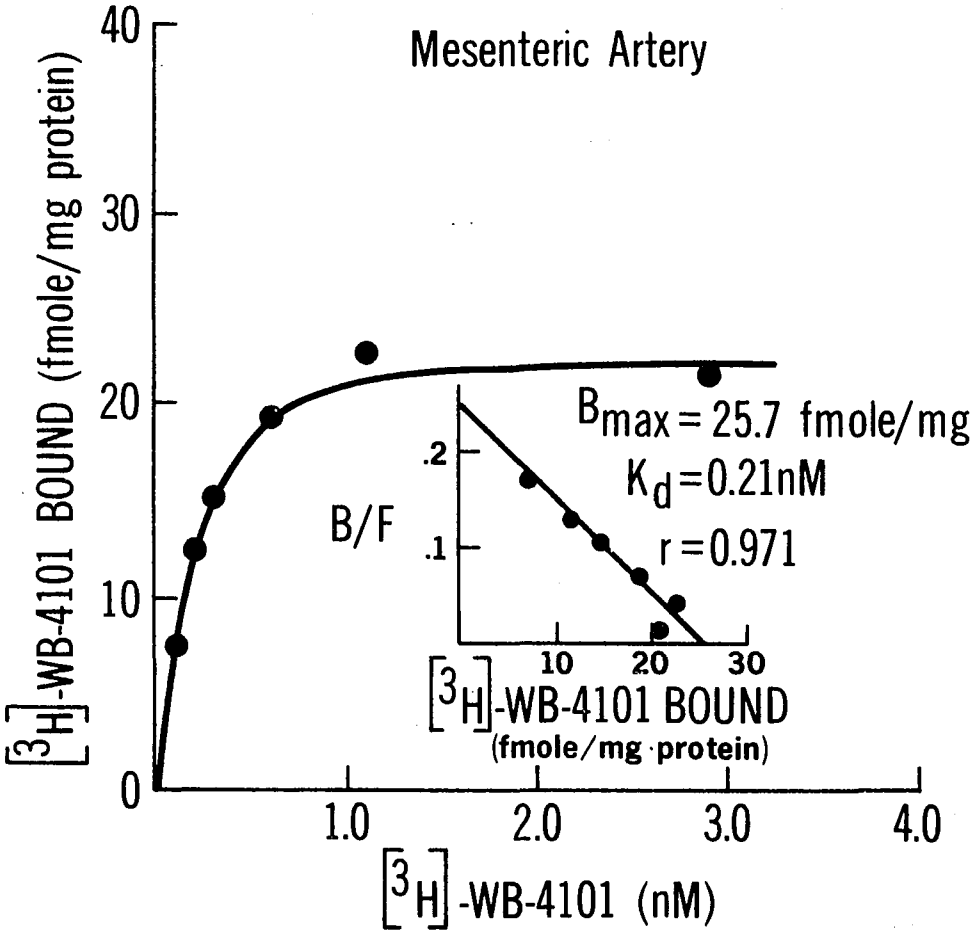

Uterine Artery
[3H]-WB-4101 BOUND (fmole/mg protein)
40
30
20
10
0
1.0
2.0
3.0
4.0
[3H]-WB-4101 (nM)
Bmax=31.7 fmole/mg
Kd=0.33nM
r=0.980
B/F
.3
.2
.1
10
20
30
[3H]-WB-4101 BOUND
(fmole/mg protein)

Figure 2. Representative saturation curve for binding of ^{3}H-WB-4101 by membrane preparations from mesenteric arteries of gilts. Inset: Scatchard analysis of saturation curve


Mesenteric Artery
[3H]-WB-4101 BOUND (fmole/mg protein)
40
30
20
10
0
1.0
2.0
3.0
4.0
[3H]-WB-4101 (nM)
Bmax = 25.7 fmole/mg
Kd = 0.21nM
r = 0.971
B/F
.2
.1
10
20
30
[3H]-WB-4101 BOUND
(fmole/mg protein)

MO] and antagonists [WB-4101, Amersham, Arlington Heights, IL; prazosin -HCl, Pfizer, Brooklyn, NY; yohimbine -HCl, Sigma, St. Louis, MO] were added along with 0.70 nM ^{3}H-WB-4101 to a membrane preparation from a pool of gilt uterine arteries. The Ki (inhibition constant) values for nonradioactive agonists and antagonists were calculated by the method of Cheng and Prusoff (1973). Catecholamines competed with ^{3}H-WB-4101 for binding sites with the classic α-adrenergic order of potency (Bevan et al., 1980): (-)-epinephrine (Ki = 0.60 μM) > (-)-norepinephrine (Ki = 1.50 μM) > (-)-isoproterenol (Ki = 120.00 μM). Unlabeled WB-4101 displayed a Ki of 0.36 nM. The α_1-selective antagonist prazosin (Ki = 1.10 nM) was 118 times more potent in competing for uterine arterial binding sites than the α_2-selective antagonist yohimbine (Ki = 130.00 nM). Potencies of these selective α-receptor antagonists indicate that this assay measures primarily the α_1-adrenergic receptor subtype, which is thought to mediate post-synaptic α-adrenergic effects (Berthelsen and Pettinger, 1977).

Concentrations of NE in uterine and mesenteric arterial tissues were determined in the laboratory of Dr. R. K. Bhatnagar, Univ. of Iowa, using a radiochemical enzymatic assay as previously published (Clark et al., 1978).

Concentrations of P_4, E_1 and $E_2\beta$ in vena cava blood were measured by using radioimmunoassays previously validated in this laboratory, as reported by Magness and Ford (1982). For each hormone, all samples were analyzed in a single assay. The intra-

assay coefficients of variation were 6.5%, 5.4% and 9.2% for P_4, E_1 and $E_2\beta$, respectively.

Statistical analysis

Differences in uterine arterial contractility between treatment groups were analyzed statistically by split-plot analysis for a completely randomized design, with perfusion periods as the subplot (Kirk, 1968). Data for BPP, BMAX, Kd, NE and steroid hormones were analyzed by analysis of variance for a completely randomized design. For comparing BMAX, Kd and NE in uterine *vs.* mesenteric arteries a factorial analysis of variance was utilized (Kirk, 1968). Differences between means were evaluted by using orthogonal contrasts (Kirk, 1968). Correlations between hormone concentrations and uterine arterial contractility at the end of saline perfusions (periods 1 and 3), BMAX, Kd and NE were calculated for each treatment group. All data are reported as the mean ± SEM.

Results

Uterine arterial segments for *in vitro* perfusion were obtained from only 4 gilts in each treatment group. The BPP did not differ ($P>0.10$) between treatment groups, averaging 26 ± 1 mm Hg. Within a treatment group, uterine arterial contractility was similar at the end of both saline perfusion periods, indicating continued viability of the preparations, and the response to electrical stimulation at the end of both saline perfusions (periods

1 and 3) have therefore been averaged in Fig. 3. Contractility at the end of saline and NE perfusion periods was greater ($P<0.01$) for uterine arteries from LP gilts than for FP and P gilts, which were similar (Fig. 3). The contractile response to electrical stimulation at the end of NE perfusion was greater ($P<0.05$) than at the end of saline perfusions for uterine arteries from LP and P, but not for FP gilts (Fig. 3).

Although the contractility of uterine arteries from LP gilts was greater than for uterine arteries from P gilts, their BMAX (fmole ^{3}H-WB-4101 bound/mg protein) was similar, and was greater ($P<0.05$) than that of FP gilts (Fig. 4). There were no differences between treatment groups in the uterine arterial Kd for ^{3}H-WB-4101 or uterine arterial concentrations of NE. For mesenteric arteries, treatment groups did not differ in their BMAX for ^{3}H-WB-4101, (Fig. 4). In addition, there was no difference between treatment groups in Kd or the concentrations of NE in mesenteric arterial tissue. However, mesenteric arteries from all treatment groups had a lower BMAX ($P<0.01$) and Kd ($P<0.05$) than for uterine arteries (30.5 ± 2.6 vs. 42.8 ± 2.9 fmole/mg protein and 0.32 ± 0.03 vs. 0.44 ± 0.04 nM, respectively). In addition, the concentrations of NE were greater ($P<0.01$) for mesenteric than for uterine arteries across all treatment groups (0.98 ± 0.12 vs. 0.34 ± 0.03 ng/mg tissue, respectively).

Samples of vena cava blood were not obtained for 2 P gilts before sacrifice. The concentrations of E_1 in vena cava plasma

Figure 3. Change in perfusion pressure from baseline in response to electrical stimulation at the end of saline and norepinephrine perfusion periods for uterine arteries from gilts. Means ± SEM

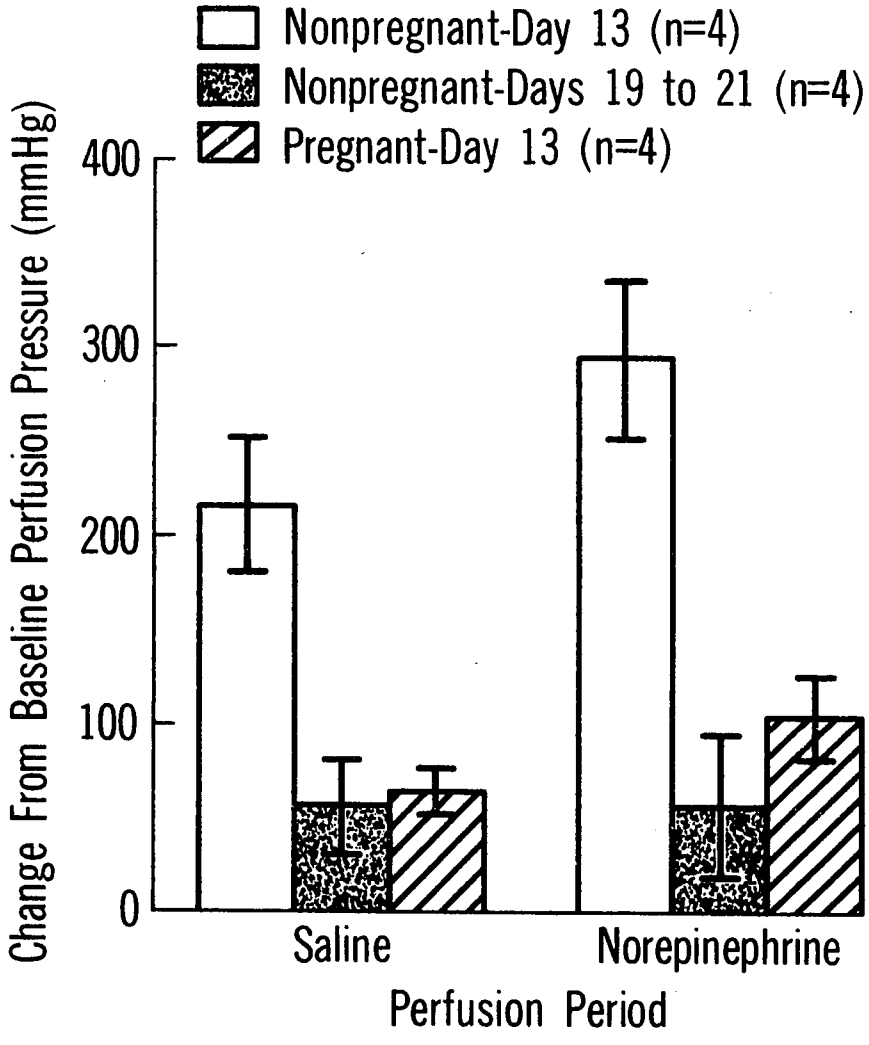
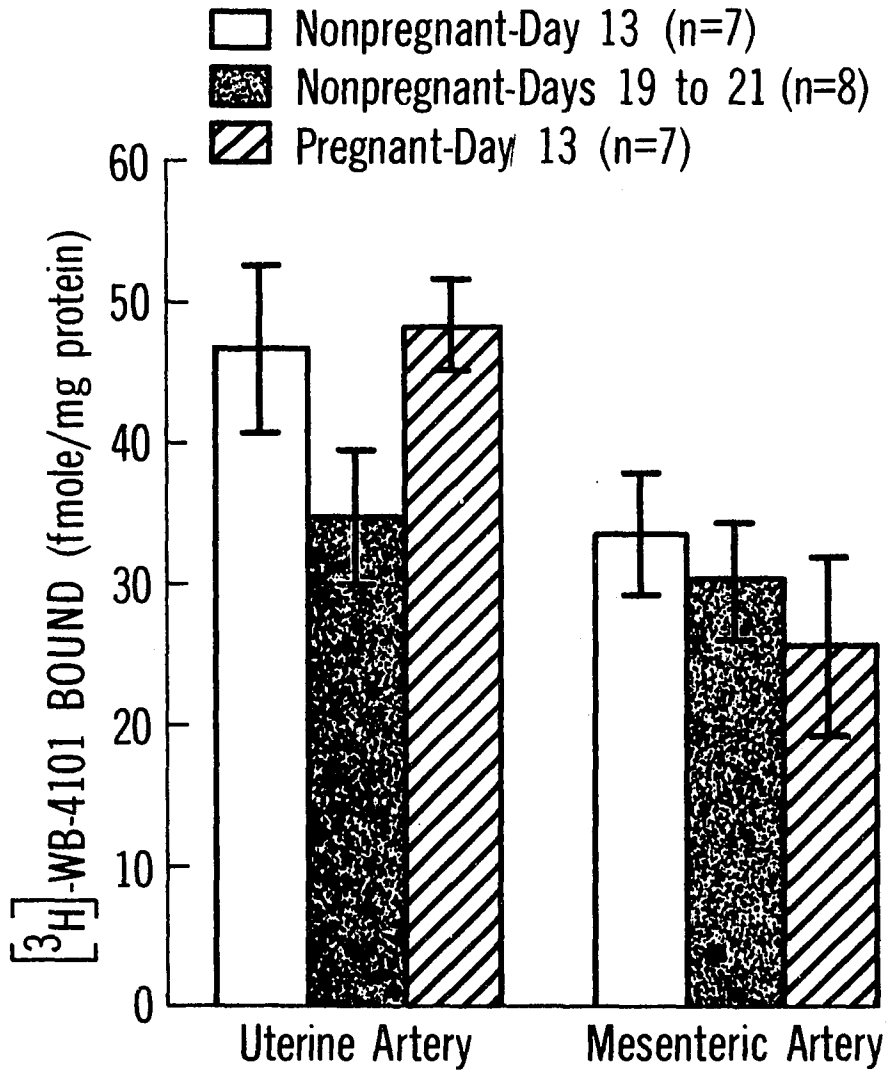

Nonpregnant-Day 13 (n=4)
Nonpregnant-Days 19 to 21 (n=4)
Pregnant-Day 13 (n=4)
Change From Baseline Perfusion Pressure (mmHg)
400
300
200
100
0
Saline
Norepinephrine
Perfusion Period

Figure 4. BMAX (fmole /mg protein) for uterine and mesenteric arterial tissue from gilts. Means ± SEM


Nonpregnant-Day 13 (n=7)
Nonpregnant-Days 19 to 21 (n=8)
Pregnant-Day 13 (n=7)
[3H]-WB-4101 BOUND (fmole/mg protein)
60
50
40
30
20
10
0
Uterine Artery
Mesenteric Artery

were not different between treatment groups, but $E_2\beta$ was greater ($P<0.05$) for FP than LP and P gilts (Table 1). The concentrations of P_4 were less ($P<0.01$) for FP than for LP and P gilts (Table 1). This difference in concentrations of $E_2\beta$ and P_4 resulted in a greater ($P<0.01$) $E:P_4$ ratio in systemic blood of FP than LP and P gilts (Table 1).

For all treatment groups, BMAX of uterine arteries was positively correlated with concentrations of P_4 ($r = 0.49$; $P<0.03$) and negatively correlated with the $E:P_4$ ratio ($r = -0.53$; $P<0.03$) in systemic blood. Uterine arterial contractility in response to electrical stimulation at the end of saline perfusion was positively correlated with P_4 ($r = 0.74$; $P<0.05$) and negatively correlated with the $E:P_4$ ratio ($r = -0.70$; $P<0.05$) only during the estrous cycle (FP and LP gilts). During early pregnancy, significant correlations between steroid concentrations in systemic blood and uterine arterial contractility were not observed.

Discussion

For nonpregnant gilts in this study, uterine arterial contractility and concentrations of α-adrenergic receptors were greatest during the luteal phase and lowest during the follicular phase. In addition, both uterine arterial contractility and α-receptor binding were positively correlated with P_4 and negatively correlated with the $E:P_4$ ratio in systemic blood. These data agree with those of Ford et al. (1977b), who observed that the contractility of ovine uterine arterial segments was greatest during the luteal

Table 1. Concentrations of steroids and ratio of estrogen: progesterone in vena cava plasma

Treatment group	No. of gilts	P_4 (ng/ml)	E_1 (pg/ml)	$E_2\beta$ (pg/ml)	$E:P_4$ [1]
Nonpregnant (Day 13)	7	34.12 ± 1.93^a	9.5 ± 0.4^a	14.5 ± 1.6^a	0.7 ± 0.1^a
Nonpregnant (Days 19 to 21)	8	0.89 ± 0.10^b	11.8 ± 1.8^a	27.5 ± 6.7^b	50.5 ± 14.0^b
Pregnant (Day 13)	5	34.18 ± 1.82^a	11.1 ± 1.9^a	14.3 ± 1.4^a	0.6 ± 0.1^a

[1] $E_1 + E_2\beta$ (pg/ml)/P_4 (ng/ml).

[a,b] Means (± SEM) within a column with different superscripts differ ($P<0.05$ for $E_2\beta$; $P<0.01$ for $E:P_4$).

phase and least during the follicular phase of the estrous cycle. Ford et al. (1977c) demonstrated that these differences were due to the effects of the ovarian steroids on the uterine PSVN because treatment of ovariectomized ewes with P_4 increased while $E_2\beta$ decreased in vitro contractility of uterine arterial segments to NE or electrical stimulation compared with arterial segments from control ovariectomized ewes. When these ewes were treated with estrogen plus P_4, uterine arterial contractility was similar to that of controls, indicating an antagonistic effect of estrogen and P_4 in controlling the activity of uterine PSVN. Barton et al. (1974) observed that the uterine vascular bed of estrogen-treated ovariectomized ewes was approximately tenfold less sensitive to the vasoconstrictor effects of exogenous NE than that of control ovariectomized ewes, suggesting that estrogen may affect postsynaptic α-adrenergic receptors. Recently, it was demonstrated that the vasodilatory response of the bovine uterus to phentolamine (a competetive α-adrenergic antagonist) or $E_2\beta$ was not additive when equimolar amounts of each were infused simultaneously into the uterine artery, suggesting that $E_2\beta$ may increase UBF by interacting with α-adrenergic receptors (Ford and Reynolds, 1983a). Data from the present experiment agree with these observations and indicate that the primary mechanism by which estrogen and P_4 induce changes in UBF during the estrous cycle is by altering the levels of postsynaptic uterine arterial α-adrenergic receptors.

The interaction of estrogens with α-adrenergic receptors would be more easily explained if the estrogens had a structure similar to the catecholamines. Estrone and $E_2\beta$ can be hydroxylated to yield 2- or 4-hydroxyestrone and 2- or 4-hydroxyestradiol, respectively (Ball and Knuppen, 1980). Intra-arterial injection of the 2-hydroxy derivatives of both E_1 and $E_2\beta$ induced uterine vasodilation in ovariectomized ewes (Rosenfeld and Jackson, 1982). In addition, porcine uterine arteries are capable of synthesizing 2-hydroxyestrone from E_1 *in vitro* (Van Orden et al., 1983a). Recently, 2-hydroxyestrone and 2-hydroxyestradiol have been shown to have a moderate ability to inhibit binding of ^{3}H-WB-4101 (Ki = 30 μM) to uterine arterial α-adrenergic receptors (D. B. Farley and D. E. Van Orden, Univ. of Iowa, and S. P. Ford, Iowa State Univ., unpublished observations). Thus, estrogens may be converted to a hydroxylated form, which then interacts with α-adrenergic receptors.

The concentrations of α-adrenergic receptors and NE in mesenteric arteries did not change with stage of the estrous cycle, indicating that mesenteric PSVN are not controlled by systemic ovarian steroids. Concentrations of NE in uterine arterial tissue also were not influenced by stage of the estrous cycle. Significant differences were observed, however, in the levels of α-receptors and NE between uterine and mesenteric arteries. Even though concentrations of α-adrenergic receptors were lower in mesenteric than in uterine arteries, the low Kd of mesenteric arterial α-adrenergic receptors indicates that their affinity for neurotransmitter may be greater

than that of uterine arterial α-adrenergic receptors. The concentration of NE in arterial tissue has been used as a quantitative measure of innervation density (Bevan et al., 1980). The ratio of NE concentration to postsynaptic (α_1) adrenergic receptor binding sites has also been used as a measure of sympathetic innervation of arteries (Bobik, 1982). Using either of these criteria, the density of sympathetic innervation is greater for mesenteric than for uterine arteries of gilts. These data agree with those of Bobik (1982) who found the ratio of NE concentration to α-adrenergic receptors was greater for mesenteric than for aortic, renal or femoral arterial tissue of dogs. In addition, the low sympathetic innervation density and high levels of α-adrenergic receptors in uterine arterial tissue may indicate that the uterine vascular bed is more responsive to circulating catecholamines than the mesenteric vasculature (Bobik, 1982).

Uterine arterial contractility was reduced on Day 13 of pregnancy compared with the same day of the estrous cycle. A similar decrease in the contractility of the ovarian vascular bed of gilts has been observed on Day 13 of pregnancy (Reynolds and Ford, 1982), in association with a transient increase in blood flow to the corpora lutea (Ford et al., 1982c). Although contractility was reduced, the levels of uterine arterial α-receptors were similar for P and LP gilts. Thus, the conceptus may induce the transient increases in uterine and ovarian blood flow observed on Days 12-14 postmating by affecting the function

of arterial smooth muscle cells, independent of effects on arterial α-adrenergic receptors. In support of this proposal, Reynolds and Ford (1982) found the response of the ovarian vascular bed to 0.1 M KCl, which causes nonspecific depolarization of vascular smooth muscle (Sparks, 1980), was markedly reduced on Day 13 in pregnant compared with nonpregnant gilts. The lack of significant correlations between uterine arterial contractility of P gilts and systemic steroid levels indicates a local effect of the conceptus to increase UBF. If estrogens, which are secreted by the gravid porcine uterus (Ford et al., 1982a, Magness and Ford, 1982), are responsible for uterine vasodilation during early pregnancy, they do not appear to reduce α-adrenergic receptor concentrations as during the follicular phase of the estrous cycle.

EXPERIMENT II. ROLE OF PERIARTERIAL SYMPATHETIC NERVES IN ALTERING OVARIAN BLOOD FLOW DURING THE ESTROUS CYCLE AND EARLY PREGNANCY IN GILTS

Introduction

The arteries of the uterine and ovarian vascular beds are innervated by post-ganglionic sympathetic neurons which become incorporated into the walls of these arteries at their points of origin and traverse their entire length (Shabanah et al., 1964). Vasoconstriction, mediated by α-adrenergic (sympathetic) receptors, predominates in the vessels of the uterus and ovaries (Innes and Nickerson, 1970). Electrical stimulation of periarterial sympathetic vasoconstrictor nerves (PSVN), or exogenous norepinephrine (NE), reduces uterine blood flow (UBF) of pregnant or nonpregnant ewes nearly to zero (Greiss and Gobble, 1967; Barton et al., 1974).

Elevated estrogen levels in systemic blood of intact or ovariectomized ewes and heifers are associated with decreased vasoconstrictor activity of the uterine and ovarian arteries in response to exogenous NE and electrical stimulation of PSVN (Barton et al., 1974; Kuhl et al., 1974; Ford et al., 1977b,c). Estrogen administration results in increased UBF in the ewe (Huckabee et al., 1970), cow (Roman-Ponce et al., 1978) and sow (Dickson et al., 1969) and increased ovarian blood flow (OBF) of ewes (Rosenfeld, 1980). Progesterone (P_4) increases the contractility of uterine and ovarian arteries (Kuhl et al., 1974; Ford et al., 1976, 1977a,b,c) and antagonizes the uterine vasodilatory effect of estrogen (Greiss and Anderson, 1970a; Caton et al., 1974).

During luteal regression, decreased ovarian P_4 secretion is associated with decreased blood flow through the corpora lutea (CL) in the ewe (Niswender et al., 1976; Brown et al., 1980), cow (Ford and Chenault, 1981) and sow (Magness et al., 1983). The luteolytic activity of prostaglandin (PG) $F_{2\alpha}$ may be partially due to its decreasing luteal blood flow (Pharriss, 1970; Nett and Niswender, 1981). Prostaglandin $F_{2\alpha}$ enhanced the in vitro contractility of uterine arteries from nonpregnant, but not pregnant, ewes and cows in response to nerve stimulation (Ford et al., 1976). Intraluminal perfusion of conceptus brei or uterine flushings from pregnant animals prevented the vasoconstrictor effect of $PGF_{2\alpha}$ on uterine arteries from nonpregnant animals (Ford et al., 1976; Ford, 1978). Transient increases in uterine, oviductal and CL blood flow have been observed on Days 11 to 13 postmating in sows (Ford et al., 1982c), coincident with maternal recognition of pregnancy in this species (Dhindsa and Dziuk, 1968; Ford et al., 1982a).

Since the PSVN of the ovarian artery are continuous with those of the arteries and arterioles within the ovary (Unsicker, 1974), steroid and conceptus-induced changes in OBF may be mediated by changes in the activity of the PSVN of the ovarian vascular bed. Thus, this study was conducted to determine the association between ovarian vascular contractility (OVC) and systemic concentrations of P_4, estrone (E_1) and estradiol-17β($E_2\beta$) at two stages of the estrous cycle and during early pregnancy in gilts.

Materials and Methods

Experimental procedures

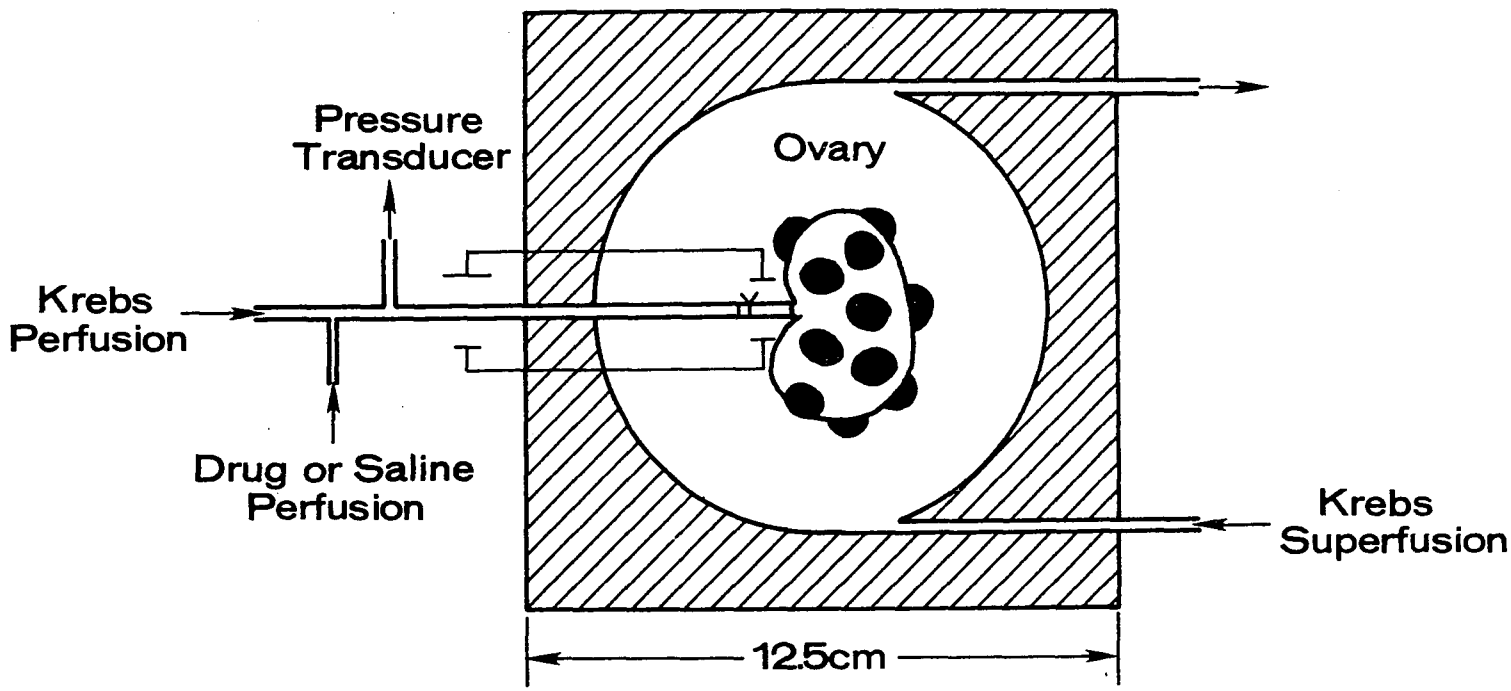
Yorkshire gilts of similar age and weight (10 mo, 150 ± 5 kg) that had exhibited at least 3 estrous cycles of normal duration (20.6 ± 0.3 days) were assigned randomly to be sacrificed during the follicular phase (FP, Days 20 to + 1; n = 5) or luteal phase (LP, Days 11 to 13; n = 4) of the estrous cycle, or on Day 13 of pregnancy (P; n = 5). Gilts in the P group were mated to an intact boar on the first day of estrus. The first day of estrus or mating was designated Day 0. Immediately before sacrifice, a sample of blood was obtained from the anterior vena cava of each gilt. Plasma was frozen at -20°C until assayed for P_4, E_1 and $E_{2\beta}$ by radioimmunoassay. Pregnancy was verified by flushing each uterine horn of P gilts with 0.9% saline to recover conceptus tissue.

One randomly selected ovary with its attached vasculature was excised from each reproductive tract and placed into a container of oxygenated (95% O_2, 5% CO_2), Krebs-Ringer (KR) solution (22°C) of the following millimolar composition: NaCl, 118.1; $NaHCO_3$, 25.0; KCl, 4.7; $CaCl_2 \cdot 6H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; glucose, 11.1. At the laboratory, each ovary was immediately transferred to a container of continuously oxygenated KR solution and prepared for perfusion. The perfusion system was similar to that used for uterine arteries in experiment I and by Ford et al. (1977a) for ovarian arteries, but was modified as follows: For each ovary, one of the 2 to 4 major branches of the ovarian artery (Ginther, 1976) was cannulated as it entered the ovarian hilus, using polyethylene tubing. Ten milliliters of 0.9% saline were infused slowly

into the arterial cannula to flush blood from the vascular bed, followed by 1 ml of Evans Blue dye (750 μg/ml) to visually determine the extent of vascular perfusion. Within 1 hr of collection, each ovary was mounted in a chamber, which had been specially designed for ovarian perfusion; as depicted in Fig. 1. The intraluminal perfusion rate was adjusted during a 60-min equilibration period, by which time all ovaries had established a constant perfusion pressure of ∿80 millimeters of mercury (mm Hg), and this was defined as the baseline perfusion pressure (BPP). The extraluminal superfusion flow rate was 40 ml/min.

At the end of the 60-min equilibration period, intraluminal flow rate and BPP were recorded for each ovary. Each ovary maintained this flow rate and BPP for the duration of perfusion. After equilibration, drugs were perfused for 20 min (perfusion periods 1-3) or 10 min (periods 4-8) into the perfusate at a rate of 0.07 ml/min using a Harvard syringe infusion pump, and were flushed from the perfusion chamber by the superfusion flow. The order of perfusion and final concentration of each drug in the perfusion fluid were: 1) vehicle (0.9% saline); 2) $PGF_{2}\alpha$-tromethamine salt, 1 ng/ml; 3) saline; 4) DL-norepinephrine (NE), 100 ng/ml; 5) phentolamine HCl (PHEN), 100 ng/ml; 6) NE, 100 ng/ml; 7) NE, 100 ng/ml; 8) NE, 100 ng/ml. At the end of each perfusion period, vasoconstriction was induced by transmural electrical stimulation (ES) of the PSVN of the ovarian vascular bed, using bipolar platinum electrodes placed on both sides of the cannulated ovarian artery and separated by 1 cm (Fig. 1). The electrical stimulus was identical to that used in experiment I. Ovarian vascular contractility was quantified as a

Figure 1. Schematic drawing of ovarian perfusion chamber


Pressure
Transducer
Ovary
Krebs
Perfusion
Drug or Saline
Perfusion
Krebs
Superfusion
12.5cm

change in perfusion pressure (mm Hg) in response to ES. After the last perfusion of NE (period 8), KCl was infused into the perfusion fluid at a final concentration of 7.4 mg/ml, and the maximum perfusion pressure attained was recorded. Finally, 1 ml of Evans Blue dye was again infused into the arterial catheter as described above to determine if changes in the extent of ovarian vascular perfusion had occurred.

The rationale for the sequence of drug perfusions and the drugs used is presented below. Responses of the ovarian vascular bed to ES of the PSVN at the end of saline perfusions served as controls with which to compare the effects of $PGF_2\alpha$ and NE on OVC to ES. It was assumed that $PGF_2\alpha$ would enhance vasoconstriction to ES in ovaries from nonpregnant, but not pregnant, sows, as reported previously for uterine arteries of ewes and cows (Ford et al., 1976). Perfusion of NE was used to provide the PSVN with a maximal amount of releasable NE, since sympathetic nerve terminals take up and store exogenous NE *in vitro*, and release it upon ES (Steinsland et al., 1973). The initial perfusion of NE served as a control with which to compare the effects of subsequent perfusions of NE. Phentolamine (a reversible competitive inhibitor of NE for α-adrenergic receptors) was perfused to verify that the effects of ES were mediated by α-adrenergic receptors. Ovaries were subjected to repeated perfusions of NE after PHEN to determine whether displacement of this competitive α-adrenergic antagonist would result in a return of vasoactivity. The final concentration used for each drug (in the perfusion fluid) was based upon preliminary data, and was the maximal

concentration that did not affect BPP. Prostaglandin $F_{2\alpha}$ (and thus the accompanying saline control periods) was perfused for 20 min to maximize its possible effect on OVC in response to ES.

Concentrations of P_4, E_1 and $E_{2\beta}$ in vena cava blood were measured by using radioimmunoassay procedures described for experiment I. For each hormone, all samples were analyzed in a single assay. The intra-assay coefficients of variation were 6.5%, 5.4% and 9.2% for P_4, E_1 and $E_{2\beta}$, respectively.

Statistical analysis

Differences in ovarian vascular contractility between treatment groups were analyzed statistically by split-plot analysis for a completely randomized design, with perfusion periods as the subplot (Kirk, 1968). Data for BPP, flow rates, contractile responses to KCl and steroid hormones were analyzed by analysis of variance. Differences between means were evaluated by using orthogonal contrasts (Kirk, 1968). Correlations between hormone concentrations and OVC at the end of saline perfusion (periods 1 and 3) were calculated for each treatment group.

Results

Although BPP was similar for all treatment groups, the intraluninal flow rates for ovaries from LP and P gilts were greater ($P<0.05$) than for FP gilts (Table 1).

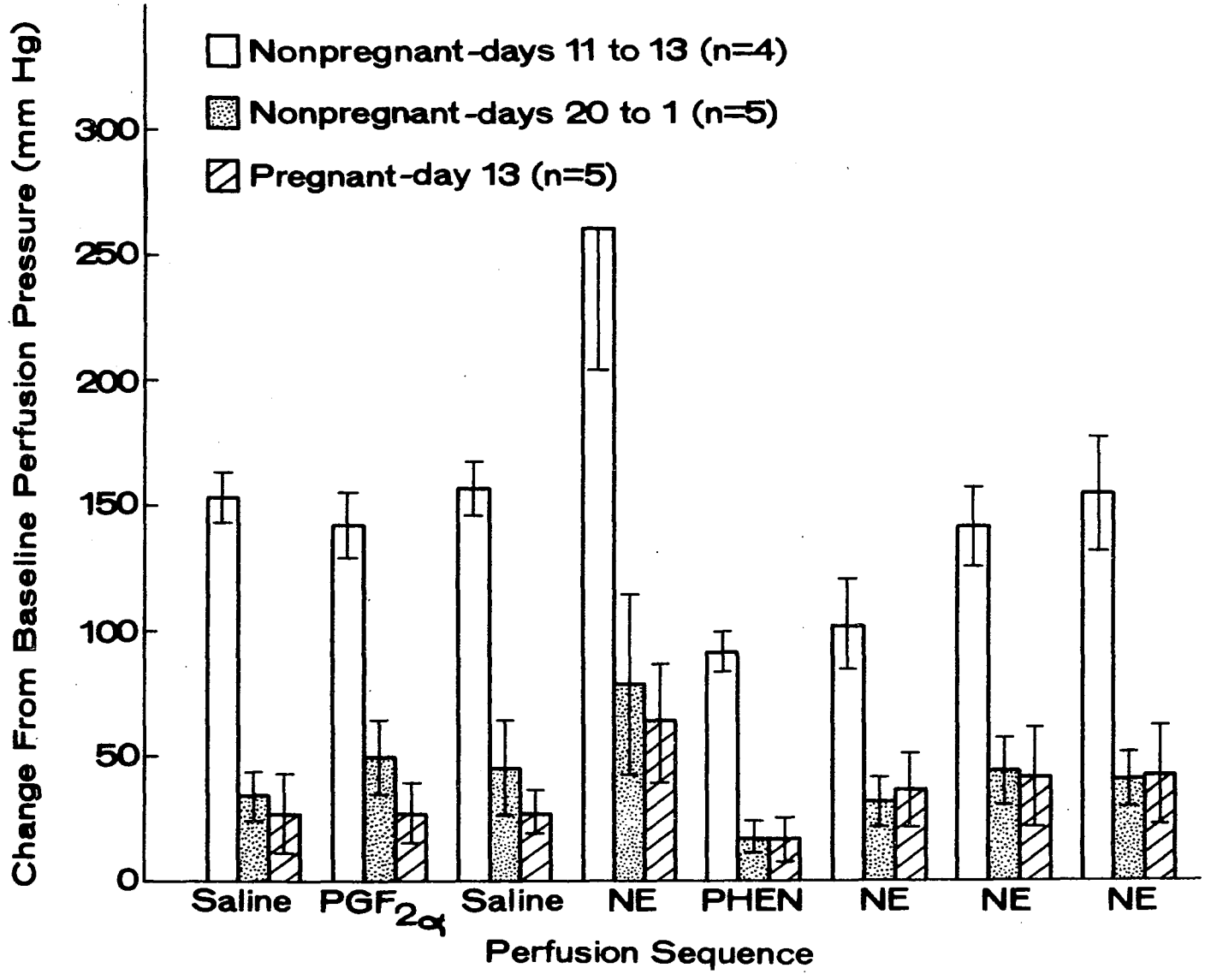
Within a treatment group, OVC in response to ES was similar ($P>0.10$) for periods 1, 2 and 3 (saline, $PGF_{2\alpha}$ and saline, respectively; Fig. 2). Ovarian vascular contractility at the end of periods 1, 2 and 3, however,

Table 1. Baseline perfusion pressures and flow rates for ovaries 1 hr after placement in perfusion chambers

Treatment group	No. of gilts	Baseline perfusion pressure (mm Hg)	Flow rate (ml/min)
Nonpregnant (Days 11 to 13)	4	79 ± 2[a]	7.7 ± 3.7[a]
Nonpregnant (Days 20 to 1)	5	88 ± 3[a]	1.5 ± 0.5[b]
Pregnant (Day 13)	5	84 ± 3[a]	4.2 ± 1.2[a]

[a,b]Means (± SEM) within a column with different superscripts differ ($P<0.05$).

Figure 2. Change in perfusion pressure from baseline (∿ 80 mm Hg) in response to electrical stimulation at the end of each perfusion period for ovaries from gilts. Means ± SEM


Nonpregnant-days 11 to 13 (n=4)
Nonpregnant-days 20 to 1 (n=5)
Pregnant-day 13 (n=5)
Change From Baseline Perfusion Pressure (mm Hg)
300
250
200
150
100
50
0
Saline
PGF2α
Saline
NE
PHEN
NE
NE
NE
Perfusion Sequence

was greater ($P<0.01$) for ovaries from LP gilts than for FP and P gilts (150 ± 6 vs. 43 ± 14 and 27 ± 12 mm Hg, respectively). Although perfusion of NE (period 4) increased ($P<0.05$) OVC in response to ES for all ovaries, OVC was again greater ($P<0.01$) for ovaries from LP gilts compared with FP and P gilts (260 ± 57 vs. 78 ± 36 and 64 ± 24 mm Hg, respectively). Blockade of α-adrenergic receptors by PHEN reduced ($P<0.05$) OVC to ES by approximately 50% for all ovaries when compared to saline perfusions (periods 1 and 3). Repeated perfusions of NE (period 6, 7 and 8) after PHEN resulted in a return of contractility for all ovaries to levels that were not different ($P>0.10$) from those observed after saline perfusions (Fig. 2). The maximum perfusion pressure attained during perfusion of 7.4 mg/ml (10^{-1} M) KCl was greater ($P<0.05$) for ovaries from LP and FP gilts than for P gilts (539 ± 39 and 425 ± 75 vs. 259 ± 59 mm Hg, respectively).

Samples of vena cava blood were not obtained for 2 FP gilts before sacrifice. Although concentrations of E_1 and $E_2\beta$ in vena cava plasma did not differ between treatment groups, concentrations of P_4 were greater ($P<0.01$) for LP and P than for FP gilts (Table 2). This difference in concentrations of P_4 resulted in a greater ($P<0.01$) estrogen (E_1 + $E_2\beta$) to P_4 ratio ($E:P_4$; pg/ml: ng/ml) in systemic blood of FP than LP and P gilts (Table 2).

Ovarian vascular contractility in response to ES at the end of saline perfusions (periods 1 and 3) was positively correlated ($r = +0.90$; $P<0.01$) with concentrations of P_4 and negatively correlated ($r = -0.99$; $P<0.01$) with $E:P_4$ in systemic blood of gilts during the estrous cycle

Table 2. Concentrations of steroids and ratio of estrogen: progesterone in vena cava plasma

Treatment group	No. of gilts	P_4 (ng/ml)	E_1 (pg/ml)	$E_2\beta$ (pg/ml)	$E:P_4$[1]
Nonpregnant (Days 11 to 13)	4	18.98 ± 1.76[a]	15.0 ± 4.0[a]	8.0 ± 0.6[a]	1.2 ± 0.2[a]
Nonpregnant (Days 20 to 1)	3	1.02 ± 0.16[b]	14.0 ± 2.5[a]	7.3 ± 0.5[a]	21.7 ± 3.4[b]
Pregnant (Day 13)	5	19.79 ± 0.07[a]	15.9 ± 3.0[a]	12.8 ± 1.4[a]	1.4 ± 0.2[a]

[1]$E_1 + E_2\beta$ (pg/ml)/P_4 (ng/ml).

[a,b]Means (± SEM) within a column with different superscripts differ ($P<0.01$).

(Table 3). However, during early pregnancy, significant correlations between steroid concentrations in systemic blood and OVC were no longer observed.

Discussion

These data demonstrate that OVC is greatest during the luteal phase of the estrous cycle and is highly correlated ($P<0.01$) with P_4 concentrations in systemic blood. This is in agreement with previous observations that exposure to P_4 augmented *in vitro* constriction of ovarian (Kuhl et al., 1974) and uterine (Ford et al., 1977b,c) arteries to nerve stimulation. Progesterone may enhance vasoconstriction by directly affecting the activity of PSVN. In experiment I, it was observed that uterine arteries of LP gilts exhibited greater *in vitro* contractility to ES, in association with greater numbers of α-adrenergic receptors, than uterine arteries from estrous gilts.

In contrast, OVC was negatively correlated ($P<0.01$) with $E:P_4$ in systemic blood, being lowest during the follicular phase. As mentioned above, the reduced *in vitro* contractility of uterine arteries from FP gilts when compared to LP gilts was associated with reduced arterial concentrations of α-adrenergic receptors. Estrogens may have a direct effect on PSVN terminals since estradiol has been shown to reduce the content of NE in uterine PSVN of ovariectomized rats coincident with increased uterine blood flow (McKercher et al., 1973). Thus, the ratio of $E:P_4$ in systemic blood appears to regulate vasoconstriction, and therefore blood flow, to the uterus and ovaries during the estrous cycle

Table 3. Association between ovarian vascular contractility at the end of saline perfusions[a] and concentrations of steroids in vena cava plasma[b]

	Estrous cycle[c]	Early pregnancy
Progesterone	+ 0.90**	- 0.83
Estrone	- 0.08	+ 0.01
Estradiol	+ 0.38	+ 0.05
$E:P_4$[d]	- 0.99**	+ 0.39

[a]Average of perfusion periods 1 and 3.

[b]Correlation coefficients.

[c]LP + FP gilts.

[d]$E_1 + E_{2\beta}$ (pg/ml)/P_4 (ng/ml).

**($P<0.01$).

by altering the quantity of neurotransmitter to which the vascular smooth muscle is exposed, as well as the number of α-adrenergic receptors on smooth muscle cells.

Intraluminal flow rates were higher for ovaries from LP and P than FP gilts even though BPP were similar (Table 1). This probably reflects the higher rates of OBF observed for gilts during the luteal phase of the estrous cycle and early pregnancy compared with gilts at estrus (Magness et al., 1983). Ovarian blood flow is highly correlated with P_4 concentrations in systemic blood throughout the estrous cycle of the ewe (Brown et al., 1980), cow (Ford and Chenault, 1981) and sow (Magness et al., 1983). Since blood flow to the CL represents 90% of OBF in the sow (Ford et al., 1982c), the positive correlation between OBF and systemic P_4 may simply reflect the large increase in the size of the ovarian vascular bed during the luteal phase of the estrous cycle. After luteolysis, the size of the ovarian vascular bed is tremendously reduced, which may explain the apparent negative association between estrogens and OBF (Magness et al., 1983) even though OVC is lowest during the follicular phase.

On Day 13 of pregnancy, which corresponds to maternal recognition of pregnancy for this species (Dhindsa and Dziuk, 1968; Ford et al., 1982a), OVC was reduced, which may explain the 60% increase in luteal blood flow previously observed on the same day of pregnancy (Ford et al., 1982c). Since a reduction in blood flow to the CL may be a primary event during natural and $PGF_2\alpha$-induced luteolysis (Niswender et al., 1976; Nett and Niswender, 1981), this increase in luteal blood flow

could be part of the embryonic antiluteolytic/luteotropic signal. In support of this proposal, Magness et al. (1983) observed a transient increase in systemic P_4 concentrations on Days 13 and 14 of pregnancy in the pig. Since OVC of Day 13 pregnant gilts was not significantly correlated with systemic concentrations of ovarian steroids, local production of a vasodilatory substance by the gravid uterus is suggested. Estrogens (E_1 and $E_2\beta$) are produced by porcine conceptuses, and are secreted from the gravid uterus as early as Day 11 postmating (Perry et al., 1976; Ford et al., 1982a; Magness and Ford, 1982). In addition, infusion of $E_2\beta$ into an isolated uterine horn on Days 11 to 15 postestrus was able to preferentially stimulate P_4 secretion by CL on the side of infusion (Ford et al., 1982b). Thus, a conceptus-associated vasodilatory substance, possibly estrogen, appears to act locally to reduce OVC, which leads to an increase in OBF and ovarian P_4 secretion during early pregnancy.

An interesting observation was that ovaries from Day 13 pregnant gilts had a lower response to KCl than ovaries from gilts during either stage of the estrous cycle. At the dose infused in this study, KCl causes a nonspecific depolarization of smooth muscle membranes and should induce maximal vasoconstriction (Sparks, 1980). Ovaries from P gilts appear to have a reduced capacity for vasoconstriction, suggesting an effect of the embryonic vasodilatory factor(s) on the contractile components of ovarian vascular smooth muscle, independent of effects on the PSVN. In support of this concept, no differences in α-receptor numbers or NE content of uterine arteries from Day 13 pregnant or

nonpregnant gilts were observed in experiment I, even though *in vitro* contractility was markedly reduced on Day 13 of pregnancy.

The lack of effect of $PGF_{2}\alpha$ on OVC in this study was unexpected since the same dose of $PGF_{2}\alpha$ augmented contractility of uterine (Ford et al., 1976) and ovarian (Ford et al., 1977a) arteries *in vitro*. However, $PGF_{2}\alpha$ has been shown to dilate the ovarian stromal vascular bed (Janson et al., 1975; Varga and Folly, 1977). Since all cannulated ovarian arteries in the present study perfused both luteal and nonluteal tissue, it is possible that any vasoconstrictor effect of $PGF_{2}\alpha$ was offset by concomitant stromal vasodilation. The observation that each artery perfused a large proportion of the ovary is consistent with reports describing the complex network of arteries within the porcine ovary (Andersen, 1926; Nunez and Getty, 1969).

EXPERIMENT III. UTERINE BLOOD FLOW DURING EARLY PREGNANCY IN EWES: INTERACTION BETWEEN THE CONCEPTUS AND THE CORPUS LUTEUM-BEARING OVARY

Introduction

The embryonic "signal" that initiates luteal maintenance occurs on about Day 12 postmating in the ewe and sow (Moor and Rowson, 1966; Dhindsa and Dziuk, 1968) and Day 16 postmating in the cow (Northey and French, 1980). Coincident with pregnancy recognition, a transient increase in blood flow (BF) is observed only for the uterine horn containing the conceptus(es) in cows (Ford et al., 1979), and unilaterally pregnant sows (Ford and Christenson, 1979). This increase in BF to the gravid uterine horn is associated with elevated estradiol-17β ($E_2\beta$) levels in uterine luminal fluid and venous blood of both species (Ford et al., 1981). Administration of $E_2\beta$ induces uterine vasodilation in cows (Roman-Ponce et al., 1978) and sows (Van Orden et al., 1983b).

Estradiol-17β also stimulates increased uterine BF in ewes (Killam et al., 1973). Production of estrogens by ovine conceptuses during early pregnancy could not be demonstrated (Gadsby et al., 1980). However, $E_2\beta$ levels in the uterine lumen and uterine arterial (UA) and uterine venous (UV) blood have not been evaluated.

For ewes on Day 15 postmating, Ford et al. (1976) observed a diminished vasoconstrictor response of uterine arteries ipsilateral but not contralateral to the corpus luteum (CL)-bearing ovary, suggesting a local influence of the CL on mediating conceptus-induced uterine vasodilation. Greiss and Anderson (1970b) observed a transient increase

in uterine BF on Days 13 to 15 postmating for some of the ewes in their study. Others, however, have been unable to demonstrate this transient increase in uterine BF (Fleet and Heap, 1982). These variable results may have been obtained because BF was only measured for one uterine horn, and was not evaluated with respect to the location of the CL-bearing ovary or conceptus.

This study was therefore conducted to determine the influence of the conceptus and the CL-bearing ovary on BF to each uterine horn, and to determine UA, UV and uterine luminal concentrations of $E_2\beta$ before, during and after maternal recognition of pregnancy in the ewe.

Materials and Methods

Experimental procedures

Multiparous crossbred ewes were checked for estrus twice daily (0730 hr and 1730 hr) using a vasectomized ram, and only ewes exhibiting estrous cycles of normal duration (15-18 days) were used. Twenty ewes (Group 1) were assigned randomly, in equal numbers, to surgery on Day 9, 11, 13 or 15 postestrus (day of estrus = Day 0). Feed and water were withheld from ewes for 24 hr before surgery. At surgery, a catheter was inserted into a jugular vein and general anesthesia was induced by infusion of pentobarbital sodium (26 mg/kg; Fort Dodge Labs., Inc., Fort Dodge, IA). Surgical anesthesia was maintained by supplemental infusion of pentobarbital (32-65 mg) as necessary. The uterus and ovaries were exposed through a midventral incision and the location of CL was recorded. An electromagnetic flow transducer was implanted around the

middle uterine artery supplying each uterine horn, as previously described (Ford and Christenson, 1979). Blood flow (ml/min) for each uterine horn was recorded at 15-sec intervals throughout a 10-min period and the average of these values taken as an estimate of BF for that uterine horn. After BF measurements were taken, the electromagnetic transducer was removed from the artery. A polyvinyl catheter was then placed in a branch of the uterine artery and a branch of the uterine vein supplying and draining each uterine horn. Simultaneous samples of UA and UV blood were obtained from each horn, and plasma was stored at -20°C. After surgery, ewes in Group 1 were checked for estrus twice daily throughout the experiment. These ewes were allowed 1 intervening estrous cycle and were then mated to an intact ram at their second post-surgical estrus. Each ewe received surgery on the same day of pregnancy as during her previous nonpregnant treatment cycle. Surgical procedures used to obtain uterine BF measurements and UA and UV blood samples were identical to those described for the nonpregnant cycle. After collection of UA and UV blood samples, each uterine horn was isolated with a tissue clamp at its junction with the uterine body and flushed with 5.0 ml of sterile 0.9% saline to obtain conceptuses and uterine flushings. Conceptus tissue was recovered from 17 of 20 mated ewes. Because of the fragile nature of the tissue, conceptuses were not separated from uterine flushings, but were dispersed by repeatedly filling a syringe and forcing the uterine flushings through a 20-gauge hypodermic needle. Uterine flushings were stored at -20°C. Samples and BF recordings obtained from the 3 mated ewes (one Day 9 and two Day 11) from which no conceptus tissue was

recovered were discarded. Samples of UA and UV blood were not obtained from 1 ewe on Day 9 of pregnancy.

To avoid the possibility of uterine infection and maximize conception rates, uterine flushings were not obtained from the 20 ewes in Group 1 at their nonpregnant treatment surgery. A second group of 20 ewes (Group 2) was therefore assigned randomly, in equal numbers, to surgery on Day 9, 11, 13 or 15 postestrus. Procedures for induction and maintenance of surgical anesthesia were identical to those described above. The uterus and ovaries were exposed through a midventral incision. A uterine horn ipsilateral to a CL-bearing ovary was then flushed, in a manner identical to that described for mated ewes in Group 1, and flushings were stored at -20°C. No further data were collected from these ewes.

Two milliliters of UA or UV plasma were extracted, chromatographed and assayed for $E_2\beta$ exactly as previously described and validated for porcine and bovine plasma in this laboratory (Magness and Ford, 1982; Ferrell et al., 1983). The sensitivity of the assay, defined as the $E_2\beta$ standard that yielded 95% of the counts/min in the buffer control tubes, was ≈ 2 pg. The precision and accuracy of this procedure were evaluated by adding 2.5, 5.0, 12.5 and 25.0 pg of $E_2\beta$ to a pool of plasma from ovariectomized ewes on a pg/ml basis. Each standard plasma was assayed four times and the $E_2\beta$ concentration of the plasma blank was subtracted. The resulting $E_2\beta$ concentrations (pg/ml ± SEM) were 2.8 ± 0.4, 4.6 ± 0.4, 12.7 ± 0.6 and 26.9 ± 0.7. Within-assay variability was determined by assaying replicates (n = 5) of a pool of systemic plasma from ewes in one assay. The resulting concentration (± SEM)

of $E_2\beta$ was 4.2 ± 0.1 pg/ml (coefficient of variation, CV = 6.8%). Between-assay variability was determined by assaying a pool of plasma from ovariectomized ewes, to which $E_2\beta$ (20.0 pg/ml) had been added, in each assay (n = 7). The resulting concentration (± SEM, after subtraction of the plasma blank) was 21.6 ± 1.1 pg/ml (CV = 13.5%).

Two milliliters of each uterine flushing of pregnant (P; Group 1) and nonpregnant (NP; Group 2) ewes were extracted, chromatographed and assayed for $E_2\beta$ by the same procedure used for plasma. Precision and accuracy were evaluated by adding 10.0, 25.0, 50.0 and 100.0 pg of $E_2\beta$ to a uterine flushing pool on a pg/ml basis. Each standard flushing was assayed four times and the $E_2\beta$ concentration of the flushing blank substracted. The resulting $E_2\beta$ concentrations (pg/ml ± SEM) were 11.5 ± 0.1, 25.2 ± 0.8, 49.0 ± 1.3 and 106.4 ± 2.4. Within-assay variability was determined by assaying replicates (n = 4) of a uterine flushing pool in one assay, and between-assay variability was determined by assaying the same flushing pool in each assay (n = 3). The resulting concentrations (± SEM) and CV for within- and between-assay variability were 10.2 ± 0.3 pg/ml (CV = 5.8%) and 10.0 ± 0.6 pg/ml (CV = 10.4%), respectively. The concentration of $E_2\beta$ in each flushing was adjusted from pg/ml of flushing to pg/uterine horn.

The concentration of P_4 in UA plasma of ewes was determined using a procedure previously reported and validated in this laboratory for porcine and bovine plasma (Magness and Ford, 1982; Ferrell et al., 1983). Sensitivity of the assay was defined as the amount of P_4 standard that yielded 95% of the counts/min in the buffer control tubes and ranged

from 50 to 80 pg. The precision and accuracy of the procedure were evaluated by adding 0.50, 1.00, 2.50, 5.00 and 10.00 ng of P_4 to a pool of plasma from ovariectomized ewes on a ng/ml basis. Each standard plasma was assayed four times and the P_4 concentration of the plasma blank was subtracted. The resulting P_4 concentrations (ng/ml ± SEM) were 0.67 ± 0.44, 1.30 ± 0.54, 3.10 ± 0.80, 6.27 ± 0.21 and 10.97 ± 0.21. Within-assay variability was determined by assaying replicates (n = 4) of the plasma pool from ovariectomized ewes in one assay. The resulting concentration (± SEM) was 0.29 ± 0.01 ng/ml (CV = 7.0%). Between-assay variability was determined by assaying the same plasma pool, to which P_4 (10.00 ng/ml) had been added, in each assay (n = 3). The resulting concentration (± SEM) and CV were 9.15 ± 0.25 ng/ml and 3.9%.

Statistical analysis

Data for UBF, and UA and UV concentrations of steroid hormones for ewes during the estrous cycle and early pregnancy (Group 1) were analyzed by split-plot analysis of variance (Kirk, 1968). Differences between means were evaluated using orthogonal contrasts (Kirk, 1968). Content of $E_2\beta$ in uterine flushings of P (Group 1) and NP (Group 2) ewes was compared using a combined analysis of variance with conservative degrees of freedom (Cochran and Cox, 1957). All data are reported as the mean ± SEM.

Results

There was no effect of surgery on estrous cycle lengths of ewes in Group 1. Pre-surgical cycles averaged 16.2 ± 0.1 days, whereas

surgical and post-surgical estrous cycle lengths averaged 16.3 ± 0.1 days.

During the estrous cycle of ewes in Group 1, BF (ml/min) was not different between uterine horns contralateral or ipsilateral to a CL-bearing ovary, and did not differ across the days measured, averaging 6.5 ± 0.4 (Table 1). During the subsequent pregnancy, BF to uterine horns contralateral to a CL-bearing ovary on all days measured, and BF to uterine horns ipsilateral to a CL-bearing ovary on Day 9, was not different from BF to either uterine horn during the estrous cycle, averaging 6.8 ± 0.6. However, on Days 11, 13 and 15 of pregnancy, BF to uterine horns ipsilateral to CL-bearing ovaries averaged 13.3 ± 0.9 and was elevated ($P<0.01$) by twofold compared with BF of ipsilateral uterine horns on Day 9 or BF of contralateral uterine horns on any of the days studied (Table 1).

This elevation in BF to ipsilateral uterine horns on Days 11, 13 and 15 was observed regardless of whether embryos were found ipsilateral or contralateral to CL-bearing ovaries. Four ewes on each of Days 13 and 15 postmating had 2 CL, and in all cases an embryo was present in each uterine horn. For those ewes with both CL located on the same ovary ($n = 6$), BF was only elevated ($P<0.01$) for the ipsilateral and not the contralateral uterine horn (13.9 ± 1.2 vs. 5.8 ± 0.8). When 1 CL was located on each ovary (2 ewes), BF was not different between uterine horns and averaged 8.5 ± 1.2.

Concentrations of $E_2\beta$ were similar in UA and UV plasma (Table 2) and were not different between uterine horns contralateral or ipsilateral

Table 1. Blood flow (ml/min) for uterine horns contralateral or ipsilateral to a corpus luteum-bearing ovary during the estrous cycle and early pregnancy of ewes[1]

Day postestrus or postmating[2]	Estrous cycle		Early pregnancy	
	Contralateral	Ipsilateral	Contralateral	Ipsilateral
9	6.9 ± 1.6(4)[3,a]	6.8 ± 1.0(6)[a]	6.4 ± 2.6(2)[a]	6.8 ± 1.3(6)[a]
11	4.5 ± 0.5(5)[a]	7.5 ± 1.8(5)[a]	5.0 ± 0.4(3)[a]	14.6 ± 1.0(3)[b]
13	5.4 ± 2.1(3)[a]	6.3 ± 1.0(7)[a]	7.4 ± 0.6(4)[a]	12.5 ± 1.4(6)[b]
15	5.6 ± 1.1(5)[a]	8.6 ± 0.9(5)[a]	7.5 ± 1.8(4)[a]	13.5 ± 1.5(6)[b]

[1]Means ± SEM.

[2]First day of estrus or mating = Day 0.

[3]Numbers in parenthesis = no. uterine horns.

[a,b]Means with different superscripts differ ($P<0.01$).

Table 2. Concentrations (pg/ml) of estradiol-17β in uterine arterial and venous plasma during the estrous cycle and early pregnancy of ewes[1]

Days postestrus or postmating[2]	Estrous cycle			Early pregnancy		
	No. of ewes	Uterine artery	Uterine vein	No. of ewes	Uterine artery	Uterine vein
9	5	4.2 ± 0.4[a]	4.5 ± 0.5[a]	3	5.2 ± 0.5[a]	5.8 ± 1.2[a]
11	5	7.6 ± 0.3[b]	8.0 ± 0.3[b]	3	7.4 ± 0.3[b]	6.9 ± 0.5[b]
13	5	8.6 ± 0.6[c]	9.4 ± 0.6[c]	5	8.9 ± 0.5[c]	8.7 ± 0.4[c]
15	5	9.1 ± 0.6[c]	9.1 ± 0.6[c]	5	8.9 ± 0.5[c]	8.6 ± 0.5[c]

[1]Means ± SEM.

[2]First day of estrus or mating = Day 0.

[a,b,c]Means with different superscripts differ ($P<0.05$).

to CL-bearing ovaries. In addition, the magnitudes and patterns of $E_2\beta$ in plasma were similar during the estrous cycle and early pregnancy (Table 2). Concentrations of $E_2\beta$ increased ($P<0.01$) from Day 9 to Day 11, increased further ($P<0.05$) from Day 11 to Day 13, then remained constant to Day 15 postestrus or postmating. During the estrous cycle, concentrations of P_4 in UA plasma were similar on Days 9, 11 and 13, then declined ($P<0.01$) by Day 15 (Table 3). Concentrations of P_4 in UA plasma on Days 9 and 11 of the subsequent pregnancy were not different from those observed on Days 9 and 11 of the estrous cycle. However, on Days 13 and 15 of pregnancy the levels of P_4 in UA plasma were elevated ($P<0.05$) compared to Days 9 and 11, and were greater ($P<0.01$) than levels observed on Days 13 and 15 of the estrous cycle (Table 3).

For the content of $E_2\beta$ in uterine flushings, there was no effect of day and no day x status (NP vs. P) interaction. Content of $E_2\beta$ in uterine flushings of P ewes did not differ between uterine horns, and was greater ($P<0.05$) than levels of $E_2\beta$ in flushings of NP ewes (35.2 ± 2.1 vs. 16.7 ± 2.4 pg/uterine horn).

Discussion

In this study, blood flow to uterine horns ipsilateral to CL-bearing ovaries exhibited a twofold increase by Day 11 postmating, which is approximately 24 hr before maternal recognition of pregnancy (Moor and Rowson, 1966) and is similar to the twofold to threefold increase in uterine BF observed at the time of pregnancy recognition in sows (Ford and Christenson, 1979) and cows (Ford et al., 1979). Mapletoft et al. (1976) demonstrated that the luteotropic effect of the ovine conceptus

Table 3. Concentrations (ng/ml) of progesterone in uterine arterial plasma during the estrous cycle and early pregnancy of ewes[1]

Day postestrus or postmating[2]	Estrous cycle		Early pregnancy	
	No. of ewes	Progesterone concentration	No. of ewes	Progesterone concentration
9	5	3.84 ± 0.21[a]	3	4.63 ± 0.22[a]
11	5	3.53 ± 0.29[a]	3	4.76 ± 0.37[a]
13	5	4.10 ± 0.78[a]	5	6.06 ± 0.52[b]
15	5	0.62 ± 0.16[c]	5	5.09 ± 0.64[a,b]

[1]Means ± SEM.

[2]First day of estrus or mating = Day 0.

[a,b,c] Means with different superscripts differ ($P<0.05$).

was exerted through a local vascular pathway from the gravid uterus to the ovary. Thus, the increase in uterine BF at or just before maternal recognition of pregnancy may function to enhance transport of a conceptus-induced luteotropin to the CL-bearing ovary. This concept is supported by the observation that P_4 levels in UA plasma were elevated in P compared to NP ewes by Day 13. A similar increase in systemic P_4 levels coincident with maternal recognition of pregnancy has been reported for sows (Magness et al., 1983) and cows (Ford et al., 1979).

The content of $E_2\beta$ in the uterine lumen of P ewes was elevated by Day 9, which was before the increase in uterine BF was observed. Estrogens stimulate increased uterine BF in the sow, cow and ewe (see Ford, 1982 for review). Estrogens in the uterine lumen may, therefore, be responsible for the increased uterine BF observed during early pregnancy. Although no venous-arterial difference in $E_2\beta$ was observed in the present study, that does not eliminate the possibility that estrogens may be transported from the gravid uterus by another route. Magness and Ford (1982) reported that concentrations of estrone and $E_2\beta$ were greater in lymph draining uterine horns of pregnant gilts than in non-pregnant gilts. In addition, estrogens may be leaving the gravid uterus in a form other than $E_2\beta$. Willis et al. (1981) found that cultured ovine conceptuses were able to synthesize conjugated estrogens from C-19 and C-21 precursors as early as Day 14 postmating. The endometrium may be an additional source of estrogens since these authors also demonstrated greater estrogen synthesis <u>in vitro</u> when ovine conceptuses were co-cultured with endometrium.

Blood flow to uterine horns of P ewes was only elevated ipsilateral to CL-bearing ovaries, regardless of location of the conceptus and even though $E_2\beta$ content was similar between uterine horns. Pope et al. (1982) observed greater concentrations of P_4 in uterine arterial tissue of cows on the side ipsilateral to the CL-bearing ovary compared with the contralateral side. In addition, exposure to P_4 has been shown to augment the contractility of vascular smooth muscle (Kalsner, 1969; Ford et al., 1977c). Ford et al. (1976) observed that uterine arterial segments removed ipsilateral to the CL-bearing ovary of nonpregnant ewes or cows exhibited greater vasoconstriction to sympathetic nerve stimulation than arteries from the contralateral side, even though BF was similar for uterine horns ipsilateral or contralateral to the CL-bearing ovary of nonpregnant ewes (present study) or cows (Ford et al., 1979). During early pregnancy, the vasoconstrictor response to nerve stimulation was reduced only for uterine arteries ipsilateral to a CL-bearing ovary (Ford et al., 1976). In addition, these researchers observed that intraluminal perfusion of a conceptus brei was able to reduce vasoconstrictor activity only for ipsilateral uterine arteries. Thus, only uterine arteries that had been exposed to elevated local concentrations of P_4, and therefore had a high level of sympathetic tone, were able to respond to the conceptus-induced vasodilatory factor(s). The increase in BF to uterine horns ipsilateral to the CL-bearing ovary of P ewes in this study is similar to the increase in uterine BF previously observed for sows (Ford and Christenson, 1979) and cows (Ford et al., 1979) during maternal recognition of pregnancy. As discussed previously,

this local increase in uterine BF may function to transport a luteotropic substance from the gravid uterus to CL-bearing ovaries and thus ensure the maintenance of luteal function.

EXPERIMENT IV. EFFECTS OF INTRAUTERINE INFUSION OF ESTRADIOL-17β AND PROSTAGLANDIN E_2 ON LUTEAL FUNCTION IN NONPREGNANT HEIFERS

Introduction

The embryonic "signal" that results in the maintenance of luteal function occurs on about Day 16 postmating in the cow (Northey and French, 1980) and Day 12 postmating in the ewe (Moor and Rowson, 1966) and sow (Dhindsa and Dziuk, 1968; Ford et al., 1982a). However, the identity of the molecule(s) that comprises this embryonic signal remains unknown. The bovine conceptus is capable of synthesizing estradiol-17β ($E_2\beta$) *in vitro* by Day 13 postmating (Shemesh et al., 1979; Chenault, 1980), while porcine conceptuses can produce estrone and $E_2\beta$ by Day 12 (Perry et al., 1976). In addition, estrogens are elevated in uterine flushings and venous blood of cows (Ford et al., 1981) and sows (Ford et al., 1982a) at the time of maternal recognition of pregnancy for each species. Intrauterine infusion of near physiological concentrations of $E_2\beta$ results in an extension of the functional lifespan of corpora lutea (CL) in the sow (Ford et al., 1982b). Prostaglandin (PG) E_2 is produced by cultured bovine blastocysts as early as Day 13 postmating (Shemesh et al., 1979; Lewis et al., 1982), and by ovine blastocysts by Day 14 (Lacroix and Kann, 1982; Hyland et al., 1982). Uterine luminal and venous concentrations of PGE_2 have been shown to be elevated on Day 15 in pregnant ewes (Ellinwood, et al., 1979). In addition, infusion of PGE_2 into the uterus of nonpregnant ewes prolongs the functional lifespan of the CL (Magness et al., 1981).

The purpose of this experiment was to determine the effects of intrauterine infusion of $E_2\beta$, PGE_2 or both hormones on the function of CL in nonpregnant heifers.

Materials and Methods

Experimental procedures

Sixteen nonpregnant Angus or Hereford x Angus heifers exhibiting estrous cycles of normal duration (18-23 days) and of similar age and weight (1½-2 yrs; 320-360 kg) were used. Heifers were checked for estrus activity twice daily throughout the experiment (0730 hr and 1630 hr) using a vasectomized bull, and were trained to stanchions at least 1 month before surgery to adjust them to handling and confinement. Feed and water were removed from heifers 24 hr before surgery, which was performed on Day 9, 10 or 11 postestrus (day of estrus = Day 0). Induction and maintenance of general anesthesia were as described by Ford et al. (1979). The uterus and ovaries were exposed through a midventral incision, and the size and location of ovarian structures were recorded. A catheter was inserted into the lumen of the uterine horn ipsilateral to the corpus luteum-bearing ovary, through a small incision at the tip of the horn. The intrauterine catheter was similar to one previously used in this laboratory (Ford et al., 1982b). The catheter had a 15-cm silastic tubing tip (O.D. = 1.65 mm) that was sealed at the end and perforated at 3-cm intervals to ensure delivery of hormones to the entire uterine horn (simulating hormone delivery by an elongated blastocyst). The

catheter was exteriorized through a small flank incision and maintained in a cloth pouch glued to the flank area.

After surgery, heifers were assigned randomly, in equal numbers, to receive intrauterine infusions of vehicle (VEH) consisting of 2.0% ethanol in sterile 0.9% saline plus 1.0% combiotic (Pfizer, Inc., New York, NY; v/v), $E_2\beta$ (150.0 ng), PGE_2 (250.0 μg) or $E_2\beta$ + PGE_2 every 6 hr from 1200 hr on Day 13 to 0600 hr on the day of subsequent estrus if it occurred before Day 21 or 0600 hr on Day 21. Each intrauterine infusion consisted of 0.8 ml VEH or hormone solution, followed by a 0.8-ml VEH flush (volume of catheter = 0.8 ml). A stock solution of PGE_2 in ethanol was stored at -20°C, and solutions for intrauterine infusion were prepared daily (0600 hr) in a sterile vial. The daily doses of hormones were derived by multiplying the maximal venous-arterial difference in $E_2\beta$ (Ford et al., 1981) across a gravid uterine horn times daily uterine arterial blood flow (Ford et al., 1979), for cows on Days 14-18 of pregnancy. The daily dose of PGE_2 was chosen to be one-tenth the effective dose for consistently maintaining luteal function in beef cows (J. R. Chenault, Upjohn Co., personal communication).

For each heifer, a sample of jugular blood was obtained by venipuncture once daily from Day 13 to Day 21 postestrus. Plasma was obtained and stored at -20°C until assayed for progesterone (P_4). All heifers receiving PGE_2 treatment plus 1 animal from the remaining 2 treatment groups (VEH and $E_2\beta$) were sacrificed on Day 21 (0800 hr - 1000 hr), and the uterus and ovaries were obtained. To minimize expense, the remaining 6 heifers were ovariectomized by midventral laparotomy between

0800 hr and 1000 hr on Day 21. Placement of intrauterine catheters was verified at sacrifice or laparotomy.

Radioimmunoassay for P_4 was identical to that previously reported and validated for cow plasma in this laboratory (Ferrell et al., 1983). Two hundred µl of plasma were extracted in triplicate using benzene:hexane (1:2;v/v) with one of the replicates receiving 12,000-15,000 dpm of 1,2,6, 7-[^{3}H] (N)-P_4 (97.0 ci/mmole, New England Nuclear Corp., Boston, MA) to serve as the individual recovery for that set of duplicates. Recovery of ^{3}H-P_4 averaged 86.5 ± 0.3% (SEM) and all values were corrected for procedural losses. Plasma extracts were assayed for P_4 using a fully characterized antibody (GDN-337; Niswender, 1973). Sensitivity of the assay was defined as the amount of P_4 standard that yielded 95% of the counts/min in the buffer control tubes and ranged from 50 to 80 pg. With this method, mean blank value for plasma from an ovariectomized cow was 0.21 ± 0.02 ng/ml (SEM, n = 10). The precision and accuracy of the procedure were evaluated by adding 0.10 (n = 4), 0.25 (n = 4), 1.00 (n = 4), 2.50 (n = 4), 5.00 (n = 4), and 10.00 (n = 4) ng P_4 to plasma from the same ovariectomized cow on a ng/ml basis. These standard plasmas were assayed, and the P_4 concentration of the plasma blank was subtracted. The resulting P_4 concentrations (± SEM) were 0.15 ± 0.01, 0.30 ± 0.01, 1.17 ± 0.03, 2.55 ± 0.09, 4.87 ± 0.17, and 10.81 ± 0.52 ng/ml, respectively. Within-assay variability was determined from replicates (n = 10) of a plasma pool from luteal-phase cows. The resulting concentration (± SEM) was 13.97 ± 0.21 ng/ml, and coefficient of variation (CV) was 4.9%. All plasma samples were assayed in a single assay.

Corpora lutea were homogenized in 5.0 ml 0.9% saline using a Polytron (Brinkmann Instruments, Inc., Westbury, NY), and the resulting homogenate was diluted with saline to a final volume of 15.0 ml. Aliquots of these homogenates were diluted 1:4 and 1:400 (v/v) with sterile bottled H_2O (Eli Lilly and Co., Indianapolis, IN), and 50-µl aliquots of these diluted homogenates were extracted and assayed as described for plasma. Mean recovery (± SEM) of ^3H-P_4 was 87.9 ± 0.3%, and all values were corrected for procedural losses. The mean blank value for a homogenate of a corpus albicans from a cow on Day 4 postestrus was 0.79 ± 0.04 ng/ml (SEM, n = 8). The precision and accuracy were evaluated by adding 1.00 (n = 6), 5.00 (n = 6), 10.00 (n = 6), and 20.00 (n = 6) ng P_4 to the same luteal homogenate on a ng/ml basis. Homogenate plus standards were assayed, and the resulting P_4 concentrations (after subtaction of the homogenate blank) were 0.97 ± 0.08, 6.17 ± 0.10, 12.27 ± 0.21 and 22.78 ± 0.27 ng/ml, respectively. Within-assay variability was determined from replicates (n = 8) of a luteal homogenate from a luteal-phase cow. The resulting concentration (± SEM) was 12.92 ± 0.23 ng/ml (CV = 5.1%). All CL were assayed in a single assay. The concentration of P_4 in each sample was adjusted from ng/ml of homogenate to µg/CL and µg/g of luteal tissue.

Statistical analysis

Changes in jugular venous P_4 concentrations were analyzed by split-plot analysis of variance for repeated measures (Kirk, 1968). Content and concentration of P_4 in CL, as well as diameters and weights of CL on Day 21 postestrus were analyzed by factorial analysis of variance. Differences between means were evaluated using orthogonal contrasts (Kirk,

1968). Differences between treatment groups in the proportion of heifers exhibiting estrus by Day 21 were analyzed by a Chi-Square test, and the lengths of the pretreatment and treatment cycles were compared using a paired t-test (Steel and Torrie, 1960). All data are reported as the mean ± SEM.

Results

All intrauterine catheters were still in place on Day 21 postestrus. For heifers from which uteri were obtained, no infection or inflammation of the endometrium was apparent by gross observation, and the treated and nontreated horns were similar in appearance.

For the VEH-, $E_2\beta$- and PGE_2-treated heifers, 3 of 4, 3 of 4 and 4 of 4 were observed in standing estrus by Day 21, respectively. For heifers exhibiting estrus, there was no difference ($P>0.10$) between the lengths of the pretreatment and treatment cycles (20.3 ± 0.4 vs. 19.2 ± 0.4 days). The proportion of heifers in the $E_2\beta$ + PGE_2 group that exhibited estrus by Day 21 (0 of 4) was less ($P<0.03$) than for the other 3 treatment groups.

All heifers used in this study had a single CL at surgery on Days 9, 10 or 11 postestrus, which averaged 24.2 ± 1.0 mm in diameter. Diameters and weights of CL of $E_2\beta$ + PGE_2-treated heifers were greater ($P<0.01$) than for the other 3 groups on Day 21 (Table 1). Seven of 12 heifers in the VEH, $E_2\beta$ and PGE_2 groups had corpora hemorrhagica (CH) present on Day 21. The remaining 5 heifers had a large (15.2 ± 1.0 mm)

Table 1. Characteristics of corpora lutea on Day 21

Treatment group	No. of heifers	Diameter (mm)	Weight (g)	Progesterone content (μg/CL)	Progesterone concentration (μg/g tissue)
VEH	4	13.0 ± 1.2[a]	1.1 ± 0.4[a]	2.22 ± 2.06[a]	1.12 ± 0.94[a]
$E_2\beta$	4	15.8 ± 1.3[a]	1.6 ± 0.3[a]	4.61 ± 2.06[a]	2.92 ± 1.60[a]
PGE_2	4	13.0 ± 1.1[a]	0.8 ± 0.1[a]	0.82 ± 0.32[a]	0.93 ± 0.25[a]
$E_2\beta$+ PGE_2	4	21.2 ± 1.3[b]	3.4 ± 0.4[b]	64.60 ± 33.58[b]	19.52 ± 10.83[b]

[a,b]Means (± SEM) within a column with different superscripts differ ($P<0.01$ for CL diameter, weight and progesterone content; $P<0.05$ for CL progesterone concentration).

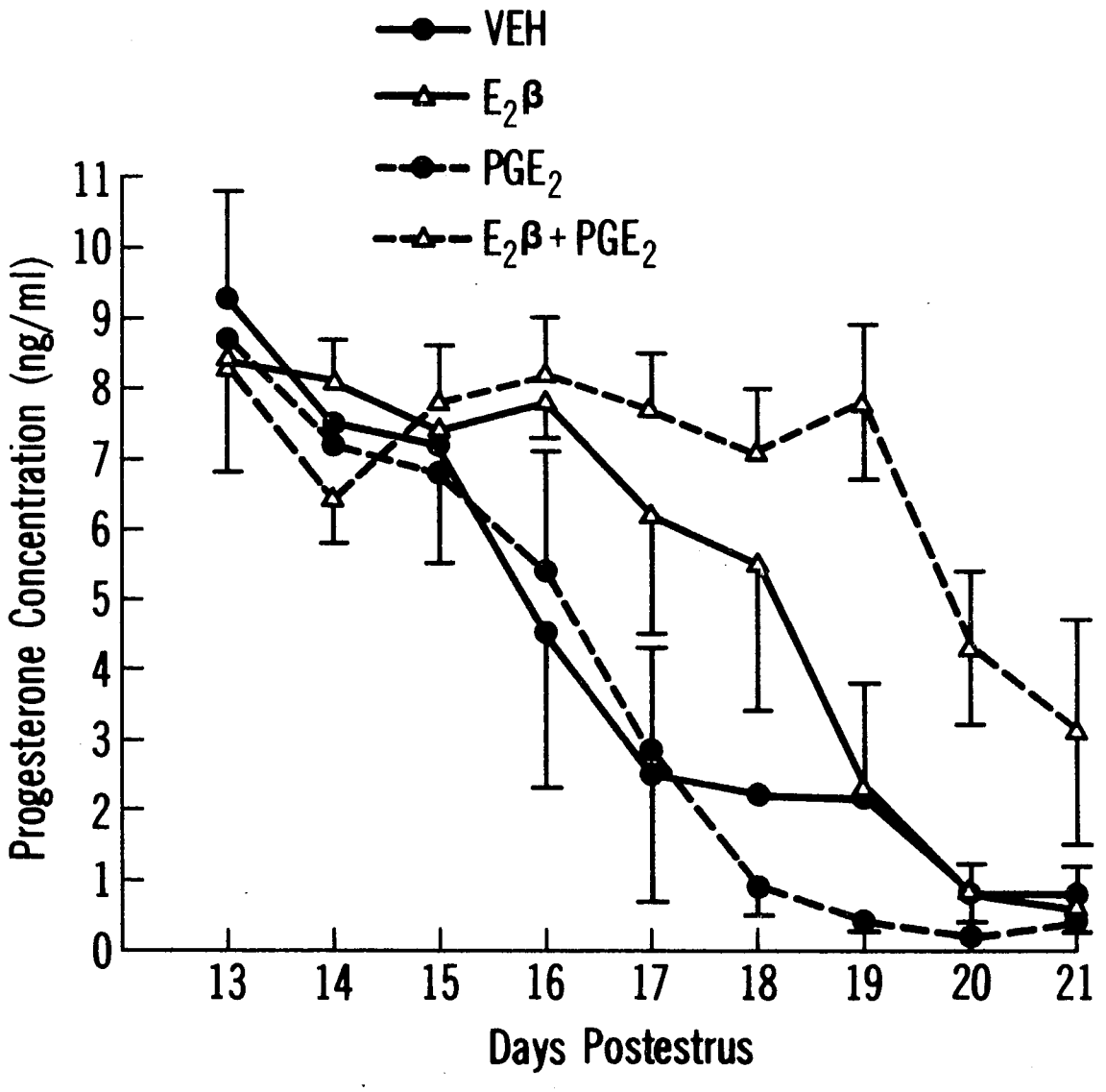
preovulatory follicle. None of the $E_2\beta$ + PGE_2-treated heifers had CH, and their largest follicle averaged 10.2 ± 0.8 mm.

Luteal concentration and content of P_4 were greater for CL of heifers in the $E_2\beta$ + PGE_2 group than for CL of heifers in the other 3 groups (Table 1). The concentrations of P_4 (ng/ml) in jugular venous plasma were similar ($P>0.10$) for all treatment groups from Days 13-15 postestrus (Fig. 1). From Days 16-18, plasma P_4 concentrations for VEH- and PGE_2-treated heifers declined to basal levels and were less ($P<0.01$) than for the $E_2\beta$ or $E_2\beta$ + PGE_2 groups, which were similar. Jugular P_4 concentrations of $E_2\beta$-treated heifers decreased on Days 19 and 20 to levels similar to those observed for the VEH and PGE_2 groups and were less ($P<0.01$) than for $E_2\beta$+ PGE_2-treated heifers. Although systemic P_4 concentrations of $E_2\beta$ + PGE_2-treated heifers had declined by Day 21, they remained elevated ($P<0.05$) when compared with the other 3 groups (3.12 ± 1.57 vs. 0.58 ± 0.12; Fig. 1).

Discussion

Data from this experiment clearly demonstrate a synergism between $E_2\beta$ and PGE_2 in maintaining luteal function in heifers. Progesterone in systemic blood of VEH- and PGE_2-treated heifers began to decrease 3-4 days before estrus, which is similar to previous reports for cycling cows (Henricks et al., 1971; Ford et al., 1979). Intrauterine infusion of $E_2\beta$ in the present study was able to extend luteal P_4 secretion for 3 days even though the occurrence of estrus for $E_2\beta$-treated heifers was similar to that of VEH-treated heifers. When both $E_2\beta$ and PGE_2

Figure 1. Concentrations of progesterone (ng/ml) in jugular venous blood of heifers receiving intrauterine infusions of vehicle (VEH), estradiol-17β ($E_2\beta$), prostaglandin E_2 (PGE_2) or $E_2\beta$ + PGE_2 from Day 13 to Day 21 postestrus (n = 4 per group; means ± SEM)


VEH
E2β
PGE2
E2β + PGE2
Progesterone Concentration (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
11
13
14
15
16
17
18
19
20
21
Days Postestrus

were infused, luteal weights and P_4 levels on Day 21 were similar to values reported previously for cows during the luteal phase of the estrous cycle and pregnancy (Erb and Stormshak, 1961; Bowerman and Melampy, 1962). In addition, jugular venous P_4 concentrations in $E_2\beta$ + PGE_2-treated heifers were maintained for an additional 5-6 days compared with VEH-treated heifers, averaging 3.12 ng/ml on Day 21 postestrus. Northey and French (1980) observed a similar maintenance of systemic P_4 levels when bovine conceptuses were flushed from the uterus on Days 17-19 postmating or when conceptus homogenates were infused into the uterus of nonpregnant cows from Days 14-18 postestrus. Thus, intrauterine infusion of $E_2\beta$ plus PGE_2, both of which are produced by the bovine conceptus and secreted from the gravid uterus, was able to successfully extend luteal function in the absence of the conceptus.

The mechanism of action of $E_2\beta$ and PGE_2 in maintaining luteal function remains unknown. Del Campo et al. (1980) demonstrated that the luteotropic effect of the bovine conceptus was exerted through a local vascular pathway. A local increase in blood flow to gravid uterine horns occurs coincident with maternal recognition of pregnancy in the sow (Ford and Christenson, 1979), ewe (Reynolds et al., 1982) and cow (Ford et al., 1979). Estrogen administration has been shown to increase uterine blood flow (UBF) of sows (Dickson et al., 1969), ewes (Killam et al., 1973) and cows (Roman-Ponce et al., 1978). Elevated UBF is associated with an increase in the amount of lymph draining the uterine horn (Fabian, 1981). The concentrations of steroid hormones in uterine lymph have been shown to reflect

concentrations in the uterine lumen and uterine venous blood (Magness and Ford, 1982; Ford et al., 1982a). Kotwica (1980) suggested that uterine lymphatics may be involved in the transport of prostaglandins from the uterine horn to the adjacent ovary. This agrees with the observation that the concentration of PGE_2 in ovarian arterial tissue of ewes was elevated on Day 14 of pregnancy compared with the same day of the estrous cycle (Lewis et al., 1978). Thus estrogens, produced by the conceptuses, may stimulate increased UBF, which could be important for the transport of substances such as prostaglandins from the lumen of the gravid uterus to the ovary.

Administration of estrogen also stimulates increased ovarian blood flow (OBF) in ewes (Rosenfeld et al., 1976; Rosenfeld, 1980). Infusion of estrogen into an isolated uterine horn of nonpregnant sows preferentially stimulates P_4 secretion from the ipsilateral ovary (Ford et al., 1982b), and intrauterine infusion of $E_2\beta$ in the present study extended systemic P_4 levels for 3 days. Estrogens of embryonic origin may, therefore, enhance luteal function indirectly by causing increased blood flow to the ovary, with a subsequent increase in P_4 secretion. In support of this hypothesis, a transient increase in OBF and P_4 secretion has been observed at the time of maternal recognition of pregnancy in sows (Magness et al., 1983) and cows (Ford et al., 1979; Ford and Chenault, 1981). A direct effect of estrogens on luteal function is unlikely because $E_2\beta$ has been shown to inhibit LH-induced P_4 secretion by cultured bovine luteal cells (Williams and Marsh, 1978; Ursely and Leymarie, 1979).

Prostaglandin E_2 directly stimulates P_4 secretion and cyclic AMP production of bovine luteal cells in vitro, in a manner similar to that of luteinizing hormone (LH) (Speroff and Ramwell, 1970; Marsh, 1971). In addition, PGE_2 has been shown to block $PGF_2\alpha$-induced luteolysis when both prostaglandins were infused simultaneously into the ovarian artery (Henderson et al., 1977) or ovarian vascular pedicle (Reynolds et al., 1981) of nonpregnant ewes. This may be a direct luteotropic effect on the CL because $PGF_2\alpha$ has been shown to inhibit LH-induced, but not PGE_2-induced, accumulation of cyclic AMP by cultured luteal cells (Khan et al., 1979). In addition, PGE_2 was able to inhibit $PGF_2\alpha$ -induced loss of luteal LH receptors in nonpregnant ewes (Reynolds et al., 1981).

Although the present study indicates that both $E_2\beta$ and PGE_2 are necessary for the maintenance of luteal function during early pregnancy in cows, the role of each hormone remains unknown. As suggested, $E_2\beta$ may stimulate an increase in lymph and venous blood draining the gravid uterus and thus enhance the transport of substances such as PGE_2 from the uterine lumen to the ovary. Prostaglandin E_2 may then have a direct effect on the luteal cells to maintain P_4 secretion.

GENERAL DISCUSSION

Previous studies have demonstrated a transient increase in uterine blood flow (UBF) coincident with maternal recognition of pregnancy in cows (Ford et al., 1979) and sows (Ford and Christenson, 1979). Results of experiment III confirm the observation of Greiss and Anderson (1970b) that a similar increase in UBF occurs at or just before pregnancy recognition in the ewe. This increase in UBF occurs before the secondary rise in UBF that is associated with implantation (Greiss and Anderson, 1970b; Ford et al., 1979; Ford and Christenson, 1979). In addition, the early, transient increase in UBF occurs concomitant with a transient increase in ovarian blood flow (OBF) in cows (Ford and Chenault, 1981) and sows (Magness et al., 1983).

It has been demonstrated that the maintenance of luteal function during early pregnancy in the ewe (Mapletoft et al., 1976) and cow (Del Campo et al., 1980) occurs by local vascular transport of a luteotropic substance from the gravid uterus to the ovaries. A similar mechanism of transport may exist in sows because intrauterine infusion of estradiol-17β ($E_2\beta$), which is luteotropic in the sow (Gardner et al., 1963), extends luteal progesterone (P_4) secretion in nonpregnant sows (Ford et al., 1982b). Thus, increased UBF and OBF may function to enhance transport of a conceptus-induced luteotropin from the gravid uterus to the luteal-bearing ovary. This proposal is supported by the observation that systemic P_4 levels were elevated coincident with pregnancy recognition and increased UBF in ewes (experiment III), cows (Ford et al., 1979) and sows (Ford et al., 1982a; Magness et al., 1983).

As discussed earlier, a primary cause of natural and prostaglandin-$F_{2\alpha}$ ($PGF_{2}\alpha$)-induced luteolysis may be a reduction in blood flow to the corpora lutea (CL; Niswender et al., 1976; Nett and Niswender, 1981). Ford et al. (1982c) found that the increase in OBF during early pregnancy in sows resulted from increased blood flow to the CL, and not to the rest of the ovary. In addition, luteal P_4 secretion is highly correlated with OBF in ewes, cows and sows (Niswender et al., 1975; Ford and Chenault, 1981; Magness et al., 1983). Ovarian vasodilation coincident with maternal recognition of pregnancy may, therefore, inhibit luteolysis by maintaining luteal blood flow, and thereby maintaining luteal P_4 secretion.

The vasoconstrictor response of the ovine uterus to norepinephrine (NE) is reduced during mid to late pregnancy compared with that observed during the estrous cycle (Ladner et al., 1970; Greiss, 1972). This reduction is primarily due to a diminished effect of NE on the caruncular (maternal placental) vascular bed, and not to a reduced effect on the endometrial or myometrial vascular beds (Anderson et al., 1977; Rosenfeld and West, 1977). The transient increase in UBF during early pregnancy, however, occurs before implantation and development of the placenta, and therefore must result from dilation of nonplacental vascular beds. Uterine arterial contractility was reduced at the time of maternal recognition of pregnancy in ewes and cows (Ford et al., 1976) as well as sows (experiment I). However, the levels of uterine arterial NE and α-adrenergic receptors, and the estrogen to progesterone ratio ($E:P_4$) in systemic blood were similar to those observed during the luteal phase of the estrous cycle (experiment I). In experiment II, the response of

the ovarian vascular bed to KCl (a nonspecific vasoconstrictor) was reduced on Day 13 of pregnancy compared with the same day of the estrous cycle, even though the $E:P_4$ ratios in systemic blood were similar. It is therefore suggested that the levels of NE and the activity of α-adrenergic receptors in the uterine and ovarian vascular beds are maintained during early pregnancy, and that the conceptus induces vasodilation by a nonspecific effect on the uterine and ovarian vascular smooth muscle. This proposal would explain the reduced contractility of uterine arteries to $PGF_2\alpha$ plus nerve stimulation during maternal recognition of pregnancy in ewes and cows (Ford et al., 1976), and would allow for the uterine arteries to rapidly regain their sympathetic tone once the conceptus-induced vasodilatory factor was withdrawn. A similar situation may occur for UBF during late gestation and parturition. Uterine blood flow does not decrease until delivery of the fetus and placenta in cows (Ford et al., 1982d) and sows (Ford and Reynolds, 1983b). The high levels of UBF during late pregnancy may partially be due to a nonspecific effect of a vasodilatory factor, produced by the conceptus, on the uterine vascular bed, rather than a direct effect on the periarterial sympathetic vasoconstrictor nerves. This would enable the vasoconstrictor ability of the uterine vascular bed to be regained rapidly once the fetus and placenta were delivered, thereby allowing for the rapid decrease in UBF.

The identity of the conceptus-associated factor(s), which induce uterine and ovarian vasodilation during maternal recognition of pregnancy, have not been elucidated. However, several lines of evidence suggest a role

for $E_2\beta$ and prostaglandin E_2 (PGE_2) in these events. Estradiol-17β is a potent dilator of the uterine vascular bed in ewes (Killam et al., 1973), cows (Roman-Ponce et al., 1978) and sows (Van Orden et al., 1983b), and stimulates increased OBF in pregnant and nonpregnant ewes (Rosenfeld et al., 1976; Rosenfeld, 1980). Synthesis of $E_2\beta$ from labelled precursors has been demonstrated for porcine conceptuses by Day 12 and bovine conceptuses by Day 13 postmating (Perry et al., 1976; Shemesh et al., 1979; Chenault, 1980). In addition, $E_2\beta$ is elevated in uterine venous blood of cows and sows (Ford et al., 1981) coincident with pregnancy recognition in each species. Synthesis of $E_2\beta$ could not be demonstrated for ovine conceptuses during early pregnancy (Gadsby et al., 1980), and $E_2\beta$ was not elevated in uterine venous blood of pregnant ewes in experiment III. The content of $E_2\beta$, however, was elevated in uterine flushings of ewes at the time of maternal recognition of pregnancy (experiment III), which confirms previous observations in cows and sows (Ford et al., 1981). Willis et al. (1981) presented evidence for an interaction between the ovine conceptus and endometrium in synthesizing estrogens by Day 14 postmating. In contrast, the conceptus appears to be the primary source of estrogens during early pregnancy in cows (Eley et al., 1983) and sows (Gadsby et al., 1980), with little or no synthesis of estrogens by the endometrium. Thus, the ovine conceptus may induce estrogen synthesis by endometrial tissue, whereas bovine and porcine conceptuses are capable of estrogen synthesis. Magness and Ford (1982) suggested that exposure of uterine periarterial sympathetic nerves to estrogens may be enhanced by the elevated estrogen levels

they observed in uterine lymph of sows on Days 11 to 15 postmating. In addition, lymph may serve as a means for transport of estrogens from the gravid uterus to the ovaries, where they may then induce an increase in OBF. This proposal is supported by the observation that the transient increase in UBF of sows was observed on Day 11 postmating, whereas oviductal and luteal blood flow did not increase until 24-48 hr later (Ford et al., 1982c). Infusion of $E_2\beta$, in amounts normally secreted from the gravid uterus during maternal recognition of pregnancy, into an isolated uterine horn of nonpregnant sows (Ford et al., 1982b), or into the uterus of nonpregnant heifers (experiment IV) maintained luteal function for several days. However, $E_2\beta$ had no effect on P_4 secretion by porcine CL <u>in vitro</u> (Watson and Maule-Walker, 1978), and inhibited LH-induced P_4 secretion by cultured bovine luteal cells (Williams and Marsh, 1978; Ursely and Leymarie, 1979). Thus, a direct effect of $E_2\beta$ in stimulating luteal P_4 production is unlikely, which serves to emphasize its probable role as a uterine and ovarian vasodilator produced by the early conceptus and (or) gravid uterus in ewes, cows and sows. As discussed in experiment I, uterine arteries from sows are capable of producing 2-hydroxyestrone from E_1 (Van Orden et al., 1983a), and 2-hydroxyestrone is an effective competitor for α-adrenergic receptors. It is conceivable that $E_2\beta$, secreted from the gravid uterus, is converted to a 2-hydroxy derivative which then competes with NE for uterine and ovarian arterial α-adrenergic receptors.

Administration of PGE_2 has been shown to stimulate increased UBF in ewes (Resnik and Brink, 1978) and cows (W. W. Thatcher, Univ. of Florida, unpublished observation). However, PGE_2 is much less potent than $E_2\beta$ in causing uterine vasodilation (Resnik and Brink, 1978). Conceptus and endometrial tissues produce PGE_2 *in vitro* by Day 14 postmating in ewes (Lacroix and Kann, 1982; Hyland et al., 1982) and Day 13 postmating in cows (Shemesh et al., 1979; Lewis et al., 1982). In addition, PGE_2 is elevated in uterine flushings of cows (Lewis et al., 1982) and sows (Geisert et al., 1982), and uterine flushings and venous blood of ewes (Ellinwood et al., 1979) coincident with pregnancy recognition in each species. Intrauterine infusion of large quantities of PGE_2 extends the functional lifespan of the CL in nonpregnant ewes (Magness et al., 1981) and cows (J. R. Chenault, Upjohn Co., unpublished observation). The antiluteolytic effect of PGE_2 may result from its ability to directly stimulate P_4 secretion by luteal cells (Speroff and Ramwell, 1970). In support of this proposal, PGE_2 has been shown to inhibit $PGF_2\alpha$-induced luteolysis when both prostaglandins were infused simultaneously into the ovarian artery (Henderson et al., 1977) or ovarian vascular pedicle (Reynolds et al., 1981) of nonpregnant ewes. Thus PGE_2, derived from the early gravid uterus and conceptus, may have a direct effect to stimulate P_4 secretion by the CL. Infusion of a low dose of PGE_2 into the uterus of sows (Schneider et al., 1982) or cows (experiment IV) had no effect on luteal P_4 secretion. However, when intrauterine infusion

of PGE_2 was combined with $E_2\beta$ in experiment IV, the extension of luteal P_4 secretion was greater than that observed for $E_2\beta$ alone. Thus, uterine and ovarian vasodilation, possibly caused by estrogens of conceptus origin, may be necessary for transport of PGE_2 from the gravid uterus to the CL-bearing ovary. Prostaglandin E_2 may then have a direct effect to stimulate luteal P_4 secretion. This does not eliminate a role for PGE_2 in stimulating UBF and OBF. Kadowitz et al. (1971) observed a vasodilatory effect of PGE_2 on the uterine vascular bed, independent of its effect on NE release from perivascular sympathetic nerves. This is similar to the nonspecific effect of the conceptus on uterine and ovarian vascular smooth muscle suggested by experiments I and II, and suggests the possibility that PGE_2 may be partially responsible for the transient increases in UBF and OBF at the time of maternal recognition of pregnancy in ewes, cows, and sows.

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