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Interrelationships between blastocyst development, maternal recognition of pregnancy and intrauterine migration in the ewe

Nephew, Kenneth Patrick, Ph.D.

The Ohio State University, 1991
INTERRELATIONSHIPS BETWEEN BLASTOCYST DEVELOPMENT, MATERNAL RECOGNITION OF PREGNANCY AND INTRAUTERINE MIGRATION IN THE EWE

D I S S E R T A T I O N

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University

By
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* * * * *

The Ohio State University
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Once again, to my beautiful wife, Linda
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Chapter I

Introduction

In most mammals in which the length of gestation exceeds that of the menstrual or estrous cycle, one of the requirements of a successful pregnancy is extension of the lifespan of the corpus luteum (CL) of the ovary and therefore continued progesterone production. Progesterone acts on the uterine lining, known as the endometrium, to maintain a milieu appropriate for the developing conceptus (the embryo plus its surrounding membranes). Prevention of luteal regression is crucial and depends upon a chemical signal(s) produced by the conceptus. This phenomenon, described as "maternal recognition of pregnancy" by Short (1969), is one of the first series of coordinated interactions that occur between the conceptus and the mother as pregnancy proceeds.

Hysterectomy (removal of the uterus) in ewes and many other species extends the lifespan of the CL (Moor and Rowson, 1964), indicating that the uterus is the source of the substance that causes luteal regression. Evidence has accumulated to suggest that the release of the hormone prostaglandin \( F_2\alpha \) (PGF\(_2\alpha\)) by the uterus during the late luteal phase of the estrous cycle is responsible for the destruction of the CL in domestic farm animals (Goding, 1974). In ewes, for example, the uterine endometrium begins to produce and release PGF\(_2\alpha\) on d 12 (d 0 = estrus) of the 17-day estrous cycle (Zarco et al., 1988a). At about the same time, concentrations of progesterone in the blood begin to fall and the animal
returns to estrus. In pregnancy, by contrast, the release of PGF$_2$α is attenuated (Zarco et al., 1988b) and the CL continues to function.

Embryo transfer experiments by Moor and Rowson (1964; 1966a,b,c) demonstrated that conceptus product(s) must first be produced around d 12 to 13, because transfers of embryos to nonpregnant ewes up to that time had a high rate of success and, vice-versa, removal of embryos from pregnant ewes after that time delayed a return to estrus. In addition, homogenates from day 14 or 15 ovine conceptuses infused into the uterine lumen prolonged luteal function in nonpregnant ewes (Rowson and Moor, 1967). The active ingredient was heat labile, sensitive to protease treatment and not produced by the conceptus after about day 21 (Rowson and Moor, 1967). This substance, which was later identified as a protein (Godkin et al., 1982a) and named ovine trophoblast protein-1 (oTP-1; Godkin et al., 1984a), is related to the interferon family of proteins (Imakawa et al., 1987). Thus, oTP-1/ interferon is now regarded as a critical substance produced by the sheep conceptus that prevents luteal regression in early pregnancy.

In addition to signalling its presence to the maternal system within about two weeks after estrus, the ovine embryo also undergoes a process termed intrauterine migration. Intrauterine migration results in the approximately equidistant spacing of embryos in polytocous species. In sheep, intrauterine migration of embryos into the uterine horn contralateral to an ovary containing multiple ovulations occurred in nearly 90% of ewes examined (Casida et al., 1966; Scanlon, 1972; Reimers et al., 1976). In contrast, when a single ovulation occurred in one or each ovary, migration into the uterine horn not associated with the CL or
into an already occupied horn was rare (Casida et al., 1966; Scanlon, 1972). The timing of embryonic migration in sheep was reported to occur between day 12 to 14 of gestation (Cummins, 1979). Taken together, these studies indicate that migration of ovine embryos is not a passive and random event.

The signal(s) to induce embryonic migration in farm animals is not fully understood. Increased activity of uterine smooth muscle (Boving, 1971; Pope et al., 1982a), which may be stimulated locally by the embryo (Cloud and Casida, 1969; Pope et al., 1982b), were associated with migration. Embryonic synthesis of estradiol (Perry et al., 1973) has been strongly implicated as a stimulus for migration in the pig (Pope et al., 1982b) and mare (Zavy et al., 1979). However, minced cells from ovine embryos were unable to synthesize estrogens (Gadsby et al., 1980), and little information is available regarding intrauterine migration in sheep.

Critical events that the ovine embryo must accomplish during the first two to three weeks of gestation include maternal recognition of pregnancy and intrauterine migration. The objective of this dissertation was to examine if supplementing the putative signal for maternal recognition of pregnancy directly and/or stimulating the conceptus to synthesize greater amounts of cTP-1 interferon before initiation of luteolytic mechanisms would increase fecundity in sheep. It was also of interest to examine the timing of embryonic migration relative to maternal recognition of pregnancy, and to investigate if potential mechanisms associated with embryonic migration in other species, such as conceptus synthesis of estradiol, might also occur in sheep.
Chapter II

LITERATURE REVIEW

Reproductive failure is one of the most costly and limiting factors in the livestock industry. The overall economic impact, although difficult to assess, was estimated in 1978 to be as high as $1.4 billion per year (Gerrits et al., 1978).

Reproductive Losses in Sheep

Greater reproductive efficiency offers the primary opportunity to improve the competitive position of the U.S. sheep industry. Lambs weaned per ewe exposed for breeding is the single most important factor associated with profitability of a sheep enterprise in the U.S., and low lambing rates represent a major obstacle to the sheep industry in the U.S. A net increase in the number of lambs going to market or replacement ewes going into breeding flocks is desired; therefore, methods to increase sheep production are a high research priority at both state and national levels.

The potential for number of lambs born is affected by many components, including ovulation rate, fertilization rate, embryonic and fetal survival. The majority of reproductive losses are concentrated in the period of ovulation to implantation and in death losses of lambs born. Despite using males of proven fertility either for natural service or for semen for artificial insemination (Robinson, 1951), 30-40% of ova shed were not represented by lambs at birth (Edey, 1979). Thus, loss due to
fertilization failure is generally very low. The death of some embryos and fetuses is due to genetic factors (Bishop, 1964); however, chromosomal anomalies have been detected in only about 6% of d 2 or d 3 sheep embryos (Long and Williams, 1980), and no anomalies were found in 13 to 18-day sheep blastocysts (Long, 1977). Causes of additional reproductive wastage are unclear, but there seems to be good evidence to suggest that asynchrony between the environment of the maternal reproductive tract and the needs of the developing embryo or failure of the conceptus to signal its presence to the maternal system adequately make a greater contribution to prenatal loss than either fertilization failure or genetic aberrations (see reviews Moore, 1985; Wilmut et al., 1985a,b, 1986; Pope, 1988; Roberts et al., 1990).

Prenatal loss can be partitioned into one of two categories: 1) death of all embryos or fetuses present and termination of pregnancy, 2) only a portion of those present die and pregnancy progresses normally with no outward signs of death. Most loss, whether it be partial or complete, is embryonic rather than fetal and takes place within the first 3 to 4 weeks after mating, before there is firm attachment between embryonic membranes and the endometrium (Robinson, 1951; Moore et al., 1960). Abortion or absorption of the fetus after this time accounted for only about 4% of reproductive loss in ewes examined (Willingham et al., 1986).

Direct evidence of the maternal environment affecting prenatal survival, obtained using embryo transfer in sheep, demonstrated that normal embryo development depends upon a sequence of changes in uterine secretions (Wilmut and Sales, 1981) that are induced by a particular pattern of ovarian steroids (Miller and Moore, 1976). In sheep,
progesterone concentrations were affected by a number of environmental factors, including nutrition (Williams and Cumming, 1982; Parr et al., 1987), breeding season (Rhind et al., 1978), ovulation rate/number of corpora lutea (Hanrahan, 1980), and stress (Rhind et al., 1984). These factors, in addition to age of the ewe (Quirke and Hanrahan, 1977) and intrauterine migration of embryos (Doney et al., 1973; White et al., 1981), were associated with embryonic loss (Cumming et al., 1975; Lightfoot, 1986; Parr et al., 1987). A comprehensive review of all potential causes of early embryonic mortality, defined as the proportion of missing or necrotic embryos related to the number of corpora lutea, in sheep is beyond the scope of this thesis and available elsewhere (Edey, 1979; Moore, 1985; Wilmot et al., 1986; Pope, 1988; Roberts et al., 1990).

The second area of sizeable reproductive wastage is from lambing to weaning. An average of 16-20% of the potential lamb crop was lost from birth to weaning, with 73% of these losses occurring in the first few weeks (Turner et al., 1965; Willingham et al., 1986). Lamb death loss is largely a function of management, weather conditions, predation, disease and many other external factors that vary from one producer to the next. Diseases of lambs account for a substantial amount of loss. Lamb diseases include those that are non-infectious such as starvation and trauma, and infectious diseases such as vibriosis, chlamydiosis, toxoplasmosis, navel ill, pasteurella hemolytica, scours, enterotoxemia and white muscle disease. For a complete review in this, the reader is encouraged to see the review by Jensen and Swift (1988).
Early Embryonic Development

Within the first week of pregnancy, the mammalian embryo undergoes an ordered sequence of developmental processes, including cleavage, compaction, and cavitation, which culminates in the formation of the preimplantation blastocyst. The extent to which mammalian blastocysts expand their volume is highly variable and species dependent.

Sheep. The morphological development of fertilized sheep eggs was first documented by Green and Winters (1945). Later, Chang and Rowson (1965) recorded early development of ovine embryos in 50 ewes slaughtered at various intervals ranging from 27 h after the onset of heat to d 17 of pregnancy. Ovulation normally occurred 24 to 40 h after the onset of estrus (Whyman et al., 1979). Fertilization occurred very soon after ovulation, probably within about 2 h, in the area of the ampullary-isthmic junction of the oviduct (Green and Winters, 1945; Chang and Rowson, 1965). When recovered 27 to 46 h after the onset of heat, zygotes were in the pronuclear stage of development (Chang and Rowson, 1965). The first cleavage was estimated to occur 12 to 22 h and the second cleavage at about 22 to 36 h after ovulation, while the third occurred at about 35 to 45 h following ovulation (Chang and Rowson, 1965). Early development in many species, including the ewe, is dependent upon products which are synthesized by the oocyte during oogenesis and then activated during maturation and cleavage (see Moor and Gandolfi, 1987 for a review of molecular and cellular changes associated early embryo development in sheep).
The ovine embryo normally enters the uterus on d 4 at the 8 to 16 cell (termed morula) stage of development (Green and Winters, 1945; Chang and Rowson, 1965) and were usually recovered from the tip of the uterine horn at this time. Blastocysts of 50 to 70 cells within the zona pellucida (zona) were recovered on d 5 to 6 (Chang and Rowson, 1965). The thickness of the zona progressively decreased from 13.3μ for an 8-cell embryo to 6.8μ for a d 4 blastocyst (Green and Winters, 1945). Enlarged blastocysts (about 300μ in diameter) without a zona were recovered on d 9 to 11 (Chang and Rowson, 1965). After the zona is shed on d 8 to 9 (termed hatching), the ovine blastocyst undergoes rapid and spectacular morphological change, elongating from a 0.1 to 1 mm diameter sphere on d 10 to 11 to a 3 to 5 mm tubular form usually found on d 13 of pregnancy (Bindon, 1971). By d 15 to 17 of gestation, the conceptus is filamentous (thread-like) in form and has increased to 150 to 250 mm (Flechon et al., 1986). This spectacular growth of the trophoblast does not seem to result from hypertrophy and cellular rearrangement as in the pig (Geisert et al., 1982a), but rather from cell multiplication, as indicated by an increase in trophoblast deoxyribonucleic acid (DNA) content (Flechon et al., 1986). Furthermore, the presence of the embryonic disc is not essential to blastocyst elongation; twelve-day old ovine blastocysts, cut into pieces and cultured for 24 h in vitro, gave rise to structures called trophoblastic vesicles (blastocyst without the embryonic disc) that elongated to 182 mm after transfer in vivo (Flechon et al., 1986).

Early histological studies (Green and Winters, 1945; Amoroso, 1952; Boshier, 1969; Short, 1969) and recent ultrastructural studies on the ovine blastocyst (Wintenberger-Torres and Flechon, 1974; Guillomot et al.,
1981; Carnegie et al., 1982, 1985; King et al., 1982; Wooding, 1984) have shown that endoderm cells spread out from beneath the inner cell mass (ICM) at about d 7 to 8 and, by d 9 to 10, completely line the cuboidal and polygonal trophectodermal cells that surround the blastocoele, thereby forming the trophoblast. The endoderm cells are thinner than those of the trophoderm, particularly at the abembryonic pole. The outer surface of the ICM remains covered by trophoderm until the hatched blastocyst begins to elongate on about d 11 to 13.

Mesodermal cells, which also differentiate from the ICM, begin to migrate out between the trophoderm and endoderm at about d 13 to 14 and separate into two layers. The outer layer of mesoderm lines the trophoderm to form the chorion, while the inner layer covers the endoderm to form the yolk sac. The space between the two mesoderm layers is called the coelom. The allantois subsequently buds into this space by about d 18.

Ultrastructurally, the trophoderm results from polarization of the outer cells of the compacted morula, which is the first differentiated epithelium of the blastocyst. Its plasma membrane is highly differentiated. The apical surface is covered with microvilli. The lateral membranes have junctional complexes of the tight and adherens-type, as well as desmosomes and interdigitations. The basal membrane, before differentiation into endoderm, is flat except for a few projections into the blastocoele. A basal lamina separates the endoderm from the trophoderm. The trophoderm contains cytoskeletal components that are typical of epithelial cells, such as microfilaments, microtubules and tonofilaments (bundles of intermediate filaments).
Trophectodermal cytoplasm contains the usual organelles, including mitochondria with transverse cristae, ribosomes and polyribosomes, smooth and rough endoplasmic reticulum and golgi sacules. The presence of both endocytotic and coated vesicles indicates that the epithelium is actively engaged in transport activity and phagocytosis. Lysosomes and lipid droplets are also present within the cytoplasm.

Histological and histochemical changes of the trophoblast and uterine epithelium during implantation in the ewe has been well described (Boshier, 1969; Short, 1969; Wooding, 1984; Smith et al., 1990). The embryonic membranes are loosely associated with the uterine endometrium on d 15 to 16. Definitive attachment of the conceptus occurred by interdigitation of trophoblast microvilli and uterine epithelium at d 18 of gestation. Implantation was associated with high levels of acid and alkaline phosphatase activity in the distal surfaces of the cells of the trophoblast and uterine epithelium, presumably due to the release of lysosomal hydrolases for modification of the maternal epithelium and carbohydrate metabolism for production of the mucopolysaccharide present between embryonic/maternal tissues, respectively.

Cow. Development of the bovine embryo has been recently reviewed (Betteridge and Flechon, 1988). Cleavage of the zygote into two blastomeres occurred 24 to 28 h after ovulation, so that most embryos recovered on d 1 to 2 are at the 2-cell stage. By d 5, bovine embryos are usually described as morula (16-cell stage) and can usually be recovered from the uterus. At about the 32-cell stage, the individual blastomeres undergo the process of compaction (loss of their sharp outline), which is generally regarded as a prerequisite for the formation of the blastocele.
The transformation from compact morula to early blastocyst (about 100 cells and 160μ in diameter) usually occurs on d 7 of gestation. Fully expanded blastocysts, still within the zona but on the verge of hatching, contain about 160 cells. The hatching process in cattle has been observed on d 7 to 8 but more commonly occurs on d 9 to 10, when the embryo is about 200 cells. On d 11 to 12, blastocysts are spherical, about 375μ in diameter and about 1000 cells. The shape of hatched blastocysts then changes from spherical to ovoidal before the obvious elongation phase that begins between d 12 to 14. The conceptus is usually filamentous and about 70 to 100 mm by d 16. Definitive attachment of embryonic and uterine tissues occurs on d 18 to 22 of gestation. By d 24, the conceptus occupies both uterine horns.

**Pig.** The pig blastocyst is generally recognized as representing the extreme case of morphogenetic differentiation and expansion prior to implantation. The embryo is moved to the ampullary-isthmic junction within a few hours of fertilization and remains there for about 36 h (Dhindsa et al., 1967). Pig embryos enter the uterus on d 3.5 to 4 (about 48 h after ovulation) at both the 4- and 8-cell stage (Perry and Rowlands, 1962; Oxenreider and Day, 1965; Broeermann et al., 1990). They remain near the tip of the uterine horn until about d 6 when they move towards the body of the uterus. After hatching occurs on about d 7 (Oxenreider and Day, 1965), pig blastocysts undergo marked morphological change between d 10 to 16 of pregnancy (Anderson, 1978). The most rapid period of blastocyst elongation is between d 10 to 12, when changes from spherical (3 to 10 mm diameter) to tubular (10 to 50 mm long) to filamentous (>100 mm long) forms occur, attaining lengths of 700 to 1000
mm by d 16 (Perry and Rowlands, 1962; Anderson, 1978). Estimates of blastocyst growth were 0.25 mm/h for development of spherical blastocysts from 4 to 9 mm in diameter, compared to 30 to 45 mm/h for blastocyst elongation after reaching 10 mm (Geisert et al., 1982a). Growth of spherical blastocysts from less than 1 mm in diameter to 8 mm in diameter appears to be due to rapid cellular hyperplasia, based on a positive correlation between embryo diameter and embryonic DNA content (Geisert et al., 1982a). Blastocyst elongation, in contrast, is accomplished through cellular migration and remodelling and not cellular hyperplasia, based on no significant change in DNA content and a slight decrease in the mitotic index during the transition form 10 mm spherical blastocysts to tubular and filamentous forms (Geisert et al., 1982a). The role of microfilaments, especially the filamentous actin cytoskeleton, have been strongly implicated in stability and contractility (elongation) of the trophectoderm (Mattson et al., 1990). Initial attachment of trophoblast to the maternal uterine epithelium begins on approximately d 13 of pregnancy; completion of attachment by interlocking microvilli to form the epitheliochorial placenta occurs on d 18 (Perry et al., 1973). Placentation in the pig is therefore noninvasive (Amoroso, 1952).

Mare. The equine embryo spends more time in the oviduct than embryos of other farm species, and it is already at the blastocyst stage when it enters the uterus on d 5 to 6 (Webel et al., 1977). Only fertilized eggs enter the uterus; unfertilized ova remain in the isthmus where they slowly degenerate over many months (Betteridge et al., 1979). The precise mechanism involved in this phenomenon is unknown, but embryonic secretion of PGE₂, a known relaxant of oviductal musculature, may be involved
(Betteridge et al., 1979). Horse embryos do not undergo rapid elongation of the trophectoderm as do sheep, cow and pig conceptuses, but expand rapidly as spherical organisms (generally termed vesicles) between d 8 and 20 of gestation. Ginther (1983) reported a 17 mm diameter vesicle on d 13 that retained its spherical shape until at least d 50. Attachment to the uterine endometrium occurs between d 15 to 17 (Ginther, 1983).

**Conceptus Secretory Products**

Conceptuses of domestic animals produce steroids, prostaglandins, specific proteins and possibly other unidentified agents which are intimately involved in the synchronous growth and development of the conceptus and the uterine endometrium. These factors will be reviewed. The possible physiological roles of these embryonic products in the maternal recognition of pregnancy and intrauterine migration will be discussed later.

**Sheep: Steroids and Proteins.** At the present time, there is only indirect evidence that the ovine conceptus has the capability to produce steroids, mainly estrogens. Minced trophoblastic tissues from d 16 to 18 ovine conceptuses were incapable of significant conversion of labeled adrostenedione to estrone or estradiol-17\(\beta\) (Gadsby et al., 1980). Dwyer and Robertson (1980) reported that levels of estrone sulfate were detectable in uterine venous plasma on d 16 of gestation in ewes. Higher, but non-significant, concentrations of estrogen in samples of utero-ovarian venous blood from pregnant and nonpregnant ewes were also observed on d 13 and 15 (Ellinwood, 1978; Reynolds et al., 1982), approximately the time of embryonic elongation (Chang and Rowson, 1965). Likewise, Willis
et al. (1979) reported aromatase activity in endometrium from ewes on d 20 of pregnancy.

Synthesis of prostaglandins by the ovine embryo has been examined. When incubated with radioactive arachidonic acid, d 12 and d 14 to 16 sheep blastocysts released radiolabelled 6-keto-PGF\(_{1\alpha}\) (a major metabolite of PGL\(_2\)), PGF\(_{2\alpha}\), PGE\(_2\), PGFM and several unidentified compounds (Marcus, 1981; Lacroix and Kann, 1982; Lewis, 1989). Triglycerides are probably an important source of arachidonic acid for conceptus synthesis of prostaglandins (Lewis, 1989).

Ashworth and Bazer (1989) characterized the secretory protein profile from conceptuses collected from mated ewes on d 10, 12, 14, and 16 by two-dimensional polyacrylamid gel electrophoresis (2D-PAGE) and fluorography. The dominant conceptus secretory protein on each of these days had the same molecular weight (MW) and isoelectric point (pI) as ovine trophoblast protein-1 (oTP-1). Western blotting using a specific anti-oTP-1 antibody confirmed that this protein was secreted in increasing quantities as pregnancy progressed was oTP-1.

**Purification and Characterization of oTP-1.** oTP-1 is the major secretory protein produced by the peri-attachment sheep conceptus. This low molecular weight acidic protein was first purified by Godkin et al. (1982a). Production of oTP-1, as detected by 2D-PAGE, was maximal between d 13 and 21. By using a more sensitive radioimmunoassay, however, Ashworth and Bazer (1989) reported that oTP-1 is produced as early as d 8 and 10 of gestation. Analysis of changes in oTP-1 messenger ribonucleic acid (mRNA) levels during this period by dot-blot and Northern techniques demonstrated a marked increase in oTP-1 mRNA beginning on d 13 or 14 or
pregnancy followed by a decline between d 16 and 22 (Hansen et al., 1988; Stewart et al., 1989). Farin et al. (1989) utilized the more sensitive method of in situ hybridization to confirm the pattern of change in oTP-1 mRNA concentrations from d 13 to 23 of pregnancy, to demonstrate that the dramatic increase in oTP-1 gene expression on d 13 was correlated closely with the morphological transition of the ovine conceptus from a spherical to an elongated form, and to show that oTP-1 mRNA was confined to trophoderm of ovine conceptuses.

OTP-1 can be purified from the medium after culturing d 13 to 21 ovine embryos in vitro for 24 h in serum free-medium (Godkin et al., 1982a). The protein is only a minor component of tissue extracts. A single d 16 conceptus can produce from 100 to 500 µg of oTP-1 in a 30-h culture period (Ashworth and Bazer, 1989). The absence of added serum in the medium allows oTP-1 to be purified in two chromatographic steps: DEAE-cellulose ion exchange chromatography and gel filtration on Sephacryl S-200. When purified by 2D-PAGE, it consists of 3 to 4 nonglycosylated, polypeptide isoforms of approximately 19,000 MW with an pI between 5.3 and 5.8 (Godkin et al., 1982a).

**Molecular Cloning of oTP-1.** Complementary DNA (cDNA) libraries were constructed from d 15 to 17 sheep embryonic poly(A)* RNA in the bacteriophage λgt11 (Imakawa et al., 1987, 1989). These libraries were screened with antiserum to oTP-1, and about .05% of the clones gave a positive immunological reaction. All of the recombinant bacteriophage isolated by this procedure contained segments of cDNA representing regions close to the carboxyl terminal end of oTP-1. Primer extension procedures were then used to make new libraries containing cDNA,
complementary to the 5'-end of the mRNAs. However, rescreening the original λgt11 libraries with these longer cDNA probes allowed the full length cDNAs to be isolated for OTP-1.

Complementary cDNAs for OTP-1, after being subjected to nucleotide sequencing, demonstrated considerable sequence similarity to alpha interferons (IFN-α's; Imakawa et al., 1987, 1989), proteins secreted by lymphocytes that are generally regarded as the first line of resistance against viruses (Morris, 1988). Specifically, the mRNAs for OTP-1 were about 1 kb in length and possessed a 585-base open reading frame that coded for a 172-amino acid polypeptide which contained a 23-residue signal sequence. The cleavage point for the signal sequence was verified by NH2-terminal amino acid sequencing of purified OTP-1 (Imakawa et al., 1987; Stewart et al., 1987; Charpigny et al., 1988). The inferred length of the mature polypeptide (172 residues) and the degree of nucleotide and amino acid sequence similarity placed OTP-1 in the IFN-αII family described in the bovine (Capon et al., 1985) rather than in the better known IFN-αI whose members are 165 or 166 residues long. However, the cDNA and amino acid sequence for OTP-1 more closely resembled that of the major secretory product of the bovine conceptus, bTP-1 (Imakawa et al., 1989) more than bovine IFN-αII. It is likely that OTP-1 and bTP-1 represent a further subgroup with the very large IFN-α family of genes (Roberts, 1989).

The sequence studies have also shown that multiple mRNAs and presumably multiple genes exist for both OTP-1 and bTP-1 (Imakawa et al., 1987, 1989). At least 10 to 15 genes for bovine IFN-αII have been identified on Southern blots (Capon et al., 1985), but it is not clear
how many of those genes were functional, and it is uncertain whether the highly stringent conditions for Southern hybridization used by those investigators would detect bTP-1 genes that differ significantly from bovine IFN-αII's at their 3' ends (Imakawa et al., 1989). No ovine IFN has been isolated, nor has any ovine IFN gene been cloned or sequenced; therefore, it has not been possible to compare oTP-1 with ovine IFN.

**Primary Structure of oTP-1.** OTP-1 has cysteines at positions 1, 29, 99 and 139 that are conserved in all IFN-α's and form the disulfide bonds (1-99; 29-139) necessary for full biological activity, as is a Cys-Ala-Trp-Ile-Val-Arg sequence (residues 139 to 145; Imakawa et al., 1989). Predictions of the secondary structures demonstrated that oTP-1 may possess a considerable amount of helical structure, as do other IFNs (Roberts, 1989). Hydrophilicity profiles by Roberts (1989) demonstrated no obvious distinction between the embryonic IFNs and bovine IFN-αI and IFN-αII.

Other conceptus secretory proteins include ovine placental lactogen, which is first detectable on d 16 (Martal et al., 1977) at the time binucleate cells in the trophoblast appear (Boshier, 1969) and may be involved in the process of attachment of the trophoblast to the caruncular epithelium (Martal, et al., 1977). Recently, Wiltbank et al. (1990a) reported a 45,000 MW factor that is heat labile and has been partially purified to electrophoretic homogeneity. This protein appears to have a direct, luteotrophic effect on the CL.

**Cow: Steroids and Proteins.** During the period of elongation, cow conceptuses produce limited quantities of progesterone, androstenedione, estradiol, PGE₂ and PGF₂α (Shemesh et al., 1979; Eley et al., 1983), as
well as 5α-reduced androgens and progestagens (Thatcher et al., 1985). Day 13 to 18 bovine conceptuses produce a small (<10,000 MW), heat labile, lipid-soluble, luteotrophic substance which was adsorbed by dextran-coated charcoal, suggesting that is probably a lipid and perhaps a steroid (Hickey and Hansel, 1987).

The major secretory glycoprotein synthesized by the bovine conceptus is bTP-1. It is produced maximally between d 17 and 22 of gestation and is one of several major products between d 24 and 29 (Godkin et al., 1988). bTP-1 consists of multiple isoforms of molecular weights between 22,000 and 24,000 (pI 5.8-6.7). bTP-1 was localized by immunocytochemistry in the cytoplasm of both mono- and binuclear trophoderm cells of d 20 bovine conceptuses, confirming that it is a product of the trophoblast (Lifsey et al., 1989). Significant amino acid sequence homology between bTP-1, cTP-1 and human and bovine IFN-α has been demonstrated (Helmer et al., 1987; Imakawa et al., 1987; Stewart et al., 1987; Imakawa et al., 1989). Messenger RNA for bTP-1 was detected as early as d 12 of pregnancy, with a marked increase in expression occurring on d 15 to 16 of pregnancy and continuing through at least d 25 (Farin et al., 1990). The increase in expression of bTP-1 mRNA on d 15 to 16 occurred coincident with elongation of the blastocyst (Farin et al., 1990), and during the reported period of maternal recognition of pregnancy in this species (Northey and French, 1980).

In addition to bTP-1, the bovine conceptus produces other factors that might be useful for early pregnancy detection, such as pregnancy-specific protein B (PSPB; Butler et al., 1982), which can be measured in the sera of pregnant cattle by radioimmunoassay after d 24 of gestation
Characterization of PSPB showed that it was an acidic glycoprotein with an apparent molecular weight of 78,000 and several isoelectric variants with pIs of 4.0 to 4.4 (Sasser et al., 1989). Alpha-fetoprotein, which is an immunosuppressive agent, can be detected by d 14 in the bovine blastocyst (Janzen et al., 1982). Heat-shock proteins have been identified in bovine embryos at d 17 (Putney et al., 1987) and may therefore be useful in monitoring the adverse effects of stress on fertility. Placental lactogen has been detected in bovine conceptuses between d 17 and 25, shortly after appearance of binucleate cells in the bovine trophectoderm but after attachment or implantation (Flint et al., 1979).

**Pig: Steroids and Proteins.** Steroid hormone production by pig blastocysts has been well documented (Perry et al., 1973; Gadsby et al., 1980; Geisert et al., 1982b). Specifically, porcine blastocysts secrete estrogens beginning on d 12 of pregnancy (Perry et al., 1973; Gadsby et al., 1980), at the 10 mm spherical stage. Production increases during rapid conceptus elongation on d 11 to 12, declines on d 13 to 14, followed by a second, sustained increase on d 15 to 25-30 of gestation (Zavy et al., 1980; Geisert et al., 1982b; Fischer et al., 1985). Spherical conceptuses, 7 mm in diameter, were able to convert progesterone to estrone and estradiol-17β (Fischer et al., 1985), and the porcine conceptus appears to have a high capacity to convert estrogens to catecholestrogens (Ford and Stice, 1985; Chakraborty et al., 1988;). Estrogens have been localized in the trophectoderm and endoderm of the conceptus on d 12, with the greatest amounts in yolk sac endoderm on d 14 to 16 (King and Ackerley, 1985). In addition, porcine conceptuses
synthesize substantial amounts of \( \text{PGE}_2 \) and \( \text{PGF}_2\alpha \) (Lewis and Waterman, 1983).

Preimplantation pig conceptuses produce a number of characteristic proteins during early gestation (Godkin et al., 1982b; Gries et al., 1989), including plasminogen activator (Fazlebas et al., 1983a), but the nature of the many specific proteins is largely unknown. The pig conceptus secretes low molecular weight, acidic polypeptides in culture between d 10.5 and 16 of pregnancy (Godkin et al., 1982b; Mirando et al., 1990a). One of these proteins (MW 22,000 to 24,000) has recently been reported to be an interferon-like protein, but not immunologically related to oIFN-1 or bIFN-1 (Cross and Roberts, 1989). The highest levels of antiviral activity was observed in uterine flushings from pregnant gilts on d 14 to 15 (Mirando et al., 1990a). Furthermore, while the ovine conceptus can stimulate synthesis of some endometrial secretory proteins and inhibit synthesis of others in sheep (Vallet et al., 1987; Ashworth and Bazer, 1989), it appears that the pig endometrium may influence secretion of antiviral proteins from pig conceptuses (Beers et al., 1990).

**Mare: Steroids and Proteins.** The horse conceptus produces substantial amounts of estrogen from d 10 to 20 of gestation (Flood et al., 1979). After a 90 min incubation in vitro, preimplantation equine conceptuses secreted up to 20 ng of estrone and 100 ng of estradiol (Zavy et al., 1979) and androgen (Flood et al., 1979). Aromatase activity, as measured in vitro by the incorporation of \(^3\text{H}\)-labelled androstenedione into phenolic compounds, has been demonstrated in the mare conceptus (Heap et al., 1982).
Fazleabas and McDowell (1983b) and McDowell et al. (1990) reported that d 12 to 14 horse conceptuses secrete a unique array of at least five proteins with molecular weights ranging from 50,000 to 400,000; however, those proteins were not primary secretory products after d 15 to 16. From d 15 or 16 to 28, proteins of yolk sac origin, i.e. transferrin and alpha fetoprotein, were dominant. The chorioallantois also produces an array of acidic proteins at this time, with the two dominant groups having estimated molecular weights of 28,000 and <15,000 and pIs of 6.4 and <4.0, respectively (McDowell et al., 1990).

**Uterine Endometrial Secretions**

Uterine secretions provide a source of nutrients essential for normal conceptus development. Identification and characterization of endometrial specific proteins are requisite to understand the regulation of conceptus development and pregnancy maintenance.

**Sheep.** The uterine endometrium, under the control of ovarian steroids (Miller and Moore, 1976), secretes proteins from the epithelium into the uterine lumen. Functions ascribed to these proteins include conceptus growth and development, fetal nutrition, enzyme action and inhibition, and immunoprotection of the conceptus.

The secretory protein profile from uterine endometrium of pregnant ewes has been characterized extensively by Ashworth and Bazer (1989). Ewes received daily injections of corn oil or progesterone (50 mg) d 4 to 9. Endometrial tissue was collected on d 8 and 10 and cultured with $^{35}$S-methionine. The medium was analyzed for total and trichloroacetic acid-precipitable radiolabeled proteins. Levels of specific proteins were determined after protein separation by 2D-PAGE and estimation of the
radioactivity associated with radiolabeled proteins on fluorographs. Thirty endometrial proteins were investigated. All 30 proteins were present in endometrial cultures from pregnant ewes on d 8 and 10, but thirteen of these proteins were absent from control ewes. Progesterone treatment increased the levels of ten of these proteins.

Recently, a progesterone-modulated, low-molecular weight (14 kd) protein from the uterus was characterized (Kazemi et al., 1990). Immunogold labeling in conjunction with electron microscopy revealed that this "14K protein" mainly accumulated in membrane-bound, needle-shaped crystal structures in the uterine epithelium; however, the 14K protein was localized within the trophectoderm of d 16 ovine conceptuses as well (Kazemi et al., 1990). Presumably, the 14K protein is synthesized by the surface and upper glandular epithelium of the maternal uterus and becomes accessible for uptake by the conceptus only after it has been secreted.

Endometrial explants from ewes released several polypeptides into the culture medium in response to treatment with oTP-1 (Godkin et al., 1984a; Vallet et al., 1987; Sharif et al., 1989). Purified oTP-1 has been shown to increase the levels of 11 and to decrease the levels of six endometrial secretory proteins on d 12 (Vallet et al., 1987). Two acidic proteins, p70 (70,000 MW; pI 4.0) and p15 (15,000 MW; pI 5.7), were particularly sensitive to oTP-1 treatment. These were produced between d 12 and 24 of pregnancy and d 12 and 16 of the estrous cycle (Vallet et al., 1987; Sharif et al., 1989). Conversely, the secretion of oTP-1 by d 16 sheep conceptuses was enhanced when co-cultured with endometrium from d 16 pregnant ewes (Ashworth and Bazer, 1989), suggesting differential regulation of individual conceptus secretory proteins by the endometrium.
Curiously, estrogen treatment stimulated synthesis of proteins by the endometrium during the luteal phase of the ewe (Salamonsen et al., 1985), although synthesis of this steroid by the ovine conceptus has not been demonstrated.

Under the influence of progesterone, the sheep uterus secretes two major glycoproteins, called "uterine milk proteins" (UIMP) (Bazer et al., 1979; Hansen et al., 1987; Ing et al., 1989). The UIMP are a pair of basic glycoproteins with similar molecular weights of 57,000 and 55,000 and identical pIs (Moffat et al., 1987). They have been shown to be closely related to each other in N-terminal primary sequence (Hansen et al., 1987; Ing and Roberts, 1989) and peptide mapping (Hansen et al., 1987). Western blotting of uterine flushes and of endometrial explant culture medium, endometrial RNA analyses on dot and Northern blots, and immunocytochemistry performed on uterine tissue sections demonstrated the presence of low levels of UIMP production and secretion in intact ewes at d 16 of the estrous cycle and at d 14 of pregnancy (Ing et al., 1989). The source of the UIMP appears to be the uterine glands of the intercaruncular endometrium (Moffat et al., 1987; Ing et al., 1989).

The uterine endometrium synthesizes enzymes for intermediary metabolism and steroid hormone metabolism (Findlay et al., 1981). There are differences in enzyme activities between regions of the endometrium. The lysosomal enzymes, acid phosphatase and β-glucuronidase, have a greater activity in caruncular than in intercaruncular tissue, and reach their maximum activities during the luteal phase (Findlay et al., 1981). Other secretory proteins, such as mitogens, binding and transport proteins, and protease inhibitors have yet to be isolated and characterized in sheep.
Cow. Protein secretion by the bovine endometrium has also been characterized. As in the ewe, synthesis of specific proteins was affected by the conceptus. Treatment of endometrial explants from pregnant or cyclic cows on d 17 with bovine conceptus secretory proteins selectively induced secretion of proteins in a 10,000 and 13,000 MW range (Bartol et al., 1981; Gross et al., 1988a). A 15,000 MW secretory protein was detected in uterine flushings from pregnant cows at d 19 that was not present in flushings from cyclic cows at d 19 (Bartol et al., 1981). Furthermore, Bartol et al. (1985) reported secretion of a 14,000 MW endometrial protein (pI>7.2) by endometrial explants from pregnant cows and from cows that were bred but subsequently found to be nonpregnant on d 17 post-estrus. Geisert et al. (1988) also described the secretion of a group of low molecular weight endometrial proteins (14,000 to 16,000; pI range 6.8 to 7.2); the amount of these proteins increased to d 17 of pregnancy. Culturing endometrial tissue with ³²P-leucine and then characterizing the acidic and basic polypeptides present in the culture medium by fluorography and silver-staining of 2D-PAGE gels, Garret et al. (1988) demonstrated that early secretion of these proteins could be induced by treatment with progesterone on d 1 to 4 of pregnancy.

Pig. The endometrium of the pig synthesizes a diverse group of proteins in response to progesterone. The most studied component of pig uterine secretions is uteroferrin. Uteroferrin is a 31,000 MW glycoprotein synthesized and secreted by the uterine glandular epithelium of the pig (Roberts and Bazer, 1980). The proposed function of uteroferrin is that of a transplacental iron transporter. The protein can bind two atoms of iron per molecule and is presumed to act as a major iron
carrier from the uterus to the developing fetus (Roberts et al., 1986). Uteroferrin is transported to the fetus via the umbilical vein and then sequestered by the fetal liver and spleen which are sites for iron metabolism. Highest concentrations of uteroferrin mRNA are observed at mid- and late pregnancy (Simmen et al., 1988a).

Using immunological reagents and specific cDNA probes, the tissue origins and temporal patterns of mRNA have been elucidated for several uterine-derived proteins (see review by Simmen and Simmen, 1990). Insulin-like growth factor-I (IGF-I), a potent mitogen structurally related to insulin (Zapf and Froesch, 1986), is synthesized by the uterine endometrium and released into the lumen (Simmen et al., 1989). Maximal concentrations of IGF-I in uterine luminal fluids were detected on d 10 to 12 of pregnancy (Simmen et al., 1989), which coincides with blastocyst estrogen synthesis and elongation. The primary amino acid sequence of porcine IGF-I has been determined. Hybridization analysis of IGF-I mRNAs in pig uterine tissues by using cloned cDNAs as probes has revealed that the highest concentrations occur on d 12 of pregnancy (Tavakkol et al., 1988), but the tissue content of the IGF-I protein remains constant (Letcher et al., 1989a). Type I IGF receptors were localized in uterine tissues and in preimplantation-stage pig blastocysts (Simmen and Simmen, 1990), implicating IGF-I as a potential mediator of endometrial and embryo growth and development. Uterine mRNAs for IGF-II, a mitogen that has been classically considered a fetal growth factor (Zapf and Froesch, 1986), are low during early pregnancy (preimplantation), increase by 10-fold by d 30 and decline at d 90 to 110 of pregnancy (Simmen and Simmen, 1990). Binding of IGF's to receptors is modulated by
a class of proteins called the IGF binding proteins (Nissley et al., 1985). A small increase in uterine mRNAs for one such binding protein, BP-3A, was observed on d 12 of pregnancy, and BP-3A mRNAs were abundant from d 30 to 120 of gestation (Simmen and Simmen, 1990). This pattern of BP-3A mRNA expression coincides with that for IGF-I and II transcripts, blastocyst elongation and synthesis of estrogen, strongly suggesting a relationship between these phenomena.

The major growth factor component of uterine luminal fluids from pregnant sows on d 8 to 12 has been termed uterine luminal fluid mitogen (ULFM) (Simmen et al., 1988b). ULFM stimulates DNA synthesis in cultured pig uterine stromal cells (Simmen et al., 1988b). Other small molecular weight mitogens (MW <10,000) found in d 12 uterine luminal fluids include epidermal growth factor (EGF; Simmen et al., 1988b). Specific binding of radiolabelled EGF to d 12 and 16 pig conceptuses has been reported (Letcher et al, 1989b). The biological function and autocrine and paracrine mechanisms of action of these growth factors are currently unknown. A group of plasmin/trypsin inhibitors has been demonstrated in uterine secretions of pigs during the luteal phase that presumably controls the activity of the protease plaminogen activator, whose substrate plasminogen is also present in uterine secretions (see review, Roberts and Bazer, 1988). An antileukoproteinase-like protein, which can inhibit the enzymes elastase and cathepsin, has also been identified in pig uterine tissue during early, mid and late pregnancy (Simmen and Simmen, 1990). Lysozymes, hydrolytic enzymes that function as antibacterial agents, and retinol binding protein, which may be involved
in vitamin A transport to the fetus, have both been identified in pig uterine secretions (Roberts and Bazer, 1988).

**Mare.** Endometrial samples obtained from pony mares on d 12 to 18 of pregnancy released an array of non-dialysable polypeptides during a 24 h incubation in vitro that were characterized by 2D-PAGE and fluorography (McDowell et al., 1990). Uteroferrin was one of these proteins.

**Luteolysis**

Luteal regression in nonpregnant farm animals is initiated on d 12 to 15 in the ewe (Zarco et al., 1988a), d 17 to 19 in the cow (Hansel et al., 1973), d 14 to 18 in the sow (Diehl and Day, 1973) and d 14 to 15 in the mare (Kindahl et al., 1982). Hysterectomy extended luteal lifespan in sheep (Moor and Rowson, 1964), cattle (Wiltbank and Casida, 1956), pigs (Spies et al., 1958), and horses (Ginther and First, 1971), implicating uterine involvement in the luteolytic process. The effect of the uterus on the ovary was due to a local rather than a systemic action. Surgical removal of the uterine horn adjacent to the ovary bearing the CL in sheep (Inskeep et al., 1966; Moor and Rowson, 1966a; McCracken et al., 1969), cattle (Ginther et al., 1967), and pigs (Mesnil and Buisson, 1966) prevented luteal regression. However, systemic regulation of luteal lifespan by the uterus was apparent in the pig (Anderson et al., 1966), as the presence of an unoccupied uterine horn regressed CL (Christenson and Day, 1971). In the mare, luteolysis occurred normally following removal of the uterine horn ipsilateral to the CL (Ginther and First, 1971); therefore, the uterine luteolysin(s) appears to travel from the uterus to the ovary bearing the CL via a systemic route in horses (Ginther, 1981).
Anatomical differences in the arrangement of the utero-ovarian vasculature were apparent between those species which exhibited a local versus systemic utero-ovarian relationship for luteolysis. In sheep, cattle and pigs the ovarian artery is highly convoluted and lies in close apposition with the ovarian vein which drains both the uterine horn and adjacent ovary (Del Campo and Ginther, 1973; Mapletoft et al., 1976). In contrast, the ovarian artery of the horse is relatively straight and remains separated from the ovarian vein (Ginther, 1981). Thus, the uterus appears to produce a blood borne product(s) that is transported from the uterine venous drainage into the ovarian arterial supply and initiates the process of luteolysis in a local manner in the sheep, cow and pig. In the mare, luteolysis appears to occur via a systemic pathway.

The luteolytic activity of PGF$_2$α has been documented in sheep (Goding, 1974), cows (Hansel et al., 1973; Lauderdale, 1974), pigs (Diehl and Day, 1973) and horses (Douglas and Ginther, 1972). Several lines of evidence support the possibility that PGF$_2$α is the endogenous agent responsible for luteolysis in domestic livestock. Elevated concentrations of PGF$_2$α in the uterine venous drainage, uterine tissue, and uterine flushings coincided with the period of expected luteolysis (Thorburn et al., 1973; Moeljono et al., 1977; Douglas and Ginther, 1976; Zarco et al., 1988a). Inhibition of uterine prostaglandin with indomethacin blocked spontaneous luteal regression (Troxel and Kesler, 1984). Immunization against PGF$_2$α prevented luteolysis (Fairclough et al., 1981; Ronayne et al., 1990). Evidence for the local exchange of PGF$_2$α from the uterine venous drainage into the ovarian arterial supply has been demonstrated in sheep (McCracken et al., 1972) and cattle (Hixon and Hansel, 1974).
Collectively, these data strongly support the hypothesis that PGF$_2\alpha$ of uterine origin is responsible for luteolysis at the end of the estrous cycle. Mechanisms of action of PGF2 (effects on luteal blood flow or on different luteal cell types at the morphological or cellular level), endocrine regulation of PGF$_2\alpha$ production, potential involvement and interactions of other hormones (such as estrogen and oxytocin), additional sources of PGF$_2\alpha$ (such as the CL itself), and the possible involvement of the immune system are currently being examined by many investigators.

**Comparative Aspects of Maternal Recognition of Pregnancy in the Ewe, Cow, Sow and Mare**

For pregnancy to proceed beyond its very earliest stages, biochemical communication must occur between the mother and the developing conceptus within the first two to three weeks in order to prevent the normal cyclic regression of the corpus luteum and to ensure production of progesterone. This phenomenon, which requires the conceptus to signal its presence to the mother in some manner, was termed maternal recognition of pregnancy by Short (1969). Failure of this process results in loss of pregnancy. Maternal recognition of pregnancy occurs on d 12 to 13 in sheep (Moor and Rowson, 1966a,b,c), d 15 to 17 in cattle (Northey and French, 1980), d 10 to 12 in pigs (Dhindsa and Dziuk, 1968ab), and d 14 to 15 in horses (Hershman and Douglas, 1979). Failure of this processes results in loss of the pregnancy.

Conceptuses of sheep, cows, pigs and horses produce proteins, steroids, and prostaglandins during early pregnancy that may be involved in protecting the CL from the luteolytic effects of PGF$_2\alpha$ produced by the uterine endometrium. These secretory products may have either a
lutetrophic effect on the CL or an antiluteolytic on the uterus or perhaps both. They may act directly in a paracrine/autocrine manner on the endometrium or in an endocrine manner on the CL. Available data suggests that most of these agents are antiluteolytic agents. Conceptus secretory products are also involved in a number of activities during early pregnancy, including those associated with the survival of the conceptus allograft, vasodilation and angiogenesis to increase uterine blood flow and substrate delivery to the pregnant uterus, transport of nutrients to the uterine lumen from the maternal system, intrauterine migration and placentation. Current concepts of how the conceptus acts to protect the CL and allow for its continued production of progesterone will now be discussed.

**Conceptus secretory proteins and the establishment of pregnancy.** In the sheep and cow, the conceptus products cTP-1 and bTP-1 are structurally similar (Imakawa et al., 1989) and generally considered to be antiluteolytic paracrine hormones which modify endometrial secretion of PGE₂ to allow for maternal recognition of pregnancy. These antiluteolytic proteins may also be biologically similar. Maintenance of CL occurred in some recipient cows and ewes after interspecies reciprocal transfer of trophoblastic vesicles which secreted these factors (Heyman et al., 1984), and antiserum to cTP-1 crossreacted immunologically with bTP-1 (Helmer et al., 1987).

The endometrium of cyclic ewes releases PGE₂ in a pulsatile manner on d 12 to 17, and about five episodes of PGE₂ in a 24 h period are required for luteolysis (Zarco et al., 1988a). Although basal concentrations of PGE₂ were higher in pregnant versus cyclic ewes
(Ellinwood et al., 1979a; Silvia et al., 1984; Zaroo et al., 1988b), the number of pulses of PGF$_2$α was reduced significantly between d 12 to 17 (Zaroo et al., 1988b). Intrauterine infusion of cTP-1 extended the interestrous interval in sheep (Godkin et al., 1984b; Vallet et al., 1988) presumably by interacting with its endometrial receptors (Knickerbocker and Niswender, 1989) and inhibiting uterine secretion of PGF$_2$α in response to estradiol and oxytocin (Fincher et al., 1986; Vallet et al., 1988a). The proposed mechanism of action was by inhibiting activation of the phosphatidylinositol cycle (Mirando et al., 1990b), the second messenger system within the endometrium which is associated with oxytocin-stimulated PGF$_2$α release (Flint et al., 1986). Similarly, infusion of bTP-1 into the uterine lumen of cyclic cows extended CL lifespan (Knickerbocker et al., 1986a) and blocked the estradiol-induced increase in PGF$_2$α secretion (Knickerbocker et al., 1986b). The in vitro synthesis of PGF$_2$α by endometrium collected at d 17 of pregnancy and cultured with bTP-1 was lower than that for endometrium collected on d 17 of the estrous cycle (Gross et al., 1988a), presumably due to increased activity of a proteinaceous, intracellular endometrial inhibitor of prostaglandin synthesis induced by bTP-1 (Gross et al., 1988b). Although these trophoblastic proteins did not stimulate progesterone secretion by dispersed luteal cells in vitro (Godkin et al., 1984a), and considerable evidence suggests that they have an antiluteolytic role, there are also data suggesting that these or other conceptus secretory proteins have a luteotrophic role. Both cTP-1 and bTP-1 are interferons of the alpha II family and possess antiviral properties characteristic of these interferons (Pontzer et al., 1988; Roberts et al., 1989). Antiviral
activity was detected in the uterine vein of pregnant ewes (Schalue-Francis et al., 1990), suggesting that oTP-1 leaves the uterus and that its action may not be confined to the uterus. High affinity binding sites for oTP-1 in the CL have been reported (Knickerbocker and Niswender, 1989), oTP-1 binds specifically to luteal membrane preparations (Godkin et al., 1984a), and the ovine and bovine conceptus produced a proteinaceous substance that stimulated progesterone synthesis by luteal slices (Godkin et al., 1978). Furthermore, a positive relationship between luteal resistance to PGF2α and the amount of oTP-1 within the uterus on d 13 has been suggested (Silvia and Niswender, 1984), and intramuscular injection of recombinant bovine interferon during the period of maternal recognition of pregnancy increased reproductive performance in pregnant ewes (Schalue-Francis et al., 1989, 1990; Davis et al., 1990). Other proteins produced by the conceptus have been shown to act directly on luteal tissue. Wiltbank et al. (1990a) demonstrated that a 45,000 MW protein produced by the conceptus blocked PGF2α-inhibited lipoprotein utilization for steroidogenesis by ovine luteal cells.

In contrast to the sheep and cow, infusion of conceptus secretory proteins into the uterine lumen of cyclic gilts had no effect on the interestrous interval, but increased concentrations of PGF2α and PGE2α in plasma and uterine flushings (Harney et al., 1989). It appears that porcine conceptus secretory proteins do not have an antiluteolytic function in early pregnancy; rather, they stimulate uterine production of prostaglandins considered important for the establishment and maintenance of pregnancy in this species (Harney et al., 1989).
The equine conceptus must migrate throughout the entire uterus in order to block uterine release of \( \text{PGF}_2\alpha \) (McDowell et al., 1985). Migration ceased on d 16 to 17 (Ginther, 1983), around the time of maternal recognition of pregnancy in the mare (Hershman and Douglas, 1979). It appears that during migration, the conceptus exposes the entire uterus to an antiluteolysin. If this process fails, the endometrium to which the conceptus has no access may cause luteolysis. McDowell et al. (1985) reported that restriction of the horse conceptus to one uterine horn resulted in luteal regression in 7 of 8 mares and presumably loss of uterine histotroph before death and resorption of the conceptus. However, when fed a synthetic progesterone (altrenogest, 2.2 mg per 50 kg BW) daily from d 8 or 9 after ovulation, 5 of 7 unilaterally pregnant mares maintained their pregnancies until d 32 to 42. Uterine luminal \( \text{PGF}_2\alpha \) was lower in pregnant mares (Zavy et al., 1984), and coincubation of horse conceptus and endometrium from d 13 to 14 of pregnancy reduce endometrial production of \( \text{PGF}_2\alpha \) (Sharp et al., 1989). The horse conceptus secretes at least 5 proteins from d 12 to 14; however, their function is presently unknown.

**Conceptus steroids and the establishment of pregnancy.** During the period of maternal recognition of pregnancy, both sheep and cow conceptuses produce limited quantities of progesterone, androstenedione, prostaglandins, and estrogen (cow only). Higher concentrations of \( \text{PGE}_2 \) were associated with more morphologically developed conceptuses (Vincent et al., 1986); however, Pratt et al. (1979) were able to extend the ovine estrous cycle by only two days with infusions of \( \text{PGE}_2 \). Thus, the role of these steroids in early pregnancy remains unclear. They may act alone or
in concert as part of the luteotrophic-antiluteolytic complex, or perhaps they are involved in other events during early pregnancy, such as intrauterine migration of embryos. Hickey and Hansel (1987) reported that a lipid-like luteotrophic substance is produced by d 13 to 18 bovine conceptuses. This material may be analogous to the biologically active phospholipid, embryo-derived platelet activating factor (O'Neill, 1987), which, when incubated with endometrium from d 17 of the bovine estrous cycle, caused a decrease in PGF2α secretion and an increase in PGE2. An increase in the ratio of PGE2:PGF2α in utero-ovarian venous plasma has been observed on d 13 of pregnancy in sheep (Silvia et al., 1984).

The role of conceptus steroids in the establishment of pregnancy in the pig has been investigated extensively (see reviews by Bazer et al., 1989; Geisert et al., 1990). A current hypothesis is that conceptus estrogens alter transport of secretion of PGF2α from an endocrine direction, toward the uterine venous drainage, to an exocrine direction, toward the uterine lumen. PGF2α is thereby sequestered and prevented from reaching the CL to cause luteal regression (Bazer and Thatcher et al., 1977). The in vitro secretion of PGF2α and PGE2 from pregnant and estrogen-treated porcine endometrium was greater towards the luminal side (exocrine), but secretion of these prostaglandins was more toward the myometrial side (endocrine) for d 10 and d 14 cyclic gilts (Gross et al., 1988c). There is evidence to suggest that exocrine secretion of prostaglandins requires an interaction between estrogen, prolactin and conceptus secretory proteins (Mirando et al., 1988; Young and Bazer, 1988; Harney et al., 1989). The complete establishment of pregnancy in the pig requires biphasic synthesis and release of conceptus estrogens (Geisert et
al., 1990). The initial release of estrogen during embryonic elongation on d 11 to 12 stimulates the release of calcium and various uterine proteins, but failed to extend the interestrous interval beyond 30 days. A second increase in estrogen after d 14 is necessary for prolonging CL function beyond 30 days. This second increase was not observed in cyclic gilts. The concentration of endometrial receptors for estrogen also follows a similar pattern, increasing on d 12 and then again on d 18. A direct involvement of estrogen in programming secretion and morphological changes in the uterine epithelium necessary for conceptus attachment and survival has been demonstrated (Geisert et al., 1990).

The horse conceptus produces substantial quantities of estrogens (Zavy et al., 1979) during the period of maternal recognition of pregnancy. Some (Berg and Ginther, 1978) but not all studies (Sharp et al., 1984) demonstrated prolonged CL maintenance for mares treated with estrogen. An luteotrophic/antiluteotytic role for conceptus steroids in the establishment of pregnancy in the mare is unknown.

Intrauterine Migration of the Embryo

Intrauterine migration and the approximate equidistant spacing of embryos are natural phenomena in all polyovular species. Initially, there was confusion as to whether the embryo migrated transabdominally (external migration) or from one horn to the other through the uterine body (internal migration). Corner (1921) confirmed this latter observation in sows. The importance of intrauterine migration has been well documented. Failure of embryos to become spaced may be associated with early embryonic mortality, (see reviews Pope and First, 1985; Dziuk, 1985) and ultimate crowding and fetal loss (Knight et al., 1977). Intrauterine migration in
most domestic species maximizes the amount of uterine luminal environment for each embryo, thereby optimizing litter size, fetal growth and survival (Bazer et al., 1969). Migration also results in the distribution of embryos sufficient to maintain pregnancy in some species (Dhindsa and Dziuk, 1968b; McDowel et al., 1985). The frequency, timing and possible mechanisms of intrauterine migration in sheep, cows, pigs and horses will be reviewed.

Sheep. Intrauterine migration of embryos, a frequent occurrence in ewes with two ovulations in one ovary, was observed in 97% (31/32) and 88% (105/120) of ewes with unilateral twin CL (Scanlon, 1972 and Reimers et al., 1973, respectively). In contrast, when a single ovulation was experienced in one or both ovaries, migration into the uterine horn not associated with the CL, or migration into an already occupied horn, was rare. For example, the migration frequency range was 4 to 8% and 0 to 0.9% in single ovulating ewes or those with one CL in each ovary, respectively (Casida et al., 1966; Scanlon et al., 1972). Little information is available on intrauterine migration in ewes with three or more ovulations, but, in general, there was a strong tendency towards equality in the distribution of fetuses (Robinson, 1951; Rhind et al., 1980). For example, the most frequent distribution for quadruplets was two in each horn, regardless of ovulation pattern (Rhind et al., 1980).

The timing of embryonic migration in sheep was reported to occur between d 12 to 14 of gestation (Cummins, 1979). Physical attachment between the embryo and uterine epithelium was not achieved until d 16 to 17 of gestation (Chang and Rowson, 1965; Short, 1969); therefore, ovine embryos are free to move within the uterine lumen prior to this time.
However, little is known regarding mechanisms associated with embryonic migration in the ewe. Cloud and Casida (1969) examined the role of uterine motility in the movement of embryos. On d 3, pregnant and nonpregnant ewes with CL in only one ovary received 1 mm pellets cut from rubber bands. All ewes were slaughtered on d 14. The distance traveled by the pellets was used as a measure of uterine motility. In pregnant ewes, the uterine horn containing the embryo displayed the greatest motility, suggesting that an embryo can stimulate uterine motility locally before d 14. No difference between the distance pellets travelled in the ipsilateral and contralateral uterine horns of unbred ewes was detected, suggesting no local effect of the ovary containing the CL upon uterine motility.

Products produced by the conceptus, especially estrogen, have been strongly implicated as a stimulator of intrauterine migration in the pig (Pope et al., 1982b) and mare (Zavy et al., 1979). The effect of ovine conceptus secretory products on migration, however, has not been investigated.

The prenatal growth and survival of fetuses is influenced markedly by the spacing and migration of embryos. Increased fetal mortality observed in ewes with high ovulation rates was attributed to an unequal distribution of fetuses between uterine horn, resulting presumably in decreased number of cotyledons per fetus and reduced fetal weights (Rhind et al., 1980). In addition, migration of an embryo into a uterine horn opposite the CL may be associated with early embryonic mortality. In eight experiments summarized by White et al. (1981), lambing rates were higher in ewes with bilateral ovulations compared to those with both CL in
one ovary. Survival of embryos located in the contralateral horn was lower than that of a conceptus which did not experience migration in some (Doney et al., 1973) but not all studies (Kelly and Johnstone, 1983; Torres and Sevellec, 1987). The effect(s) of migration on embryonic mortality, though presently unknown, might be associated with an asynchronous uterine environment in the contralateral horn compared with that of the ipsilateral horn, or failure of the contralateral endometrium to respond to conceptus signals (Pope, 1988).

Cow. In single-ovulating species, intrauterine migration of embryos may be the exception rather than the rule. In the cow, the frequency of natural twin births is very low (1-5%; Morris, 1984); therefore, the cow is generally considered to be a monotocous species. Migration occurred in 0.27% (7/2605) of cows with one ovulation, 2.1% of single ovulating buffalo cows (*Bos bubalis*), and in about 20% of cows with unilateral twin CL (Hafez, 1964; Scanlon, 1972; Sreenan and Diskin, 1989). Using unilaterally ovariectomized cows that had been superovulated, Hafez (1964) demonstrated that the maximum number of embryos which had migrated into the opposite uterine horn was one per animal. Development of the migrant embryo was slower than that of the embryo which remained in the ipsilateral uterine horn, and Hafez (1964) concluded that a single horn could sustain only one or two embryos. Likewise, Rowson et al. (1971) postulated that in cows with unilateral twin ovulations, failure to undergo intrauterine migration resulted in overcrowding of two embryos within one uterine horn and death of one of the embryos. Observations with recipient cows indicated that embryonic survival was greater in the
ipsilateral horn in some (Sreenan et al., 1975; Christie et al., 1979; Newcomb et al., 1980) but not all studies (Sreenan and Diskin, 1989).

Little information is available on the timing of intrauterine migration in the cow. Bovine embryos completed migration by approximately d 20 (Hafez, 1964). The possible role(s) of conceptus steroids or proteins in intrauterine migration in twin-ovulating cattle has not been investigated.

**Pig.** Upon reaching the uterus, the pig embryo migrates a substantial distance of perhaps 100 cm or more from the tip of a uterine horn to the body of the uterus and beyond. Embryos progress through the horn during d 7 and 8 and have usually entered the opposite horn by d 12 (Dhindsa et al., 1967; Anderson, 1978). After d 12, embryos can no longer successfully move to a different site (Polge and Dziuk, 1970).

Factors influencing intrauterine migration of pig embryos have been investigated. The number of embryos migrating does not appear to influence the rate of migration, as more embryos do not migrate faster than fewer (Dziuk et al., 1964). Random movement of embryos has been observed, with more embryos remaining in the horn of origin than migrating to the other horn (Dhindsa et al., 1967). During migration, embryos apparently do not repel each other nor do they influence the migration of other embryos (Dziuk et al., 1964). Migration appeared to be influenced in part by peristaltic contractions of the uterine myometrium (Corner, 1921), and Wislocki and Guttamacher (1924) concluded that the porcine embryo can maintain increased uterine activity. Evidence that intrauterine migration in pigs is not completely passive and random can be found in a series of experiments by Pope et al. (1982a,b; 1986). Small, spherical beads of
Silastic glue, containing physiological amounts of estradiol-17β, have been observed to stimulate migration as compared to cholesterol-impregnated beads in control gilts (Pope et al., 1982b). Increased synthesis of estradiol by the porcine embryo was found to occur concomitantly with migration and increased myometrial activity in vitro (Pope et al., 1982a). These observations suggested that estrogen synthesis by the blastocyst (Perry et al., 1973) might be the signal to initiate and coordinate myometrial activity and embryonic migration. Other hormones, such as prostaglandins and histamines, may play a role in migration (Pope et al., 1982a). The extent to which these hormones might be interrelated during migration is not fully understood at this time; however, all three hormones are known to increase blood flow, and Ford et al. (1981) observed an increase in uterine blood flow associated with migration of porcine embryos.

Although migration may not be influenced directly by other embryos, distribution and spacing seem to be clearly affected by the proximity of other embryos. Ligating one uterine horn on d 4 to create crowded and uncrowded conditions on either side of the ligature reduced the distance between fetuses on d 25 to 30 (Dziuk, 1968). However, fetuses were spaced equidistant from each other and the proportion of embryos that survived to d 30 was not affected, suggesting that uterine space does not influence survival before d 30. By d 70, however, few fetuses survived in the crowded segment. Since each fetus needs about 20 cm of uterine length to survive (Knight et al., 1977), spacing and migration during early pregnancy can markedly influence the uniform distribution of embryos and subsequent prenatal growth and survival of fetuses.
The distribution of embryos in the pig is also critical to the maintenance of pregnancy. The proportion of the uterus that is unoccupied is inversely related to the probability that pregnancy will continue (Dhindsa and Dziuk, 1968b). For example, when only one half the uterus was unoccupied, pregnancy failed. When one quarter was unoccupied, 20 to 30% of pregnancies continued, and one one-eighth was unoccupied, 50 to 60% of pregnancies continued. Therefore, a minimum of four embryos, two occupying each uterine horn, is required on d 12 to maintain pregnancy in the pig.

**Mare.** Intrauterine migration of the horse conceptus is extensive. Using ultrasound, Ginther (1983) reported that the equine conceptus is highly mobile within the uterus between d 11 and 15. Ultrasound examinations 3 or 4 times per day at 2 to 4 h intervals demonstrated that the vesicle was found in opposite uterine horns 43% of the time. Mobility began to decrease by d 15 and was no longer detectable after d 17. This mobility may allow conceptus secretions, such as estrogen and proteins, to affect the entire endometrium and allow for establishment of pregnancy; restriction of the horse conceptus to one uterine horn resulted in pregnancy loss (McDowell et al., 1985). Uterine contractions, which increased during the period of migration (Cross and Ginther, 1988), may play a role in this process.
CHAPTER III

Effects of Intramuscular Administration of Recombinant Bovine Interferon-Alpha,1 During the Period of Maternal Recognition of Pregnancy in Sheep

One hundred ewes were utilized to determine the effects of interferon supplementation on the number of ewes pregnant and embryonic survival. Ewes were checked twice daily (0700 and 1600) for estrus using fertile rams. On d 12 through 16, ewes received twice daily i.m. injections of either recombinant bovine interferon alpha,1 (2 mg IFN) or vehicle. Ewes remained penned with rams and were observed for subsequent estrous activity for at least 35 d after mating. To determine the number of fetuses and corpora lutea, all ewes were subjected to one surgery during mid-pregnancy (d 45 to 80). More (P<.05) ewes were pregnant after treatment with IFN vs vehicle (45 of 49, 92% vs 37 of 49, 76%, respectively). The interestrous interval for ewes that were treated with IFN and did not conceive was longer (P<.05) than for ewes given vehicle (26 ± 1 vs 17 ± 2 d, respectively). Embryonic survival (98.2 vs 87.9%; P<.05), calculated as the number of fetuses present at the time of laparotomy and expressed as a percentage of the ovulation rate, and percentage of ewes with 100% fetal survival (96 vs 76%; P<.05) were greater after treatment with IFN. It was
concluded that supplementation of IFN increased both the number of ewes pregnant and embryonic survival.

**Introduction**

Biochemical communication must occur between mother and embryo for pregnancy to proceed. This phenomenon, referred to as maternal recognition of pregnancy by Short (1969), occurs on d 12 to 13 in ewes (Moor and Rowson, 1964, 1966b,c) and coincides with synthesis of oTP-1 by the trophectoderm of the conceptus (Godkin et al., 1982a, 1984a). Structural (Imakawa et al., 1987, 1989; Stewart et al., 1987;) and functional (Pontzer et al., 1988; Roberts et al., 1989) characteristics of oTP-1 indicate that it is an interferon (IFN) of the 172-amino acid long alpha_{II} subfamily. Furthermore, oTP-1 is closely related to both bovine IFN-α_{II} and bovine TP-1 (Imakawa et al., 1989). Interaction of oTP-1 with endometrial receptors (Godkin et al., 1984a; Hansen et al., 1989; Knickerbocker and Niswender, 1989) probably is responsible for altering secretion of endometrial polypeptides (Vallet et al., 1987; Sharif et al., 1989) and prostaglandins (Fincher et al., 1986; Salamonsen et al., 1988) and for extending luteal function (Godkin et al., 1984b; Vallet et al., 1988a). The implications of these findings and possible role(s) of IFN in early pregnancy have been reviewed by Roberts (1989).

The purpose of this experiment was to determine effects
of bovine IFN-α supplementation from d 12 to 16 after mating on the number of ewes pregnant and embryonic survival.

**Materials and Methods**

One hundred 2- to 4-year old Polypay, Targhee or Hampshire X Targhee ewes in good condition were utilized. All animals were unilaterally ovariectomized and allowed 2 to 3 mo to recover prior to mating. Intrauterine migration of ovine embryos occurs after maternal recognition of pregnancy (Nephew et al., 1989b); therefore, ewes were unilaterally ovariectomized in order to localize accumulation of a luteotrophic substance(s) produced by the embryo in the uterine horn ipsilateral to corpora lutea (CL). Furthermore, utilization of ewes with only unilateral ovulations might minimize effects associated with migration and embryonic survival (White et al., 1981).

Beginning in mid-September, animals were allotted into groups of 25 by breed and age. Each lot was penned with two fertile rams, and the number of treatment and control ewes per lot balanced. Ewes were checked at 0700 and 1600 h for fresh rump marks. Ewes exhibiting estrus (d 0) were then hand-mated to a third, isolated ram. Two ewes failed to display estrus and were omitted from the experiment. On d 12 through 16, ewes received either recombinant bovine interferon-α,1 (2 mg IFN) or vehicle (2 mg buffered mannitol solution) twice daily. The IFN was supplied as a freeze-dried powder prepared by Ciba-Geigy Limited, Basel,
Switzerland. Administration of the IFN in .8 ml sterile water was via single-site, i.m. injections that were alternated between the right and left rump areas. Ewes remained penned with rams for at least 35 d after mating; ewes with new rump coloration were placed with an isolated ram to allow further confirmation of return to estrus.

To compare ovulation rates among ewes and to examine the effect of administration of IFN on embryonic survival, all animals were subjected to one surgery during pregnancy (between d 45 to 80). After exposure of the reproductive tract through a mid-ventral abdominal incision, the ovary and uterus were examined. The number of CL and fetuses were compared. All ewes lambed; lambing dates allowed further confirmation of breeding dates.

The number of ewes that were pregnant and lambed after treatment with IFN or vehicle was compared by use of a chi-square test (Steel and Torrie, 1980). Differences in interestrous intervals, numbers of CL, fetuses, fetal survival, lambs born and weight of lambs both at birth and weaning were compared by a t-test (Steel and Torrie, 1980). Linear regression was utilized to examine the relationship between the number of fetuses present at mid-gestation and fetal survival after surgery.

Results and Discussion

A higher (P<.05) percentage of ewes that received IFN were pregnant than of ewes treated with vehicle (Table 1).
Successful maintenance of pregnancy after treatment with exogenous prostaglandin F\textsubscript{2}\textalpha{} on d 13 was greater in ewes with two embryos than in those with one embryo (Silvia and Niswender, 1984; Nephew et al., 1989a), suggesting a direct relationship between recognition of pregnancy and the amount of oTP-1 to which the uterus is exposed. Furthermore, Maurer (1988) achieved higher pregnancy rates by transferring embryos, which had been split in half, into one vs both uterine horns of ewes with bilateral ovulations. It seems likely, therefore, that when more conceptuses are present, the signal for maternal recognition of pregnancy is greater, and the likelihood that pregnancy will be maintained increases. Results from the present experiment support this concept; similar results have been reported by Schalue-Francis et al. (1989) with a less fecund group of ewes. Because only three ewes in this experiment had a single CL, we could not test the potential interaction between IFN and ovulation rate on pregnancy rate. The mechanism by which IFN supplementation improves fecundity remains unknown and requires further study.

Number of CL of pregnant ewes was higher (P<.05) in groups treated with vehicle than in groups treated with IFN (2.54 ± .04 vs 2.37 ± .04, respectively), and the number of CL were higher (P<.05) in both groups compared to those ewes that failed to conceive after initial exposure to rams (2.20 ± .07). Under natural conditions in sheep, as ovulation
rate increases, the number of fertilized ova also increases (Hanrahan, 1980). A similar screening process might have occurred in the present experiment such that only those vehicle treated ewes with higher ovulation rates, more conceptuses and, therefore, enough oTP-1/IFN, recognized pregnancy. Supplementation of this signal by treatment with IFN appears to have resulted in more pregnant ewes with a lower ovulation rate lower than the contemporary group.

Use of a recombinant source of IFN offers additional information about the biological importance of maternal recognition of pregnancy. For example, the estimate of early reproductive loss observed after treatment with IFN was 8% (100% - 92%; Table 1), similar to the 10% loss attributed to fertilization and(or) developmental anomalies by Edey (1979). However, supplemental IFN appears to have permitted survival of concepti, indicating that the remaining embryonic loss (92% - 76%= 16%) in the vehicle treated ewes might have been due to insufficient or delayed oTP-1/IFN production by blastocysts.

Ewes that were treated with IFN and did not remain pregnant had a longer (P<.05) interestrous interval than vehicle treated ewes (Table 1). Rowson and Moor (1967) first reported that infusion of homogenates from d 14 or 15 ovine conceptuses into the uterine lumen prolonged luteal function. Secretory proteins of ovine conceptuses (Godkin et al., 1984b) or oTP-1 alone (Vallet et al., 1988)
prolonged luteal maintenance. Intrauterine infusion (cows, Plante et al., 1988; sheep, Stewart et al., 1989) or injection (cows, Plante et al., 1989) of IFN during the period of maternal recognition of pregnancy extended luteal function in nonpregnant ruminants. Collectively, these results strongly implicate IFN as being antiluteolytic.

The mechanism of action whereby IFN signals pregnancy recognition is not fully understood. IFN appears to interact with endometrial receptors that bind either oTP-1 or IFN-α with somewhat similar affinity (Hansen et al., 1989; Knickerbocker and Niswender, 1989). The concentration and affinity of receptors for oTP-1/IFN-α change on d 12 to 16 in estrous and pregnant ewes; ovine luteal tissue possesses a high number of binding sites for oTP-1 (Knickerbocker and Niswender, 1989). The exogenous IFN administered on d 12 to 16 in the present study presumably bound to available oTP-1 endometrial and(or) luteal receptors to facilitate establishment of pregnancy.

IFN supplementation increased prolificacy of ewes in this experiment. The number of lambs born per ewe exposed to fertile rams and total number of lambs born were greater (P<.05; Table 1) for ewes treated with IFN. More (P<.01) ewes experienced 100% fetal survival at mid-gestation after treatment with IFN vs vehicle (43/45, 96% vs 28/37, 76%, respectively). Subsequent survival of fetuses, however, was negatively related (R² = .98; P<.001) to the number of
fetuses present at this time (i.e., ewes with more fetuses were more prone to post-surgical losses). Consequently, the number of fetuses lost later in gestation, which under normal conditions is very low (Quinlivan et al., 1966; Moore, 1985), was higher (P<.05) in the ewes treated with IFN vs those treated with vehicle (18 vs 11%, respectively). This may have been due to surgical stress. The weight of lambs at birth (4.7 ± .1 vs 4.6 ± .1 kg) or weaning (23.7 ± .7 vs 22.7 ± .9 kg) and survival of the neonates to weaning (86 vs 75%) were not different between ewes treated with IFN vs vehicle, respectively. No congenital or health defects were observed in lambs born from IFN-treated ewes.

Implications

Supplementing the putative signal for maternal recognition of pregnancy with a compound like interferon can increase pregnancy success in ewes and thereby improve the overall efficiency of sheep production. Further studies are required to evaluate the mechanism(s) by which interferon supplementation improves fecundity in mated ewes.
TABLE 1. THE EFFECTS OF INTERFERON SUPPLEMENTATION ON FECUNDITY IN SHEEP

<table>
<thead>
<tr>
<th>Observation</th>
<th>Vehicle</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ewes mated</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Number pregnant (%)</td>
<td>37(76)c</td>
<td>45(92)d</td>
</tr>
<tr>
<td>Interestrous interval for nonpregnant ewes (days)</td>
<td>17 ± 2c</td>
<td>26 ± 1d</td>
</tr>
<tr>
<td>Total number of lambs born</td>
<td>80c</td>
<td>98d</td>
</tr>
<tr>
<td>Lambs born per ewe exposed</td>
<td>1.6 ± .1c</td>
<td>2.0 ± .1d</td>
</tr>
</tbody>
</table>

*aRecombinant bovine interferon-α1.

*bEwes received twice daily injections of either 2 mg IFN or vehicle (isotonic buffered mannitol) on d 12 through 16.

*c,dMeans with different superscripts differ (P < .05).

*eMean ± SE.
CHAPTER IV

Relationship between variation in conceptus development and differences in estrous cycle duration in ewes

The objective of this study was to examine conceptus development on d 13 in ewes with estrous cycles of different durations. Ewes (n = 80) were screened according to the length of their estrous cycles. Subsequently, ewes that had either SHORT or LONG cycles were utilized (15.9 ± 0.1 or 18.6 ± 0.4 d; mean ± SE, P<.01; 10 ewes per group). Jugular blood samples were collected twice daily from d 0 to 6 after mating and then once a day until slaughter on d 13. Concentrations of progesterone in plasma and amounts of oTP-1, protein and PGE₂ and PGF₂α in uterine flushings were determined. Concentrations of progesterone were greater (d X treatment interaction, P<.01) on d 2 to 4 for ewes in the SHORT group. On d 5 and thereafter, progesterone concentrations were not different between groups. More (P<.05) oTP-1 and protein (8.1 ± 1.3 μg and 1.8 ± .3 μg vs 2.4 ± 1.3 μg and .8 ± .3 mg) were recovered from uterine flushings from ewes in the SHORT versus LONG groups, respectively. The ratio of PGE₂:PGF₂α was higher (P<.06) in flushings from ewes in the SHORT versus LONG group (1.4 ± .2 vs .9 ± .2, respectively). Conceptuses were classified by stage of morphological development. Conceptus development was accelerated (P<.01) in ewes of the SHORT
group, as filamentous conceptuses were recovered from 78 vs 0% of SHORT versus LONG ewes, respectively. These data indicate that conceptus development and production of antiluteolytic signals occur sooner in ewes with shorter estrous cycles.

Introduction

The sequence of hormonal changes during early pregnancy in sheep (Wilmut et al., 1985a,b) are important in the establishment of a uterine environment suitable for normal conceptus development (Miller and Moore, 1976). The secretory profile of uterine proteins (Ashworth and Bazer, 1989) and embryonic development (Wintenberger-Torres, 1967) was altered after administration of progesterone.

For pregnancy to proceed beyond the early stages, a series of coordinated biochemical events must occur between the maternal system and the developing conceptus. Termed maternal recognition of pregnancy (Short, 1969), this phenomenon occurs around d 12 to 13 after estrus in sheep (Moor and Rowson, 1966b,c). One substance strongly implicated in this process is oTP-1 (Godkin et al., 1984a; Vallet et al., 1988a) released by the trophectoderm during the period of conceptus elongation (Godkin et al., 1984b; Farin et al., 1989). Intrauterine administration of purified oTP-1 to cyclic ewes extended luteal function (Godkin et al., 1984a; Vallet et al., 1988a). Further, it has been suggested that a direct relationship exists between the amount of oTP-1 within the uterus on d 12 to 13 and luteal resistance to PGF2α during
early pregnancy (Silvia and Niswender, 1984; Nephew et al., 1989a).

Another factor involved in the establishment of pregnancy in sheep is PGE$_2$ (Henderson et al., 1977; Pratt et al., 1977; Mangess et al., 1981). Prostaglandin E$_2$ increases during early pregnancy (Ellinwood et al., 1979a; Silvia et al., 1984) and progesterone can advance the release of PGE$_2$ in the presence of an older conceptus (Vincent et al., 1986).

Pulsatile release of PGF$_{2\alpha}$ is attenuated in pregnant ewes (Zarco et al., 1988a); however, in nonpregnant ewes, the time at which luteolysis is initiated is a determinant of estrous cycle length (Zarco et al., 1988b). Thus, if the onset of luteolysis occurs sooner in ewes with estrous cycles of shorter duration, then initiation of mechanisms required for establishment of pregnancy might also be expected to occur earlier in those ewes. In the present study, we determined if blastocyst development was different between ewes with previous estrous cycles of short or long duration.

Materials and Methods

Animals. Eighty crossbred ewes (Hampshire X Targhee X Finnsheep), between 2 and 7 years old, and vasectomized rams were maintained in an open-fronted barn. During the mid-breeding season (October through November), ewes were checked twice daily for estrus. The first day of estrus (d 0) was confirmed by observing a vasectomized ram mate the ewe. The occurrence of estrus was recorded for three complete estrous
cycles for each ewe. Subsequently, we identified ewes within the flock that had displayed extremes in duration of the estrous cycle. These ewes were classified as having either SHORT or LONG cycles (15.9 ± 0.1 or 18.6 ± 0.4 d, mean ± SE, P<.01; 10 ewes per group). The repeatability of estrous cycle length was calculated as the intra-class correlation from the corresponding components of variance among and within ewes (Steel and Torrie, 1980). Estrous cycle length was highly repeatable (0.95, P<.01).

Ewes were then penned with fertile rams and observed for estrus at 0700 and 1900 h. Blood samples were obtained twice daily by jugular venipuncture from d 0 to 6 and then once a day until slaughter on d 13. Plasma was obtained and stored at -20°C until assayed for progesterone by RIA (Hu et al., 1990).

Ovulation rate (the number of corpora lutea in the ovaries) and luteal weight were determined at slaughter. Uterine horns were flushed separately by introducing saline (0.9% NaCl; 20 ml) into the tip of each horn and flushing towards the external bifurcation. The amount of saline recovered was recorded. After removal of conceptuses, flushings were stored at -20°C until assayed for oTP-1, PGE₂, PGF₂α, and protein. Each conceptus, upon microscopic examination, was classified by stage of morphological development in accordance with the descriptions of Green and Winters (1945): spherical, tubular or filamentous.
Assays. The antibody for progesterone (GDN #337) was graciously provided by Dr. Gordon Niswender, Colorado State University. Tritiated progesterone (1,2,6,7,21-3H-progesterone) was purchased from NEN Research Products (Boston, Mass.). Standards of progesterone (Sigma Chemical Co., St. Louis, MO) in charcoal-stripped ovine plasma (0.05 to 10 ng/ml) and unknown samples were extracted using 4 ml of a 1 benzene : 2 hexane (v:v) solution. The extraction efficiency was 84% ± 1.7%. After extraction, plasma was frozen at -18 C for 1.5 h, and solvent was decanted into assay tubes and evaporated. The extracted steroids were reconstituted in 200 μl phosphate-buffered saline containing 0.1% gelatin (PBS-gel). Progesterone antiserum (1:5000) and tritiated progesterone (35.8 pg/tube) were then added. Incubation was conducted for 24 h at 4 C. Free and bound progesterone were separated with dextran-coated charcoal (0.25% dextran and 0.625% charcoal in PBS-gel). Analysis for parallelism was performed by dilution of a pooled quality control with steroid-free plasma (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0). The resultant slope of the ln:logit curve generated by varying dilutions was -0.99, compared to a slope of -1.01 for the standard progesterone curve. The sensitivity of this assay was 30 pg/ml (6 pg/tube). Intra- and inter-assay coefficients of variation (CV) averaged 3.8 and 7.1%, respectively. The average recovery was 101.9%. The concentration of oTP-1, PGE2, and PGF2α in
uterine flushings were determined by RIA (Vallet et al., 1988a,b). Samples for oTP-1, PGE$_2$, and PGF$_2\alpha$ were measured in a single assay with intrassay CVs of 9, 10.5 and 13%, respectively. Sensitivities of the oTP-1, PGE$_2$, and PGF$_2\alpha$ assays were 200, 25, and 12.5 pg/ml, respectively. Total recoverable protein was determined (Bradford, 1976) with bovine serum albumin as the standard.

**Statistical analyses.** All data were analyzed using the Statistical Analysis System (SAS, 1986). Data for concentrations of progesterone in plasma were analyzed by least-squares analysis of variance (ANOVA) using the General Linear Models procedures. The model had the independent effects of treatment group, sheep within treatment group, day, and treatment X day. Differences between least squares means were compared using Fisher's protected least significant difference test. Differences in total uterine content of oTP-1 and protein, ratio of PGE$_2$:PGF$_2\alpha$, conceptus development, number of conceptuses, ovulation rate and luteal weight were analyzed using a one-way ANOVA. After calculating the percentage of ewes that had spherical to ovoidal, tubular or filamentous conceptuses, percent data were log transformed before analysis. Due to heterogeneity of variance, as determined by use of Bartlett's test (Steel and Torrie, 1980), data for progesterone, oTP-1 and protein were subjected to a logarithmic transformation before analysis. Least squares means are reported.
Results

Concentrations of progesterone in plasma are summarized in Table 2. No conceptus was recovered from one ewe in the SHORT group, and this ewe was excluded from the study. Concentrations of progesterone were greater (d X treatment interaction; P<.01) on d 2 to 4 for ewes in the SHORT group. On d 5 and thereafter, concentrations of progesterone were not different between groups.

As shown in Table 3, more (P<.05) oTP-1 and protein were measured in uterine flushings from ewes in the SHORT group. The ratio of PGE$_2$ : PGF$_2\alpha$ was higher (P<.06) in flushings from ewes in the SHORT group. Conceptus development was accelerated (P<.05) in ewes of the SHORT group; 78% of the ewes in the SHORT group had filamentous conceptuses compared to 0% of ewes in the LONG group. Conceptus survival to d 13, ovulation rate and luteal weight were not different between groups.

Discussion

The important finding from this study is that ewes with shorter estrous cycles have both advanced conceptus development and uterine environment by d 13 postcoitum. The rate of embryonic development in ewes can be altered by uterine environment, as administration of progesterone (Wintenberger-Torres, 1967; Lawson and Cahill, 1983) or transfer of embryos to advanced recipients (Wilmut and Sales, 1981; Lawson et al., 1983) accelerated embryonic development.
Previous studies in cows have shown that higher concentrations of progesterone during early pregnancy were associated with both enhanced uterine secretory activity (Garrett et al., 1988) and conceptus development (Boyd et al., 1969; Garrett et al., 1988). Perhaps changes in uterine secretions favorable for embryonic/conceptus development, such as specific endometrial proteins (Ashworth and Bazer, 1989), occurred sooner in the SHORT group. The greater amount of protein in flushings from these ewes supports this possibility. Alternatively, conceptuses from ewes in the SHORT group might have developed at a predetermined faster rate; therefore, conceptus-induced changes in uterine secretions occurred earlier in those ewes. We observed considerable variation in conceptus development between, but not within ewes, as did Ashworth and Bazer (1989). Further, advanced conceptuses were able to accelerate uterine function (sheep, Ashworth and Bazer, 1989; swine, Xie et al., 1990), perhaps by production of signals such as conceptus secretory proteins (Vallet et al., 1988a; Ashworth and Bazer, 1989; Mirando et al., 1990b) and estrogen (Nephew et al., 1989b). Alterations in uterine physiology might also affect the timing of other events during early pregnancy. For example, only ewes in the SHORT group with the highest content of oTP-1 in uterine flushings had experienced migration of conceptuses into the uterine horn contralateral to the ovary containing multiple ovulations on d 13.
OTP-1 has been identified as a functionally active interferon (Imakawa et al., 1987; Roberts et al., 1989). Nephew et al. (1989a) demonstrated that a positive relationship exists between successful pregnancy recognition and the amount of OTP-1 recovered in uterine flushings. Supplementation of recombinant bovine interferon during the period of maternal recognition of pregnancy improved fertility in ewes (Nephew et al., 1990). In light of these observations, we suggest that OTP-1/interferon accumulates to a threshold level in order for pregnancy to proceed through the period of recognition and that this level was reached sooner in ewes of the SHORT group. However, production of other factors by the ovine conceptus that may be luteal protective (Wiltbank et al., 1990a) cannot be overlooked.

Prostaglandin E₂ produced by the ovine conceptus (Marcus, 1981; Lacroix and Kann, 1982) and/or endometrium (Ellinwood et al., 1979a; Marcus, 1981; Lacroix and Kann, 1982) is a factor involved in maintenance of the corpus luteum of pregnancy. Concentrations of PGE₂ and ratio of PGE₂:PGF₂α in utero-ovarian venous plasma increased beginning on d 13 of pregnancy (Silvia et al., 1984) and appeared to depend upon both progesterone and the presence of a conceptus (Vincent et al., 1986). Although based upon single samples, the ratio of PGE₂:PGF₂α was higher in flushings of ewes in the SHORT group. When a more morphologically developed conceptus was present, the ratio of
prostaglandins favored PGE₂, supporting the observation by Vincent et al. (1986).

A causal relationship appears to exist between natural variability in length of the ovine estrous cycle and: 1) the time elapsed between ovulation and luteal production of significant amounts of progesterone (Wilmut et al., 1985b; Zarco et al., 1988b) and/or 2) the time between estrus and the onset of luteolysis (Zarco et al., 1988b). Uterine exposure to progesterone for a relatively fixed period of time was requisite for initiation of luteolysis (Woody et al., 1967; Warren et al., 1973; Ford et al., 1975; Ottobre et al., 1980); moreover, administration of exogenous progesterone early in the estrous cycle hastened luteal regression (Woody et al., 1967; Ottobre et al., 1980; Lawson and Cahill, 1983). Our finding that progesterone secretion increased more rapidly in ewes in the SHORT group, together with our observation that progesterone concentrations on d 5 and after were not different between groups, offer support for the causal relationship between the pattern of progesterone secretion early in the estrous cycle and cycle length. However, we consider this support to be indirect because neither the progesterone profiles during the preceding estrous cycles nor the relationship between estrus and timing of ovulation were assessed in these ewes. Furthermore, as cycle length is perhaps genetically determined (Zarco et al., 1988b), so too might be rates of luteal development.
Implications

Based on results from the present study, we conclude that maternal-conceptus relationships are coordinated such that blastocyst development and production of antiluteolytic signals occur sooner in ewes that experience luteolysis earlier. We further suggest that the timing of the phenomenon of maternal recognition of pregnancy is coupled to the duration of the estrous cycle.
TABLE 2. Plasma concentrations of progesterone during the first 13 days in ewes predisposed to experience SHORT or LONG estrous cycles*.

<table>
<thead>
<tr>
<th>Day</th>
<th>SHORT</th>
<th>LONG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>1</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>2**</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>3**</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>4**</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>7</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>8</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>2.9</td>
<td>3.1</td>
</tr>
<tr>
<td>10</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>11</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>12</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>13</td>
<td>2.2</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Ewes were identified as having previous estrous cycles that were 15.9 ± 0.1 d (SHORT) or 18.6 ± 0.4 (LONG).

*bIllustrated values represent samples collected at either 07:00 or 19:00 h for d 0 to 6. Estimate of the untransformed standard error, as derived from the antilogarithm of the mean-square error, was .08.

 Only 3 and 1 ewes in the SHORT and LONG groups, respectively, had detectable progesterone concentrations before d 2.

**Indicates differences between least squares means within a day (row). Day X treatment interaction (P< .01) for progesterone concentration.
TABLE 3. Comparison of various reproductive characteristics of early gestation among ewes with estrous cycles of SHORT or LONG duration*.

<table>
<thead>
<tr>
<th>Observation</th>
<th>Cycle Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHORT</td>
</tr>
<tr>
<td>oTP-1 (µg)b,c</td>
<td>8.1 ± 1.3</td>
</tr>
<tr>
<td>Protein (mg)b,c</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>[PGE₂]/[PGF₂α]</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Conceptus development(%)d</td>
<td></td>
</tr>
<tr>
<td>Spherical to ovoidal</td>
<td>11l</td>
</tr>
<tr>
<td>Tubular</td>
<td>11</td>
</tr>
<tr>
<td>Filamentous</td>
<td>78l</td>
</tr>
<tr>
<td>Conceptus survival(%)e,f</td>
<td>93</td>
</tr>
<tr>
<td>Ovulation rateb,f</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Luteal weight (mg)b,f</td>
<td>708 ± 77</td>
</tr>
</tbody>
</table>

*Ewes were identified as having previous estrous cycle that were 15.9 ± 0.1 d (SHORT) or 18.6 ± 0.4 d (LONG).

bLeast squares mean ± SE.

cThe total amount of oTP-1 or protein recovered per ewe was determined by multiplying the concentration in the flushings by the total volume of flushings.

dStage of conceptus development per ewe expressed as a percentage of total ewes.

eTotal number of conceptuses recovered on d 13: total number of corpora lutea.

fNonsignificant (P>.1)

g,hMeans within the same row without a common superscript are different (P<.05).

j,kMeans within the same row without a common superscript are different (P<.06).

l,mMeans within the same row without a common superscript are different (P<.01).
CHAPTER V

Effects of hCG on Conceptus Development,
Timing of Luteolysis and Fecundity in Ewes

One hundred and twenty-one ewes were used to examine the
effects of administration of hCG (100 IU) on d 11.5 (d 0=
estrus) on timing of luteolysis, conceptus development,
secretion of oTP-1 and fecundity. In experiment 1, treatment
of nonmated ewes with hCG on d 11.5 increased (P<.01)
concentrations of progesterone and estrogen in plasma by d 13.
The duration of the estrous cycle, however, was not different
(17.4 ± .2 vs 17.2 ± .2 days, hCG vs vehicle, respectively).
In experiments 2 or 3, pregnant ewes received hCG on d 11.5
and were either slaughtered on d 13 or allowed to lamb. In
experiment 2, plasma concentrations of progesterone and
estrogen increased (P<.01), luteal weight was greater (616 ±
15 vs 433 ± 24 mg, P<.05), embryos were longer (35 ± 14 vs 8
± 3 mm, P<.01) and concentrations of oTP-1 (10.7 vs 1.2 µg,
P<.05) and protein (5.2 vs .8 mg, P<.05) measured in uterine
flushings were greater in hCG versus vehicle treated ewes,
respectively. In experiment 3, more (P<.07) hCG-treated ewes
lambed compared to those treated with vehicle (94 vs 83%,
respectively). These results indicated that hCG administration
on d 11.5 had no effect on the duration of the estrous cycle,
but stimulated uterine secretions, conceptus growth and oTP-1
production sufficiently by d 13 of gestation to increase the number of ewes pregnant.

Introduction

Maternal recognition of pregnancy (Short, 1969), which occurs on d 12 to 13 in sheep (Moor and Rowson, 1966b,c), includes a complex network of communication involving secretions from the conceptus as well as from the uterine endometrium. Miller and Moore (1976) demonstrated that ovarian steroids, especially progesterone, stimulated and mediated changes in these essential secretions. However, administration of exogenous progesterone early in the estrous cycle hastened luteal regression (Woody et al., 1967; Ford et al., 1975; Ottobre et al., 1980; Lawson and Cahill, 1983).

A conceptal factor strongly implicated in pregnancy recognition is oTP-1 (Godkin et al., 1984a; Vallet et al., 1988a), which is an interferon (IFN) both in structure (Imakawa et al., 1987, 1989) and function (Pontzer et al., 1988; Roberts et al., 1989). Nephew et al. (1990) administered recombinant bovine IFN during the period of maternal recognition of pregnancy and increased fecundity in ewes, presumably by supplementing the oTP-1 signal released by the conceptus. Results of that study further suggested that pregnancy failure may be partially due to insufficient or delayed oTP-1 production by the conceptus.

The objectives of the present study were to examine the effects of hCG administration on d 11.5 (d 0= estrus) on the
timing of luteolysis in nonpregnant ewes, and embryonic
development, oTP-1 production and fecundity in pregnant ewes.

**Materials and Methods**

**Experiment 1.** The objective of this experiment was to examine the effect of hCG on the timing of luteolysis. Nonmated, crossbred ewes (n = 16) were checked for estrus at 0700 and 1900 h using brisket-painted rams. Ewes received a single injection of hCG (100 IU, i.m.; Sigma Chemical Co., St. Louis, MO) or vehicle (saline, 0.9% NaCl) on d 11.5 and were allowed to return to estrus. Jugular blood samples were collected before administration of hCG or vehicle and then twice daily until estrus. Plasma was obtained and stored at -20 C until assayed for progesterone (Nephew et al., 1991) and estrogen (Nephew et al., 1989c) by RIA.

**Experiment 2.** The purpose of this experiment was to examine conceptus development and oTP-1 secretion on d 13 after administration of hCG on d 11.5 of gestation. Crossbred ewes (n = 10) were penned with fertile rams and observed for estrus at 0700 and 1900 h. Estrus was confirmed by observing a fertile ram mate the ewe. On d 11.5, ewes received an i.m. injection of 100 IU hCG or vehicle and were slaughtered on d 13. Preliminary experiments had indicated that treatment with hCG on d 10, 10.5 or 12 were less effective at altering conceptus development by d 13 (data not shown). Blood samples were obtained twice daily from d 11.5 until slaughter. Reproductive tracts were collected and corpora lutea were
counted and weighed. Uterine horns were flushed separately as described previously (Nephew et al., 1991). Conceptuses were gently removed from uterine flushings, and the morphological classification (Green and Winters, 1945) and length of each intact conceptus was immediately determined. Uterine flushings were stored at -20 C until assayed for oTP-1 by RIA (Vallet et al., 1988b). Total recoverable protein in the flushings was determined (Bradford, 1976) with bovine serum albumin as the standard.

Experiment 3. The objective of this experiment was to determine the effect of hCG administration on pregnancy rate. Ninety-five 2- to 6-year-old Targhee ewes were randomly allotted into five groups by age. The number of treatment and control ewes per lot was balanced. Each lot of 10 ewes was penned with a fertile ram. Ewes were checked twice daily and those exhibiting estrus were hand-mated to an isolated ram. On d 11.5, ewes received either hCG (100 IU) or vehicle via an i.m. injection. Ewes remained penned with rams for at least 35 d after mating and were checked twice daily for return to estrus. All ewes lambed, and lambing dates allowed further confirmation of breeding dates.

Data were analyzed using the Statistical Analysis System (SAS, 1986). Hormone concentrations in plasma were analyzed by least squares analysis of variance (ANOVA) using the General Linear Models procedures. Fisher's least significant difference test was used to determine differences between
least squares means after a day X treatment interaction was determined to be significant. Differences in total uterine content of oTP-1 or protein, conceptus length, luteal weight, number of CL or conceptuses were analyzed using a one way ANOVA. Data for oTP-1 and protein were transformed to logarithms during to heterogeneity of variance, as determined by Bartlett's test (Steel and Torrie, 1980). Least squares means are reported. The number of ewes that were pregnant and lambed after treatment with hCG or vehicle was compared using a chi-square test (Steel and Torrie, 1980).

**Results and Discussion**

In experiment 1, concentrations of progesterone and estrogen in nonmated ewes increased (day X treatment interaction, $P<.01$) after administration of hCG (Table 4). Luteolysis, as indicated by decreasing concentrations of progesterone, began on d 15 in both groups. Likewise, estrous cycle duration was not different ($P> .60$) between hCG versus vehicle treated ewes ($17.4 \pm .2$ and $17.1 \pm .2$, respectively). Progesterone administered early in the estrous cycle of the ewe caused premature release of PGF$_2\alpha$ (Ottobre et al., 1980) and a shortened cycle length (Woody et al., 1967; Ottobre et al., 1980; Lawson and Cahill, 1983). Similarly, administration of estrogen during the luteal phase increased uterine secretion of PGF$_2\alpha$ (Ford et al., 1975) and caused premature luteal regression (Stormshak et al., 1969; Warren et al., 1973; Kittok and Britt, 1977). Results from the present
study demonstrated that the transient increase in ovarian steroids late in the luteal phase failed to induce premature regression of the CL. Furthermore, the decreasing concentrations of progesterone in plasma and subsequent display of behavioral estrus indicated that treatment with hCG did not induce the formation of accessory ovulations.

In pregnant ewes (experiment 2), progesterone and estrogen concentrations increased (day X treatment interaction, P<.01) after treatment with hCG and remained elevated until d 13 (Table 5). In addition, CL from hCG-treated ewes were heavier (P<.05) than those from vehicle ewes (Table 6). Increased plasma progesterone (Kittok et al., 1983; Gamboni et al., 1984; Farin et al., 1988) and luteal weight (Gambone et al., 1984; Farin et al., 1988) following hCG administration have been reported, apparently due to hypertrophy of both steroidogenic and nonsteroidogenic cell types (Farin et al., 1988) and/or increased blood flow to the CL (Niswender et al., 1976).

Conceptuses recovered on d 13 from hCG treated ewes were longer (P<.05), secreted more (P<.05) αTP-1 and were associated with flushings that contained more (P<.05) protein than ewes in the control group (Table 6). A uterine environment advanced by treatment with exogenous progesterone altered conceptus development (Wilmut and Sales, 1981). Administration of progesterone to ewes advanced uterine secretions such that a d 6 uterine environment was suitable
for the development of a 10-day-old embryo (Lawson and Cahill, 1983; Vincent et al., 1986), altered production of specific endometrial proteins (Ashworth and Bazer, 1989) and accelerated conceptus development (Wintenberger-Torres, 1967). Furthermore, higher concentrations of progesterone in plasma were associated with an advanced uterine environment (sheep, Nephew et al., 1991; cows, Garret et al., 1988) and enhanced conceptus development (Boyd et al., 1969; Garret et al., 1988; Nephew et al., 1991). Results from the present study suggest that administration of hCG transiently increased concentrations of estradiol and progesterone. Perhaps progesterone induced changes in uterine secretions that directly or indirectly stimulated conceptus growth and development. No binding sites for hCG were detected in the ovine conceptus (Ellinwood et al., 1979b); therefore, it seems unlikely that hCG stimulated conceptus growth directly.

In experiment 3, after treatment with hCG on d 11.5, the proportion of pregnant ewes increased (94%, 44 of 47 vs 83%, 40 of 48, hCG vs vehicle, respectively; P< .07). The number of CL (Silvia and Niswender, 1984), which presumably corresponded to the number of embryos, and amount of oTP-1 within the uterus on d 13 (Nephew et al., 1989a) were associated with successful pregnancy recognition. Supplementation of a structural homolog of oTP-1, recombinant bovine IFN, during the period of maternal recognition of pregnancy increased the number of pregnant ewes (Nephew et
al., 1990). Since luteal regression is initiated on d 12 to 14 in nonpregnant ewes (Zarco et al., 1988a), it seems possible that early synthesis and release of oTP-1, a primary antiluteolytic signal in sheep (Vallet et al., 1988a), might increase the chance of pregnancy success. Thus, the greater amount of oTP-1 on d 13 (Table 6) after treatment with hCG on d 11.5 may have contributed to the increase in pregnancy rate.

Results from the present study may help to partially explain the inconclusive results reported by some groups utilizing supplemental progesterone as a means of improving reproductive performance in ewes (Table 7). Exogenous progesterone administered within one week after mating either decreased (Hecker et al., 1974) or had no effect (Diskin and Niswender, 1989) on pregnancy rate. However, administration of progesterone (Peterson et al., 1984; Davis et al., 1986; McMillan, 1987; Parr et al., 1987), hCG (Kittok et al., 1983), or recombinant bovine IFN (Nephew et al., 1990) after this time increased pregnancy rates in ewes. Perhaps this later exposure of the reproductive tract to progesterone or hCG was able to uncouple embryonic development from luteolysis and improve reproductive performance.

Implications

Administration of hCG could potentially be used to increase embryonic development, production of pregnancy recognition signal(s) and pregnancy rate. However, the labor required to observe estrous behavior daily and administer
injections would probably preclude this treatment from being a practical means of improving reproductive efficiency. Furthermore, subsequent doses of hCG would have to be altered in order to adjust for antibody production to this protein by the immune system of sheep. Additional studies are required in order to investigate alternative mechanisms that stimulate conceptus growth and development and uncouple luteolytic from antiluteolytic mechanisms.

TABLE 4. CONCENTRATIONS OF PROGESTERONE (NG/ML)\(^a\) AND ESTROGEN (PG/ML)\(^b\) IN PLASMA OF NONMATED EWES AFTER ADMINISTRATION OF HCG (100 IU) ON D 11.5 (D 0= ESTRUS)

<table>
<thead>
<tr>
<th>Day</th>
<th>Progesterone Vehicle</th>
<th>Progesterone Treatment</th>
<th>Estrogen Vehicle</th>
<th>Estrogen Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2.0</td>
<td>1.9</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>12</td>
<td>2.2</td>
<td>3.0**</td>
<td>&lt;2.0</td>
<td>4.5 ± .6</td>
</tr>
<tr>
<td>13</td>
<td>2.1</td>
<td>3.3**</td>
<td>&lt;2.0</td>
<td>5.5 ± .6</td>
</tr>
<tr>
<td>14</td>
<td>2.0</td>
<td>2.7</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
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<tr>
<td>15</td>
<td>1.1</td>
<td>1.5</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
</tr>
</tbody>
</table>

\(^a\)Overall SE from the analysis was .2.

\(^b\)<2.0= Plasma concentrations of estrogen below the sensitivity of the assay.

**Asterisks indicate a significant day X treatment interaction (P<.01).
TABLE 5. CONCENTRATIONS OF PROGESTERONE (NG/ML)\textsuperscript{a} AND ESTROGEN (PG/ML)\textsuperscript{b} IN PLASMA OF PREGNANT EWES AFTER ADMINISTRATION OF HCG (100 IU) ON D 11.5 (D 0= ESTRUS)

<table>
<thead>
<tr>
<th>Day</th>
<th>Progesterone</th>
<th>Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Treatment</td>
</tr>
<tr>
<td>11</td>
<td>2.2</td>
<td>2.3</td>
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<tr>
<td>12</td>
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<td>3.1**</td>
</tr>
<tr>
<td>13</td>
<td>2.4</td>
<td>3.6**</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Overall SE from the analysis was .2.
\textsuperscript{b}<2.0= Plasma concentrations of estrogen below the sensitivity of the assay.
\textsuperscript{**}Significant day X treatment interaction (P<.01).

TABLE 6. REPRODUCTIVE CHARACTERISTICS OF PREGNANT EWES ON D 13 AFTER ADMINISTRATION OF HCG (100 IU) ON D 11.5 (D 0= ESTRUS)

<table>
<thead>
<tr>
<th>Observation</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>oTP-1 (µg)</td>
<td>1.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Protein (mg)</td>
<td>.8\textsuperscript{a}</td>
</tr>
<tr>
<td>Conceptus length (cm)\textsuperscript{e}</td>
<td>.8 ± .3\textsuperscript{c}</td>
</tr>
<tr>
<td>Embryonic survival (%)\textsuperscript{f}</td>
<td>84</td>
</tr>
<tr>
<td>Luteal weight\textsuperscript{e}</td>
<td>433 ± 24\textsuperscript{a}</td>
</tr>
<tr>
<td>Ovulation rate\textsuperscript{e}</td>
<td>2.4 ± .2</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b}Numbers within a row with different superscripts differ (P<.05).
\textsuperscript{c,d}Numbers within a row with different superscripts differ (P<.001).
\textsuperscript{e}Mean ± SE.
\textsuperscript{f}Ratio of the total number of embryos recovered on d 13:total number of CL.
TABLE 7. A REVIEW OF PREGNANCY RATES IN EWES FOLLOWING VARIOUS HORMONAL TREATMENTS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pregnancy Rate (%)</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Treatment</td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1 to 4</td>
<td>98</td>
<td>68*</td>
</tr>
<tr>
<td>d 6 to 50</td>
<td>86</td>
<td>83</td>
</tr>
<tr>
<td>Mid/Late</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 7 to 12 or d 9 to 14</td>
<td>56</td>
<td>79*</td>
</tr>
<tr>
<td>d 8 to 14</td>
<td>48</td>
<td>68*</td>
</tr>
<tr>
<td>d 10 to 16</td>
<td>67</td>
<td>95*</td>
</tr>
<tr>
<td>d 10 to 16</td>
<td>54</td>
<td>66</td>
</tr>
<tr>
<td>hCGa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 11 to 13</td>
<td>29</td>
<td>58*</td>
</tr>
<tr>
<td>d 11.5</td>
<td>83</td>
<td>94*</td>
</tr>
<tr>
<td>IFNb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 12 to 16</td>
<td>76</td>
<td>92*</td>
</tr>
</tbody>
</table>

*a100 IU, i.m.

bRecombinant bovine interferon alpha1, 2 mg per day, i.m.

*Indicates statistical significance.
CHAPTER VI

Accumulative Recognition of Pregnancy in Sheep

The hypothesis that an embryo, either alone or in combination with another embryo within one uterine horn, prevents luteolysis by accumulatively producing a threshold amount of oTP-1 by d 13 was examined in thirty ewes. Pregnant and nonmated ewes with two corpora lutea (CL) in one ovary and one CL in the other ovary served as either sham operated controls or were luteectomized on d 4 (d 0 = estrus) such that one CL remained in each ovary. On d 13, ewes were injected (i.m.) with a subluteolytic dose of PGF₂α (6 mg/58 kg BW) and slaughtered 0 or 6 h postinjection. Jugular blood samples were collected at both of these times and analyzed for progesterone. Reproductive tracts were flushed, three conceptuses (two from one horn and one from the other) were recovered and assessed by stage of development. The weight, progesterone content, in vitro production of progesterone and 6-keto-PGF₁α (the stable, inactive metabolite of prostacyclin, PGI₂) by individual CL, and amounts of oTP-1 in uterine flushings were determined. Treatment with PGF₂α decreased (P<.01) concentration of progesterone in plasma of nonmated ewes and pregnant ewes with spherical or tubular embryos, but not of those with filamentous conceptuses. Corpora lutea ipsilateral to a uterine horn containing two conceptuses and
more (P<.001) oTP-1 (12,480 vs 2503 ng, respectively) were heavier (503 vs 457 mg, respectively; P<.05), contained more progesterone (12.9 vs 7.4 µg, respectively; P<.05) and produced more progesterone and 6-keto-PGF₁α in vitro (P<.05 and P<.001, respectively). Luteal resistance to PGF₂α and the amount of oTP-1 present on d 13 were positively related; therefore, these data support the hypothesis that the antiluteolytic effect(s) of early pregnancy in sheep is an accumulative event.

Introduction

Protection of the CL from the luteolytic activity of PGF₂α of uterine origin (Goding, 1974) is a key event in the process of pregnancy recognition in sheep. Pregnant ewes were more resistant to the luteolytic actions of exogenously administered PGF₂α on d 13 after estrus than nonpregnant ewes (Pratt et al., 1977). Silvia and Niswender (1984) demonstrated that pregnant ewes with two CL, which presumably corresponded to the number of conceptuses, were more likely to maintain pregnancy after exposure to exogenous PGF₂α on d 13 than ewes with one embryo. A factor produced by the ovine conceptus that plays an important role in extension of CL function is oTP-1 (Godkin et al., 1984a; Vallet et al., 1988a). Administration of recombinant bovine interferon, a protein closely related to oTP-1 (Imakawa et al., 1989), increased pregnancy rates in ewes (Nephew et al., 1990). Collectively, those studies suggested that the amount of oTP-
1/interferon which is secreted during early pregnancy might be related to luteal resistance to PGF$_2\alpha$ and subsequently influence the chance of pregnancy recognition.

Prostacyclin (PGI$_2$) has been implicated as part of the luteotropic/antiluteolytic complex in sheep (Marcus, 1981). Production of PGI$_2$ by bovine luteal tissue diminished as the age of the CL advanced, unless the CL was maintained by pregnancy (Milvae and Hansel, 1983). A positive relationship between luteal biosynthesis of PGI$_2$ and progesterone was demonstrated (Milvae and Hansel, 1983), suggesting that PGI$_2$ production was an indicator of normal luteal function (Milvae and Hansel, 1985).

The purpose of the present experiment was to examine the relationships between the number of embryos, accumulation of oTP-1 within one uterine horn and luteal resistance to exogenous PGF$_2\alpha$ on d 13 of pregnancy.

**Materials and Methods**

Thirty crossbred ewes were utilized in experiments conducted during the autumn of 1988 and 1989. Ewes were mated to fertile rams at the onset of estrus (d 0) and every 12 h until the end of estrus. Ewes assigned to the nonpregnant group were checked for estrous behavior with vasectomized rams twice daily. All ewes were subjected to a midventral laparotomy on d 4 to identify those with two CL in one ovary and one CL in the other. Ewes were then assigned randomly to one of four groups. Two groups of pregnant and one group of
nonpregnant ewes were lutectomized on d 4. One of the two CL on the same ovary, chosen at random, was gently excised from the connective tissue of the ovarian cortex. The tunica albuginea was sutured using 3/0 chromic catgut (Newhausen, Switzerland). A third group of pregnant ewes served as sham-operated controls; a suture was placed in the tunica albuginea of the ovary containing two CL.

On d 13, all ewes received an i.m. injection of PGF$_2$α (6 mg/58 kg BW; Lutalyse, The Upjohn Co., Kalamazoo, MI). In a dose-response study by Silvia and Niswender (1984), this dosage of PGF$_2$α induced partial, but not complete, luteolysis in pregnant ewes on d 13. Lutectomized pregnant and nonpregnant ewes were slaughtered or 6 h after treatment with PGF$_2$α. The group of sham-operated ewes was slaughtered 6 h after PGF$_2$α treatment. Blood samples were collected by jugular venipuncture before PGF$_2$α administration and at slaughter. Plasma was obtained and stored at -20 C until assayed for progesterone by RIA (Nephew et al., 1991).

Reproductive tracts were collected within 10 min of slaughter. Uterine horns were flushed separately as described previously (Nephew et al., 1991). Recovery of three conceptuses was requisite for inclusion in the study. After removal of conceptuses, flushings were stored at -20 C until assayed for oTP-1 by RIA (Vallet et al., 1988b). The morphological stage of development was determined for each
conceptus according to the descriptions of Green and Winters (1945): spherical, tubular or filamentous.

Luteal weight (Denamur et al., 1973) and content of progesterone (Stormshak et al., 1963) are used as indicators of the functional state of CL, and a positive correlation between these variables and plasma progesterone levels has been demonstrated (Stormshak et al., 1962). Corpora lutea from lutectomized pregnant ewes slaughtered 6 h after administration of PGF₂α were decapsulated, weighed and sliced individually into approximately 1.5 mm slices (18-20 mg). A center slice of each CL was homogenized in phosphate-buffered saline containing 0.1% gel (PBS-gel; 1 ml/20 mg tissue). Homogenates (10 mg tissue equivalents/0.5 ml PBS) were extracted twice with 5 ml petroleum ether as described by Knickerbocker and Niswender (1989). Solvent extracts were pooled and dried under air. The extract was reconstituted in 1.5 ml PBS-gel and analyzed for progesterone by RIA. The remaining tissue slices were incubated in 12 X 75 mm disposable culture tubes containing 3 ml Hamm's F-12 medium (Sigma Chemical Co., St. Louis, MO) at 37 C in a Fisher metabolic shaker under a humidified atmosphere of 95% air, 5% CO₂. Each tube contained one slice of tissue. After a 10 min pretreatment incubation period, an aliquot (1 ml per tube; 0 h) was removed, and the tissue was incubated for 2 h in the presence or absence of LH (NIADDK-oLH-25; National Hormone and Pituitary Program, Baltimore, MD; 100 ng/ml). At the end of
the incubation period, tissue slices were separated from their corresponding incubation medium. The medium was stored at -20 C until analyzed for concentrations of progesterone and 6-keto-PGF \(_\alpha\) (Milvae and Hansel, 1983). Net biosynthesis of progesterone and 6-keto PGF \(_\alpha\), the stable metabolite of PGI\(_2\), in medium represented the difference between concentrations at 0 and 2 h.

All data were analyzed using the Statistical Analysis System (SAS, 1986). Data for concentrations of progesterone and PGI\(_2\) were analyzed by least squares analysis of variance (ANOVA) using General Linear Models procedures. Differences between least squares means were compared using Fisher's protected least significant difference test. Differences in total uterine content of oTP-1, luteal weight, tissue content of progesterone and concentrations of progesterone in plasma were analyzed using a one-way ANOVA. After calculating the percent decline in plasma progesterone, percent data were log transformed before analysis. Due to heterogeneity of variance, determine using Bartlett's test (Steel and Torrie, 1980), data for oTP-1 and luteal weight were subjected to a logarithmic transformation before analysis.

**Results**

After injection of PGF\(_2\alpha\), plasma progesterone decreased (P < .01) in nonpregnant ewes and in pregnant ewes that had either spherical or tubular embryos (Figure 1), but not in ewes with filamentous conceptuses (P > 0.10).
More \( (P < .01) \) oTP-1 was measured in uterine flushings that contained two versus one conceptus (2647 vs 1024 \( \mu g \), respectively), and this relationship was most pronounced in flushings from ewes with filamentous conceptuses (Figure 2). For pregnant, lutectomized ewes slaughtered 6 h after PGF\(_2\alpha\), luteal weight (Table 8) and content of progesterone (12.9 ± 1.4 vs 7.4 ± 1.4 \( \mu g \)) were greater \( (P < .05) \) for CL ipsilateral to a uterine horn containing two versus one conceptus, respectively. Furthermore, content of progesterone was greater \( (P < .05) \) for CL ipsilateral to two versus one filamentous conceptus, but not for CL adjacent to spherical or tubular conceptuses (Figure 3). Weight and progesterone content of individual CL of unilateral twin ovulations are normally less than that of CL of single ovulating ewes (Silvia and Niswender, 1984).

The unstimulated, in vitro, production of progesterone and PGI\(_2\) by tissue slices is shown in Figure 4. Secretion of progesterone and PGI\(_2\) by CL ipsilateral to filamentous conceptuses producing more oTP-1 were greater \( (P < .05 \) and \( P < .001 \), respectively) compared to values for CL adjacent to spherical or tubular embryos secreting less oTP-1. Correlation coefficients between these two hormones were 0.97, 0.92 or 0.82 for CL ipsilateral to filamentous, tubular or spherical conceptuses, respectively. Milvae and Hansel (1983) reported a correlation coefficient of 0.92 between PGI\(_2\) and progesterone. The addition of LH did not significantly
increase basal progesterone or PGI₂ production from luteal slices.

**Discussion**

Results from this study demonstrate that CL adjacent to a uterine horn containing multiple conceptuses are resistant to the luteolytic action(s) of PGF₂α on d 13. Exogenously administered PGF₂α was less effective as a luteolysin in pregnant than in nonpregnant ewes on d 13 post-estrous (Pratt et al., 1977). Silvia and Niswender (1984) demonstrated that luteal resistance to PGF₂α on d 13 was greater in ewes with two CL than ewes with one CL. Improved fertility for ewes with two versus one CL was reported (Bradford and Quirke, 1986). Higher pregnancy rates were achieved by transferring embryos, which had been split in half, into one versus both uterine horns of ewes with bilateral CL (Maurer, 1988). Collectively, these data suggest that the presence of multiple conceptuses in one uterine horn augments CL resistance to luteolysis, and offer additional information regarding the biological importance of the high frequency of unilateral twin ovulations in ewes (Casida et al., 1966) and migration of ovine embryos after maternal recognition of pregnancy (Nephew et al., 1989b). Perhaps by remaining in the uterine horn ipsilateral to CL, conceptuses accumulatively enhance the signal(s) for pregnancy recognition.

Pregnancy recognition is mediated by products released from the conceptus (Rowson and Moor, 1967), and oTP-1 has been
strongly implicated in this process (Godkin et al., 1984b; Vallet et al., 1988a). Nephew et al. (1990) administered a related interferon, recombinant bovine interferon (Imakawa et al., 1989), during the period of maternal recognition of pregnancy and increased fecundity in ewes. In the present study, luteal resistance to exogenous PGF$_2$α and amount of oTP-1 in uterine flushings were positively related (Figure 1). Furthermore, conceptus development and synthesis of oTP-1 were related to estrous cycle duration; ewes that normally experienced luteolysis earlier in the cycle accumulated more oTP-1 by d 13 of pregnancy (Nephew et al., 1991). Taken together, these observations suggest that maternal recognition of pregnancy in sheep is an accumulative event; a threshold concentration of oTP-1 must be produced in order to resist luteolysis.

Based on luteal weight, content of progesterone and in vitro function, CL ipsilateral to a uterine horn containing advanced conceptuses and more oTP-1 were resistant to exogenous PGF$_2$α. These results suggest a local effect of a factor(s) from the ipsilateral uterine horn and(or) conceptus on the CL. Contents from the gravid uterine horn reach the ipsilateral ovary through a local venoarterial pathway (Mapletoft et al., 1976); however, it has not been completely resolved whether a factor(s) produced by the conceptus affects the CL directly. A proteinaceous substance produced by the ovine conceptus stimulated luteal function in vitro (Godkin et
Specific binding of oTP-1 to luteal membrane preparations (Godkin et al., 1984a) and high affinity binding sites for oTP-1 in the CL (Knickerbocker and Niswender, 1989) have been demonstrated. OTP-1 is related to the interferon alpha family (Imakawa et al., 1987, 1989) and possesses antiviral properties characteristic of these interferons (Pontzer et al., 1988; Roberts et al., 1989), and antiviral activity was detected in the uterine vein of pregnant ewes (Schalue-Francis et al., 1990). These observations suggest that oTP-1 may exit the uterus and act locally.

Other factors associated with advanced embryonic development may also induce luteal resistance to PGF₂α. A conceptus secretory protein of a different molecular weight than oTP-1, for example, decreased the ability of PGF₂α to inhibit utilization of lipoprotein by ovine luteal cells (Wiltbank et al., 1990). Ashworth and Bazer (1989) reported increased secretion of ten endometrial proteins and decreased secretion of another protein at this stage of early pregnancy. These and other factors produced by the gravid uterus could have acted locally to maintain CL. Concentrations of PGE₂ and PGI₂ (Silvia et al., 1984) and the ratio of PGE₂:PGF₂α in the utero-ovarian vein (Silvia et al., 1984; Vincent et al., 1986) or uterine flushings (Ellinwood et al., 1979a; Nephew et al., 1991) increase during early pregnancy. Similar changes in uterine physiology may be augmented by the presence of multiple conceptuses in one uterine horn.
Implications

Luteal resistance to exogenous PGF$_2$α on d 13 was related to the number of conceptuses, stage of conceptus development and amount of oTP-1 in uterine flushings. Furthermore, a local relationship between a factor(s) produced by the ipsilateral uterine horn and(or) conceptus and resistance of CL to PGF$_2$α was apparent. We propose that the antiluteolytic effect(s) of early pregnancy is an accumulative event; therefore, accumulation of uterine/conceptus factor(s) before luteolysis may assist pregnancy recognition.
TABLE 8. EFFECT OF NUMBER OF CONCEPTUSES ON WEIGHT OF CL\(^a\) OF PREGNANT OR NONMATED EWES ON D 13 (DAY 0 = ESTRUS)

<table>
<thead>
<tr>
<th>Group(^b)</th>
<th>n</th>
<th>1 conceptus</th>
<th>2 conceptuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant, 0 h(^c)</td>
<td>15</td>
<td>457(^g)</td>
<td>503(^h)</td>
</tr>
<tr>
<td>Pregnant, 6 h(^d)</td>
<td>5</td>
<td>510</td>
<td>465</td>
</tr>
<tr>
<td>Nonmated, 6 h(^e)</td>
<td>5</td>
<td>401</td>
<td>376</td>
</tr>
<tr>
<td>Pregnant-Sham, 6 h(^f)</td>
<td>5</td>
<td>467</td>
<td>440</td>
</tr>
</tbody>
</table>

\(^a\)CL were adjacent to a uterine horn containing one or two embryos at slaughter on d 13.

\(^b\)Ewes received PGF\(_2\)\(\alpha\) (6 mg/58 kg BW; i.m.) on d 13 of gestation.

\(^c\)Pregnant ewes with three CL (two in one ovary and one in the other) on d 4 were lutectomized such that one CL remained in each ovary. Ewes were slaughtered 6 h after PGF\(_2\)\(\alpha\).

\(^d\)Pregnant ewes with three CL (two in one ovary and one in the other) on d 4 were lutectomized such that one CL remained in each ovary. Ewes were slaughtered 0 h after PGF\(_2\)\(\alpha\).

\(^e\)Nonmated ewes with three CL (two in one ovary and one in the other) on d 4 were lutectomized such that one CL remained in each ovary. Ewes were slaughtered 6 h after PGF\(_2\)\(\alpha\).

\(^f\)Pregnant ewes with three CL (two in one ovary and one in the other) served as sham-operated controls and were slaughtered 6 h after PGF\(_2\)\(\alpha\). The total luteal weight per ovary was greater (P<.05) for the ovary containing two vs one CL (751 vs 467 mg, respectively).

\(^g,h\)Numbers within a row with different superscripts differ (P<.05).
Figure 1. Percent decline in plasma progesterone 6 h after administration of Prostaglandine F2 on d 13. Bars with Different letters differ (P<.05).
Figure 2. Uterine content of oTP-1 on d 13. Each uterine horn contained one or two conceptuses. Bars with different letters differ (P<.05).
Figure 3. Content of progesterone in CL ipsilateral to a uterine horn containing one or two conceptuses. Bars with different letters differ (P<.05).
Figure 4. Production of progesterone and 6-keto-PGF1 by luteal slices in vitro. Bars with different letters differ for progesterone and 6-keto-PGF1 (P<.05 and P<.001, respectively).
CHAPTER VII

Embryonic Migration Relative to Maternal Recognition of Pregnancy in Sheep

Twenty-five crossbred ewes were utilized to examine the timing of embryonic migration relative to maternal recognition of pregnancy. These ewes also were utilized to examine whether ovine embryos synthesized estradiol-17β in association with embryonic elongation and intrauterine migration. Embryos were flushed on d 11 through 15 from hemiovariectomized ewes. Recovery of embryos from the uterine horn contralateral to the remaining ovary indicated that migration occurred. Ewes subsequently were returned with rams to determine their interestrous interval. Recovered embryos were classified morphologically, their length determined and individually incubated. Changes in estradiol within the medium were determined after a 6 h incubation. Embryonic migration began on d 14 (P<.05); on consecutive days from 11 through 15, 0, 0, 0, 60 and 100% of ewes examined, respectively, had an embryo in the contralateral horn. Extended estrous cycles (>20 d) were observed in 0, 0, 40, 80 and 100% of ewes examined (P<.05) following removal of embryos on d 11 through 15 of the cycle. Ovine embryos were longer (P<.05) on d 14 (4.8±1.1 cm length) compared with d 13 (.2±.1 cm) and increased further (P<.05) on d 15 (7.8±1.1 cm). Incidence of intrauterine
migration was correlated with embryonic length \((r = 0.83; P<0.01)\) and estradiol synthesis \((r = 0.77; P<0.01)\). Embryonic length also was correlated with estradiol synthesis \((r = 0.86; P<0.01)\). Intrauterine migration of ovine embryos was associated with embryonic elongation and synthesis of estradiol. Embryonic migration began on d 14, was completed by d 15 and occurred after maternal recognition of pregnancy.

**Introduction**

Equidistant spacing of embryos in polytocous species is accomplished partially by intrauterine migration. In sheep, for example, intrauterine migration of embryos into the uterine horn contralateral to an ovary containing multiple ovulations occurred in nearly 90% of ewes examined (Casida et al., 1966; Scanlon, 1972), indicative of a well controlled and efficient process. However, the timing of intrauterine migration relative to maternal recognition of pregnancy has not been investigated in sheep.

The signal(s) to induce embryonic migration also is not fully understood. Peristaltic contractions of the myometrium (Boving, 1971; Pusey, 1980; Cross and Ginther, 1988), which may be stimulated locally by the embryo (Cloud and Casida, 1969; Pope et al., 1982a), were associated with migration. Estradiol synthesis by porcine embryos (Perry et al., 1973; Gadsby et al., 1980) appeared to stimulate activity of uterine smooth muscle and intrauterine migration (Pope et al., 1982a). Likewise, increased production of estradiol by equine embryos
occurred coincident with the period of migration (Zavy et al., 1979). Estradiol synthesis also has been observed by bovine embryos (Shemesh et al., 1979), but minced cells from ovine embryos were unable to synthesize estrogens (Gadsby et al., 1980).

The present experiment was conducted to determine the time of embryonic migration relative to maternal recognition of pregnancy. In addition, we examined whether ovine embryos could synthesize estradiol in association with intrauterine migration.

**Materials and Methods**

Twenty-five crossbred ewes were hemiovariectomized in the spring, before allocation to experimental groups in the fall. One ovary, chosen at random, was removed to ensure initial entry of embryos into one uterine horn. In the fall, ewes were mated at the onset of estrus (d 0) and every 12 h until the end of estrus.

**Embryo Removal.** On d 11 through 15 of pregnancy, embryos were obtained by exposing the uterus via a midventral incision in the abdomen and surgically flushing each uterine horn. A blunt incision was made below the external bifurcation, and a glass cannula, with a smooth, flared end, was inserted into the uterine lumen. Medium (modified Dulbecco's, 10 ml) was injected into the tip of the ipsilateral uterine horn; the medium plus embryo(s) were flushed toward the cannula and placed immediately into an incubator at 39 C. The
contralateral horn was flushed in the same manner. Each horn was flushed twice with 10 ml of medium. Recovery of an embryo from the uterine horn contralateral to the remaining ovary indicated that embryonic migration had occurred. Only ewes displaying multiple ovulations were accepted, and 100% recovery of embryos was requisite for inclusion in the study. Ewes were returned to a pen with marked rams, and blood samples were collected daily for subsequent progesterone analysis to assist in determination of the interestrous interval. Blood samples were centrifuged at 4 C and serum was stored at 20 C until assayed for progesterone by RIA, as described by Hild-Petito et al. (1987). Serum progesterone less than 1 ng/ml, accompanied by estrus, was indicative of luteolysis.

**Embryo Culture.** Recovered embryos were gently removed from the uterine flushings, and the morphological classification and length of each intact embryo were immediately determined. Each embryo was washed individually in Krebs Ringer Bicarbonate Medium (37 C, pH 7.3 to 7.4, 287 to 293 mOs, filter sterilized) supplemented with fetal calf serum (10%) and an antibiotic/antimycotic (100 IU/ml). Washed embryos were cultured individually in dishes containing 4 ml of medium. One ml of medium was removed at the initiation of culture (0 h sample) and frozen (-20 C) until analysis by RIA. Embryos were incubated for 6 h at 37 C in a 95% air and 5% CO₂ humidified atmosphere. At 6 h, embryos were removed from the
culture dish; the remaining medium was frozen for determination of the total amount of estradiol synthesis per embryo.

The concentration of estradiol 17-β in the medium sampled at 0 and 6 h was quantified directly by methods reported previously (Butcher et al., 1974). The antiserum crossreacted only with estriol (3%) and estrone (12%). Sensitivity of the assay was 5.0 pg. Pools of media containing low and high concentration of estradiol 17-β were established, consistent with the samples, which allowed for determination of the intra- and inter-assay coefficients of variation (4.3 and 0.6%, respectively). The slope of the curve generated by using aliquots of pooled media (-2.29 ± 3.1) did not differ (P = .77) from the linear portion of the standard curve (-2.40 ± .13).

Preliminary attempts to verify estradiol synthesis by recovered embryos were conducted by two methods. Embryos (n= 3) were incubated by (125I) testosterone. Extracted medium subsequently was eluted on a Sephadex LH-20 column with benzene-methanol (85/15, vol/vol). Coalescence of label and estradiol indicated partial incorporation of testosterone into estradiol.

Second, d 15 embryos (n= 2) were transected in half, and half-embryos were placed in culture, as described. Extraction of half-embryos (4 ml diethyl ether) at both 0 and 6 h yielded less than 5 pg of estradiol. Extraction of culture medium
yielded 85 pg of estradiol after 6 h in culture, an indication that estradiol in the medium was a result of synthesis and release by the embryo, as opposed to accumulation of maternally derived steroid.

**Statistical Analysis.** All data were analyzed by least squares analysis of variance using the General Linear Models procedure of SAS (1982). Binomial observations, embryonic migration and maternal recognition of pregnancy, were coded. A code of 1 represented ewes with embryos recovered from the contralateral uterine horn. Ewes experiencing no embryonic migration were coded as 0. If ewes displayed in interestrous interval greater than 20 d (4 standard deviations beyond the normal 16.5 ± 0.8 d cycle length observed in these ewes), they were considered to have experienced maternal recognition of pregnancy and were coded as 1. Ewes with a cycle length of less than 20 d received a code of 0. Correlation coefficients were calculated (Steel and Torrie, 1980) to examine the relationship between the occurrence of intrauterine migration, embryonic length and in vitro estradiol synthesis. Differences between means were tested by a least significant difference test (Snedecor and Cochran, 1980).

**Results**

Migration into the contralateral uterine horn by ovine embryos was not evident until d 14 (Figure 5), at which time 60% of ewes examined had experienced (P<.05) embryonic migration. By d 15, 100% of ewes examined had embryos
recovered (P<.05) from the contralateral horn. Embryos were spherical between d 11 and 13, except for two embryos recovered from one ewe on d 13 that were tubular in shape (8 to 10 mm). Elongation of ovine embryos of the filamentous stage began (P<.05) on d 14 (4.8 ± 1.1 cm) and increased (P<.05) on d 15 (7.8 ± 1.1 cm). Some embryos synthesized detectable quantities of estradiol after 6 h in culture of d 11 and 13 (Table 9). It was not until d 14 and 15, however, that embryos began to synthesize detectable (P<.05) amounts of estradiol in vitro. All three variables were highly and positively correlated. Intrauterine migration was correlated with both embryonic length (r= .83; P<.01) and in vitro estradiol synthesis (r= .77; P<.01). Embryonic length also was correlated with estradiol synthesis (r= .86, P<.01).

Removing embryos had no effect on the interestrous interval when done on d 11 or 12 (Figure 6). However, embryo removal on d 13, 14 and 15 resulted in 40, 80 and 100% (P<.05) of ewes examined experiencing extended estrous cycles, respectively. When embryos were recovered on d 13, the percentage of ewes with an extended cycle length was greater (P<.05) than the percentage that had experienced embryonic migration and suggested that recognition of pregnancy occurred before embryonic migration. Embryos from one of the two ewes that failed to experience embryonic migration also failed to synthesize estradiol in vitro (Table 9). Interestingly, this is also the ewe that had a normal cycle.
Discussion

When two ovulations occurred in one ovary, migration of one embryo into the contralateral uterine horn was observed in nearly 90% of ewes examined (Casida et al., 1966; Scanlon, 1972). This high frequency of embryonic migration also was observed in our study; 100% of ewes with twin ovulations had an embryo recovered from the contralateral horn when examined on d 15. In contrast, when a single ovulation was experienced in one or each ovary, migration into the uterine horn not associated with the corpus luteum, or migration into an already occupied horn, was rare (Casida et al., 1966; Scanlon, 1972).

The timing of embryonic migration in sheep was reported to occur between d 12 to 14 of gestation (Cummins, 1979). In the present study, ovine embryos began and completed intrauterine migration on d 13 to 14 and d 15, respectively. Physical attachment between the embryo and uterine epithelium was not achieved until d 16 to 17 of gestation (Chang and Rowson, 1965; Short, 1969), suggesting that ovine embryos were free to move within the uterine lumen prior to this time. Polge and Dzuik (1970) demonstrated that filamentous embryos were not able to undergo intrauterine migration in the pig. In the present study, migration of elongated embryos was completed by d 15; this suggests that embryos have a limited and critical period to experience intrauterine mobility.
Ovine embryos synthesized and released estradiol. Gadsby et al. (1980) observed that trophoblastic tissues from ewes on d 16 to 18 were incapable of estrogen production, based on conversion of labeled androstenedione to estradiol. However, those investigators incubated minced tissue, compared with incubation of intact embryos in our study. Dwyer and Robertson (1980) reported that levels of estrone sulfate were detectable in uterine venous plasma on d 16 of gestation in ewes. Higher, but non-significant, concentrations of estrogen in samples of utero-ovarian venous blood from pregnant and nonpregnant ewes also were observed on d 13 and 15 (Ellinwood, 1978; Reynolds et al., 1982), approximately the time of embryonic elongation (Chang and Rowson, 1965). Likewise, Willis et al. (1979) observed in vitro synthesis of estradiol by ovine conceptus membranes at this time. Initial estrogen production was concomitant with elongation for by the porcine (Perry et al., 1973, 1976) and bovine (Shemesh et al., 1979) embryos; maximal estrogen synthesis occurred as porcine embryos became filamentous and declined shortly thereafter (Geisert et al., 1982b). Perhaps ovine embryos have a capacity for steroid production that is limited to a defined stage of development. Increased synthesis of estradiol appeared to begin on d 14, coincidental with embryonic elongation. This observed increase in estradiol synthesis by ovine embryos occurred at the time of intrauterine migration, suggesting that estradiol production might influence embryonic
migration, perhaps by mechanisms similar to those reported for swine (Pope et al., 1982b) and mares (Zavy et al., 1979). Together, these observations suggested that embryos attain a critical size before developing the capacity to synthesize estrogen in amounts adequate to influence intrauterine migration.

Luteotrophic extension of the interestrous interval was not detected until d 13, the period of maternal recognition of pregnancy in sheep; this result is consistent with previous observations (Moor and Rowson, 1966a,b). Recognition of pregnancy occurred after intrauterine migration of embryos in the sow (Dhindsa et al., 1967) and mare (Ginther, 1983). However, our study suggested that recognition of pregnancy in sheep occurred before embryonic migration. The significance of this finding is unclear. Maurer (1988) reported that recipient ewes, with bilateral ovulations, experienced higher pregnancy rates when transected embryos were transferred into one vs both uterine horns. By remaining in the ipsilateral uterine horn, embryos may have enabled more of these recipient ewes to experience recognition of pregnancy. This suggests that embryos cumulatively enhanced the luteotrophic signal before migration into the contralateral horn.

The higher incidence of embryonic loss observed with multiple unilateral, compared with single and bilateral, ovulating ewes (Doney et al., 1973) was associated with intrauterine migration (White et al., 1981). The effect(s) of
migration on embryonic mortality though presently unknown, might be associated with an asynchronous environment in the contralateral uterine horn compared with the ipsilateral horn, or failure of the contralateral endometrium to respond to an embryonic signal. In cows, Pope et al. (1982c) observed a higher content of progesterone within ipsilateral vs contralateral uterine tissue. Such a subtle difference between uterine horns in ewes might be exaggerated by d 14 and 15, as asynchrony became more critical later in gestation (Wilmut and Sales, 1981). Although recipients with unilateral and bilateral ovulations experienced similar lambing rates (Torres and Sevellec, 1987), observations with recipient cows clearly indicated greater embryonic survival in the ipsilateral uterine horn (Sreenan el al., 1975; Christie et al., 1979; Newcomb et al., 1980). In addition, embryonic production of ovine trophoblast protein-1 (Godkin et al., 1984a) might have preferentially altered the uterine environment of the ipsilateral horn. The observed homology between oTP-1 and bovine interferon (Inakawa et al., 1987) suggested that the ipsilateral horn might be a more immunologically privileged site for embryonic development that the contralateral horn. Consequently, the contralateral uterine horn may not be exposed to oTP-1 early enough to result in a uterine environment conducive to survival of an embryo following migration.
Implications

Intrauterine migration of ovine embryos was associated with both embryonic elongation and synthesis of estradiol. Migration began on d 14, was completed by d 15 and occurred after maternal recognition of pregnancy. Perhaps, unique to sheep, ipsilaterally located embryos may cumulatively enhance luteal maintenance before equidistant spacing/embryonic migration. Further investigations are necessary to elucidate the mechanisms of embryonic migration and subsequent mortality of embryos once positioned into the contralateral horn.

TABLE 9. ESTRADIOL SYNTHESIS BY OVINE EMBRYOS RECOVERED ON DAYS 11 TO 15 OF GESTATIONa

<table>
<thead>
<tr>
<th>Days of Gestation</th>
<th>Estradiol synthesis</th>
<th>Detectable amounts of estradiolb</th>
<th>Estradiol production, pgc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Detectable amounts of estradiolb</td>
<td>2/5</td>
<td>2/5</td>
<td>1/5</td>
</tr>
<tr>
<td>Estradiol production, pgc</td>
<td>11±18</td>
<td>14±21</td>
<td>13±9</td>
</tr>
</tbody>
</table>

aCultured for 6 h in vitro.

bRatio of ewes with embryos that synthesized detectable amounts of estradiol (6 h > 0 h) to the total number of ewes examined.

cMean ±SE for five embryos from each day of gestation examined.
Figure 5. The relationship between embryonic migration, embryonic length and synthesis of estradiol on d 11 to 15 of pregnancy. Each bar represents the mean and SE of five ewes. Standard errors were calculated from the appropriate error mean squares.
Figure 6. Timing of intrauterine migration relative to maternal recognition of pregnancy (mean of five ewes per bar).
CHAPTER VIII

Influence of the Embryo on Intrauterine Migration in Sheep

Mechanisms of intrauterine migration were examined in eighty-four ewes. In the first experiment, unilaterally ovariectomized ewes had corpora lutea removed on d 4 (d 0 = estrus) and pregnancy maintained with progesterone. In the second experiment, the reproductive tract was altered surgically such that embryos initially entered the uterine horn contralateral to the site of ovulation. In the third experiment, ewes received beads of silastic glue releasing either cholesterol or estradiol-17β (estradiol) in an attempt to mimic embryonic synthesis of estradiol. In experiment 4, unilaterally ovariectomized ewes were superovulated and the subsequent equidistant spacing of embryos examined. Ewes were slaughtered on d 15 and recovery of embryos or beads from each uterine horn indicated that migration had occurred. All ewes in experiment 1 and 2 experienced embryonic migration. Beads impregnated with estradiol migrated farther (P<.01) than cholesterol-containing beads (27.6 ± 4.3 vs 12.5 ± 1.6 cm, respectively). In experiment 4, all ewes had only one conceptus recovered from the contralateral horn. These results demonstrated that 1) embryonic migration occurred after local or systemic exposure to progesterone, 2) embryos migrated to the unoccupied horn regardless of initial horn of
entry 3) estradiol may be involved in stimulating migration and 4) conceptuses were not equally distributed between horns.

**Introduction**

Intrauterine migration, which tends to equally distribute the number of embryos within the uterus, results in a greater intrauterine area for development and growth of each embryo. In sheep, failure of intrauterine migration has been associated with embryonic mortality (Doney et al., 1973; White et al., 1981). Likewise, fetal mortality in ewes with high ovulation rates was attributed to failure to equally distribute embryos between uterine horns (Rhind et al., 1980).

In ewes with a single corpus luteum (CL) in one or both ovaries, embryos remained predominately in the uterine horn adjacent to the site of ovulation (Casida et al., 1966; Scanlon et al., 1972; Reimers et al., 1973). Intrauterine migration, however, was a frequent occurrence when multiple ovulations occurred in one ovary (Casida et al., 1966; Scanlon, 1972; Reimers et al., 1973; Nephew et al., 1989b), suggesting that migration is not a passive, random event but rather an active, well controlled process. The timing of embryonic migration in sheep has been documented (Nephew et al., 1989b); however, little information is available on possible mechanisms associated with this phenomenon. Migration appeared to be induced by the embryo (Cloud and Casida, 1969), associated with both embryonic elongation and synthesis of estradiol (Nephew et al., 1989b) and may be
influenced by a local uterine environment (Weems et al., 1988).

The present experiments were examined 1) if localized effects of progesterone on migration differ from systemic, exogenous exposure, 2) if the initial horn of entry affects migration of embryos into the opposite horn, 3) if estradiol is a possible stimulus for intrauterine migration of ovine embryos and 4) how efficiently sheep space three or more embryos which initially enter only one uterine horn.

**Material and Methods**

**Experiment 1.** The objective of this experiment was to examine the effects of local versus systemic exposure of progesterone on the timing and extent of embryonic migration. Ten crossbred ewes were unilaterally ovariectomized in the summer and allowed at least one estrous cycle of normal length (15-18 d) before assignment to the experiment. Ewes were subjected to a mid-ventral laparotomy on d 4 (d 0 = 1st day of mating) and served as either sham-operated controls or had all CL removed. Pregnancy was maintained in the latter group by a regimen of i.m. injections of progesterone in corn oil twice daily on d 4 to 8 and once a day on d 9 to 15 (Miller and Moore, 1976). Ewes in the control group received injections of vehicle. All ewes were slaughtered on d 15. Pregnancy was verified by flushing conceptuses from the uterus. Recovery of a conceptus from the uterine horn contralateral to the
remaining ovary indicated that embryonic migration had occurred.

**Experiment 2.** The objective of experiment 2 was to determine if the initial uterine horn of entry influenced migration of embryos into the opposite horn. Thirty crossbred ewes were randomly assigned to one of two groups. During the non-breeding season, all ewes were subjected to a mid-ventral laparotomy. Ewes in the control group were unilaterally salpingo-ovariectomized and the fimbria was sutured to the mesovarium of the ovary (Figure 7a). Ewes assigned to the treatment group were unilaterally ovariectomized and salpingectomized on the side opposite the ovariectomy. The remaining fimbria was extended dorsally over the uterine body and sutured to the mesovarium of the remaining (opposite) ovary to ensure entry of oocytes only into the uterine horn contralateral to the site of subsequent ovulations (Figure 7b).

In the autumn, ewes were observed for at least one estrous cycle of normal duration and then mated to fertile rams. Six ewes subjected to these surgical procedures and slaughtered on d 13 had not yet experienced embryonic migration (control, n= 4; treatment, n= 2); therefore, all remaining ewes (control, n= 11; treatment, n= 13) were slaughtered on d 15. The incidence of migration was assessed as described previously. Since the presence of a non-gravid uterine horn ipsilateral to CL could result in a return to
estrus (Moor and Rowson, 1966a), progesterone (12 mg, i.m.) was administered on d 13 and 14 to assist pregnancy maintenance. Results of experiment 1 indicated that this progesterone treatment had no effect on embryonic migration. **Experiment 3.** The objective of experiment 3 was to examine the possible role of estradiol-17β in intrauterine migration of the ovine embryo. Twenty-four crossbred ewes, having estrous cycles of normal duration, were penned with vasectomized rams and monitored for estrous activity twice daily. Ewes were assigned randomly to receive three Silastic beads (polydimethylsiloxane, medical adhesive silicone type A, Dow Corning) via a mid-ventral laparotomy in the order in which they displayed estrus. Within a ewe, these beads contained either cholesterol or estradiol-17β (estradiol). Beads were introduced surgically via a 1 ml injection of physiological saline into the tip of one uterine horn on d 13 of the estrous cycle. To preclude any potential IUD/luteolytic action(s) of the beads that might result in a premature return to estrus, all ewes were bilaterally ovariectomized at the time of bead insertion, and progesterone (12 mg, i.m.) was administered once a day until slaughter on d 15. The reproductive tracts were recovered at this time and opened longitudinally. The distance beads migrated was determined by summing the distance between each bead and the site of introduction.
Beads were prepared as previously described by Pope et al. (1982b). Beads contained either 10 mg of cholesterol or estradiol per ml glue. This dosage was based on the amount of estradiol synthesized and released by filamentous ovine conceptuses after a 6 h incubation in vitro (Nephew et al., 1989b) and represents approximately 200 pg of cholesterol or estradiol per bead. Beads were 1 mm in size and spherical in shape.

**Experiment 4.** The purpose of this experiment was to examine how efficiently sheep space three or more embryos that initially enter only one uterine horn. Twenty cyclic crossbred ewes were unilaterally ovariectomized via mid-ventral laparotomy and allowed one estrous cycle to recover. In order to increase the ovulation rate of ewes in this experiment, a single injection (i.m.) of 400 IU pregnant mare serum gonadotropin (PMSG; Equinex, Ayerst Laboratories) was administered on d 13. Ewes were mated to fertile rams at the following estrus and every 12 h until the end of estrus. All ewes were slaughtered on d 15. The incidence of embryonic migration was assessed as described previously. Only ewes that had three or more CL and a corresponding number of embryos were accepted into the study.

**Statistical Analysis.** Data for experiments 1 and 2 were analyzed by least squares analysis of variance using the General Linear Model procedure of the Statistical Analysis System (SAS, 1986). Embryonic migration was analyzed as a
binomial observation; a code of 1 represented ewes with conceptuses recovered from the uterine horn opposite the side of entry. Ewes that did not experience embryonic migration were coded as 0. For experiment 3, the distance beads migrated was summed for each ewe. Distances were compared using a t-test. In experiment 4, the distribution of conceptuses between horns was compared using a chi-square test (Steel and Torrie, 1980).

Results and Discussion

All ewes in experiments 1 and 2 experienced embryonic migration by d 15 (Figures 8 and 9), which agrees with previous observations on the timing of intrauterine migration of ovine embryos (Nephew et al., 1989b). In experiment 2, the fimbria had become adhered to the ovary of two and eight ewes in the control and treatment groups, respectively. These ewes failed to become pregnant and were excluded from the study. The high frequency of intrauterine migration in ewes with multiple CL in one ovary reported in other studies (Casida et al., 1966; Scanlon, 1972; Reimers et al., 1973; Nephew et al., 1989b) was also observed in this study.

The timing and frequency of intrauterine migration was not affected by local versus systemic exposure to progesterone (experiment 1). Furthermore, embryos migrated from a uterine horn opposite CL into the horn adjacent to the site of ovulation (experiment 2). Taken together, these observations suggest that a local source of progesterone is not requisite
for ovine embryos to undergo intrauterine migration. Although inequalities in progesterone content existed between uterine tissue ipsilateral versus tissue contralateral to the ovary containing CL (sheep, Weems et al., 1988; cattle, Pope et al., 1982c), such differences did not have a direct effect on intrauterine migration per se, suggesting that the ovine embryo stimulates the migratory process locally. Furthermore, ewes with a single ovulation in one or each ovary rarely experience embryonic migration (Casida et al., 1966; Scanlon, 1972; Reimers et al., 1973); therefore, it appears that multiple embryos must be present within one uterine horn and opposite an unoccupied horn in order for migration to occur.

Beads impregnated with estradiol (experiment 3) migrated farther (P<.01) than cholesterol impregnated beads (27.6 ± 4.3 vs 12.5 ± 1.6 cm, respectively; Figures 10 and 11). Furthermore, only estradiol-releasing beads were recovered from the uterine horn contralateral to the site of introduction (Figure 11). Pope et al. (1982b) reported essentially similar results using cholesterol or estradiol releasing beads in swine. Synthesis and release of estradiol by ovine embryos occurred concomitantly with the time of intrauterine migration (Nephew et al., 1989a), suggesting an involvement of estradiol in migration, perhaps by mechanisms similar to those reported for swine (Pope et al., 1982a,b) and mares (Zavy et al., 1979). Estrogenic induction of bead/embryo migration may result from activation of the
uterine myometrium, which is thought to play a primary role in intrauterine migration (Boving, 1971). Perhaps uterine musculature must first be exposed to a threshold level of a conceptal factor(s), such as estrogen, before migration can proceed. The uterus of the nonpregnant ewe was relatively quiescent during the luteal phase (Hawk, 1975). However, the greater uterine motility observed in pregnant versus nonpregnant ewes with unilateral ovulations, which was not due to a local effect of the ovary containing CL (Cloud and Casida, 1969), may be due to local stimulation by the ovine embryo. Estrogen stimulates uterine contractions (Hawk, 1975), possibly by increasing uterine blood flow (Greiss and Anderson, 1970; Anderson et al., 1977; Ford et al., 1977; Pope et al., 1979) and(or) acting through or in concert with maternal/conceptus production of prostaglandins (Ellinwood et al., 1979; Marcus et al., 1981) to stimulate myometrial activity (Pope et al., 1982a). The ratio of PGE$_2$:PGF$_2\alpha$ in uterine flushings was higher for ewes that had completed embryonic migration before d 15 compared to those which had not yet experienced migration (Nephew et al., 1991). The potential influence of these factors on intrauterine migration requires further study.

The distribution of conceptuses between uterine horns on d 15 (experiment 4) is depicted in Figure 12. In ewes that had four embryos initially occupying one uterine horn, only one embryo was recovered from the opposite horn. Little
information is available regarding the efficiency of distribution of embryos between uterine horns in sheep. Ewes with two CL in one ovary balance the number of embryos/fetuses between uterine horns (Casida et al., 1966; Scanlon, 1972; Reimers et al., 1973; Nephew et al., 1989); however, this tendency toward equal distribution of embryos was not the case in the present experiment.

Implications

Embryonic migration occurred after local or systemic exposure to progesterone, and, regardless of the initial horn of entry, embryos migrated into the unoccupied uterine horn. Estrogen may be involved in this process. It appeared that sheep are not as efficient as other polytocous species at equidistant spacing of embryos.
Figure 7. Schematic representation of the reproductive tract following surgical reconstruction in experiment 2 to allow initial ipsilateral (a; control) or contralateral (b; treatment) entry of embryos.
Figure 8. Distribution of conceptuses within the uterus on d 15 of pregnancy after exposure to local (top) or systemic (bottom) progesterone. Each "x" represents the relative location of a conceptus within a uterine horn.
Figure 9. Distribution of conceptuses on d 15 of pregnancy after entry into a uterine horn ipsilateral (top) or contralateral (bottom) to the ovary remaining after surgical reconstruction. The circle represents the remaining ovary. The arrow represents the initial horn of entry.
Figure 10. Distribution of silastic beads (three per horn) impregnated with cholesterol. Beads were inserted on d 13 of the estrous cycle and recovered on d 15. The arrow represents the original point of insertion.
Figure 11. Distribution of silastic beads (three per horn) impregnated with estradiol. Beads were inserted on d 13 of the estrous cycle and recovered on d 15. The arrow represents the original point of insertion.
<table>
<thead>
<tr>
<th>Number of CL</th>
</tr>
</thead>
</table>
| 2            | x
|              | \[\text{\_\_}\] x
| 3            | x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x
| 3            | x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x
| 4            | x \ x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x
| 4            | x \ x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x
| 4            | x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x
| 4            | x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x
| 5            | x \ x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x
| 5            | x \ x
|              | \[\text{\_\_}\] \[\text{\_\_}\] x

Figure 12. Relative distribution of conceptuses on d 15 of pregnancy after initial entry of multiple embryos into one uterine horn. Each "x" represents the relative location of a conceptus in the uterus. For illustrative purposes, the initial horn of entry is next to the number of CL.
BIBLIOGRAPHY


Godkin, J. D. 1974. The demonstration that PGF₂α is the uterine luteolysin in the ewe. J. Reprod. Fertil. 38:261.


