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Understanding and exploiting the physiology and endocrinology of reproduction to enhance reproductive efficiency in cattle

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ABSTRACT

The bovine conceptus induces an array of physiological and biochemical responses that lead to the establishment of pregnancy. Among the most studied of these is secretion of prostaglandin (PG) $F_{2\alpha}$ (F) from the uterine endometrium. Pregnancy appears to be associated with laterations in many of the regulatory systems that cause PGF secretion leading to luteolysis in cyclic animals. The conceptus secretes an antiluteolytic protein (bovine trophoblast protein-1 complex [bTP-1]) that appears to induce an inhibitor of prostaglandin synthesis. The bTP-1 antiluteolytic protein is an α_{II} interferon. The secondary effects associated with interferon-like activities (antiviral, antiproliferative, altered gene expression, immunosuppressive) may complement development and sustenance of the conceptus. Both ovarian CL function and follicular activity are altered in early pregnancy. Changes in follicular activity may reinforce the antiluteolytic signals from the conceptus leading to maintenance of the CL. Potential management systems to improve reproductive efficiency that are based on supplementation with bTP-1-like molecules, controlled ovarian follicular management, and progesterone therapy are discussed.

Keywords Cattle; conception; follicles; bovine trophoblast protein-1; interferon; progesterone; oxytocin; GnRH agonist.

INTRODUCTION

The field of animal reproduction is experiencing major and exciting changes because of advancements in our understanding of oocyte maturation, *in vitro* fertilization, cloning of embryos, production of transgenic animals, sexing of sperm and embryos, and routine transfer of frozen embryos. In spite of these major advancements, low conception rates due to high rates of embryonic mortality continue to be a major cause of reproductive failure. It has been estimated that rates of fertilization are 90% or greater. However, only 50% of cattle inseminated produce an offspring (Diskin and Sreenan, 1980). Based on collection of specimens at various stages after breeding, early embryonic deaths accounted for 75 to 80% of pregnancy losses. The greatest losses occurred between days 8 to 16 (Diskin and Sreenan, 1980; Roche *et al.*, 1981). Such losses could be attributed to many causes including intrinsic failure of embryonic development, improper uterine environment, insufficient secretion of signals produced by the conceptus (embryo plus extraembryonic membranes) including those necessary to maintain the *corpus luteum* (CL) of pregnancy, CL dysfunction as a result of inadequate preovulatory follicle development and maturation, and perhaps follicular development during early pregnancy that antagonizes CL maintenance.

The focus of this review is to describe the physiological, endocrinological and biochemical dialogue that occurs between the conceptus and maternal unit that permits maintenance of the CL; examine how ovarian follicular function may complement or antagonize the processes associated with initiation of pregnancy; and describe how our understanding of these basic reproductive principles might be exploited in reproductive management systems to enhance reproductive efficiency. While major emphasis will be on cattle, similar mechanisms exist in sheep (Bazer, 1989).

UTERINE-CONCEPTUS CONTROL OF THE *CORPUS LUTEUM* (CL)

With utilization of embryo transfer, reproductive losses

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due to fertilization failure and inadequate embryonic development to the blastocyst stage will be eliminated. With current advancements in reproductive technology, on-farm transfer of frozen embryos could become as routine as current artificial insemination techniques. Following embryo transfer, establishment of pregnancy involves interactions between the conceptus and uterus. The period from initial elongation of the trophoblast until definitive microvillar attachment of the trophoblastic membrane to the maternal endometrium is associated with high losses of conceptuses (Thatcher *et al.*, 1984a). A successful interaction between conceptus and maternal tissues likely involves many alterations in uterine and conceptus function. One of the most noticeable and important is the conceptus-driven change in endometrial and ovarian function responsible for maintenance of the CL and progesterone secretion to sustain a histotrophic uterine environment essential for growth and development of the conceptus.

Uterine Secretion of Prostaglandin $F_{2\alpha}$ (PGF).

Associated with regression of the CL during the estrous cycle are luteolytic pulses of PGF secretion. Luteolysis is exerted through a veno- arterial pathway involving veins that drain the uterus and ipsilateral ovarian artery (Thatcher *et al.*, 1986a). Estradiol (E_2) secretion from ovarian follicles stimulates secretion of PGF from the uterus. Destruction of follicles reduces concentrations of E_2 in the utero-ovarian vein, delays CL regression and prevents full CL regression in response to an injection of PGF (Hughes *et al.*, 1987). Thus, non-ovulatory ovarian follicles during mid- to-late diestrus are necessary for CL regression in cyclic cows. Injections of E_2 will stimulate uterine secretion of PGF in cyclic cattle at day 13 (Thatcher *et al.*, 1986b). McCracken *et al.*(1984) proposed that E_2 acts by stimulating synthesis of oxytocin receptors in the endometrium of sheep and that oxytocin from either the neurohypophysis or CL then stimulates secretion of PGF. Oxytocin is secreted by the bovine CL (Wathes *et al.*, 1983) and stimulates secretion of PGF from the uterus of cyclic cattle when administered either *in vivo* (Lafrance and Goff, 1985) or *in vitro* (Gross *et al.*, 1988a). If the model proposed by McCracken *et al.* (1984) is applicable to cattle, then oxytocin-induced secretion of PGF will decrease secretion of progesterone and perhaps stimulate secretion of additional oxytocin that continues pulsatile secretion of PGF until final demise of the CL (Figure 1).

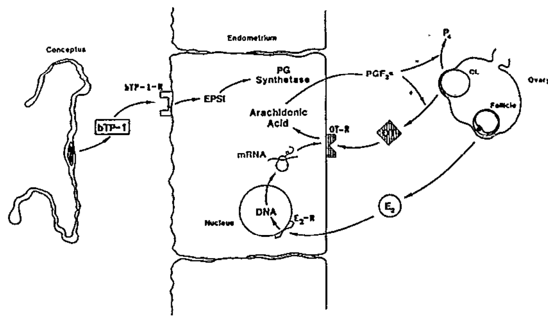

FIG 1 Conceptual dialogue between conceptus, endometrium and ovary relative to the regulation of PGF secretion and maintenance of the CL (antiluteolytic effect). CL, *corpus luteum*; OT, oxytocin; E2, estradiol; R, receptor; PG, prostaglandin; EPSI, endometrium prostaglandin synthetase inhibitor; bTP-1, bovine trophoblastic protein-1 complex (*Theriogenology 31*: 149-164).

Based upon the model of McCracken *et al.* (1984), induction of PGF secretion by oxytocin is dependent upon the presence of oxytocin receptors in the uterine endometrium. Endometrial oxytocin receptors are present in high concentrations during early phases of the estrous cycle (days 1 to 7), low in the mid-luteal phase (days 8 to 17), and show a large progressive increase from days 18 to 21 associated with CL regression, progesterone withdrawal and proestrous follicular development (Meyer *et al.*, 1988). Interpretation of experimental PGF responses must be integrated relative to availability of estradiol and oxytocin, induction and/or presence of oxytocin receptors, and whether the uterus has been exposed to progesterone for a sufficient period of time to have the responsive elements necessary to synthesize PGF from arachidonic acid (phospholipid pool, cyclooxygenase, phospholipase-2, etc.). For example, Lafrance and Goff (1988) demonstrated that a 7-day period of progesterone priming was necessary before oxytocin-induced secretion of PGF could occur in cattle. Presence of a conceptus attenuates endometrial secretion of PGF in cattle. When pregnant cows are injected with either oxytocin (Lafrance and Goff, 1985) or E_2 (Thatcher *et* *al.*, 1984), PGF release from the uterus is reduced compared to the response of cyclic cows at the same day after estrus. Likewise, perifused endometrial tissue of pregnant cows collected at day 17 after estrus secretes less PGF than that for cyclic cows and, unlike endometrium from cyclic cows, endometrium from pregnant cows is unresponsive to treatment with oxytocin (Gross *et al.*, 1988a). Reduced secretion of PGF in early pregnancy is due to several physiological and biochemical differences in endometrial tissue. Recently, Fuchs *et al.* (1990) demonstrated that endometrial oxytocin receptors remained low in pregnant cows during the period when receptor concentrations increased in cyclic cows (day 17 to 21 postestrus). Whether this is due to sustained progesterone concentrations associated with CL maintenance or a direct effect of the conceptus on oxytocin receptor synthesis is not known. The inositol phosphate-diacylglycerol second messenger system has been implicated in mediating oxytocin-stimulated pulsatile secretion of PGF (Flint *et al.*, 1986) and oxytocin-stimulated inositol phosphate turnover is reduced in pregnant ewes compared with cyclic ewes on day 16 (Mirando *et al.*, 1990a). Mirando *et al.* (1990) found that basal and oxytocin stimulated inositol phosphate turnover was greater for cyclic than pregnant cows at day 17 after estrus. A reduction of PGF secretion in response to oxytocin in pregnant cows (Lafrance and Goff, 1985) may be consistent with the reduction in oxytocin receptors within endometrial tissues of pregnant cows.

The reduced secretion of PGF in bovine endometrial tissue of early pregnancy is likely due, in part, to an intracellular, endometrial prostaglandin synthesis inhibitor (EPSI; Gross *et al.*, 1988b; Basu and Kindahl, 1987; Figure 1). Activity of EPSI was greater in the endometrial tissue from day 17 pregnant than cyclic cows. The inhibitor(s) is present in the 100,000 g cytosolic supernatant, proteinaceous, exists at apparent molecular weights of 70,000 and 35,000, and acts in a non-competitive manner with respect to arachidonic acid. Presence of EPSI in endometrium is sustained throughout the major part of pregnancy (Thatcher *et al.*, 1989a). What needs to be determined is the local distribution of EPSI in endometrium (e.g., epithelium and stromal cells).

In addition to the reduction of oxytocin receptors and the presence of an inhibitor to prostaglandin synthesis, there are multiple biochemical differences between endometrial tissues of cyclic and pregnant cattle at day 17 postestrus in responsiveness to agents regulating prostaglandin synthesis (Danet-Desnoyers *et al.*, 1990). Basal secretion of PGF is lower in endometrium of pregnancy in the presence or absence of Ca^{++}. Calcium ionophore (A23187) stimulates PGF secretion in endometrium from pregnant cows in the presence or absence of Ca^{++}, whereas endometrium of cyclic cows is more responsive to the ionophore in the presence of Ca^{++} (Figure 2). Availability of arachidonic acid (AA) substrate in endometrium from pregnant cows appears to be limiting since addition of phospholipase A-2 (PLA-2) to endometrial explant cultures stimulated PGF secretion more than for endometrium from cyclic cows in the presence or absence of Ca^{++}. Stimulation of endometrial tissue from pregnant cows with PLA-2 was reduced in the presence of calcium ionophore, but this inhibition was not detected in endometrium of cyclic cows. That the availability of AA is limited is supported by marked stimulation of PGF secretion by endometrial tissues from pregnant and cyclic cows following addition of AA (Figure 2). The increase in PGF secretion in response to AA was less for endometrium of pregnancy, possibly due to presence of EPSI. Endometrial tissues from pregnant cows (day 17) have less AA bound to phospholipids than endometrium from day 17 cyclic cows (66.4 versus 143.8 μg arachidonic acid in phospholipids per gram of endometrium; J.C. Curl and W.W. Thatcher, unpublished observations). Perhaps this decrease is associated with the concurrent development of extraembryonic membranes in which AA from the endometrial pool is utilized. It is clear that the endometrium of early pregnancy is programmed towards an antiluteolytic mode of secretion in which the secretion of PGF is reduced.

Antiluteolytic Effect of Bovine Trophoblast Protein-1.

The bovine conceptus causes maintenance of the CL by blocking luteolysis beginning on days 16-17 (Thatcher *et al.*, 1986). The conceptus factor responsible for CL maintenance is bovine trophoblast protein-1 complex (bTP-1). Advancements in this area were based on the purification and chemical characterization of ovine trophoblast protein-1 (oTP-1) from sheep conceptuses (Bazer, 1989). Ovine trophoblast protein-1 is a nonglycosylated 19 kDa acidic protein containing at least 3 isomeric forms (pI's of 5.3 to 5.7) secreted by mononuclear cells of the trophectoderm. The oTP-1 binds to endometrial receptors and alters protein secretion by the endometrium with amplification of at least five proteins. When purified oTP-1 was introduced into the uterine lumen between days 12 to 14 after estrus, it reduced estradiol- and oxytocin- induced secretion of PGF and extended the interestrous interval.

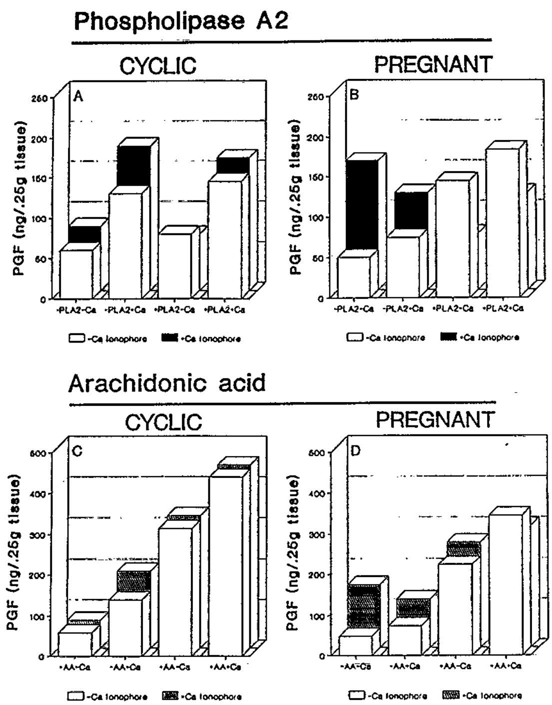

FIG 2 **Panels A and B**: Interactive effects of calcium (Ca^{++}; $CaCl_2$-$2H_2O$,.265 mg/ml), phospholipase A_2 (PLA_2;1U/ml), and calcium ionophore A23187 (CaI; 7.5 ug/ml) on mean PGF in media from endometrial explants (0.25 g in 7.5 ml MEM; incubated at 37°C for 24 hrs under an atmosphere of 45% O_2, 50% N_2, 5% CO_2) of cyclic and pregnant cows. There was a Ca^{++} x PLA_2 x CaI interaction for each reproductive status. **Panels C and D**: Interactive effects of calcium (Ca^{++}), arachidonic acid (AA; 13.3 ug/ml) and calcium ionophore A23187 (CaI) on mean PGF in media from endometrial explants of cyclic and pregnant cows. Results expressed as ng/0.25g tissue.

The potential similarity between sheep and cow antiluteolytic proteins was anticipated since CL lifespan in recipient cows and ewes is extended following interspecies reciprocal transfer of trophoblastic vesicles (Martal *et al.*, 1984). The bTP-1 was defined originally as secretory proteins of the bovine conceptus crossreacting immunologically with antiserum to oTP-1 (Helmer *et al.*, 1987). Chemically, bTP-1 differs from oTP-1 in that it contains various amounts of N- linked carbohydrate (Anthony *et al.*, 1988; Helmer *et al.*, 1988), giving rise to molecular weight variants of 22 kDa, 24 kDa and 26 kDa. Only a single glycosylation site exists on bTP-1 (Imakawa *et al.*, 1989). The differences in molecular weight between the 22 kDa and 24 kDa forms of bTP-1 arise because the 22 kDa form contains a single high mannose oligosaccharide chain and the 24 kDa form contains a larger complex type oligosaccharide (Helmer *et al.*, 1988). The several isoelectric variants (3-4) of each molecular weight class are derived from different mRNA (Anthony *et al.*, 1988). It is not known whether chemical differences between the bTP-1 complex and oTP-1 (nonglycosylated) are related to differences in their catabolism or binding characteristics to endometrial receptors.

Immunocytochemical localization of bTP-1 in day 20 bovine conceptus tissue revealed specific staining in the cytoplasm of both mono- and binuclear cells of trophectoderm (Lifsey *et al.*, 1989). Utilizing an oTP-1 cDNA probe, Farin *et al.* (1989) detected bTP-1 mRNA in trophectoderm of bovine conceptuses as early as day 12. Total production of bTP-1 increases with age and elongation of the conceptus (Geisert *et al.*, 1988; Bartol *et al.*, 1985; and Godkin *et al.*, 1988a).

The antiluteolytic role of bTP-1 (Figure 1) is supported by both *in vivo* and *in vitro* experiments. When infused into the uterine lumen of cyclic cows between days 15.5-21, bTP-1 extended the estrous cycle and functional lifespan of the CL, while also decreasing within-animal variability in concentrations of PGF in plasma of the posterior vena cava (Helmer *et al.*, 1989a). When endometrial explants from day 17 of the estrous cycle were incubated with either bovine conceptus secretory proteins (Gross *et al.*, 1988c) or bTP-1 (Helmer *et al.*, 1989b) for a 24-h period, secretion of PGF was reduced compared to appropriate control incubations. Furthermore, endometrial tissue of bCSP- and bTP-1-treated cows had higher amounts of EPSI activity. This suggested that bTP- 1 exerted its antiluteolytic action by inducing an intracellular inhibitor of PGF synthesis (Figure 1). The inhibitor appears to act by inhibiting cyclooxygenase since, in cell-free systems of cotyledonary microsomal preparations rich in prostaglandin-synthesizing enzymes, the inhibitor blocks conversion of arachidonic acid to both PGF and PGE-2 (Gross *et al.*, 1988b). Although EPSI inhibits secretion of both PGF and PGE-2 in a cell-free system, the regulatory role of bTP-1 on endometrial explants is to decrease PGF secretion and increase PGE-2 (Helmer *et al.*, 1989b). It is conceivable that EPSI (induced by bTP-1) is compartmentalized within epithelial cells of the endometrium. During the estrous cycle, the major source of endometrial PGF is epithelial cells while the major source of PGE-2 is stromal cells (Fortier *et al.*, 1988). Godkin *et al.* (1988b) demonstrated that bTP-1 inhibited PGF secretion from dispersed epithelial cells. Additional studies are needed to examine the differential response of endometrial cell types and their interactions to regulatory agents such as bTP-1 and oxytocin.

Recently, bTP-1 and oTP-1 have been shown to be members of the IFN-αII sub- family (Imakawa *et al.*, 1989). Both bTP-1 and oTP-1 contain 172 amino acids and share around 70% amino acid sequence homology with BoIFN-αII. Bovine TP-1 has an inferred amino acid sequence homology of 82% with oTP-1. Endometrial receptor binding analysis has further documented the similarities between oTP-1 and IFN- α. Binding of HuIFN-α or recombinant BoIFN-α to membrane receptors from uteri of cyclic ewes is inhibited by purified oTP-1 (Hansen *et al.*, 1989; Stewart *et al.*, 1987). With bTP-1 and oTP-1 being classified as trophoblastic interferons, the recognized roles of interferons in inhibiting cell proliferation, versus replication and lymphocyte blastogenesis, as well as effects on gene expression, are intriguing relative to potential functions of conceptus interferons related to embryonic differentiation and immunoprotection during early pregnancy. Indeed, oTP-1 and bTP-1 have potent antiviral activity (Pontzer *et al.*, 1988; Godkin *et al.*, 1988b), and oTP-1 can inhibit lymphocyte proliferation *in vitro* (Newton *et al.*, 1989a).

OVARIAN FOLLICULAR DYNAMICS DURING EARLY PREGNANCY

Follicular development occurs in periodic waves throughout the estrous cycle (Knopf *et al.*, 1989; Savio *et al.*, 1988; Fortune *et al.*, 1988). The frequency distribution of follicular waves is such that either three or two waves occur during each cycle. Those waves occurring at the time of luteolysis lead to the subsequent ovulatory follicle. Follicular waves occurring during diestrus, in the presence of a functional CL, lead to follicular atresia of a large dominant follicle. Undoubtedly, development of an estrogenic follicle during the luteal phase of the estrous cycle initiates the luteolytic process via the secretion of E_2 (Fogwell *et al.*, 1985). Follicle induced luteolysis occurs because the estrogenic effect 'is imposed upon a progesterone-primed endometrium capable of synthesizing and secreting PGF. A logical question is whether follicular development is altered during early pregnancy to complement the antiluteolytic process.

A reasonable hypothesis would be that follicular development is attenuated on the CL-bearing ovary ipsilateral to the uterine horn bearing the developing conceptus. Guilbault *et al.* (1986) examined the population of ovarian follicles on the ovary bearing the CL during early pregnancy (17 and 34 days) and compared results to those from non-pregnant cattle (day 17 of cycle and day 34 post-estrus in hysterectomized cows). Pregnancy altered the distribution of follicles. The proportion of small non-atretic follicles in class I (0.16-0.28 mm) was lowered while that of non-atretic follicles in class II (0.29-0.67 mm) was increased. This represented a more rapid turnover of follicles from smaller to larger classes. However, among all atretic follicles in a class, the percentage in a state of late atresia in class V (3.68-8.56 mm) was higher in pregnant than in non-pregnant cows (63.6% > 25.0%). Thus, presence of a conceptus induced a more rapid turnover of follicles from small to larger size classes but limited further growth by increasing atresia. This histological approach at a fixed point in time does not permit an assessment of follicular dynamics on a day-to-day basis.

Utilizing ultrasonography, Pierson and Ginther (1987) and Driancourt *et al.* (1990) demonstrated that the number of follicles greater than 7 mm was reduced on the ovary ipsilateral to the CL after approximately day 22 of pregnancy. Furthermore, size of the largest follicle was greater on the ovary contralateral to the CL-bearing ovary. Ginther *et al.* (1989) demonstrated that follicular waves occur during pregnancy, but that recurring waves 3 to 10 occur primarily on the ovary contralateral to the CL. Presence of a conceptus is likely to be the physiological factor attenuating follicular development on the ovary with the CL. For example, follicular development is sustained on the ovary bearing the CL in hysterectomized cows in which CL function was sustained (Figure 3; Thatcher *et al.*, 1990). In contrast, follicular development was attenuated on the CL-bearing ovary during the same period in pregnant heifers.

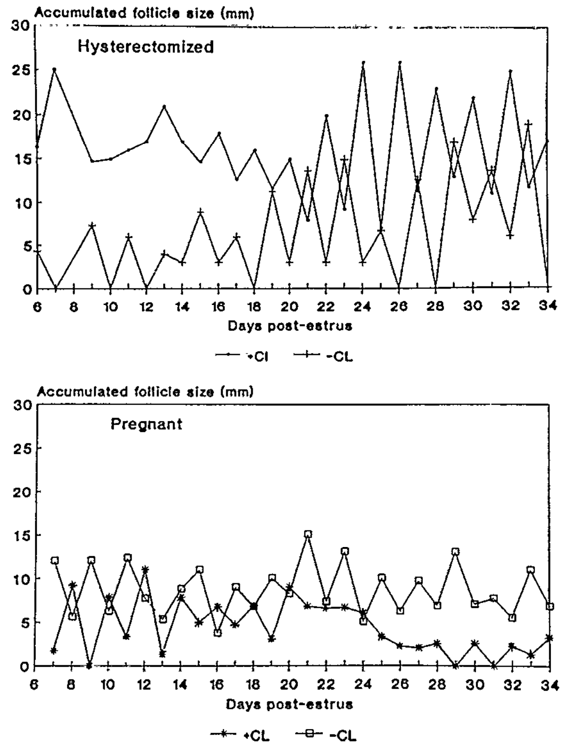

FIG 3 Accumulated follicle size (mm; sum of all follicular sizes measured) on the corpus lutuem (+CL) and non-*corpus luteum* bearing (-CL) ovaries of pregnant (n=7) or hysterectomized (n=4) heifers. Following surgery, between days 9 and 14 of the estrous cycle, hysterectomized heifers were induced into estrus by injection of PGF. Daily follicular development between days 6 to 34 post-estrus was monitored by ultrasonography.

In a series of cows in which follicular aromatase activity was examined on day 17 of the estrous cycle and pregnancy, differences in aromatase activity between the largest and second largest follicles varied with reproductive status (Figure 4; Thatcher, W.W. and Badinga L., unpublished observations). In cyclic cows, the largest follicle had appreciably more aromatase activity than did the second largest follicle. However, aromatase activity of the largest follicle from pregnant cows was less than that of cyclic cows. However, in pregnant cows the second largest follicle became the estrogen-active follicle and it occurred with a higher frequency (71.4%) on the ovary contralateral to the ovary bearing the CL. These changes in aromatase activity were reflected by estrogen concentrations in follicular fluid (Figure 5). Furthermore, the poor status of the largest follicle in pregnant cows was supported by the high progesterone concentrations in follicular fluid (Figure 5). This change in follicular estrogen activity associated with pregnancy would change local production of estradiol. Weems *et al.* (1989) showed that endometrial concentrations of progesterone were higher in endometrium ipsilateral to the ovary bearing the CL and that a local countercurrent movement of steroids occurred in the ovarian- uterine vascular bed. Perhaps a similar analogy exists with regard to secretion of estrogen. As a consequence, endometrium adjacent to the developing conceptus may be exposed to lower concentrations of estradiol which would support conceptus-mediated antiluteolytic effects of paramount importance to maintain the CL. The local steroidal system would also favor a high progesterone: estradiol ratio that would antagonize the induction of oxytocin receptors. In this regard, it is interesting that at days 17 and 21 of pregnancy in cattle, oxytocin receptors are depressed compared to those of the estrous cycle (Fuchs *et al.*, 1990). Whether this is due to some direct effect of the conceptus, lack of local estradiol exposure or sustained effects of progesterone on endometrium, needs to be determined.

Collectively, these results indicate that the conceptus, either directly or indirectly (e.g., via the endometrium), decreases local intraovarian follicular development. Such an effect is supportive of maintaining pregnancy since presence of an active estrogenic follicle and localized production of estradiol would otherwise stimulate the secretion of PGF from the uterine endometrium. This localized attenuation of ovarian follicular development by the conceptus complements effects of the conceptus which attenuate endometrial secretion of PGF (via bTP-1 secretion and induction of EPSI). As a consequence, cattle in which follicular development is sustained on the ovary ipsilateral to the uterine horn bearing the conceptus may have higher rates of embryonic mortality during this critical period. Additional research is warranted to examine the local amounts of estradiol, progesterone, and receptors for estradiol, progesterone and oxytocin between uterine horns ipsilateral and contralateral to the conceptus in cattle.

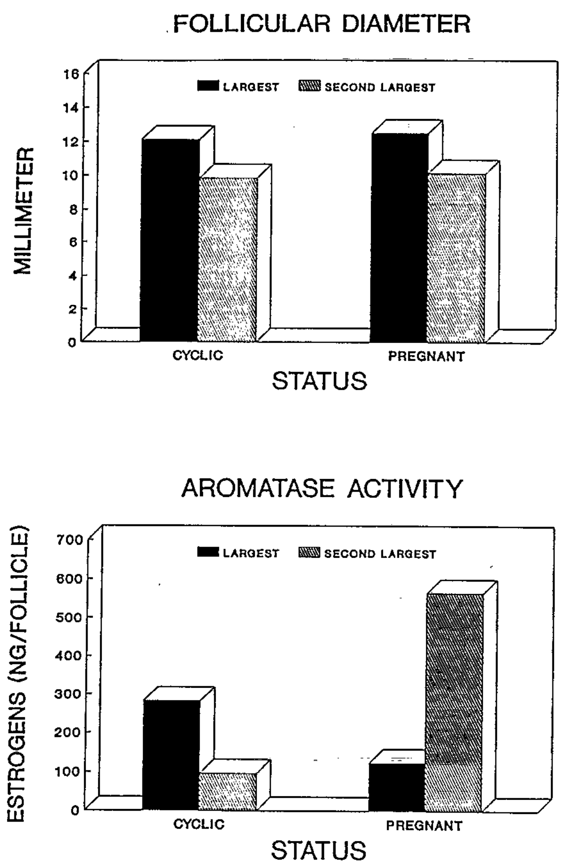

FIG 4 Follicular diameter (mm) and aromatase activity for the largest and second largest follicles recovered from cows at day 17 of the estrous cycle or pregnancy. Aromatase activity was determined by incubation of hemispheres of follicular walls in 4 ml of Medium 199 supplemented with 0.5 uCi [1ß,2ß-3H]-testosterone (2.7 ng) for 3 h at 38.5°C in an environment of 95% air-5% CO_2. Aromatase activity was assessed by measuring production of tritiated water; system validated by isolation of radioactive estradiol following HPLC.

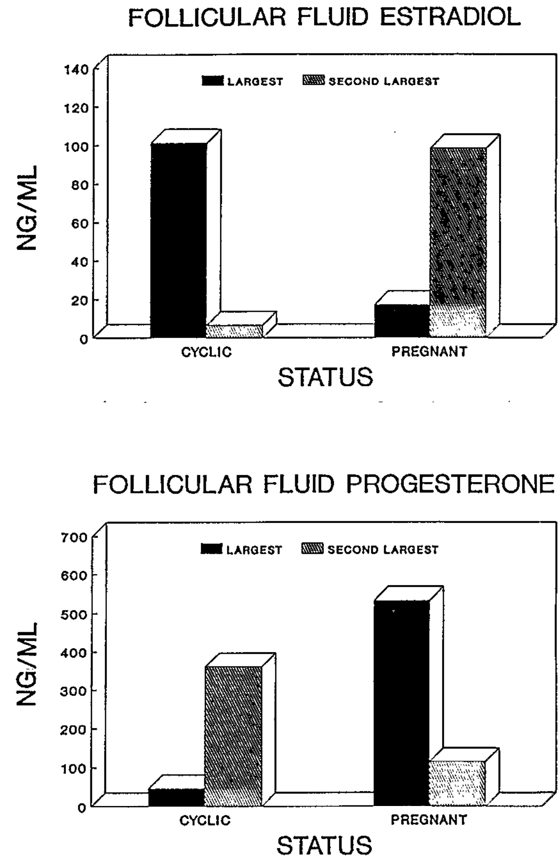

FIG 5 Follicular fluid concentrations of estradiol and progesterone for largest and second largest follicles recovered from cows at day 17 of the estrous cycle or pregnancy. Steroids measured by radioimmunoassay.

POTENTIAL APPLICATION OF bTP-1 AND RECOMBINANT BOVINE ALPHA-INTERFERON TO IMPROVE EMBRYONIC SURVIVAL

A major question is whether a portion of the early embryonic deaths in cattle are associated with inadequate secretion of bTP-1. This inability to exert an antiluteolytic effect would lead to regression of the CL. Our research in the area of heat stress suggests that regulation of bTP-1 secretion is critical to embryonic survival. *In vivo* heat stress, either on day 0 for a 10-h period prior to ovulation (Putney *et al.*, 1989a) or daily from days 1 to 7 post-insemination (Putney *et al.*, 1988a), increased the incidence of abnormal, retarded and dead embryos recovered from superovulated heifers on day 7. One strategy to bypass this early period of embryo sensitivity to hyperthermia is to transfer good quality embryos at day 7 of development to cattle that are exposed to heat-stressed conditions. This concept was recently tested in a trial conducted in the summer in Florida (Putney *et al.*, 1989b). Pregnancy rates at day 21 for lactating dairy cows receiving embryos at day 7 (n = 113) were higher than pregnancy rates for control cows bred by artificial insemination (47.6 versus 18%). This difference decreased to 29.2 and 13.5% by day 40 for embryo transfer and artificial insemination groups, respectively. The marked reduction in estimated pregnancy rate between days 21 and 40 for lactating cows receiving day 7 embryos, indicates that heat stress may have continued to alter conceptus function and cause conceptus deaths.

When cows were subjected to a severe heat stress between days 8 to 16 of pregnancy (Biggers *et al.*, 1987), conceptus weight was reduced by 50% in comparison to conceptuses collected from control cattle. *In vitro* heat stress of day 17 conceptuses caused a large decrease in bTP-1 secretion (Putney *et al.*, 1988b). Collectively, these results indicate that compromised conceptus function (reduced growth of conceptus and decreased secretion of bTP-1) may lead to CL regression and termination of pregnancy. In fact, when oxytocin was injected on day 17 post-insemination (Putney *et al.*, 1989c), the induced response of PGF secretion was greater in cows containing small conceptuses than for cows containing larger conceptuses. Presumably, the larger conceptuses secreted more bTP-1 and were better able to inhibit oxytocin-stimulated PGF secretion.

Infertility may also be higher for cows having normal conceptuses, but initiating luteolysis earlier than usual (short cycle) and before the conceptus can produce sufficient amounts of bTP-1 (Hinshelwood *et al.*, 1982). Early supplementation with bTP-1 may enhance the antiluteolytic signal and improve pregnancy rates in these cows. The transfer of bovine trophoblastic vesicles at the time of normal embryo transfer has been shown to increase pregnancy rates (Heyman *et al.*, 1987), possibly because these vesicles produce bTP-1 and other conceptus signals which supplement that produced by the transferred embryo to enhance the antiluteolytic physiological response.

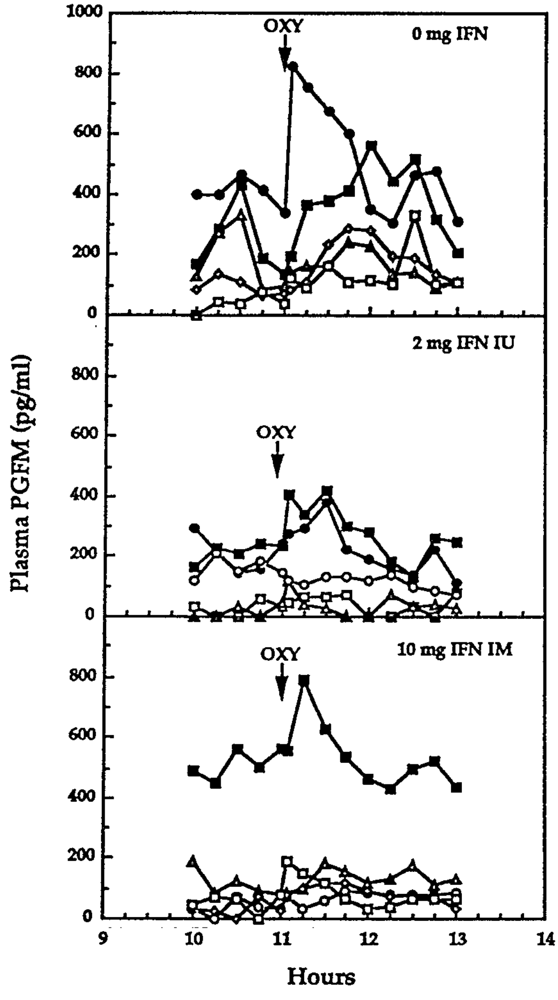

FIG 6 Individual profiles of plasma PGFM from cows receiving a control BSA solution, 2 mg recombinant bovine interferon alpha (rBoIFN-α) in utero or 10 mg rBoIFN-α intramuscularly. Cows were challenged at 1100h with 100 U of oxytocin (OXY). Oxytocin caused an increase in PGFM concentrations. Based on heterogeneity of regression ($P<0.05$), the increase was lower for cows treated with rBoIFN-α as compared to control cows.

At the present time, neither native nor

recombinant bTP-1 is available in sufficient amounts to evaluate its potential effects on fertility. It is reasonable to assume that administration of molecules with properties similar to bTP-1 may also amplify the antiluteolytic signal. Recently it was reported that the gene for bTP-1 has 69% homology with the gene for bovine interferon-αII (Imakawa *et al.*, 1989). Of potential functional importance is the observation that radiolabelled human α-interferon binds to membrane receptors of endometrium of cyclic sheep and that binding can be inhibited by purified oTP-1 (Stewart *et al.*, 1987). Furthermore, secretion of both PGF and PGE_2 by cultured ovine endometrial cells can be inhibited by both oTP-1 and human interferon-α (Salamonsen *et al.*, 1989). The homology between bTP-1 and α-interferons led us to test possible antiluteolytic effects of recombinant bovine interferon class I, type 1 (interferon-α_I1) that was produced in Escherichia coli by recombinant DNA techniques and purified to homogeneity (Capon *et al.*, 1985). The interferon-α_I1 was generously provided by CIBA-GEIGY, Basle, Switzerland. Twice-daily intrauterine infusion of interferon-α_I1 (520 µg per infusion) from day 15.5 to 21 after estrus delayed luteolysis (Plante *et al.*, 1988). Interferon-α_I1- treated cows had a mean interestrous interval of 26.8 ± 1.4 days, which was longer than the 22.8 ± 0.8 day interestrous interval for control cows. Measurements of serum progesterone confirmed that the longer interestrous intervals were due to an increase in CL lifespan following treatment with interferon. In other studies, twice-daily intramuscular injections of 0, 2.5, 5 or 10 mg/injection of recombinant bovine interferon-α_I1 from day 14 to day 21 after estrus to heifers increased interestrous intervals and functional luteal lifespans (Plante *et al.*, 1989). Interestrous intervals were 22.0 ± .7, 24.0 ± 1.1, 24.6 ± 1.2 and 25.4 ± 1.0 days for 0, 2.5, 5 and 10 mg, respectively. It appears that interferon-α_I1 does mimic the antiluteolytic actions of bTP-1 on uterine secretion of PGF since administration of interferon-α_I1, either intramuscularly or into the uterine lumen, reduced oxytocin-induced release of PGFM in cattle (Figure 6; Plante *et al.*, 1990).

These results offered the potential for using a recombinant molecule to mimic the actions of the endogenous antiluteolysin (bTP-1) produced by the conceptus. Administration of interferon-α_I1 might be one means of decreasing embryonic mortality by supplementing the reduced secretion of bTP-1 by retarded or small conceptuses. To be practical, alternate delivery routes must be developed that would be less damaging to embryos than the intrauterine route of administration. Intramuscular administration of interferon-α_I1 (1.25 to 20 mg) caused rectal temperatures to increase with average peak responses of greater than 40°C at 6 h after treatment (Newton *et al.*, 1989b; Barros *et al.*, 1990). Temperatures returned to baseline between 12 and 16 h. The increase in rectal temperatures was temporally associated with a decrease in serum progesterone concentrations (Newton *et al.*, 1989b; Plante *et al.*, 1990). Because of these effects on body temperature and progesterone secretion, additional investigations are necessary to develop delivery systems that minimize peripheral deleterious effects of exogenous interferons but induce local effects within the uterus that mimic actions of the natural antiluteolysin bTP-1.

PHARMACOLOGICAL MANIPULATION OF OVARIAN FOLLICULAR DEVELOPMENT

Since ovarian follicular development is critical to regression of CL lifespan and follicular dynamics are altered during early pregnancy, pharmacological management of follicular growth could be used in a reproductive management program to enhance reproductive efficiency. Indeed, an injection of Buserelin (a synthetic GnRH agonist) on day 12 of the estrous cycle altered follicular growth that led to recruitment of a new potentially-ovulatory follicle 7 days later (Thatcher *et al.*, 1989a). Repeated injections of Buserelin at 3-day intervals extended CL lifespan due to the repeated luteinization of follicles that would normally cause luteolysis. Macmillan *et al.* (1986) found that a single injection of Buserelin between 11 to 13 days post-insemination increased pregnancy rates of cows by 10%. Such an effect was likely due to the actions of Buserelin in luteinizing the follicle that initiates luteolysis. This would permit a longer period of conceptus development prior to the time an antiluteolytic effect must be implemented by the conceptus. The positive effects shown by Macmillan *et al.* (1986) have not been repeated by Jubb *et al.* (1990) in which fertility in Buserelin-treated cows was comparable to control cows when given Buserelin between days 11 and 13.

An alternative approach to improve embryonic survival has been direct supplementation with progesterone. Garrett *et al.*, (1988) demonstrated that injection of progesterone (100 mg) on days 1,2,3, and 4 of pregnancy advanced development of conceptuses recovered on day 14. These conceptuses were greater in length and produced a greater array of conceptus secretory proteins, including bTP-1, into conditioned medium following a 24-hour culture. Progesterone treatment also appeared to alter secretion of polypeptides from endometrial tissues collected at day 5 compared to endometrium of control-treated cows. However, number of cows per group at day 40 were too small (approximately 10 per group) to truly examine potential differences in pregnancy rate.

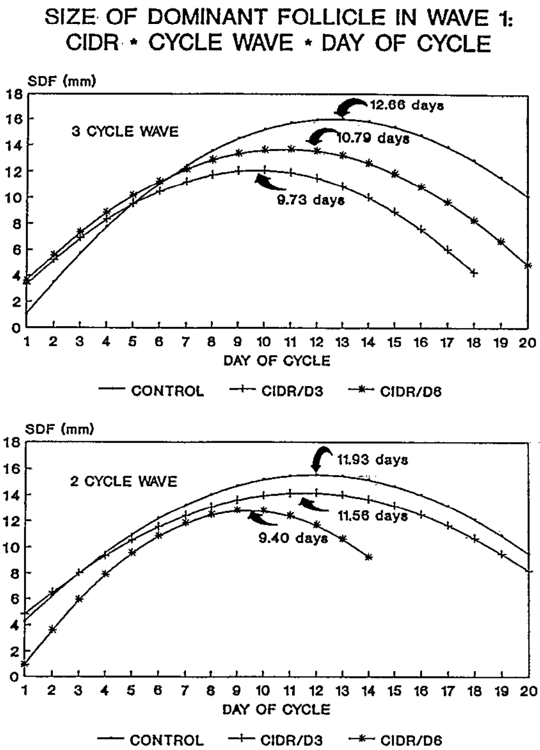

FIG 7 Size of dominant follicles during the first follicular wave for cows experiencing either three or two follicular waves. Cows (n=18) were assigned randomly to receive a Controlled Internal Drug Releasing (CIDR) device, containing 1.9 grams of progesterone, on day 3 or 6 of the estrous cycle or no CIDR device (control). CIDR devices remained in place for a 9 day period. A Treatment (control, CIDR on day 3, CIDR on day 6) x Day x Cycle (three versus two wave cycles) interaction (P<.01) was detected.

More rapid development of conceptuses was induced by progesterone treatment of cows. However, this would be essential if uterine maturation also is advanced leading to earlier release of PGF (Garrett *et al.*, 1988b). Van Cleeff *et al.* (1989) inserted an intravaginal Controlled Internal Drug Releasing (CIDR; Carter Holt Harvey, Hamilton, New Zealand) device on day 1 (Experiment 1:Control, n = 69; CIDR, n = 59) or 2 (Experiment 2: Control, n = 36; CIDR, n = 28) following estrus, and left the CIDR in place for a 7 day period. Conception rate was depressed when the CIDR was inserted on either Day 1 (18.6% versus 42%) or Day 2 (17.2% versus 52.8%) compared to control cows. It is possible that the abrupt increase in circulating or tissue progesterone achieved with the CIDR device versus a more gradual increase due to injections of progesterone induced differential physiological effects. For example, ova transport may have been adversely altered. Robinson *et al.* (1989) detected an increase in conception rates when a progesterone-releasing intravaginal device (PRID) was present in the vaginas of lactating dairy cows between days 5 and 12 (n = 28) or days 10 and 17 (n = 27) after insemination. Treatments with the PRID increased pregnancy rate to 60% versus 30% for untreated control cows (n = 30). Undoubtedly, conception rates were low in the control group and suggests that progesterone supplementation benefited cows of low fertility. More recently, Macmillan *et al.*, (1990) examined the effect of progesterone supplementation on conception rates by insertion of CIDR devices at various stages post-insemination (4 to 17 days). Supplemental progesterone via CIDR insertion into the vagina increased conception rates when treatment was initiated from 6 to 8 days after estrus and insemination (79.2% [n = 240] versus 65.7% for control [n = 245] cows). The positive effect on fertility, when progesterone is administered at this time in fertile animals, is very encouraging and the mechanism by which this effect is manifested is of interest. Potential influences on rate of embryonic development, altered uterine environment, enhanced progesterone block to reduce induction of oxytocin receptors, and altered follicular development are possible responses to progesterone.

Insertion of a CIDR device either on day 3 (n = 6) or day 6 (n = 6) of the estrous cycle versus control cows (n = 6) reduced size of the dominant follicle during the first follicular wave of three follicular wave cycles (Figure 7). This response was not so apparent during the first follicular wave of cows experiencing cycles with two follicular waves. Rates of follicular growth were not altered but time period of follicular growth was reduced in CIDR-treated cows. Thus, the effects of progesterone on conceptus, endometrial and ovarian interrelationships at a critical period may result in an integrated response to enhance embryonic survival.

CONCLUSION

Our basic understanding of endocrine, biochemical and physiological factors controlling reproductive function during pregnancy has improved considerably over the last 10 years. As a consequence, the potential to optimize reproductive management systems in cattle will be improved. Current strategies are based on a foundation of knowledge that unveils the biological dialogue between the developing conceptus and maternal unit.

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