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LATE EMBRYONIC AND FETAL MORTALITY IN THE EWE

Alison Brown Dixon

Dissertation submitted to the
Davis College of Agriculture, Forestry, and Consumer Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy
in
Reproductive Physiology

E. Keith Inskeep, Ph.D., Chair
Robert A. Dailey, Ph.D.
Paul E. Lewis, Ph.D.
Robert L. Goodman, Ph.D.
Matthew E. Wilson, Ph.D.

Division of Animal and Veterinary Sciences

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ABSTRACT

Late embryonic and fetal mortality in the ewe

Alison Brown Dixon

Timing and factors associated with late embryonic and fetal loss in the ewe were examined with ultrasonography. In study one, pregnancy diagnosis and counts of embryos were conducted on days 25, 45, 65, and/or 85 of gestation and compared to lambs born at term. Preliminary results indicated that more potential offspring were lost during fetal development than during late embryonic development. Approximately 18% of ewes experienced late embryonic and fetal loss and 20% of embryos or fetuses were lost from day 25 to term. Losses of potential offspring were continuous throughout gestation and approximately 3% of embryos present on day 25 were lost during each 20-day interval of pregnancy beyond that point. More ewes lost one, but not all, embryos or fetuses. The pattern of late embryonic and fetal losses during the late breeding season was similar to anestrous or transitional periods. Breeding season and service period did not affect losses during any stage of pregnancy. Late embryonic and fetal mortality was not related to temperature-humidity index. Breed differences affected the proportion of embryos or fetuses lost and concentrations of progesterone, estradiol, and VEGF. A threshold concentration of maternal progesterone was necessary for maintenance of pregnancy and survival of individuals within a litter might be related to a role of vascular endothelial growth factor in placentation. In study two, pregnancy diagnosis was conducted on days 18 and 19 of gestation to determine effects of allantois expansion on late embryonic and fetal mortality. Pregnancy retention from day 18 to term and the number of lambs born per ewe pregnant on day 18 did not differ between ewes with embryos having an expanded or non-expanded allantois. Number of lambs born per ewe lambing tended to be greater in ewes with one non-expanded embryo on day 18. Allantoic expansion on day 18 was not associated with location of corpora lutea. A single embryo was more likely to be lost if it failed to undergo allantoic expansion by day 18 of gestation.

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REVIEW OF LITERATURE

Introduction

In the sheep industry, embryonic and fetal mortality contribute to a large, but frequently unrecognized economic loss. Embryonic and fetal losses were estimated to be approximately 30% when comparing the number of offspring born to corpora lutea (Bolet, 1986). These losses are often overestimated due to the lack of separation between fertilization failure and early embryonic mortality (Bolet, 1986). However, fertilization failure tends to be very low, accounting for 5 to 10% of total potential offspring (Quinilivan et al., 1966; Restall et al., 1976; Long and Williams, 1980; Armstrong et al., 1983). The greatest amount of loss in the ewe occurred before day 18 of gestation (before embryonic attachment to the maternal epithelium) and accounted for the majority of the total loss (Moor and Rowson, 1960; Quinlivan et al., 1966). Most authors concluded that the majority of loss in the ewe occurs during the critical period of maternal recognition of pregnancy, attachment, and initial placental development (Cross, 2001). Late embryonic and fetal mortality between day 18 and lambing has been estimated at 9.4% (Hulet et al., 1956). Moor and Rowson (1960) observed a minimal amount of loss after day 18 and suggested that the majority of those losses occurred post-attachment, but during late embryonic development. The pattern of loss and the period of gestation in which most late embryonic or fetal mortality occurs in the ewe is unknown.

Many factors play a role in embryonic and fetal loss in the ewe. Bolet (1986) suggested these losses are due to one of three components: a) the male

by the “quality” of semen, b) the female by the “quality” of the ova and uterine environment, or c) the embryo itself. Environmental factors that might influence late embryonic or fetal survival include internal factors such as uterine physiology and hormone secretion from the uterus or embryo, or external factors such as ambient temperature, nutrition, and disease. Genetic factors that originate from either the male or female gametes might result in improper embryonic or placental development or function that lead to embryonic or fetal loss.

Most studies concerning embryonic or fetal loss in the ewe have been focused upon early embryonic mortality, particularly during maternal recognition of pregnancy and the period prior to attachment during the first 18 days of pregnancy. Few authors have studied late embryonic and fetal mortality in the ewe extensively. Estimates of late embryonic and fetal mortality between day 30 and term were low (Robinson 1951; Quinlivan et al., 1966) but varied with increasing ovulation rate (Quinlivan et al., 1966; Rhind et al., 1980a). A higher rate of embryonic loss on day 18 was observed in ewes with twin ovulations rather than single (Quinlivan et al., 1966). Loss from day 50 of gestation to term was estimated to be 78% in ewes ovulating six or more oocytes compared to 19% in ewes ovulating five or fewer oocytes (Rhind et al., 1980a). In these studies, however, the number of offspring observed at certain periods of late embryonic or fetal development was compared to the number of corpora lutea; fertilization failure was not separated from embryonic or fetal loss. Evidence of fetal loss has been observed in the ewe, primarily in the form of decaying membranes, or discoloration of caruncles. These signs were interpreted as

failure or loss of fetal attachment to the maternal interface and subsequent resorption; in other cases, loss was evident in the form of mummified skeletal remains (Rhind et al., 1980a). Numerous authors have documented spontaneous abortions due to infectious disease (reviewed by Roberts, 1971).

Partial rather than complete late embryonic or fetal loss might occur, depending in part on the number of potential offspring. Several authors have reported the loss of individual embryos without the total loss of pregnancy (Rhind et al., 1981; Schrick and Inskeep, 1993). Approximately 54% of ewes with twin ovulations had only one embryo by day 18 of gestation, while an average of 3.9% of ewes had evidence of complete loss of all embryos (Qunilivan et al., 1966). Physiological factors associated with late embryonic or fetal loss in the ewe have not been studied extensively.

Embryonic Development

Pre-attachment (Day 0 to 18) Embryogenesis in the sheep occurs in the first six weeks of pregnancy and fetal development extends from the end of embryogenesis to term (approximately 146 days). Embryogenesis begins with fertilization, cleavage, followed by the formation of a blastocyst, and the initiation of attachment during maternal recognition of pregnancy.

The pre-attachment stage begins at fertilization; the sheep embryo remains in the junction of the ampulla and isthmus of the oviduct for approximately two days before entering the uterus (Robertson, 1977). Once in the uterus, the embryo undergoes cellular differentiation and produces stage

specific proteins. The first mitotic division of the embryo is achieved, around 24 hours after fertilization in the sheep resulting in a two-cell embryo (Moore, 1959; Lawson, 1970). In sheep, a change from maternal to embryonic genomic control began at the 8- to 16-cell stage of development (Calarco and McLaren, 1976; Crosby et al., 1988). Changes in gene expression included a change from the translation of products of maternal origin to those of embryonic origin. This process was required for completion of preimplantation morphogenesis beyond the 8- to 16-cell stage (Kidder and McLaughlin, 1985; Telford, 1990).

An ovine morula develops by days three and four after fertilization and differentiates into a blastocyst with an inner cell mass surrounded by a single layer of trophectoderm by approximately day five after mating. In the mouse, formation of a blastocoel began with the osmotic transport of water and ions through a Na^+/K^+ pump (Benos and Biggers, 1981). Others have determined that tight junctions formed between the outer cells of the embryo to prevent further ion transport and reduce permeability to antibodies (Ducibella et al., 1975).

Compaction in the mouse blastocyst occurring at a fixed time after fertilization resulted in the loss of all observable cell boundaries (Ducibella and Anderson, 1975) and was thought to involve the molecule E-cadherin. Gap junctions formed between blastomeres, allowing cell to cell communication (Lo and Gilula, 1979a,b; Goodall and Johnson, 1982). These cell to cell junctions in mice were thought to involve a calcium-dependent cell adhesion system (Ogon et al., 1982; Bird and Kimber, 1984).

The pre-attachment sheep blastocyst is spherical until day eight, when it begins to transform from a 1 mm blastocyst into an 11.7 mm filiform trophoblastic vessel by day 11 (Rowson and Moor, 1966) and continues to grow on days 13 and 14 of gestation (Guillomot et al., 1981). The conceptus at this stage is located in the uterine horn ipsilateral to the CL. It invades the opposite horn on day 13 and might fill the horn more than one-half by day 17 (Green and Winters, 1946; Chang and Rowson, 1965; Rowson and Moor, 1966; Bindon, 1971).

In a study by Reimers et al. (1973), 93% of embryos remained in the ipsilateral uterine horn in ewes with one CL. In twin pregnancies, one embryo migrated to the contralateral uterine horn in 31 of 32 ewes that had two CL on the same ovary (Reimers et al., 1973). Intrauterine migration of embryos is thought to be due to uterine contraction, possibly by local effects of the early embryo. Using rubber pellets to measure uterine motility, Cloud and Casida (1969) found that the uterine horn containing an embryo had the greatest motility. In non-pregnant ewes that unilaterally ovulated, there was no difference in the distance that the pellets traveled between the ipsilateral and contralateral uterine horns, thus a difference in uterine motility between horns could not be traced to a local effect of the CL (Cloud and Casida, 1969). The authors suggested that the greater activity of the ipsilateral uterine horn in pregnant ewes might be due to a local stimulus on uterine motility by the embryo during early stages of pregnancy.

After elongation of the embryonic trophoblast, the mesodermal layer begins to develop from endoderm of the embryonic disc to form a thin sheet of tissue between the ectoderm and the underlying endoderm (Perry, 1981). The

mesoderm eventually splits, forming two layers, one overlying the endoderm, (somatopleure), the other underlying the ectoderm of the embryonic disc (splanchnopleure). The somatopleure and the outer trophoblastic layer eventually fuse to form the outermost extra-embryonic layer, the chorion. The inner splanchnopleure, and the ectoderm fuse to form the yolk sac, in which form blood islands that eventually differentiate into a rudimentary blood vessels by approximately day 18 after mating (Perry, 1981). It is at this stage that the allantois expands and fuses with the chorion, initiating the formation of the vascularized chorio-allantoic placenta (Perry, 1981).

Contact between the conceptus and the uterine epithelium is first evident on approximately day 15 of gestation (Boshier, 1969) when the conceptus might measure up to 15 to 19 cm in length (Chang and Rowson, 1965; Rowson and Moor, 1966). This process involves the attachment of the outer extra-embryonic layer of the conceptus to the maternal caruncular epithelium. By approximately day 18 after mating, progressive interdigitation of microvilli of the chorion and the maternal caruncular epithelium has occurred, forming an intimate association within the developing placentome (Weitlauf, 1988). During this process, the microvilli become shorter, more blunted, and irregular (Harding and Bocking, 2001).

The connection of the apical ends of the endometrial epithelial cells and the extra-embryonic membranes that disrupt the surface of the uterine lumen is referred to as the attachment reaction (Harding and Bocking, 2001). Actual adhesion of the chorion to uterine caruncular epithelium occurs around days 16

to 18 in sheep (Guillomot et al., 1981) and might involve various cell surface glycoproteins and extracellular matrix molecules and cell adhesion/substrate-adhesion molecules (Enders and Schlafke, 1972; Hakansson and Sundquist, 1975; Hewitt et al., 1979; Chavez et al., 1985). In sheep, the superficial-epitheliochorial type of attachment is non-invasive to the uterine tissue, making direct contact with the epithelium without destroying integrity of the surface epithelium, unlike invasive placentation. Thus, for the developing sheep conceptus, attachment is the appropriate term, rather than “implantation” or “nidation” (Cook and Hunter, 1978).

Post-maternal recognition of pregnancy (Day 18 to 45) This period includes the beginning of placentation and continues into fetal development around day 45 in the sheep. Proper establishment of maternal-embryonic connections is necessary to ensure normal development and function of the fetus. The chorion contains villi that are restricted to a number of well-defined circular or oval areas of the chorionic sac, which are separated by less specialized areas of smooth chorion (Stevens, 1975). The trophoblast of ruminants has binucleate cells that are interspersed throughout the mantle of the chorionic villi (Ramsey, 1982). Ovine placental development begins between days 22 to 25 of gestation with projections of fetal membranes into maternal caruncles (Davies and Wimsatt, 1966; Boshier, 1969). These projections are incipient villi, which will come to interdigitate with depressions or crypts on the surface of the caruncles (Wimsatt, 1950; Amoroso, 1952). Placentomes form when the endometrial tissue rises

above the surface of the uterine wall in the form of a cup into which the chorionic villi dip (Ramsey, 1982), only in those parts of the chorion that overlie the caruncles (Stevens, 1975).

In the developing intercotyledonary areas of the sheep placenta, the fetal and maternal epithelia are simply apposed, without interlocking microvilli (Perry, 1981). By the sixth week of gestation, the chorionic villi become firmly interlocked within the folds of the caruncular endometrium (Ramsey, 1982). The villi increase in length, and branch in various directions, growing into the maternal tissue (Stevens, 1975). At the base of the chorionic villi, maternal blood leaks into the spaces between the maternal tissues and the conceptus (Perry, 1981).

The long maternal caruncular cellular epithelium of the endometrium undergoes a transformation and loses the basement membrane, forming a syncytium, which lies in the crypts of the caruncle (Lawn et al., 1969). Making up 15 to 20% of the chorion, binucleate cells migrate into the uterine epithelium and fuse with the maternal cells to form multinucleated plaques (Rurack, 2001). Each syncytial plaque contains 20 to 25 nuclei and is connected to adjacent plaques by tight junctions (Rurack, 2001). The molecular and cellular aspects of this event are poorly understood.

Placentomes serve as key areas of placental exchange between the mother and fetus. Blood flow from the dam is supplied by the utero-ovarian arteries. The secondary branches of the utero-ovarian arteries coil and run along the ventral and dorsal surfaces of the uterus to form the arcuate arteries (Stevens, 1975). The arcuate arteries pass circumferentially to the anterior and

posterior uterine walls to meet the arcuate vessels from the other side (Rurack, 2001), sending small branches to the myometrium and larger radial branches to the endometrium and placentomes (Stevens, 1975). The radial arteries at the base of the placentome branch into arterioles (Stevens, 1975). The maternal vessels originate from the periphery of the placentome and extend to the central depression, while fetal branches from the umbilical artery supply each cotyledon with one to three arteries that enter the central depression of the cotyledon, divide, and send their terminal branches into the villi (Stevens, 1975).

Each villus is supplied by a single, centrally-located artery that penetrates to its tip, extending to the surface of the mesodermal core, and forming an extensive capillary plexus beneath the trophoblast (Stevens, 1975). By mid-gestation (approximately day 70), proliferation of blood vessels at the maternal-fetal attachment site is nearly complete (Rosenfeld et al., 1974).

In a single pregnancy, the uterine vascular trunks are of unequal length, the longer trunks leading to the cotyledonary attachments in the non-pregnant horn, while the shorter ones are restricted to the pregnant horn (Stevens, 1975). In a twin pregnancy, the umbilical vessels of one fetus might extend well into the opposite horn, encroaching upon the vascular territory of the second fetus and restricting its growth (Stevens, 1975). Twin pregnancies might develop a smaller number of placentomes per fetus due to the restriction of placental size (Stevens, 1975).

Fetal Development (Day 45 to 150)

Fetal growth For most mammals, the later part of gestation is characterized predominantly by rapid growth and development of the fetus (Ford, 1995). The typical growth pattern of the fetus follows a sigmoid-shaped curve. Development occurs at a slow rate, increases exponentially during mid to late gestation, and concludes with slow growth near term. In the sheep, the daily increment of crown rump length is five to six mm before day 120, four to five mm per day between day 120 and 130, and less than three mm thereafter (Hay, 1999). The prepartum decline in fetal growth rate reflects a switch from tissue accretion to tissue differentiation in preparation for life outside the uterus (Rurack, 2001).

Placental Development and Function The intimate contact between fetal membranes and the uterine endometrium is responsible for the exchange of all respiratory gases, nutrients, and wastes of the fetus (Ramsey, 1982; Faber and Thornburg, 1983; Reynolds and Redmer, 1995). The importance of the transplacental exchange for fetal growth is well known (Assheton, 1906; Needham, 1934; Ramsey, 1982; Faber and Thornburg, 1983; Morriss and Boyd, 1988; Reynolds and Redmer, 1995).

Vascular growth in the ovine endometrium exhibits a two fold increase in the uterine horn containing the embryo proper by day 24 of gestation, followed by an increase in vascular growth in the non-gravid uterine horn by day 30 (Reynolds and Redmer, 1992). Rapid proliferation of blood vessels primarily

occurs in the placentome (Ford, 1994). The vascular density of the maternal placental tissues increases slowly throughout gestation (Stegeman, 1974). However, the vascular density of the fetal cotyledons is consistent through mid-gestation and increases dramatically during the last third of gestation in association with the increased growth of the fetus (Barcroft and Barron, 1946; Stegeman 1974).

The exponential growth of the fetus during the last half of gestation depends upon the growth of the placental vascular beds (Meschia, 1983; Reynolds and Redmer, 1995), concomitant with increased rates of uterine and umbilical blood flow (Makowski et al., 1968). Therefore, angiogenic factors that affect the development of placental vasculature might have a dramatic effect upon fetal growth and development (Alexander, 1974; Meschia, 1983; Reynolds et al., 1985; Reynolds and Redmer, 1995; Harrington et al., 1997).

Follicular development

Follicular growth in the ewe is thought to occur in a wave-like pattern. In sheep, many authors using India ink, slaughter, concentrations of estradiol, or laparotomy suggested that there were two (Brand and de Jong, 1973; Dailey et al., 1982) or three (Smeaton and Robertson, 1971; Mattner et al., 1972) waves of follicular growth and atresia during an estrous cycle. More recent studies using ultrasonography have produced similar results (Ginther et al., 1995; Souza et al., 1996; Bister et al., 1999; Bartlewski et al., 1999). Wave-like patterns of follicular growth were plotted for follicles 3 mm in diameter that progressed to 5 mm.

Bartlewski et al. (1999) concluded that waves did occur, but that ovulatory follicles came from both the ultimate and penultimate waves. Evans et al. (2000) suggested that follicular dominance might occur in the sheep. Within each follicular wave, one follicle grew significantly larger than the next largest follicle. The largest follicles of the first and second waves contained a higher estrogen:progesterone ratio and had higher concentrations of estradiol than other follicles in the same wave. Evans et al. (2000) observed that the follicles in the ovulatory wave emerged about the same time as the largest follicle from the previous wave stopped growing. They suggested that there might be a relationship between the demise of the follicles of one wave and the emerging follicles of the subsequent wave.

The idea of follicular dominance in the ewe, however, is questionable. Schrick et al. (1993) observed small follicles continuously entering an antral pool of follicles, growing, and undergoing atresia, supporting the thought that follicular dominance does not occur in the ewe (Driancourt et al., 1991). Greater follicular growth seemed to occur in two periods, one during the first eight days of the estrous cycle and the other during luteal regression, both during periods of low progesterone. Follicles that ovulated were detected at 2 or 3 mm in diameter as early as day 9 or 10 and as late as day 15. Schrick et al. (1993) observed several follicles greater than 4 mm throughout the estrous cycle, thus ovulation might occur in response to treatment with gonadotropin (Inskeep et al. 1963) resulting in luteal regression during any time during the estrous cycle (Deaver et al., 1986).

Timing of the LH surge and LH pulse frequency during follicular development

Hormonal events involved in the timing of estrus and ovulation might affect oocyte competence and subsequent embryonic development. These events might play a role in the establishment of a uterine environment that is suitable for gamete transport (Allison and Robinson, 1972) and normal embryonic development (Miller and Moore, 1983).

Embryonic survival decreased in sows as the interval between the peak concentration of estradiol and the peak concentration of LH increased during estrus (Blair et al., 1993). In the sow, the number of CL represented by viable embryos on day 30 of gestation decreased as the interval between peak estradiol and peak LH increased (Soede et al., 1994). A longer interval between the peak of estradiol and peak of LH might affect follicular development and embryonic development via hormonal effects upon the transport of gametes within the oviduct, timing of fertilization, and/or synchrony between the uterus and the embryo (Soede et al., 1994).

Madill et al. (1994) observed that the number of transferable embryos decreased when superovulated heifers were given a GnRH antagonist to delay the LH surge and ovulation. Suppression of LH pulses one day prior to ovulation using a GnRH antagonist decreased plasma concentrations of estradiol and embryonic development (Oussaid et al., 1999). The treatment did not affect fertilization rates, but a smaller proportion of embryos developed beyond the 16-cell stage and the overall percentage of embryos developing to the blastocyst

stage was decreased compared to those receiving exogenous pulses of LH (Oussaid et al., 1999). In cattle, blastocyst development was correlated to follicular fluid concentrations of estradiol (Van de Leemput et al., 1998). Follicles with increased aromatase activity were more likely to contain oocytes capable of developing to the blastocyst stage (Driancourt et al., 1998).

Patterns of LH pulse frequency and follicular development depend upon concentrations of progesterone during the estrous cycle. Treatments that lowered concentrations of progesterone in the normal luteal phase in cattle resulted in the persistence of the largest follicle, associated with an increase in pulse frequency of LH and led to greater concentrations of peripheral estrogen (Roberson et al., 1989; Sirois and Fortune, 1990; Savio et al., 1993ab; Wehrman et al., 1993). Taft et al. (1996) showed that persistence of the largest follicle was due to the increase in LH pulse frequency.

Ovulation or atresia of a dominant follicle depended upon LH pulse frequency (Cupp et al., 1993; Savio et al, 1993b, Stock and Fortune, 1993; Anderson and Day, 1994; Taylor and Rajamahendran, 1994). When the concentrations of progesterone decreased below those observed during the luteal phase, the pulse frequency of LH increased (Roberson et al., 1989; Kojima et al., 1992; Stock and Fortune, 1993), maintaining the largest follicle, and concentrations of estrogen increased (Sirois and Fortune, 1990; Savio et al., 1993a,b; Wehrman et al., 1993; Ahmad et al., 1995). Once progesterone increased to a concentration similar to that observed during the luteal phase, LH pulse frequency decreased within six hours (Bergfeld et al., 1996), and continued

high concentrations of progesterone resulted in the regression of the largest follicle (Savio et al., 1993b; Stock and Fortune, 1993; Anderson and Day, 1994; Taylor and Rajamahendran, 1994).

Hormones Affecting Embryonic / Fetal Loss

Progesterone Concentrations of steroids might affect embryonic loss, particularly before and during maternal recognition of pregnancy. Several physiological and environmental factors contribute to embryo loss in sheep (Edey, 1969) and some might alter peripheral concentrations of progesterone, disrupting the relationship between the uterus and embryo (Wilmut et al., 1985a, b).

Embryonic survival tended to be lower in ewes with lower concentrations of progesterone during the luteal phase (Wilmut et al., 1986). On days three and six after mating, cows with normally-developing embryos had higher concentrations of peripheral progesterone than cows with degenerating embryos (Maurer and Echterncamp, 1982). Pregnancy rates were correlated to peripheral concentrations of progesterone before maternal recognition of pregnancy (Henricks et al., 1971; Lukaszewska and Hansel, 1980; Lee and Ax, 1984; Lamming et al., 1989; Albihn et al., 1991).

Regional differences in concentrations of progesterone were observed in uterine tissues of non-pregnant, cycling ewes (Weems et al., 1989) and cows (Weems et al., 1988). Concentrations of progesterone were higher in the cranial third than in the middle or caudal thirds of the uterine horn ipsilateral to the

corpus luteum or in the entire contralateral horn (Weems et al., 1989). Weems et al. (1989) suggested that the regional differences were evidence that progesterone might have been delivered to the uterus by local mechanisms and that these differences might contribute to the variable effects that concentrations of progesterone have upon early embryonic survival.

In contrast, Abecia et al. (1996) reported that concentrations of progesterone in the ovarian vein were 300-fold higher than those in the peripheral circulation in pregnant ewes during early embryonic development, while non-pregnant ewes had similar concentrations of progesterone in jugular and ovarian veins. Pregnant ewes were thought to have a higher endometrial content of progesterone than non-pregnant ewes with no evidence of an interaction between regions of the uterine horn or side of collection (Abecia, 1996).

Decreased concentrations of progesterone were associated with increased (Parr et al., 1987; Branca et al, 2000) or decreased (Gombe and Hansel, 1973; Apgar et al., 1975) levels of nutrition, which led to decreased embryonic survival during early pregnancy. Reduced concentrations of progesterone due to poor nutrition decreased embryonic survival during early pregnancy (Edey, 1976; Rhind et al., 1989). Overfeeding ewes significantly reduced embryonic survival (Cumming et al., 1975) or pregnancy rate (Parr et al., 1982). Peripheral progesterone concentrations after mating were related inversely to feed intakes (Williams and Cumming, 1982; Parr et al., 1982) and short-term increases in dietary intake before mating decreased embryonic

survival in superovulated ewes (Wallace, 1996). High dietary intakes that suppressed progesterone concentrations during the period of follicular recruitment, oocyte maturation, and ovulation resulted in embryos that were developmentally retarded, both upon recovery four days after insemination and after 72 hours in culture (McEvoy et al., 1995). Effects of dietary intake upon pregnancy rate were not observed in ewes eight days after mating (Abecia et al., 1997) and ewes did not experience embryonic loss when fed maintenance rations and given high exogenous doses of progesterone on day 11 after mating (Parr et al., 1982).

Lowered concentrations of progesterone might be due to increases in metabolism of the hormone as a result of increased feeding, frequency of feeding, or diet composition (Rabiee, 2000). Increases in metabolism resulted in a higher clearance rate of progesterone, because blood flow to the liver of ewes increased with feeding (Bensadoun and Reid, 1967). Thus, the main effect of nutrition upon progesterone in plasma might be due to an alteration in metabolic rate rather than in secretion of progesterone (Rabiee, 2000).

Progesterone supplementation during early embryonic development has given conflicting results in relation to embryonic survival. Several authors have observed an increase in embryonic survival with exogenous progesterone before day 10 of gestation compared to controls (Davis et al., 1986; McMillan et al., 1987), while others (Parr et al., 1982; Diskin and Niswender, 1989) found no effect.

Progesterone supplementation before and during maternal recognition of pregnancy can affect directly embryonic growth. Ewes receiving supplementary progesterone beginning before significant luteal secretion (day 1 after estrus) were able to accept and maintain older conceptuses after embryo transfer, possibly due to specific changes in the uterine environment (Lawson and Cahill, 1983; Vincent et al., 1986; Garrett et al., 1988). Increasing peripheral concentrations of progesterone resulted in a higher rate of growth of bovine embryos (Fox et al., 1988; Garrett et al., 1988) and uterine secretory activity (Nephew et al., 1991).

Transfer of embryos into an asynchronous uterine environment provided similar results (Albihn et al., 1991a). Longer embryos were observed in virgin heifers with high plasma progesterone on days six to eleven after mating (Albihn et al., 1991a). Smaller embryos with fewer trophoblastic cells were less successful than longer embryos of the same age in preventing luteolysis in the ewe and cow (Thatcher et al., 1984).

Increased concentrations of progesterone before day 20 of gestation might (Fonseca et al., 1983; Meisterling and Dailey, 1987; Britt et al., 1989) or might not (Wiltbank et al., 1956; Hawk et al., 1963) predict improved fertility. Parr et al. (1982) observed a positive, dose dependent effect of progesterone replacement on embryo survival rate following embryo transfer to ovariectomized recipient ewes. In a study by Ashworth et al. (1987), epostane, a 3β-HSD inhibitor, was administered to cattle on days nine, ten, and eleven of pregnancy. The inhibition of progesterone synthesis decreased embryonic survival to day

thirty of gestation. Progesterone implants reduced the effects of epostane, so it was suggested that the decreased embryonic survival was due to an alteration of concentrations of progesterone.

If adequate concentrations of progesterone are not maintained during early embryonic development, pregnancy will not continue beyond the length of the estrous cycle. In a study by Kastelic (1991), embryonic mortality in cattle was determined by observing the CL from day 10 after ovulation and detection of an embryonic heartbeat on day 22 to day 40 after mating using ultrasonography. In cows that experienced embryonic mortality before day 25, luteal regression occurred before embryonic mortality, whereas if embryonic mortality occurred between days 25 and 40 of gestation, conceptus degeneration preceded luteal regression (Kastelic, 1991). Luteolysis induced by treatment with $PGF_2\alpha$ on days 28 or 40 of pregnancy was characterized by rapid embryonic loss, minimal conceptus degeneration, and ovulation within two days after embryonic mortality (Kastelic, 1991). Embryonic death induced by colchicine injection or rupture of amnionic vessels was associated with maintenance of the corpus luteum and extensive degeneration of the conceptus (Kastelic, 1991). In recent studies in dairy cows, late embryonic mortality between days 30 and 60 of gestation was associated with low concentrations of progesterone in cows sampled once on days 28 to 37 of gestation (Starbuck, 2001).

Progesterone is secreted from both the corpus luteum and placenta in the ewe and is required throughout gestation for the maintenance of pregnancy (Casida and Warwick, 1945). Ovariectomy of ewes without progesterone

replacement before day 55 of pregnancy resulted in abortion or resorption of the fetus while ovariectomy after day 55 of pregnancy allowed gestation to continue in most ewes (Casida and Warwick, 1945; Denamur and Martinet, 1955; Ricketts and Flint, 1980). In ovariectomized ewes, concentrations of progesterone after day 60 of gestation were similar to those of ewes with ovaries that were pregnant on day 90 (Fylling, 1970; Sarda et al, 1973; Ricketts and Flint, 1980). Pregnancy was maintained after day 90 of gestation despite luteolytic dosages of $PGF_2\alpha$ (Weems et al., 1992).

Prostaglandin F_2 alpha ($PGF_2\alpha$) In the anestrous ewe, ovulation may be induced by ram introduction (Martin et al., 1986b), infusion of LH (McNeilly et al., 1982) or administration of GnRH (McLeod et al., 1982a; McNatty et al., 1982). Anestrous ewes that were not treated with progesterone before induced ovulation produced short-lived CL approximately 50% of the time (Hunter et al., 1989). Progesterone treatment (20 mg), at ram introduction or 24 hours before injection of GnRH, prevented the occurrence of short-lived CL (McLeod et al., 1982a; McLeod and Haresign, 1984; Martin et al., 1986).

The short lifespan of induced CL in postpartum cows not treated with progesterone was hypothesized to be due to the premature secretion of $PGF_2\alpha$ from the uterus (Troxel and Kesler, 1984; Zollers et al., 1989; Cooper et al., 1991). Evidence obtained by Hu et al. (1991) supported this idea in unilaterally hysterectomized ewes, in that the CL ipsilateral to the remaining uterine horn regressed prematurely but contralateral CL did not. Short-lived CL weighed less

than contralateral CL when collected on day 10 after ovulation and secreted less progesterone (Hu et al., 1991).

Hunter et al. (1989) observed an increase in PGFM in short-cycling ewes by day five of the estrous cycle whereas ewes treated with progesterone before ovulation experienced low concentrations of PGFM by day three to five of the estrous cycle. Concentrations of PGFM observed in short-cycling ewes were similar to those found during normal luteolysis (Flint and Sheldrick, 1983; Cooper et al., 1986). Lassoued et al. (1997) showed a decrease in pulses of PGFM and incidences of short-cycles when fluninxin meglumine, a cyclooxygenase inhibitor, was administered every 12 hours on days three to six after ram introduction and concluded that premature secretion of $PGF_2\alpha$ was the cause of short-lived CL in ewes.

Premature secretion of $PGF_2\alpha$ has been shown to reduce fertility in postpartum cattle despite supplementation with progesterone (reviewed by Inskeep, 1995). Pregnancy rate was decreased when $PGF_2\alpha$ was injected between days four through seven or five through eight after mating (Buford et al., 1996; Seals et al., 1998) despite supplemental progesterone. Similar effects were seen in ewes (Costine et al., 2001). Embryonic survival was not affected by administration of $PGF_2\alpha$ during maternal recognition of pregnancy in progesterone-supplemented cows (Seals et al., 1998). Embryonic loss was observed in cattle when spontaneous luteal regression occurred on days 31 to 35 of pregnancy (Schallenberger et al., 1989). However, Bridges et al. (2000) observed that naturally-occurring higher concentrations of $PGF_2\alpha$ (200-400

pg/ml) were beneficial to embryonic survival in cattle with induced replacement corpora lutea during this same time period. They proposed that $PGF_2\alpha$ might play a role in embryonic attachment.

In rodents, high concentrations of $PGF_2\alpha$ are necessary for implantation. Uterine phospholipase A2 activity and concentrations of PGF and PGE increased during implantation in the rat (Novarao et al., 1996). Prostaglandin $F_2\alpha$ is thought to mediate permeability of endometrial capillaries at implantation sites in the rat (Kennedy, 1977), and indomethacin given on day five decreased implantation site weight in the rat (Kennedy, 1977) and hamster (Evans and Kennedy, 1978). In the mouse, indomethacin blocked induced decidual cell reactions and the subsequent increase in $PGF_2\alpha$ (Rankin et al, 1979). In conclusion, high concentrations of $PGF_2\alpha$ might be detrimental during early embryonic development, but beneficial to late embryonic survival during the attachment period.

Prostaglandin E_2 (PGE_2) Prostaglandin E_2 is produced from PG endoperoxide H_2 via PGE synthase. Prostaglandin E_2 is produced by the uterus and has opposite effects of $PGF_2\alpha$. Luteal regression induced by injections of estradiol or intrauterine devices was delayed by uterine infusion of PGE_2 or PGE_1 in ewes (Pratt et al., 1977; Colcord et al., 1978; Hoyer et al., 1978; Huecksteadt and Weems, 1978). Prostaglandin $F_2\alpha$ was not as effective in regressing the CL when PGE_2 or PGE_1 was administered concomitantly (Henderson et al., 1977; Mapletoft et al., 1977; Reynolds et al., 1981).

Concentrations of PGE_2 from serum (Silvia et al., 1984) and uterine flushings (Ellinwood et al., 1979) were greater in pregnant than in non-pregnant ewes on day 13 post estrus. Higher concentrations of PGE_2 were observed in uterine venous serum, uterine flushings, and endometrial tissue from pregnant than non-pregnant ewes on days 15 and 17 (Ellinwood, et al., 1979). In contrast, Lewis et al. (1978) did not observe differences in concentrations of PGE_2 in uterine venous plasma or endometrial tissue between pregnant and non-pregnant ewes during limited sampling on days 15 and 16 post-estrus.

The conceptus has an antiluteolytic effect upon the CL and must be present in the uterus by day 12 or 13 of gestation to ensure luteal maintenance (Moor and Rowson, 1966a). The presence of the conceptus on day 12 is necessary to ensure uterine secretion of PGE_2 in the concentrations necessary for luteal maintenance. Prostaglandin E_2 might be involved in luteal mantainence through increasing concentrations of progesterone from the CL. Bovine luteal tissue secreted progesterone in vitro when subjected to PGE_2 (Marsh, 1970). In turn, progesterone is thought to regulate the ability of the uterus to release PGE_2 in response to the conceptus. In a study by Vincent et al. (1986) progesterone was administered to ewes on days 1 to 3 after estrus and day 10 blastocysts were transferred to the progesterone-treated ewes on day 6 post-estrus. Treatment with progesterone hastened development of the uterus to provide a suitable environment for the day 10 blastocyst. Secretion of PGE_2 from the uterus was advanced by 3 to 4 days and the day 10 blastocyst survived. Without early progesterone, the day 10 blastocysts did not survive and an increase in

PGE_2 was absent. The increase in PGE_2 observed in the pregnant ewe required the continued presence of progesterone with a blastocyst that is synchronous with the uterine environment (Vincent et al., 1986). For further understanding of the regulation of PGE_2, see discussion below on the effects of interferon-tau from the conceptus.

Estrogen Delayed ovulation in rats, either by use of artificial methods or light-induced six-day cycles, resulted in aged oocytes, decreased fertilization rates, increased embryonic and fetal anomalies, and an increase in embryonic resorption (Fugo and Butcher, 1966; Butcher and Fugo, 1967; Butcher et al., 1969). The increase in estrous cycle length was associated with an early increase in intrafollicular and plasma concentrations of estrogen in relation to the time of ovulation (Page et al., 1983). The effect of delayed ovulation on increased embryonic mortality and congenital abnormalities in the rat was shown to be due to prolonged exposure of the oocyte to increased concentrations of estrogen (Butcher and Pope, 1979; Butcher and Page, 1981; Page and Butcher, 1982). Reciprocal transfers of blastocysts between young rats with four-day cycles and young rats in which ovulation was delayed (six-day cycles) showed the delay produced changes in both zygotes and the intrauterine environment, resulting in a decreased implantation rate, and increased embryonic abnormalities and mortality (Butcher et al., 1969).

In sheep and cattle, several authors have concluded that estrogen remains lower during the early luteal phase (days four to seven) in pregnant

animals than in non-pregnant animals (Schallenberger et al., 1989). High E2:P4 ratios on days three to six were associated with abnormally-developed embryos in cattle (Maurer and Echternkamp, 1982). Thatcher et al. (1989) observed that elevated concentrations of estrogen in ovarian follicles during maternal recognition of pregnancy from days 14 to 17 in cattle (equivalent to days 12 to 14 in sheep) could be detrimental to maintenance of pregnancy. Decreases in conception rates in cattle were associated linearly with increased concentrations of estrogen during this period (Pritchard et al., 1994). Similarly, maintenance of pregnancy was associated with low concentrations of estrogen during days 31 to 35 in cattle (Bridges et al., 2000b).

Secretions of the Embryo and Fetus

Interferon –tau Maternal recognition of pregnancy requires communication between the conceptus and the dam during early pregnancy. Specific interactions between the conceptus and dam essentially prevent luteal regression in response to episodic secretion of $PGF_2\alpha$ from the uterine endometrium (reviewed by Bazer et al., 1997). Ovine interferon-tau (oIFN-τ) begins to be secreted on approximately day 10 of gestation from the mononuclear cells of the trophoblast and secretion increases as morphological changes occur on days 12 and 13 of pregnancy (Bazer et al., 1997).

Moor and Rowson (1966a,b) were the first to report anti-luteolytic effects of the conceptus. Luteal function was extended when day-12 to -13 conceptuses were transferred into the uteri of non-pregnant ewes (Moor and Rowson, 1966a)

or day-14 to -15 homogenized conceptuses were infused into the uteri of non-pregnant ewes on day 11 of the estrous cycle (Moor and Rowson, 1966b). Removal of the blastocyst from pregnant ewes before day 11 did not extend luteal function and the anti-luteolytic effect was absent when day-25 conceptuses were infused into non-pregnant animals (Moor and Rowson, 1966b).

In pregnant ewes, basal concentrations of $PGF_2\alpha$ are not eliminated, and concentrations of circulating PGFM are higher compared to cycling ewes (Silvia et al., 1984; Silva et al., 2000). However, luteal regression is prevented during maternal recognition of pregnancy. This is thought to be due to a change in the ratio of $PGF_2\alpha$:PGE_2, through a decrease in 9-keto-reductase and decrease in PGF synthase (Asselin and Fortier, 2000). Interferon-tau increased PGE_2 secretion in vitro and was correlated positively with induction of COX-2 and PGE synthase (Parent et al., 2002) in endometrial tissue. Enzymatic activity of PGDH, measured by the conversion of $PGF_2\alpha$ to PGFM, was greater on day 13 of pregnancy than on day 13 of the estrous cycle in ewes (Silva et al., 2000). Thus, the CL might be more capable of converting $PGF_2\alpha$ to an inactive form.

The high proportion of embryonic loss in the ewe before day 18 of gestation includes the period during which luteal regression is prevented by the conceptus, therefore, losses might be due to insufficient production of IFN-τ. However, attempts to use IFN-τ or other interferons, such as IFN-α, to improve pregnancy rates, produced inconclusive results (Thatcher et al., 2001). Ewes treated with IFN-α had an increase in both pregnancy rates (Roberts et al., 1990b, Schalue-Francis et al., 1991; Francis et al., 1991) and overall lambing

rates (Nephew et al., 1991). In cattle, delays in conceptus development and IFN-τ secretion might be due to insufficient progesterone concentrations prior to or during maternal recognition of pregnancy (Mann et al., 1999).

Pregnancy-Specific Protein B Pregnancy-specific protein B (PSPB) is a type of pregnancy associated glycoprotein and is a member of the aspartic proteinase family (reviewed by Davies, 1990). Pregnancy-specific protein B is secreted from binucleate cells of the ovine conceptus, beginning on approximately day 13 of gestation (Nagel et al., 1993) and might have a relationship with PGE_2 by regulating placental secretion of progesterone from the placenta (Weems et al., 1994). Thus, PSPB, rather than LH, might regulate steriodogenesis of the placenta (Weems et al., 1994).

Measurement of pregnancy-specific protein B has been used to detect embryonic mortality in ruminants (Humbolt et al., 1988; Szenci et al., 1998). However, detection of embryonic mortality in cattle using PSPB is limited by the extended half-life of PSPB (seven days) observed in the maternal circulation after embryonic mortality (Semambo, et al., 1992; Kiracofe et al., 1993) and in the early postpartum period (Sasser et al., 1989; Zoli et al., 1992; Mialon et al., 1993; Kiracofe et al., 1993). Concentrations of PSPB were similar to those found in cattle that remained pregnant (Humbolt et al., 1988), thus, concentrations of PSPB were not associated with embryonic mortality. In sheep, concentrations of PSPB decreased rapidly after termination of pregnancy (Ranilla

et al., 1990), but PSPB has not been studied as an indicator of embryonic mortality in sheep.

Angiogenic Factors

Vascular endothelial growth factor Angiogenesis, the growth of new blood vessels from existing vessels, involves the degradation of the underlying basement membrane and the interstitial matrix, endothelial cell migration and proliferation, alignment and differentiation into tubular structures, and establishment of a new basement membrane (Grant et al., 1994; Cameliet and Jain, 2000). Vascular endothelial growth factor (VEGF) is a specific stimulator of vascular endothelial cell proliferation, migration, and vascular permeability (Folkman and Klagsburn, 1987; Klagsburn and D'Amore, 1991; Reynolds et al., 1992; Ferrara and Davis-Smyth, 1997; Reynolds et al., 2000) and has been shown to stimulate angiogenesis in vitro and in vivo (Leung et al., 1989, Nicosia et al., 1994; Phillips et al., 1994).

Vascular endothelial growth factor has a variety of functions involving angiogenesis of many different tissues, specifically, during embryonic development and placentation (Reynolds et al., 1987; Reynolds and Redmer, 1988; Millaway et al., 1989; Zheng et al., 1995; Zheng et al., 1998). Inactivation of the VEGF gene in mice resulted in death of homozygous embryos during mid-gestation (Theiler, 1989). Mice heterozygous for the VEGF gene were retarded in growth and exhibited several developmental abnormalities (Theiler, 1989). Bogic et al. (2001) observed defects in vasculogenesis, large vessel formation,

capillary sprouting, and remodeling of the yolk sac vasculature in knock-out mice deficient in VEGF. Two types of tyrosine kinase receptors found within the endothelium bind VEGF with high affinity, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR) (Breier, 2000). The inactivation of each receptor has resulted in developmental abnormalities or embryonic mortality during mid-gestation (Shalaby et al., 1995; Fong et al., 1995). However, VEGFR-1 and VEGFR-2 have opposite activities. Vascular endothelial growth factor receptor-1 is required for maturing endothelial cells, while binding of VEGF to VEGFR-2 appears to reduce vasculogenesis (Fong et al., 1999).

In the ewe, VEGF and its receptors have been localized around the blood vessels of the placenta (Rimouche et al., 1999), and production of VEGF in placental tissues has been observed during various periods of gestation in both tissue and cell specific patterns (Zheng et al., 1995; Zheng, 1995; Redmer et al., 1998; Reynolds et al., 1998). Expression of VEGF was higher in the chorion than the amnion (Cheung et al., 1995). Vascular endothelial growth factor protein was localized in the villous cytotrophoblasts and connective tissue of the ovine placenta (Cheung and Brace, 1998), similar to expression observed in the human placenta (Jackson et al., 1994). An increase in microvascular support is necessary for the increasing demands of the growing fetus. Thus, concentrations of VEGF increased between day 60 and the end of gestation from low to high concentrations coinciding with morphological changes in the placenta during fetal development (Bogic et al., 2001).

Endothelial cells of the villous stroma differentiate into placental capillaries (Vuckovic et al., 1996). Apparently, ovine placental VEGF is produced and stored in the cytotrophoblast and exerts a paracrine effect on the endothelial cells to regulate angiogenesis (Bogic et al., 2001). Vascular endothelial growth factor expression persisted in the ovine placenta from day 62 to 142 of gestation, and Bogic et al. (2000) suggested that it regulates its own expression or induces proliferation of amniotic epithelial cells by acting through an autocrine mechanism. Vascular endothelial growth factor has been localized in smooth muscle cells around the blood vessels of the fetal villi and maternal blood vessels and might aid in maintaining the differentiated state of the underlying endothelium (Bogic et al., 2001).

Intramembranous blood vessels are a primary route of fluid exchange between the amniotic sac and fetal blood (Brace, 1995). Vascular endothelial growth factor might be involved in maintaining amniotic fluid volume by regulating growth and permeability of these vessels or the fetal membranes themselves (Bogic et al., 2001). Because growth of the near-term fetus is minimal, VEGF might function in the absorption of amniotic fluid, enhancing fluid exchange between the amniotic sac and fetal blood (Bogic et al., 2001).

Regulation of angiogenesis might be dependent upon concentrations of VEGF itself, rather than its receptor (Bogic et al., 2001). The amount of VEGF mRNA within fetal membranes increased as gestation advanced (Cheung and Brace, 1999). Apparently, development of the cardiovascular system depends upon precise concentration gradients of VEGF and a decrease in VEGF

concentration might lead to a decrease in angiogenesis with fatal consequences (Neufeld et al., 1999).

When concentrations of VEGF are increased, the concentration gradient of VEGF stimulates growth of new blood vessels from tissues producing VEGF (Neufeld et al., 1999). As concentrations of oxygen increase, production of VEGF decreases, but a threshold of VEGF is required to inhibit apoptosis of the endothelial cells and is essential for the stabilization of the newly-formed blood vessels (Neufeld et al., 1999). When tissues are exposed to high concentrations of VEGF, hyperproliferation of blood vessels and other abnormalities occur. Exposure of quail embryos to high concentrations of VEGF resulted in excessive fusion of vessels and formation of vessels with abnormally large lumens (Drake and Little, 1995). If the supply of VEGF was reduced or completely inhibited, angiogenesis was impaired, leading to abnormal or inhibited organ development (Neufeld et al., 1999).

Hypoxia stimulated secretion of VEGF from local tissues and resulted in rapid angiogenesis (Stone et al., 1995; Pierce et al., 1995). Hypoxia-induced transcription is mediated by the binding of hypoxia-inducible factor 1 (HIF-1) to an HIF-1 binding site located in the VEGF promoter (Levy et al., 1995; Liu et al., 1995). In addition to angiogenesis, hypoxia promotes stabilization of VEGF mRNA through protein binding to sequences located in the 3' and 5' untranslated regions of VEGF mRNA (Stein et al., 1998; Akiri et al., 1998).

Cytokines mediate production of VEGF to some degree. Epidermal growth factor, insulin-like growth factor-1, fibroblast growth factor-4, transforming

growth factor-β, tumor necrosis factor-α, platelet-derived growth factor, and interleukin-6, have been shown to increase VEGF expression in specific cell types (Pertovaara et al., 1994; Ryuto et al., 1996; Deroanne et al., 1997; Finkenzeller et al., 1997). However, cytokines such as interleukin-10 and interleukin-13 inhibited VEGF expression (Matsumoto et al., 1997).

Basic fibroblast growth factor (bFGF) Basic fibroblast growth factor has shown to be a potent angiogenic growth factor in vitro and in vivo (Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991; Zheng et al., 1999) in a variety of developmental processes (Gospodarowicz, 1991). Basic fibroblast growth factor promoted cell survival in several different cell types (Tilley et al., 1992; Yasuda et al., 1995; Reynolds et al., 2000) and stimulated differentiation of the embryonic mesoderm (Slack et al., 1987; Klein and Melton, 1994). During fetal development, bFGF is secreted from the maternal and fetal placental tissues throughout gestation (Zheng et al., 1995,1998; Rider et al., 1998; Maddock et al., 1999; Reynolds et al., 1999). In early gestation, expression of bFGF mRNA was greater in endometrial tissue than in fetal placental tissues, whereas in late gestation, bFGF mRNA expression was greatest in intercotyledonary fetal tissues (Reynolds and Redmer, 2001).

Estrogen Estrogen might be involved in the regulation of angiogenesis due to the presence of estrogen receptors on endothelial cells and regulation of VEGF and bFGF activity (Cullinan-Bove and Koos, 1993). Disruption of estrogen

receptors reduced bFGF-induced angiogenesis (Johns et al., 1996). Treatment with estrogen dramatically increased vascular permeability and edema of the uterus (Hechter et al., 1941; Ham et al., 1970) and VEGF mRNA (Clark and Markaverich, 1988). The endometrial expression of both VEGF and bFGF mRNA increased three to ten fold after estrogen treatment in ovariectomized ewes (Reynolds et al., 1998), along with uterine blood flow and endometrial microvascular volume (Magness, 1998; Reynolds et al., 1998).

Local effects of estrogen upon uterine blood flow might be mediated through stimulation of synthesis of endothelial nitric oxide synthase (eNOS) (Giscard et al., 1988; Van Buren et al., 1992; Mendelson and Karas,1994; Bell et al., 1995), which is believed to be important in maintaining vascular tone (Nathan, 1995). In the vasculature, nitric oxide (NO) is derived from the endothelium via the conversion of L-arginine to L-citrulline by NOS (Rosenfeld et al., 1996). Nitric oxide has been shown to maintain vascular tone (Nathan, 1995) and systemic and uteroplacental vasodilation during pregnancy (Conrad et al., 1993; Weiner et al., 1989,1994). Endothelial nitric oxide synthase increased in the uterine arteries in pregnant sheep (Magness et al., 1996). Inhibition of eNOS activity increased fetoplacental vascular resistance (Chang et al., 1992; Myatt et al., 1992; McCarthy et al., 1994; Lyall et al., 1996), therefore, decreased placental NO might lead to a reduction of fetoplacental blood flow (Boura et al., 1994; Sooranna et al., 1995).

Nitric oxide was stimulated from endothelial cells in uterine and placental arteries by VEGF and bFGF (Hood et al., 1998; Zheng et al., 1999; Bird et al.,

2000) and estrogen might mediate indirectly the production of NO (Magness et al., 1997; Sladek et al., 1997; Vagnoni et al., 1998). Estrogen might mediate bFGF production by affecting endometrial vascular function (Reynolds et al., 1998; Zheng et al., 1999). A model describing a cascade reaction among estrogen, VEGF, bFGF, and NO, stimulating endometrial angiogenesis and blood flow, has been proposed by Reynolds and Redmer (2001). Estrogen binds to its nuclear receptor within the vascular smooth muscle cell in endometrial arterioles and glandular epithelium to stimulate VEGF and bFGF production. Both VEGF and bFGF stimulate angiogenesis as well as production of NO by endothelial cells. Nitric oxide induces vasodilation by relaxation of vascular smooth muscle, stimulating production of VEGF and bFGF, establishing a positive feedback loop.

Angiopoietin 1 and 2 Angiopoietin 1 (Ang1) and angiopoietin 2 (Ang2) are angiogenic factors that regulate vascular growth and development (Suri et al., 1996; Lindahl et al., 1998; Patan, 1998). Both factors bind to a common receptor, Tie 2, but have opposing activities (Reynolds and Redmer, 2001). Angiopoietin 1 acts as a Tie 2 agonist and has been shown to be crucial to embryonic and vascular development (Suri et al., 1996; Breirer et al., 1997). Knockout-mice deficient in Ang1 die by mid-gestation due to insufficient cardiovascular development (Suri et al., 1996; Breirer et al., 1997). Angiopoietin 2, however, is a Tie 2 antagonist that might be involved in vascular regression, serving as an anti-angiogenic factor during vascular development (Maisonpierre et al., 1997; Holash et al., 1999).

Angiopoietin 1 is thought to promote microvascular organization and endothelial cell survival (Patan, 1998; Hayes et al., 1999; Kwak et al, 1999; Papapetropulous et al., 1999; Thurston et al., 1999) through the stabilization of blood vessels (Davis and Yancopoulos, 1999). Angiopoietin 1 might act with VEGF to produce endothelial cell sprouts, stimulating angiogenesis in the developing organism (Koblizek et al., 1998). Angiopoietin 2 has been shown to destabilize existing blood vessels, inhibiting actions of growth stimulatory factors upon vascular endothelium (Breier et al., 1997).

Uterine Blood Flow

By mid-gestation, the proliferation of new blood vessels at the maternal-fetal attachment site diminishes, completing placental development (Hutchenson et al., 1962; Rosenfeld et al., 1974). The oxygen and nutrient demands of the growing fetus are dependent upon the rate of uterine blood flow (Morriss et al., 1980; Vorherr et al., 1982; Ford et al., 1984) and increase as fetal growth continues throughout gestation. In early pregnancy, caruncular tissue received only 27% of the total uterine blood flow but by late pregnancy, it received 82% (Rosenfeld et al., 1974).

Throughout gestation, decreasing uterine arterial smooth muscle leads to progressive increases in arterial diameter and baseline uterine blood flow (Rosenfeld et al., 1974). Marked increases in uterine blood flow have been seen in the ewe as early as day 17 of gestation (Greiss and Anderson, 1970). The increases were associated temporally with the initiation of fetal attachment in

most domestic species (Melton et al., 1951; Perry et al., 1976). In the ewe, uterine blood flow continues to increase until day 130 of gestation (Rosenfeld et al., 1974) and diminishes at the delivery of the fetus and expulsion of the placental membranes (Assali et al., 1958).

Increased uterine blood flow during early gestation has been associated with high concentrations of estrogen. Markee (1932) was the first to demonstrate that estrogen might be a potent vasodilator in uterine tissue. Exogenous estrogen caused hyperemia of endometrial tissue transplanted into the eye of the guinea pig or monkey (Markee, 1932).

Many investigators confirmed this finding in a variety of species by measuring uterine blood flow after exogenous estrogen (Greiss et al., 1963; Killam et al., 1973; Anderson et al., 1977; Magness et al., 1993; Rosenfeld et al., 1996). In the ewe, injection of estrogen into the lumen of an isolated uterine horn resulted in a rapid unilateral increase in uterine blood flow (Greiss and Miller, 1981). Rosenfeld et al. (1974) observed significant systemic vasoactive-effects of estradiol-17β on several non-reproductive and reproductive tissues of non-pregnant, pregnant, and postpartum ewes. However, several authors suggested that uterine responses to estrogen are mediated locally (Killam et al., 1973; Clewell et al., 1980; Magness and Rosenfeld, 1989).

The adrenergic nervous system might regulate uterine blood flow. In addition to indirect involvement with the NO and VEGF production, estrogen can undergo hydroxylation at the 2 or 4 position to yield 2-and 4-hydroxyestradiol (Ball and Knuppen, 1980) and behave as a catechol (Fishman, 1963; Lloyd and

Weisz, 1978). Vasoconstriction, mediated by α-adrenergic nerves, predominates in blood vessels of the uterus and ovaries (reviewed by Ford, 1995). Catechol estrogens have the ability to bind to α-receptors on adrenergic nerves resulting in relaxation of vascular smooth muscle and vasodialation. Catechol estrogens diminish the activity of α-adrenergic receptors, increasing blood flow (Ford, 1995).

The increase in uterine blood flow observed during early pregnancy might be mediated by estrogen of conceptus origin (Greiss and Anderson, 1970). Estrone sulfate is the predominant form of estrogen present in the fetal and maternal fluids throughout gestation in the ewe (Carnegie and Robertson, 1978). Patterns of estradiol-17β are similar to those of estrone sulfate, but remain at lower concentrations (Carnegie and Robertson, 1978). The site of estrogen synthesis in the ewe is thought to be the feto-placental unit, although the uterus might modify the form of estrogen that acts upon maternal tissues (Ford, 1994).

In the ewe, estrone sulfate in the chorioallantoic fluid increased during pregnancy from day 31 to 46, declined from day 46 to 55, and increased around mid-gestation (Ford, 1995). The decline of concentrations of estradiol at mid-gestation were not associated was simultaneous decreases in uterine blood flow (Ford, 1995). However, myoendometrial blood flow in ewes showed a greater response to catecholamines (Rosenfeld et al., 1976a) and estradiol-17β (Rosenfeld et al., 1976b) during late gestation compared to the maternal placental vascular bed. Therefore, the two types of vasculature beds might

respond to various stimuli differently from and independently of each other to (Ford, 1995).

Genetic Abnormalities

Several authors estimated that the majority of embryonic loss in the ewe occurs during the first 18 days of gestation (Moor and Rowson, 1960; Quinlivan et al., 1966). Losses occurring during this period might reflect a high percentage of genetic abnormalities affecting cleavage, maternal recognition of pregnancy, attachment, or formation of the placenta. A small percentage of oocytes or embryos (1-3%) were found to have cracked zona pellucidae, possibly contributing to opportunities for embryonic loss (Long and Williams, 1980). Chromosomal abnormalities in the sheep have been reported as early as day 2 post-coitum in 14.6% of embryos observed (Long and Williams, 1980). In contrast, Long (1977) observed an absence of chromosomal abnormalities in embryos before attachment in the ewe. A high percentage of chromosomal abnormalities observed in the ewe consisted of Robertsonian translocations and reciprocal translocations (Bruere, 1979) acting at various stages of gestation (Bolet, 1986). Reciprocal translocations reduced the numbers of twins and triplets born (Glahn-Luft and Wabmuth,1980), and reduced prolificacy by as much as 10 to 45% when mating occurred between carriers of translocations (Bolet et al., 1986). Robertsonian translocations are typically not as common in sheep as in other domestic species, such as goats or pigs. However, a variety of

combinations of these types of translocations could result in a high incidence of embryonic loss (Bruere, 1979).

During very early embryonic development, modifications of the parental genomes result in the expression of maternal or paternal genes influencing specific developmental events (Duranthan and Renard, 2001). Expression of certain genes is mediated by specific actions of maternal or paternal genes though a process called genomic imprinting (reviewed by Shi et al., 2003). The maternal genome is thought to directly influence development of the embryo, whereas the paternal genome might support proper development and/or function of the extraembryonic membranes and placenta (Shi et al., 2003). Transplanting maternal or paternal pronuclei from early preimplantation embryos into unfertilized eggs did not result in embryonic survival, suggesting that both genomes are required for specific functions that result in embryonic development (Surami et al., 1986).

In recent studies in mice, Georgiades et al. (2000, 2001) developed diploid conceptuses in which both copies of chromosome 12 were inherited from either the mother or father only, or from both parents. Conceptuses that inherited copies of the chromosome from both the mother and father were the only ones that survived (Georgiades et al., 2001). Mice that inherited both copies of the chromosome from their mother exhibited embryonic and placental growth retardation based on size reduction of the placenta or died prematurely. Mice inheriting the chromosome only from their father had small crown-rump lengths, and exhibited a variety of defects, including placentomegally, and experienced

mortality in late gestation (Georgiades et al., 2000). The mice failed to retain the close association normally observed between the fetal capillaries and the innermost trophoblastic layer and only a shallow or delayed invasion of the maternal decidualized stromal cells (Georgiades et al., 2001). Volume fraction and thickness of the trophoblastic layer were increased, whereas the fetal capillaries were abnormal with a reduced volume fraction and density.

Seasonal Effects

Seasonal differences in fertility have been observed in several studies. Both fertilization failure and embryonic mortality contributed to low conception rates observed in ewes during the early breeding season (Dutt, 1954). A high percentage of oocytes failed to develop into lambs (Dutt, 1963). A higher percentage of ewes bred before September (10.2%) failed to produce live lambs compared to those bred after this period (6.5%) and fertility was thought to improve as the breeding season advanced to October (Hulet et al., 1956). Effects of season were observed in ewes bred at synchronized estrus, as fertilization rate was lower out of season (58%) than in season (80%; Lunstra and Christenson, 1981). However, embryonic mortality, expressed as the percentage of lambs not born to fertilized ova, averaged 27% for ewes synchronized out of season and 36% for ewes synchronized in season (Lunstra and Christenson, 1981).

The quality of semen produced by rams was markedly lower during summer than in other seasons of the year (McKenzie and Berliner, 1937). Ram fertility was lower before September than during subsequent fall months (Hulet et

al., 1956). In Indian breeds of sheep and goats, semen collected in autumn was of lower quality than that collected in spring (Shukla and Bhattacharya, 1952a,b). The lower fertility and higher incidence of embryonic mortality observed during summer months are thought to be due to the high ambient temperatures.

Heat Stress

Fertilization Exposing ewes to high temperatures for 12 days before breeding produced fertilization rates one-half of those of controls (Dutt et al., 1959). When ewes were exposed to high ambient temperatures on the day of breeding (estrus), fertilization rate was not significantly affected (Dutt, 1963).

Early embryonic development Continuous exposure to high ambient temperatures during early embryonic development increased embryonic loss (Alliston and Ulberg, 1961; Dutt, 1963; Smith et al., 1966; Thwaites et al., 1967a) to rates as high as 100% (Dutt et al., 1959). With exposure to high temperatures for diurnal periods (12 hour periods of daylight), embryonic mortality was only 38% (Dutt et al., 1959). The early stages of development, particularly cleavage, might be the most susceptible period to maternal heat stress. Embryonic mortality, taken into account each day, decreased as gestation advanced from day one to five in ewes exposed to continuous high ambient temperatures (90º F) for 24 days (Dutt, 1963). However, continuous exposure to high ambient temperatures resulted in a high rate of embryonic mortality (83%) by day 15 compared to a loss rate of 35% in ewes exposed to high ambient temperatures

during daylight hours only, and 19% in controls (Thwaites, 1969). Therefore, maternal exposure to high ambient temperatures might increase embryonic mortality at several stages throughout early development.

Late embryonic / fetal development Exposure to continuous high ambient temperatures during late gestation retarded fetal development, increased fetal mortality (Yeates, 1956, 1958; Shelton, 1964b; Goode, 1964), and decreased lamb birth weights (Brown et al., 1977). Elevation of maternal body temperatures by 0.3 to 1.0°C between days 64 and 141 of gestation was associated with fetal mortality, reduced neonatal viability (Bell, 1987), and depressed fetal growth (Brown et al., 1977). Maternal heat stress during late gestation might result in an increased amount of fetal resorption in utero or mummification of fetuses if dwarfed fetuses do not survive (Shelton and Hutson, 1968). Effects of continuous exposure to heat stress during late embryonic development were seen as early as day 50, whereas effects of elevated temperature exposure for 12 hours per day were observed around day 90 of gestation (Barbera,1995).

Decreased fetal growth and development during late gestation were thought to be a result of heat exposure during late embryonic development (Barbera, 1995; Galan et al, 1999). Galan et al. (1999) observed increasing amounts of fetal growth retardation in ewes exposed to heat stress for 55 days or 80 days compared to ewes that had no heat exposure. They suggested that effects of heat are non-reversible, but dependent upon duration of heat exposure during pregnancy.

Effects upon the male High ambient temperatures affected spermatogenesis and fertility of the male (Moore and Oslund, 1924; Phillips and McKenzie, 1934; McKenzie and Berliner, 1937; McKenzie and Colvard, 1938; Gunn et al., 1942; Hulet et al., 1956). Mortality from fertilization to lambing was greater (69.2%) in ewes bred to rams exposed to high ambient temperatures during the summer months than to rams kept within an air conditioned facility (41%; Dutt and Simpson, 1957). Semen quality was improved and fewer services were required to settle ewes by rams kept at lower environmental temperatures during the summer months (Dutt and Bush, 1955).

An increased scrotal temperature of 1º C reduced fertility in heat-stressed rams (Dutt and Simpson, 1957; Fowler and Dunn, 1966; Howarth, 1969; Rathorne, 1970; Braden and Mattner, 1970). Embryonic mortality was observed in ewes inseminated with semen from rams in which the scrotal temperature increased approximately 2º C for as few as four days (Mieusset et al., 1992). The proportion that remained pregnant at day 65 of gestation was significantly lower for ewes inseminated with semen from rams exposed to high ambient temperatures (Mieusset et al., 1992). The reduced fertility of females mated with heat-stressed males has been attributed to fertilization failure (Dutt and Simpson, 1957; Fowler and Dunn, 1966; Howarth, 1969; Rathorne, 1970; Braden and Mattner, 1970), normal fertilization but embryonic mortality (Howarth,1969; Burfening and Ulberg, 1968; Bellve et al., 1972), or both (Rathorne, 1970).

Heat stress reduced semen quality, with a decline in both percent motility and number of morphologically normal sperm (Howarth,1969). Motility and

concentrations of spermatozoa differed in rams exposed to high ambient temperatures during the summer months from those kept in air conditioned facilities (Dutt et al., 1954). Cellular damage in the seminiferous epithelium leading to seminal degeneration was observed when testes were exposed to elevated temperatures (Waites and Setchell, 1969). Phillips and McKenzie (1934) suggested that exposure to high ambient temperatures affects developing rather than mature spermatozoa.

Placental development Thermal heat stress has been shown to affect placental development, uterine blood flow, and subsequent fetal growth and development. The effects of heat stress upon fetal development are much more apparent than those produced by reduction in maternal feed intake (Reynolds et al., 1985). Effects of heat stress are thought to be upon the utero-placental unit, reducing both fetal and placental weights (Alexander and Williams, 1971; Bell et al., 1987). Birth weights of lambs from ewes subjected to heat stress during pregnancy were reduced by 7 to 66% (Yeates, 1958; Shelton 1964b; Alexander and Williams, 1971; Cartwright and Thwaites, 1976). Placental weights from ewes exposed to chronic heat stress were reduced to 58% of those of controls (Early et al., 1991). Alexander and Williams (1971) reported that heat exposure reduced placentome weight by 40 to 50% and fetal weight by 50%. Fetal organs that grow rapidly during development, such as the liver, kidney, heart, lung, and spleen, were affected by chronic heat stress during late gestation (Reynolds et al., 1985).

Heat stress might affect fetal development in multiple pregnancies differently than in single pregnancies. Exposure of ewes to heat during the last 2.5 months of gestation reduced weight of twin fetuses, placental size, and decreased total protein, RNA, and DNA compared to single lambs (Early et al., 1991). A within litter difference in lamb weights was seen in heat-stressed ewes; Dreiling et al. (1991) suggested that heat exposure unevenly affects the growth and development of twins. Heat stress decreased fetal weight in multiple pregnancies (Early et al., 1991) and reduced the proportion of live twin births (Bell et al., 1989).

Decreased fetal weight associated with chronic heat exposure is thought to be a consequence of the placenta's reduced ability to supply an adequate amount of oxygen and nutrients to the developing fetus (Bell et al., 1987; 1989). Carunclectomies, which reduced placental size to a similar degree as observed during heat stress, resulted in fetal growth retardation characterized by a high brain/liver weight ratio, fetal hypoxemia, and hypoglycemia (Robinson et al., 1979). Chronic changes in hormone concentrations during gestation in heat-stressed ewes were considered to be a consequence of placental stunting rather than a direct effect (Bell et al., 1989).

Placental function might be altered due to the reduction of uterine blood flow that is seen during chronic heat stress. Uterine blood flow is a major factor in dissipation of uterine metabolic heat (Abrams et al., 1971; Gwazdauskas et al., 1974a) and a primary determinant of nutrient uptake by the gravid uterus (Morriss et al., 1980; Vorherr,1982; Ford et al., 1984). Uterine temperatures

increased at a greater rate than arterial blood temperatures during periods of thermal stress (Gwazdauskas et al., 1974a). Uterine blood flow decreased 25 to 48% in response to hyperthermia in ewes (Oakes et al., 1976). Leduc (1972) observed a 46% reduction in placental blood flow in rabbits and an associated increase in the number of runts and dead fetuses during summer temperatures of 27º C. Reductions in uterine blood flow might be due to vasoconstrictive effects of catecholamines (Robson and Schild, 1938; Greiss, 1963,1972; Leduc, 1972; Abrams et al., 1971; Barton et al., 1974; Rosenfeld et al., 1976) that are released in response to thermal heat stress (Alvarez and Johnson, 1973).

Catecholamines might reduce uterine blood flow by affecting myometrial activity and/or uterine vascular resistance (Roman-Ponce et al., 1978). Regional changes in blood flow were thought to be due to regional sympathetic adrenergic activity (Simon and Riedel, 1975). However, Brown and Harrison (1981) could not abolish the decrease in uterine blood flow during acute heat exposure by ganglionic blockade with hexomethium. Thus, sources other than the nervous system might release catecholamines. There is increasing evidence that catecholamines of adrenal origin or non-adrenergic mechanisms might be responsible for the blood flow of many tissues (Hales et al., 1984; Hales, 1986).

The progressive rise in uterine blood flow during late pregnancy was not associated with concentrations of estrogen released by the conceptus (reviewed by Ford, 1995). Studies in the guinea-pig showed that uterine adrenergic nerves that bind catecholamines decreased in number (Thornbert, 1979) and sensitivity to catecholamines progressively decreased (Thornbert et al., 1978) as gestation

progressed. Thus, effects of catecholamines upon uterine blood flow might be limited to early- to mid-gestation.

Direct effects upon gametes and embryos Exposure to high temperatures within maternal tissues of heat-stressed ewes or during *in vitro* culture conditions has been shown to affect gametes and subsequent embryonic development or embryonic development directly. Elevated temperature during in vitro culture compromised oocyte function (Lenz et al., 1983; Baumgartner and Chrisman, 1987; Edwards and Hansen, 1996) and fertilization rate (Ulberg and Burfening, 1967; Lenz et al., 1983). Direct effects of temperature upon the oocyte (Lenz et al., 1983; Baumgartner and Chrisman, 1987; Edwards and Hansen, 1997; Rocha et al., 1998) and spermatozoa (Lenz et al., 1983; Chandolia et al., 1999) have been observed. Heat shock during fertilization reduced cleavage rate and developmental competence of cleaved embryos (Ulberg and Burfening, 1967; Lenz et al., 1983; Rivera and Hansen, 2001). Cytoskeletal elements have been shown to collapse and aggregate after heat shock (Welch and Suhan, 1985), possibly affecting syngamy or the first cleavage division (Rivera and Hansen, 2001).

Exposure to elevated temperatures in the uterus or oviduct may affect survival of spermatozoa (Hansen et al., 2001). Heat shock briefly applied to semen did not affect motility or viability of spermatozoa (Monterroso et al, 1995; Chandolia et al., 1999). However, rabbit embryos formed from fertilization of

oocytes with heat-shocked spermatozoa had greater embryonic loss compared to controls (Burfening and Ulberg, 1968; Howarth,1969).

Two-cell embryos might be more sensitive to direct effects of heat stress than gametes (Edwards and Hansen, 1997). Exposing two-cell embryos to temperatures similar to those observed during summer months reduced the number of embryos that developed to the four-cell (Edwards and Hansen, 1997) or blastocyst stages (Rivera and Hansen, 2001). Dutt (1963) observed a decrease in effects of heat stress upon embryos as pregnancy advanced in the ewe.

In cattle, two-celled embryos were more susceptible to heat stress in vitro than morulas (Ealy et al., 1995). The heat-shock protein 70 (HSP70) family and antioxidants such as glutathione protect early embryos by aiding in the refolding of damaged proteins and stabilizing ribosomal RNA (Duncan and Hershey, 1989; Nover and Scharf, 1991). Embryos acquired thermal resistance once they reached the morula stage (Ealy et al., 1995; Edwards and Hansen, 1997). The increased cell number found in older embryos has been associated with increased survival (Williams et al., 1982). In response to heat stress, embryos might acquire biochemical mechanisms, such as HSP68, that protect against damage from heat stress during early stages of development (Edwards and Hansen, 1996, 1997).

Cortisol as an indicator of stress

Stress from routine farm management practices, such as transportation or handling of animals, alters reproductive function. Hormones released in response to stress, such as cortisol, have been associated with inadequate reproductive function, especially blocking of ovulation (Martin et al., 1981) or reducing expression of estrus in cattle (Allrich et al., 1989). Administration of cortisol or synthetic glucocorticoids, such as dexamethasone, inhibited ovine cyclicity (Martin et al., 1981; Shutt et al., 1987; Horton et al., 1996). In the ewe, ACTH, rather than cortisol, is thought to suppress reproduction in response to stress, even though cortisol is used assess levels of stress (Martin et al., 1981; Shutt et al., 1987). Effects of stress due to sampling of blood and other basic research practices are thought to be minimal. In a study by Chernock et al. (1997), during routine blood sampling, concentrations of cortisol did not differ between sampling with venipuncture and cathertization of the jugular, and were below those observed in ewes after shearing during anestrus.

Effects of service period

The service period in which ewes were bred, particularly during seasonal anestrus, has been shown to affect embryonic mortality. Several authors observed that ewes returning to first service and conceiving to second service (thus conceived later in the summer) experienced a higher percentage of pregnancy loss. Knights et al. (2001a, b) observed a higher percentage of embryonic mortality after day 26 in ewes that conceived from second service

(28%) than from the first service (12%) after synchronization of estrus by treatment with progesterone and ram introduction to anestrous ewes. Lower lambing rates during the second service period were seen during the spring/summer breeding season (Lunstra and Christenson, 1981), as well as lower litter weights (Shelton and Hutson, 1968; Hendy and Bowman, 1974) in similar studies. Litter sizes are thought to increase gradually from early to mid-season followed by a gradual decline to the end of the breeding season (Hulet et al., 1956; Hendy and Bowman, 1974).

Effects of synchronization of estrus

Ewes experienced higher fertilization failure and embryonic mortality (combined) when estrus was synchronized with treatments that included PMSG in-season (49%) and out-of-season (58%) compared to untreated ewes mated in season (25%; Lunstra and Christenson, 1981). Embryonic mortality among treated ewes was associated with variation of stage of embryonic development within ewe, indicating that asynchronous timing of the onset of estrus, ovulation, and fertilization might have occurred in synchronized ewes (Lunstra and Christenson, 1981).

Effects of the Male

Sire effects upon prolificacy Contributions of the male to fertility and embryonic survival are not limited to fertilization. In sheep, pregnancy loss has been estimated between 20 and 30% (Edey, 1969) with variation among sires (Blockey et al., 1975). Fertilization rates did not differ among rams that had been selected

for high and low reproductive rates, but differences in embryonic survival rates were observed in ewes mated with the two types of rams (Burfening et al., 1977). There was a positive correlation of prolificacy with the percentage of ewes lambing when utilizing semen from different rams (Despierres et al., 1984). "Service sire" was a significant source of variation for the number of lambs born per ewe exposed (Burfening et al., 1977). Rams differed in the number of offspring born per ewe lambing with a range of nearly 0.8 lambs born per ewe lambing (Carr et al., 2001). However, Barker and Land (1970) found no evidence of differences in prolificacy among rams bred to ewes known to vary in prolificacy.

Genetic effects upon prolificacy Several genes have been identified that affect prolificacy in sheep (Haranahan et al., 1976; Davis et al., 1982;; Bradford et al., 1986; Radomska et al., 1988; Davis et al., 1991). The *Booroola* gene, located on chromosome six, is additive for ovulation rate and one copy increased ovulation rate by approximately 1.65 (Piper et al., 1985). The *Inverdale* gene, located on chromosome X, increased ovulation rate by approximately 1.0 and litter size by 0.6 (Davis et al., 1991). Intensive screening among flocks of New Zealand led to the establishment of highly prolific flocks, in such breeds as Coopworth, Romney, and Perendale (Davis et al., 2001). When measuring the ovulation rates of Coopworth ewes, it was observed that the *Woodlands* gene, located on chromosome X, was inherited from the ram by daughters that had high ovulation rates (Davis et al., 2001). The *Woodlands* gene was maternally

imprinted, expressed from the paternal inheritance from carrier males that were the progeny of non-expressing carrier dams (Davis et al., 2001).

Presence of a strange male Pregnancy block, or the "Bruce Effect", is a common phenomenon in mice, and is named for Hilda Bruce, who first reported it in 1959. A strange male in the presence of a pregnant female within 48 hours of coitus resulted in a failed pregnancy and the female returning to estrus within seven days (Bruce, 1959; Bruce and Parrott, 1960; Bruce and Parks, 1960).

It is known that the presence of a ram might affect physiological responses such as the release of LH (Chestworth and Tait, 1974), ovulation (Schnickel, 1954), induction of estrus in anestrous ewes (Underwood et al., 1944), and ovarian follicular growth (Atkinson et al., 1986). The Bruce Effect, however, has not been clearly identified in any other species other than mice. Al-Gubory et al. (1998) exposed pregnant ewes to vasectomized males or isolated them from males during pregnancy. The presence of rams did not affect gestation length, overall lamb mortality, or birth weights of singles, twins, or triplet lambs. However, the proportion of ewes with multiple births was greater in the control group than in the ram-exposed group.

Multiple Offspring

Increase in ovulation rate Based upon early studies, Edey (1969) suggested that nutritional effects were the cause of early embryonic mortality in highly prolific ewes. However, as laproscopic techniques improved, reproductive losses due fertilization failure from multi-ovulating ewes were discovered (Kelly et al.,

1978). As ovulation rate increased, the probability of ova success measured by litter size decreased (Robinson, 1951; Moore and Shelton, 1962; Rhind et al., 1980a, b; Meyer, 1985; Bradford et al., 1986). The proportion of ova shed that were represented by viable fetues at the time of slaughter on day 60 or 145 of gestation was related inversely to ovulation rate (Rhind et al., 1980a). Five ovulations or fewer were associated with normal rates of fertilization failure and embryonic mortality (Rhind et al., 1980a). When number of ovulations increased beyond five, litter size decreased significantly (Haranahan, 1976; Land and Wilmut, 1977; Rhind et al., 1980a,b). Rhind et al. (1980a) suggested that there is an intermediate optimum ovulation rate. "Uterine efficiency", described as the marginal response in litter size due to ovulation of an additional egg, decreased as ovulation rate increased (Meyer, 1985).

Use of gonadotropins to increase ovulation rate in ewes has produced similar findings (Robinson, 1951; Bindon et al., 1971). An increase in PMSG from 1500 IU to 2000 IU resulted in a decrease in mean litter size (Rhind et al., 1980b). In a study by Knights et al. (2003), the proportion of corpora lutea not represented by fetuses was associated linearly with increasing dosages of FSH (0, 42, 60 mg). Losses represented a combination of both fertilization failure and the failure of fertilized ova to survive to day 46 to 51 of gestation.

Site of ovulation and embryo migration Embryonic development does not always occur in the uterine horn on the same side as ovulation. There is a tendency towards balanced embryo number due to migration between horns

before attachment rather than a loss of ova (Rhind et al., 1980a). However, high incidences of fetal mortality in twin pregnancies were associated with an unequal distribution of twin fetuses (Rhind et al., 1980a). In the cow, single pregnancies were more likely to fail when embryos were transferred to the uterine horn opposite the CL (Newcomb and Rowson, 1976; Sreenan, 1976; Christie et al., 1979; Newcomb et al., 1980). Likewise, when bovine embryos were transferred into separate uterine horns, embryonic death was greater in the horn opposite the CL (Del Campo and Ginther, 1973; Ginther, 1974; Ford et al., 1976).

The earliest signs of attachment are observed around day 15 of gestation (Boshier, 1969), but some movement of embryos within the uterus might be possible up to day 19 or 20 (Rhind et al., 1980a). Initial attachment of embryos to the uterine endometrium and subsequent embryonic mortality might lead to an unequal distribution of remaining embryos (Rhind et al., 1980a) due to the unavailability of previously-attached caruncles. Mortality that occurred after the start of attachment on day 15 of gestation led to an uneven distribution of the surviving embryos between the two uterine horns, the birth of smaller lambs than expected, and a high variability in the birth weight of lambs within the same litter (Rhind et al., 1980a; McDonald et al., 1981). Variations in birth weight were thought to arise from poor distribution of embryos in the uterus and the inability of those that survived to draw nourishment from the attachment points on the uterine epithelium that were vacated by those embryos that died (Robinson, 1982). It is not known if competition for attachment sites between multiple embryos occurs or if sites are established strictly by chance.

Pregnancy Diagnosis

Rectal palpation Rectal palpation is a common method used in livestock to diagnose pregnancy. Pregnancy in cattle is often determined by the palpation of fluids from an enlarged uterine horn (Roberts, 1971), slipping of the chorioallantoic membranes (Zemjanis, 1970; Roberts, 1971) or palpation of an amniotic vesicle (Wisnicky and Casida 1948; Zemjanis, 1970; Roberts, 1971). The slipping of the chorioallantoic membranes between the thumb and forefinger (Zemjanis, 1970) was often used to assist in diagnosing pregnancy and was thought to be less damaging to the conceptus than palpation of the amniotic vesicle (Abbit et al., 1978).

Palpation of the amniotic vesicle has resulted in damage to the conceptus (Ball and Carroll, 1963; Rowson and Dott, 1963; Zemjanis, 1970) and might induce abortion or congenital defects (Bellows et al., 1975). When the membrane-slip method is used for pregnancy diagnosis in cattle before day 45, an increase in the incidence of fetal or placental trauma might occur (Thurmond, 1993). Fetal loss was greater when palpation was performed during placental development (day 28 to 42 in cattle) than if conducted after day 42 (Thurmond, 1993).

In sheep, digital rectal palpation is not possible due to the limited size of the ewe's rectum. A recto-abdominal technique for pregnancy diagnosis in the ewe was described by Hulet (1972). Ewes were kept off feed for 24 hours, placed on laparotomy cradles or tilting squeeze chutes, and a rod was placed into the rectum for examination (Hulet, 1972). The rod was then manipulated

until an obstruction was encountered and palpated against the abdominal wall, or a decision was reached that the ewe was not pregnant. This method was around 97% accurate for mid-gestational diagnosis, cheap to purchase, and required approximately 30 seconds per ewe (Memon and Ott, 1980). However, rectal trauma was observed and a small number of abortions and deaths were apparently due to the rectal damage and subsequent infections when using this technique (Hulet, 1972; Morcan, 1973; Plant and Tyrell, 1974; Turner and Hindson, 1975).

In a study by Tyrell and Plant (1979), the incidence of rectal damage did not vary among the operators or palpating rods in any experiment, and there were fewer perforated recta among pregnant than non-pregnant ewes. However, while the level of perforated recta fell with each successive experiment, the incidence of bruising and abrasions increased (Tyrell and Plant, 1979). Abortion due to abdominal recto-palpation has been reported in goats as well (Shelton, 1978).

Ultrasonography Ultrasonography is probably the most reliable technique for determining pregnancy and embryonic mortality, because the operator visualizes the embryonic vesicle and can follow its development and eventual retention or disappearance (Beghelli et al., 1986). B-mode ultrasonograophy for two-dimensional imaging was developed when A-mode, one-dimensional imaging, used to characterize loin eye and back fat, was not suitable for pregnancy diagnosis. With the Doppler technique, in which the transducer was placed upon

the abdomen, Lindahl (1971) was able to detect pregnancy from mid-gestation to term.

The development of an intra-rectal Doppler technique provided a more accurate and much earlier pregnancy diagnosis determination in ewes (Lindahl, 1971; Deas, 1977; Horvarth et al., 1978) and was approximately 90% or more accurate in mid-gestational pregnancy diagnosis (Lindahl, 1971). Modern ultrasound machines are B-mode, real time scanners. Real time imaging refers to the live or moving display in which the echos are recorded continuously and events such as fetal leg movements and heart beats can be observed as they occur (Ginther, 1986). When using a transrectal transducer with real time ultrasonography, the short distances from the rectal wall to viewing area allow this type of scanner to produce images with great detail (Ginther, 1986).

Transrectal ultrasonography using real time imaging has been used frequently for pregnancy diagnosis in ewes (Buckrell et al., 1986, 1988; Gearhart et al., 1988; Schrick and Inskeep, 1993). Gearhart et al. (1988) detected pregnancy in ewes as early as day 20 with low accuracy, but diagnosis improved as embryonic or fetal development progressed (Beardon, 1980). In a study by Schrick and Inskeep (1993), embryos could be readily observed and counted by day 25 of gestation, using a 7.5 mHz rectal probe and deaths of individual embryos were noted during five-day intervals. The effects of transrectal ultrasonography upon late embryonic and fetal mortality are unknown.

STATEMENT OF THE PROBLEM

The overall goal of sheep producers in the Eastern United States is to maximize economic profit by maximizing the number of ewes that become pregnant and the number of offspring marketed per ewe. Methods of synchronizing estrus and lambing have given producers the opportunity to increase annual yearly lamb crops. Embryonic and fetal mortality, however, contribute to a large, but frequently unrecognized economic loss. The majority of loss in the ewe is thought to occur during the first 18 days of pregnancy, which includes maternal recognition of pregnancy, attachment, and initial placental development. Most studies concerning embryonic or fetal loss in the ewe have been focused upon that period. Few authors have studied late embryonic and fetal mortality in the ewe extensively, and most estimates did not account for fertilization failure or early embryonic mortality. Therefore, estimates of late embryonic or fetal mortality are questionable.

Based upon literature reviewed, many factors might play a role in late embryonic and fetal loss in the ewe. Hormonal events involved in the timing of estrus and ovulation might affect oocyte competence and subsequent embryonic or fetal development. Concentrations of steroids from the dam and / or conceptus influence maintenance of pregnancy, uterine blood flow, and placental development. Angiogenic factors might dramatically affect fetal growth and development. Genetic abnormalities that are expressed during cleavage, maternal recognition of pregnancy, attachment, or formation of the placenta can result in late embryonic or fetal mortality. High ambient temperatures have been

shown to affect both very early embryos and fetal development. In previous work, ewes bred at the second service period had greater pregnancy loss between day 25 and term than those pregnant from first service. The influence of the male upon embryonic and fetal survival might not be limited to fertilization. Very high ovulation rates per ewe have led to reduced litter size and effects of "uterine capacity" on late embryonic and fetal survival might vary with the other variables discussed above.

Methods used in previous studies to determine late embryonic and fetal mortality required laparotomy, laparoscopy, or slaughter. Comparisons were made of the number embryos or fetuses at specific times during gestation or at term to the number of CL, as an indicator of ovulation rate. Ultrasonography has provided the opportunity for repeated, non-invasive observations during late embryonic and fetal development, and should allow more accurate estimates of mortality. To date, complete losses to day 25 and from day 25 to term have been determined, while timing of late embryonic and fetal losses and patterns of complete or partial loss remain unknown. The objectives of the present studies were to identify the pattern and timing of late embryonic and fetal mortality in the ewe and to determine possible causative factors these types of losses were associated with.

LATE EMBRYONIC AND FETAL MORTALITY IN THE EWE

INTRODUCTION

In the sheep industry, embryonic and fetal mortality contribute to a large, but frequently unrecognized economic loss. Bolet (1986) estimated embryonic and fetal loss at approximately 30%. Fertilization failure tends to be low, accounting for 5 to 10% to total potential offspring (Quinlivan et al.,1966; Restall et al., 1976; Long and Williams, 1980; Armstrong et al., 1983). The greatest amount of embryonic loss in the ewe occurred before day 18 of gestation and accounted for the majority of the total losses from breeding to term (Moor and Rowson, 1960; Quinlivan et al., 1966).

Few authors have studied late embryonic and fetal mortality extensively. Complete losses of pregnancy between day 18 and lambing were estimated at 9.4% (Hulet et al., 1956). Moor and Rowson (1960) suggested that the majority of late embryonic or fetal losses after day 18 occurred post-attachment, on approximately day 20 after mating. Late embryonic or fetal losses from day 30 to term were low (Robinson, 1951; Quinlivan et al., 1966), but varied with increasing ovulation rate. A higher rate of embryonic loss on day 18 was observed in ewes with twin ovulations than in single ovulators (Quinlivan et al., 1966). Loss from day 50 of gestation to term was estimated to be 78% in ewes ovulating six or more oocytes compared to 19% in ewes ovulating five or fewer oocytes (Rhind et al., 1980a).

Evidence of fetal loss has been primarily in the form of decaying membranes or discoloration of caruncles. These signs were interpreted as failure or loss of fetal attachment to the maternal interface and subsequent

resorption; in other cases, loss was evident in the form of mummified skeletal remains (Rhind et al., 1980a). Partial rather than complete late embryonic or fetal losses might depend in part on the number of potential offspring. Several authors have reported the loss of individual embryos without the total loss of pregnancy (Rhind et al., 1980a; Schrick and Inskeep, 1993). Approximately 54% of ewes with twin ovulations had only one embryo by day 18 of gestation, while an average of 3.9% of ewes had evidence of complete loss of all embryos (Quinilvan et al., 1966).

Dutt (1954) observed higher rates of fertilization failure and embryonic mortality to day 18 after mating during the early breeding season than during mid-season. Others noted that a high percentage of ewes did not lamb when bred before September and that fertility improved as the breeding season advanced (Hulet et al., 1956). Effects of season upon embryonic mortality were thought to be due to exposure to high ambient temperature during anestrus. Lunstra and Christenson (1981) observed a lower percentage of ewes lambing and Knights et al. (2001a, b) observed higher percentages of complete embryonic mortality after day 26 in ewes that conceived from second compared to first service during the spring/summer breeding season.

Physiological factors, such as concentrations of steroids from the dam and/or conceptus, might affect late embryonic and fetal survival. Casida and Warwick (1946) noted that progesterone was necessary for pregnancy maintenance in the ewe. Starbuck et al. (2002) showed that late embryonic

survival was less in lactating dairy cows with lower concentrations of progesterone during the fifth week of pregnancy.

Increased uterine blood flow was temporally associated with attachment in the ewe (Melton et al., 1951; Perry et al., 1976) and might be mediated by estrogen of conceptus origin (Greiss and Anderson, 1974). Estrogen might affect angiogenesis through regulation of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) activity (Culinan-Bove and Koos, 1993).

Vascular endothelial growth factor is a specific stimulator of vascular endothelial cell proliferation, migration, and vascular permeability (Folkman and Klagsburn, 1987; Klagsburn and D'Amore, 1991; Reynolds et al., 1992; Ferrara and Davis-Smith, 1997; Reynolds et al., 2000). Certain thresholds of VEGF during pregnancy might be required for proper establishment and stabilization of newly-formed blood vessels of the developing embryo and/or placenta. The effects of environmental and physiological factors on late embryonic and fetal mortality are unknown. The objectives of the current study were to characterize the pattern and the time periods during which most late embryonic and fetal losses occur, and to characterize factors associated with these types of losses in the ewe.

MATERIALS AND METHODS

Animals and Induction and Synchronization of Estrus

Group 1. A total of 916 non-lactating ewes of mixed breeding (mainly Dorset and Suffolk) from seven cooperating farms were bred either in early May and June (anestrus, season 1) or July, August or September (transition, season 2), 2000. Estrus was induced and synchronized for four flocks by controlled internal drug releasing devices (CIDR-G) containing 300 mg of progesterone (InterAg, Hamilton, NZ) in season 1. A CIDR was inserted intravaginally for five days and removed at the time of ram introduction (n =474). Ewes received 0, 42, or 62 mg of FSH (Folltropin, Vetrepharm, Inc., London, Ontario, Canada) 12 or 36 hours before CIDR withdrawal/ram introduction. In season 2, ewes in three flocks received an injection (i.m.) of 25 mg of progesterone at ram introduction followed by an injection (i.m.) of 20 mg of prostaglandin $F_{2}\alpha$ (Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) 14 days later (n = 292). Ewes in one flock received a CIDR-G device containing 300 mg of progesterone inserted for five days followed by an injection of 20 mg prostaglandin $F_{2}\alpha$ at insert removal / ram introduction or $PGF_{2}\alpha$ only (n = 150) at ram introduction. A total of 692 ewes (76%) was diagnosed pregnant on approximately day 25 after the first or second service period (table 1).

Group 2. In January 2001, 211 non-lactating ewes of mixed breeding (mainly Dorset) on one farm were synchronized for estrus with CIDR-G devices containing 300 mg of progesterone (InterAg, Hamilton, NZ) inserted for five days

followed by an injection of 20 mg prostaglandin $F_2\alpha$ at insert removal / ram introduction or prostaglandin $F_2\alpha$ only. A total of 177 ewes (84%) was diagnosed pregnant on approximately day 25 after the first or second service period (table 1).

Group 3. In May, June, and July of 2001, 459 ewes of mixed breeding (mainly Dorset and Suffolk) in four flocks received an injection (i.m.) of 25 mg of progesterone at ram introduction followed by an injection (i.m.) of 20 mg of prostaglandin $F_2\alpha$ (Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) 14 days later. A total of 264 ewes (58%) was diagnosed pregnant on approximately day 25 after the first or second service period (table 1).

Table 1. **NUMBER OF EWES SYNCHRONIZED FOR ESTRUS AND PREGNANT ON APPROXIMATELY DAY 25 AFTER FIRST OR SECOND SERVICE IN GROUPS 1, 2, AND 3**

		SEASON	FARM	METHOD	N	PREG D25 (%)
2000		ANESTRUS	JH	CIDR + FSH	120	108 (90.0)
			KM	CIDR + FSH	112	98 (87.5)
			OH	CIDR + FSH	120	56 (46.7)
	GROUP 1		DM	CIDR + FSH	122	90 (73.8)
		TRANSITIONAL	AP	P_4 + $PGF_2\alpha$	36	33 (91.7)
			DE	P_4 + $PGF_2\alpha$	155	118 (76.1)
			OH2	CIDR + $PGF_2\alpha$	150	113 (75.3)
			RB	P_4 + $PGF_2\alpha$	101	76 (75.2)
2001	***GROUP 2***	LATE BREEDING	KM	CIDR + $PGF_2\alpha$	211	177 (83.9)
		ANESTRUS	GF	P_4 + $PGF_2\alpha$	60	36 (60.0)
			W	P_4 + $PGF_2\alpha$	80	57 (71.3)
	GROUP 3		JH	P_4 + $PGF_2\alpha$	199	90 (45.2)
			OH	P_4 + $PGF_2\alpha$	120	81 (67.5)
		TOTAL			1586	1134 (71.5)

Determination of Pregnancy Loss

Initial pregnancy diagnosis and counts of embryos were done with ultrasonography using an Aloka 500 (Corometrics Medical Systems, Wallingford, CT) with a 7.5 mHz linear transrectal probe from days 25 to 30 (Schrick and Inskeep, 1993). An Oviscan 4 (BCF Technology, Ltd. Livington, Scotland) with a 3.5 mHz transabdominal sector probe was used to recheck pregnancy and recount fetuses on days 45 to 50, 65 to 70, and (or) 85 to 90 of gestation. Late embryonic or fetal mortality was determined from these counts and numbers of lambs born.

Determination of Temperature-Humidity Indices

Ambient temperature and percent relative humidity were collected from a National Weather Service Station nearest each farm during the months of May, June, July, August, and September of 2000. Daily values were pooled for each farm (N = 7) during each of six intervals before and during gestation (day –7 to 0, 0 to 7, 0 to 25, 25 to 45, 45 to 65, 65 to 85). A temperature-humidity index (THI) for each interval was determined for each farm in group 1. The index was determined from ambient temperature and percent relative humidity using a livestock THI chart (Smith et al., 1998) and classified into one of three categories (THI < 72 = 0, THI 72-74 = 1, THI > 74 = 2). Index scores of 1 and 2 represented increasing exposure to heat stress.

Blood Collection and Hormone Assays

A blood sample (5 mL) was collected by jugular venipuncture at each day of pregnancy diagnosis for groups 1 and 3. Following collection, samples were placed on ice and transported to the laboratory. Samples were refrigerated at 4ºC for 12 to 24 hours, then centrifuged for 20 minutes at 3,000 rpm. Serum was harvested and frozen at -20ºC, until concentrations of progesterone were determined by radioimmunoassay (Sheffel et al.,1982) or ELISA (Petroff et al., 1997), and estradiol-17β (Rozell and Keisler, 1990) and VEGF (Vonnahme et al., 2003) were measured by radioimmunoassay. Intra and inter-assay CV were: progesterone 9.7% and 15.3%, respectively for ELISA and 8.6% and 13.4%, respectively for RIA and estradiol 7.0% and 14.1%, respectively. Inter-assay CV for VEGF was 13%.

Percentages of Ewes Experiencing Late Embryonic or Fetal Loss

Stages of pregnancy, based upon all combinations of days of pregnancy diagnosis and term (25 to 45, 25 to 65, 25 to 85, 25 to term, 45 to 65, 45 to 85, 45 to term, 65 to 85, 65 to term, 85 to term) were examined for patterns of embryonic and fetal loss in groups 1, 2 and 3. Ewes were classified at the beginning of each stage as being pregnant with a single embryo or fetus or with multiple embryos or fetuses on a per farm basis. Ewes with a single pregnancy at examination that lost the pregnancy were classified as single complete losses. Ewes with multiple embryos or fetuses lost all embryos or fetuses (multiple

complete), lost one, but not all, embryos or fetuses (multiple partial), or lost no potential offspring.

The percentages of ewes in groups 1, 2, and 3 experiencing late embryonic or fetal loss by type of loss were determined for breeding season (anestrus, transitional, late breeding) and service period (first or second) for all possible intervals based on the combinations of days of pregnancy diagnosis and term (tables 2-6). The numbers of ewes losing late embryos or fetuses during each stage and total number of pregnant ewes at the beginning of each stage was pooled over farms (tables 2-6).

Because not all days were sampled on all farms or groups, the cumulative numbers of ewes in groups 1, 2, and 3 that lost late embryos or fetuses during each stage and total number of ewes per stage were used to construct weighted means representing loss of pregnancy up to each day of pregnancy diagnosis (45, 65, 85) and term. Weighted means were calculated by the following method: the weighted mean for the previous day x loss from each stage that ended at the particular day x number of animals observed for each stage. Values from each stage included in the calculation were then averaged and divided by the average number of animals. The final result was the weighted mean of cumulative percentages of animals that experienced each type of pregnancy loss for each day of pregnancy diagnosis or term.

Example:

D25 = 0% Loss

D45 = Loss from d25 to 45

D65
1. D25 + (loss from d25 to 65) x # animals for the interval = value 1
2. D45 + (loss from d45 to 65) x # animals for the interval = value 2
3. Sum of value 1 and 2 / Ave # of animals

D85
1. D25 + (loss from d25 to 85) x # animals for the interval = value 1
2. D45 + (loss from d45 to 85) x # animals for the interval = value 2
3. D65 minus loss from d65 to 85 x # animals for the interval = value 3
4. Sum of values 1, 2, and 3 / Ave # of animals

Term
1. D25 + (loss from d25 to term) x # animals = value 1
2. D45 + (loss from d45 to term) x # animals = value 2
3. D65 + (loss from d65 to term) x # animals = value 3
4. D85 + (loss from d85 to term) x # animals = value 4
5. Sum of value 1, 2, 3, and 4 / Ave # of animals

Table 2. **PROPORTIONS OF PREGNANT EWES AT THE BEGINNING OF EACH OF SEVERAL INTERVALS OF PREGNANCY THAT EXPERIENCED LOSS OF A SINGLE PREGNANCY DURING THAT INTERVAL FOR 8 FLOCKS STUDIED IN GROUP 1[a]**

		FARM	25-45[b]	45-65[b]	25-65[b]	65-TERM[b,c]	45-TERM[b,c]	25-TERM[c]
FIRST SERVICE	ANESTRUS	JH	4/26	1/29	5/25	3/25	3/29	7/25
		KM	0/11	0/11	0/11	0/7	0/7	0/7
		OH	1/8	1/11	2/8	0/13	1/11	2/8
		DM	N/A	0/9	N/A	0/12	0/9	N/A
	TRANSITIONAL	AP	0/6	0/7	0/6	0/8	0/7	0/5
		DE	2/19	1/18	3/10	1/8	2/9	4/10
		OH2	0/4	0/8	0/4	0/11	0/8	0/4
		RB	N/A	N/A	3/24	0/13	N/A	N/A
SECOND SERVICE	ANESTRUS	JH	0/18				1/17	1/17
		KM	0/11				0/3	0/3
		DM	1/31				4/35	5/28
	TRANSITIONAL	AP	0/1				N/A	N/A
		DE	0/9				0/8	0/8
		OH2	2/29				8/30	10/29
		TOTAL	10/173	3/93	13/88	4/97	19/173	29/144
		LOSS (%)	5.8	3.2	14.8	4.1	11.0	20.1

[a] Not all ewes were observed at all stages on all farms. Observations were made more often on ewes that conceived to first service.

[b] Number of single pregnancies observed on a farm can increase as pregnancy progresses by virtue of ewes that initially had multiple pregnancies incurring partial loss and having a viable single fetus at a later stage.

[c] Lambing records were not available on some ewes that were observed at earlier stages of pregnancy.

Table 3. **PROPORTIONS OF PREGNANT EWES AT THE BEGINNING OF EACH OF SEVERAL INTERVALS OF PREGNANCY THAT EXPERIENCED COMPLETE LOSS OF A MULTIPLE PREGNANCY DURING THAT INTERVAL FOR 8 FLOCKS STUDIED IN GROUP 1[a]**

		FARM	25-45	45-65	25-65	65-TERM[b]	45-TERM[b]	25TERM[b]
FIRST SERVICE	ANESTRUS	JH	0/53	0/45	0/53	0/36	1/43	1/51
		KM	1/45	0/44	1/45	0/27	0/27	1/28
		OH	0/49	1/45	1/49	2/41	3/45	3/49
		DM	N/A	0/19	N/A	0/16	0/30	N/A
	TRANSITIONAL	AP	0/21	0/20	0/21	0/17	0/19	0/20
		DE	0/75	1/74	1/75	0/64	1/65	1/66
		OH2	0/31	0/27	0/31	0/24	1/27	1/31
		RB	N/A	N/A	4/52	0/29	N/A	N/A
SECOND SERVICE	ANESTRUS	JH	0/10				0/10	0/10
		KM	0/31				0/10	0/10
		DM	0/30				0/25	0/30
	TRANSITIONAL	AP	0/5				0/5	0/5
		DE	0/15				0/13	0/13
		OH2	0/48				1/45	2/49
		TOTAL	1/413	2/274	7/326	2/254	6/364	9/362
		LOSS (%)	0.2	0.7	2.1	0.8	1.6	2.5

[a] Not all ewes were observed at all stages on all farms. Observations were made more often on ewes that conceived to first service.

[b] Lambing records were not available on some ewes that were observed at earlier stages of pregnancy.

Table 4. **PROPORTIONS OF PREGNANT EWES AT THE BEGINNING OF EACH OF SEVERAL INTERVALS OF PREGNANCY THAT EXPERIENCED PARTIAL LOSS OF A MULTIPLE PREGNANCY DURING THAT INTERVAL FOR 8 FLOCKS STUDIED IN GROUP 1[a]**

		FARM	25-45	45-65	25-65	65-TERM[b]	45-TERM[b]	25-TERM[b]
FIRST SERVICE	ANESTRUS	JH	9/53	7/45	16/53	7/36	13/43	22/51
		KM	0/45	3/44	4/45	8/27	8/27	8/28
		OH	4/49	4/45	8/49	10/41	13/45	17/49
		DM	N/A	11/16	N/A	13/16	5/30	N/A
	TRANSITIONAL	AP	1/21	2/20	3/21	4/17	6/19	7/20
		DE	1/75	0/74	1/75	16/64	16/65	17/66
		OH2	4/31	3/27	8/31	2/24	5/27	10/31
		RB	N/A	N/A	5/52	10/29	N/A	N/A
SECOND SERVICE	ANESTRUS	JH	0/10				2/10	2/10
		KM	2/31				5/10	5/10
		DM	5/30				14/25	19/30
	TRANSITIONAL	AP	0/5				2/5	2/5
		DE	0/15				4/13	4/13
		OH2	3/48				3/45	6/49
		TOTAL	29/413	20/271	45/326	70/254	96/364	119/362
		LOSS (%)	7.0	7.4	13.8	27.6	26.4	32.9

[a] Not all ewes were observed at all stages on all farms. Observations were made more often on ewes that conceived to first service.

[b] Lambing records were not available on some ewes that were observed at earlier stages of pregnancy.

Table 5. **PROPORTIONS OF PREGNANT EWES AT THE BEGINNING OF EACH OF SEVERAL INTERVALS OF PREGNANCY THAT EXPERIENCED THE LOSS OF PREGNANCY DURINGTHAT INTERVAL FOR 1 FLOCK STUDIED IN GROUP 2[a]**

	STAGE	25-45	45-65[b]	25-65[b,c]	65-TERM[b,c]	45-TERM[b,c]	25-TERM[b,c]
SINGLE	FIRST SERVICE	1/30	2/30	0/23	1/29	0/25	0/23
	SECOND SERVICE	1/21				0/12	0/12
	TOTAL	2/51	2/30	0/23	1/29	0/37	0/35
	LOSS (%)	3.9	6.7	0.0	3.4	0.0	0.0

	STAGE	25-45	45-65[b]	25-65[b,c]	65-TERM[b,c]	45-TERM[b,c]	25-TERM[b,c]
MULTIPLE	FIRST SERVICE	2/64	0/62	2/64	0/43	0/50	0/52
COMPLETE	SECOND SERVICE	0/11				0/5	0/5
	TOTAL	2/75	0/62	2/64	0/43	0/55	0/57
	LOSS (%)	2.7	0.0	3.1	0.0	0.0	0.0

	STAGE	25-45	45-65[b]	25-65[b,c]	65-TERM[b,c]	45-TERM[b,c]	25-TERM[b,c]
MULTIPLE	FIRST SERVICE	2/64	6/62	9/64	28/43	35/50	37/52
PARTIAL	SECOND SERVICE	4/11				0/5	4/5
	TOTAL	6/75	6/62	9/94	28/43	35/55	41/57
	LOSS (%)	8.0	9.6	9.6	65.1	63.6	72.0

* Indicates number of farms with ewes pregnant at the beginning of each period.

[a] Not all ewes were observed at all stages. Observations were made more often on ewes that conceived to the first service.

[b] **Number of single pregnancies observed on a farm can increase as pregnancy progresses by virtue of ewe that initially had multiple pregnancies incurring partial loss and having a viable single fetus at**

Table 6. **PROPORTIONS OF PREGNANT EWES AT THE BEGINNING OF EACH OF SEVERAL INTERVALS OF PREGNANCY THAT EXPERIENCED LOSS OF PREGNANCY DURINGTHAT INTERVAL FOR 4 FLOCKS STUDIED IN GROUP 3[a]**

	STAGE	25-45	25-65[b,c]	25-85[b,c]	25-TERM[b,c]	45-65[b]	45-85[b]	45-TERM[b,c]	65-85[b]	65-TERM[b,c]	85-TERM[b,c]
SINGLE	FIRST SERVICE (N)*	0/6 (1)	3/39 (3)	5/43 (4)	9/43 (4)	0/7(1)	0/10 (2)	1/10 (2)	1/44 (3)	4/44 (4)	5/59 (4)
	SECOND SERVICE (N)*	0/4 (1)	0/4 (1)	0/4 (1)	0/2 (1)	0/29 (4)	0/6 (1)	4/28 (4)	0/7 (1)	5/42 (4)	0/8 (1)
	TOTAL	0/10	3/43	5/48	9/45	0/36	0/16	5/38	1/51	9/86	5/67
	LOSS (%)	0.0	7.0	10.4	2.0	0.0	0.0	13.2	2.0	10.4	7.4

	STAGE	25-45	25-65	25-85	25-TERM[c]	45-65	45-85	45-TERM[c]	65-85	65-TERM[c]	85-TERM[c]
MULTIPLE	FIRST SERVICE (N)*	0/37 (1)	2/96 (3)	2/98 (4)	7/98 (4)	0/36 (1)	0/40 (2)	1/40 (2)	0/83 (3)	3/83 (4)	4/86 (4)
COMPLETE	SECOND SERVICE(N)*	0/34 (1)	1/34 (1)	1/34 (2)	3/34 (1)	1/16 (4)	1/31 (1)	5/61 (4)	0/30 (1)	4/61 (4)	2/29 (1)
	TOTAL	0/71	3/130	3/132	10/132	1/52	1/71	6/101	0/113	7/144	6/115
	LOSS (%)	0.0	9.6	2.2	7.5	1.9	1.4	5.9	0.0	4.9	5.2

	STAGE	25-45	25-65	25-85	25-TERM[c]	45-65	45-85	45-TERM[c]	65-85	65-TERM[c]	85-TERM[c]
MULTIPLE	FIRST SERVICE (N)*	1/37 (1)	11/96 (5)	18/98 (6)	38/98 (6)	3/36 (1)	7/40 (2)	9/40 (2)	6/83 (5)	18/83 (5)	16/86 (7)
PARTIAL	SECOND SERVICE(N)*	2/34 (1)	3/34 (1)	4/34 (2)	6/34 (1)	3/16 (4)	2/31 (1)	10/61 (4)	2/30 (1)	7/61 (6)	2/29 (1)
	TOTAL	3/ 71	14/130	22/132	44/132	6/52	9/71	19/101	8/113	25/144	18/115
	LOSS (%)	4.2	10.7	16.7	33.3	11.5	12.7	18.8	7.1	17.3	15.7

* Indicates number of farms with ewes pregnant at the beginning of each period.

[a] Not all ewes were observed at all stages on all farms. Observations were made more often on ewes that conceived to the first service.

[b] Number of single pregnancies observed on a farm can increase as pregnancy progresses by virtue of ewe that initially had multiple pregnancies incurring partial loss and having a viable single fetus at a later stage.

[c] Lambing records were not available on some ewes that were observed at earlier stages of pregnancy.

Statistical Analysis

Effects of Season and Time of Breeding

Analysis of variance using the GLM procedure of SAS (SAS Institute, 1988) was used to determine the effects of season (anestrus or transition), service period (first or second), and their interaction on the percentages of ewes in groups 1 and 3 that experienced late embryonic or fetal loss. The analysis was based on the weighted means for each day of pregnancy diagnosis and the overall number of ewes per day (table 7). Loss for each stage was then derived by subtracting the value for the latter day from the value for the previous day of pregnancy diagnosis. A separate analysis of variance determined the same effects on the number of embryos or fetuses lost during each stage and all combination of stages. The overall patterns of ewes in groups 1 and 3 that experienced late embryonic or fetal loss and of embryos or fetuses lost from approximately day 25 to term was derived from each analysis. The relationship between type of loss and the day of pregnancy was examined by multiple regression analysis.

Temperature-Humidity Index

The proportion of pregnant ewes at the beginning of each interval in group 1 that experienced late embryonic or fetal loss was calculated for each of three stages of pregnancy (approximately day 25 to 45, 45 to 65, 65 to term) in group 1. The proportion lost was determined for each type of loss on a per farm basis

Table 7. **WEIGHTED MEANS OF CUMULATIVE EMBRYONIC AND FETAL LOSSES PER EWE IN GROUPS 1, 2, AND 3 FOR EACH DAY OF PREGNANCY DIAGNOSIS SUBSEQUENT TO DAY 25 BY TYPE OF LOSS[a]**

TYPE	SEASON	SERVICE	DAY	N[b]	% LOSS
SINGLE COMPLETE	*ANESTRUS*	1	45	51	9.8
			65	67	10.8
			85*	37	10.2
			TERM**	59	22.4
		2	45	64	1.6
			65	23	1.6
			85*	7	10.5
			TERM	8	15.4
	TRANSITION	1	45	29	6.9
			65	33	10.9
			TERM	43	12.9
		2	45	39	5.1
			TERM	38	34.5
	LATE BREEDING	1	45	30	3.3
			65	29	3.4
			TERM	32	3.4
		2	45	21	4.8
			TERM	12	0.0
MULTIPLE COMPLETE	*ANESTRUS*	1	45	184	0.5
			65	189	1.3
			85*	83	1.3
			TERM	86	4.2
		2	45	71	0.0
			65	61	0.0
			85*	30	0.0
			TERM	29	0.0
	TRANSITION	1	45	127	0.0
			65	121	0.2
			TERM	134	1.4
		2	45	39	0.0
			TERM	38	3.0
	LATE BREEDING	1	45	64	3.1
			65	62	3.1
			TERM	43	3.1
		2	45	11	0.0
			TERM	5	0.0
MULTIPLE PARTIAL	*ANESTRUS*	1	45	190	7.6
			65	186	20.0
			85*	36	23.8
			TERM	83	42.3
		2	45	114	8.5
			65	61	13.4
			85*	30	20.1
			TERM	29	24.9
	TRANSITION	1	45	127	4.7
			65	121	8.9
			TERM	134	32.2
		2	45	65	4.4
			TERM	63	17.9
	LATE BREEDING	1	45	11	3.1
			65	62	9.7
			TERM	43	64.8
		2	45	11	36.4
			TERM	5	36.7

[a] Not all ewes were observed at all stages on all farms. Observations were made more often on ewes that conceived to first service.

[b] Number of ewes pregnant on that day.

* Ewes pregnancy checked on day 85 were in Anestrus and Group 3 only.

(tables 2-4). Each farm was classified into one of three categories of THI (0, 1, or 2) for each of six intervals before and during pregnancy (day –7 to 0, 0 to 7, 0 to 25, 25 to 45, 45 to 65, 65 to 85). Effects of THI on the proportions of ewes having complete loss of a single pregnancy, complete loss of a multiple pregnancy, or the partial loss of a multiple pregnancy during each stage of pregnancy that followed the respective intervals for which THI had been calculated were determined by ANOVA.

Effect of Face Color (Breed Type)

Analysis of variance using PROC GLM was used to determined effects of face color of the ewe on the number of embryos or fetuses lost during each stage of pregnancy and all combination of stages. Losses of embryos and fetuses were determined from ewes in groups 1 and 3 with a face color of black (mainly Suffolk), white (mainly Dorset), or mottled (crossbred). In a separate analysis, ANOVA was used to test effects of face color on concentrations of progesterone and estrogen on days 25, 45, 65, and 85, and VEGF on days 25, 45, and 65.

Hormones

The relationships of the percentages of ewes experiencing late embryonic and fetal loss during subsequent intervals to concentrations of hormones in jugular venous serum were examined for a single sample on days 25, 45, 65, and /or 85 from each ewe from groups 1 and 3 (progesterone and estradiol-17β) or in group 1 (VEGF on days 25, 45, and 65). In one analysis, logistic regression

was used to predict the percentages of ewes experiencing complete or partial loss during a particular stage of pregnancy based upon concentrations of hormones at an earlier day of pregnancy (e.g. day 25, 45, 65, or 85). In a separate analysis, for each sampled day of pregnancy, concentrations of progesterone, estradiol-17β, and VEGF were ranked from least to greatest, then divided into three classifications (low quarter, middle half, high quarter) and the effects on complete and partial losses of pregnancy beyond that interval of pregnancy were evaluated by ANOVA.

RESULTS

Timing of late embryonic and fetal losses during anestrus and transitional period

More ewes from groups 1 and 3 lost potential offspring during fetal development (16.1% from day 45 to term) than during late embryonic development (3.8% from day 25 to 45). Losses were continuous throughout gestation (Figure 1); 3.8% of ewes lost one or more embryos from day 25 to 45, 6.2% lost one or more fetuses from day 45 to 65, 0.5% from day 65 to 85; and 9.4% from day 85 to term (Figure 2). Based on multiple regression, losses occurred at a linear rate (Figure 3). More ewes lost one or more, but not all, embryos or fetuses from day 25 to term (36.7%) than completely lost a single pregnancy (20.5%) or a multiple pregnancy (3.8%; Figure 3). Mean losses of embryos or fetuses in groups 1 and 3 averaged 4.1% from day 25 to 45, 4.1% from day 45 to 65, 3.2% from day 65 to 85, and 10.2% from day 85 to term (Figure 2).

Timing of late embryonic and fetal losses during the late breeding season

The pattern of late embryonic and fetal losses during the late breeding season (group 2) was similar to that observed during the anestrous and transitional periods. The percentages of ewes that lost one or more embryos or fetuses were 3.2% from day 25 to 45, 4.5% from day 45 to 65, and 20.3% from day 65 to term (Figure 4). The percentages of embryos present at day 25 that were lost were 6.4% as embryos from day 25 to 45, 5.0% as fetuses from day 45 to 65, and 13.9% as fetuses from day 65 to term (Figure 4).

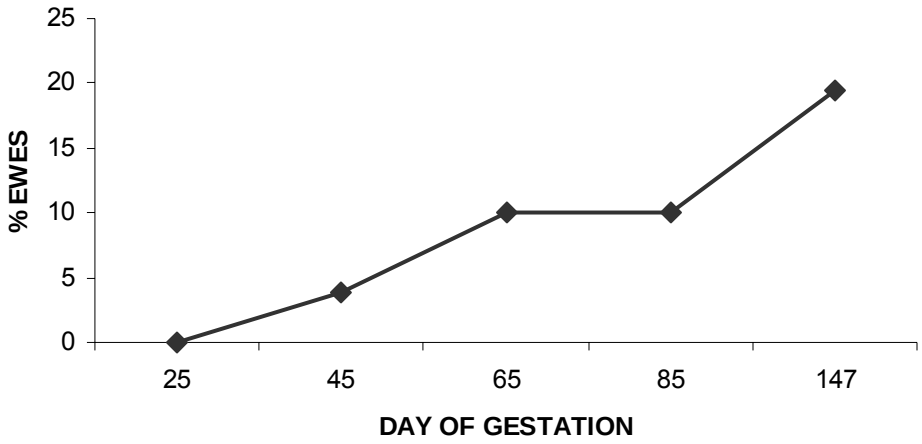

Figure 1. Percentages of ewes in groups 1 and 3 pregnant at day 25 of gestation losing some potential offspring during late embryonic or fetal development.

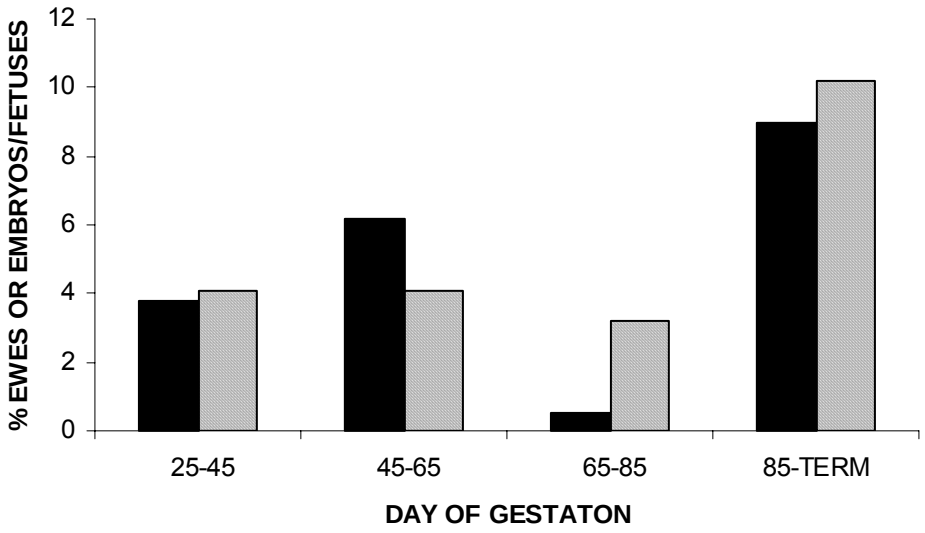

Figure 2. Percentages of pregnant ewes (■) in groups 1 and 3 that had late embryonic or fetal loss and of embryos or fetuses (▥) lost from approximately day 25 of gestation to term, by period of loss.

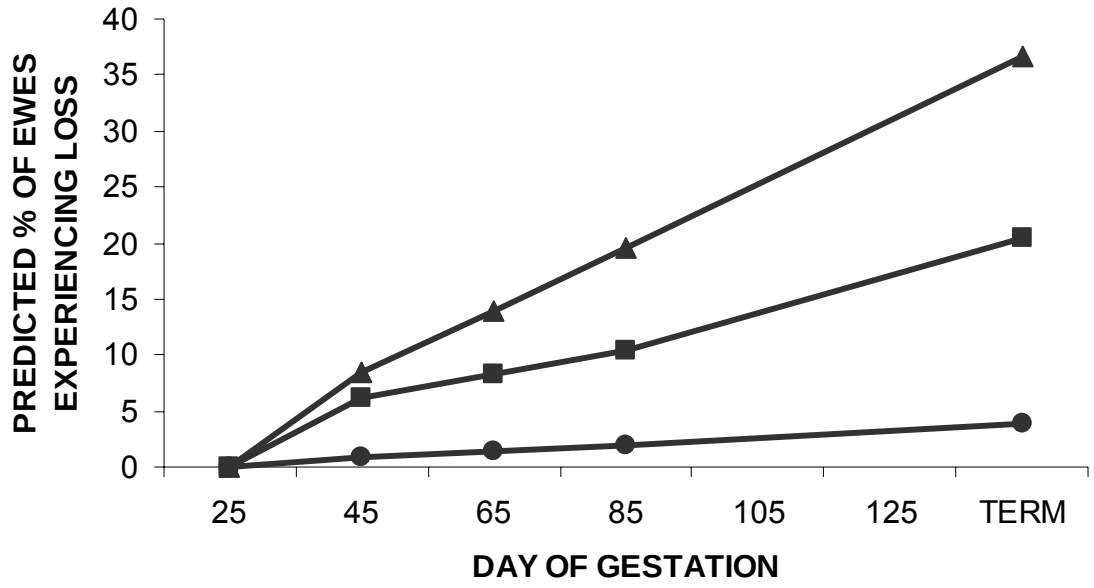

Figure 3. Estimated percentages of ewes in groups 1 and 3 that had Single [■], Multiple Complete [●], or Multiple Partial [▲] loss of embryos or fetuses from day 25 to term. Calculations were based on weighted means of observations on 12 flocks, of a total of 957 ewes pregnant on day 25.

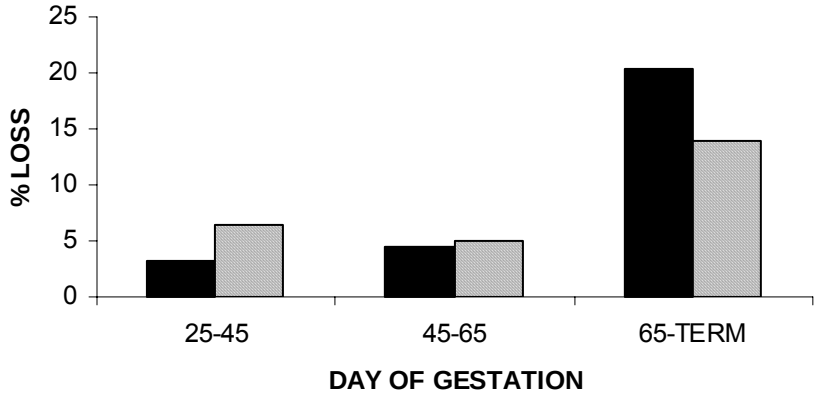

Figure 4. Percentages of pregnant ewes (■) in group 2 that lost embryos or fetuses and of embryos or fetuses (▥) lost from approximately day 25 of gestation to term during the late breeding season.

Effects of season, service period, and THI

There were no effects of season (table 8) or service period (table 9) on late embryonic or fetal mortality or percentages of ewes in groups 1 and 3 that experienced late embryonic or fetal mortality. Effect of THI were examined only for ewes in group 1. Losses from day 25 to term were expamined for effects of THI during all intervals before day 25 and day 25 to 45. Losses from day 45 to term and day 65 to term were examined for effect of THI on days 45 to 65 and 65 to 85, respectively.

THI from day – 7 to day 0 (breeding). Ewes exposed to a THI of 1 from days -7 to 0 experienced greater ($P < 0.05$) losses from day 25 to term (14.5%) than ewes exposed to a THI of 0 (6.8%) or 2 (5.3%; table 10).

THI from breeding to day 7. Ewes exposed to a THI of 0 from breeding to day 7 of gestation had a greater amount of loss ($P < 0.05$), averaging 9.9% from day 25 to term, than ewes exposed to a THI of 1 (6.0%) or 2 (4.3%; $P < 0.05$; table 10).

THI from breeding to day 25 . Ewes exposed to a THI of 0 between breeding and day 25 of gestation averaged 10.0% loss from day 25 to term, which was greater ($P < 0.05$) than the loss by ewes exposed to a THI of 1 (2.8%) or 2 (7.7%; table 10).

Table 8. **Effect of Season on Late Embryonic and Fetal Loss and Ewes Experiencing Loss on Farms Sampled in Groups 1 and 3**

Season	Number of Ewes D25	Number of Embryos D25	% Embryos/Fetuses Lost During Stage			% Ewes Experiencing Loss During Stage		
			D25-45	D45-65	D65-TERM	D25-45	D45-65	D65-TERM
Anestrus	563	989	4.4	4.7	12.4	4.5	7.0	9.9
Transitional	341	613	3.3	2.9	11.4	2.9	3.6	9.6

Table 9. **Effect of Service Period on Late Embryonic and Fetal Loss and Ewes Experiencing Loss on Farms Sampled in Groups 1 and 3**

Service Period	Number of Ewes D25	Number of Embryos D25	% Embryos/Fetuses Lost During Stage				% Ewes Experiencing Loss During Stage			
			D25-45	D45-65	D65-85	D85-TERM	D25-45	D45-65	D65-85	D85-TERM
First	569	1044	4.7	4.0	3.2	11.3	4.0	7.1	1.0	10.9
Second	334	554	3.0	2.9	1.5	5.6	3.6	0.8	3.4	5.6

Table 10. **PERCENTAGES OF EWES IN GROUP 1 EXPERIENCING LATE EMBRYONIC OR FETAL LOSS WHEN EXPOSED TO A THI OF 0, 1, OR 2 DURING SIX INTERVALS BEFORE AND DURING PREGNANCY**

	INTERVAL					
THI	*D-7 TO 0**	*D0 TO 7**	*D0 TO 25**	*D25 TO 45**	*D45 TO 65***	*D65 TO 85****
0	6.8[b]	9.9[a]	10.0[a]	6.3[ab]	5.7	9.1
1	14.5[a]	6.0[b]	2.8[b]	8.3[a]	7.1	8.1
2	5.3[b]	4.3[b]	7.7[b]	4.4[b]	4.3	7.9

[a,b] Values in the same column with different letters differ ($P < 0.05$).

* Data are losses from day 25 to term.

** Data are losses from day 45 to term.

*** Data are losses from day 65 to term.

THI from day 25 to 45. Ewes exposed to a THI of 1 during days 25 to 45 (8.3%) experienced greater losses from days 25 to term than ewes exposed to a THI of 0 (6.3%) or 2 (4.4%; $P < 0.05$; table 10).

THI from day 45 to 65 and 65 to 85. THI during these periods had no effect on fetal losses beyond that initial day of those periods (table 10).

Effects of Face Color (Breed Type)

Face color affected loss of embryos or fetuses from day 25, 45, and 64 of pregnancy to term. Ewes with black face color had greater ($P <0.05$) loss of embryos or fetuses from day 25 to term (29.0%) than either white-faced ewes (17.7%) or mottled-faced (19.2%) ewes. From day 45 to term, black-faced ewes had greater proportions of fetal loss (21.6%) than mottled- faced ewes (13.3%; $P<0.05$). Ewes with black face color lost more fetuses between days 85 and term (31.3%) than white-faced (4.6%), or mottled-faced ewes (15.8%; $P <0.01$; table 11).

Black-faced ewes had lower ($P <0.01$) concentrations of progesterone (ng/ml) on days 25 (2.3), 45 (2.6), and 65 (2.4) than those of white- (3.1, 3.0, 3.4) and mottled-faced (3.4, 3.8, 3.8) ewes, respectively (table 12). Concentrations of estradiol (pg/ml) were lower ($P < 0.05$) in white-faced ewes on days 25 than black-faced (4.0) or mottled-faced ewes (4.3), respectively (table 12). On days 45 and 65, mottled-faced ewes had the greater ($P <0.01$) concentrations of estradiol (5.0 and 5.5) than black-faced (4.1 and 4.4) or mottled-faced ewes (4.5

and 5.2), respectfully (table 12). Black-faced ewes tended (P = 0.06) to have greater concentrations of estradiol on day 85 (9.1%) than white- (5.2) or mottled-faced ewes (5.5), respectively (table 12). Concentrations of VEGF (mg/ml) were lower (P <0.01) in mottled-faced ewes on days 25 (1.0), 45 (1.0), and 65 (0.90) than in white-faced ewes (1.4, 1.5, 1.2; table 12).

Table 11. **EFFECTS OF FACE COLOR ON PROPORTIONS OF EMBRYONIC OR FETAL LOSS DURING SEVERAL STAGES OF GESTATION**[a]

	Face Color					
Stage	Black	(N)[b]	White	(N)[b]	Mottled	(N)[b]
D25-45	6.0	(83)	1.8	(173)	3.8	(283)
D25-65	11.9	(21)	5.3	(156)	9.9	(225)
D25-85	18.1	(11)	8.5	(41)	13.1	(99)
D25-term[c]	29.0[d]	(88)	17.7[e]	(165)	19.2[e]	(305)
D45-65	6.0	(42)	3.5	(109)	4.0	(199)
D45-85	0.0	(6)	N/A		5.7	(78)
D45-term[c]	21.6[d]	(104)	15.7[de]	(116)	13.2[e]	(275)
D65-85	0.0	(3)	0.0	(38)	4.7	(94)
D65-term[c]	18.0	(50)	12.8	(126)	10.9	(215)
D85-term[c]	31.3[d]	(16)	15.8[e]	(38)	4.6[f]	(97)

[a] Not all ewes were observed at all stages on all farms.

[b] Number of ewes pregnant at the beginning of each stage.

[c] Values are for ewes with lambing data only.

[d,e,f] Different letters in each row are different (P<0.05).

Table 12. **EFFECTS OF FACE COLOR ON CONCENTRATIONS OF PROGESTERONE, ESTRADIOL, AND VEGF ON DAYS 25, 45, 65, AND / OR 85 OF GESTATION**

		Face Color		
	Day	BLACK	WHITE	MOTTLED
PROGESTERONE	25	2.3[a]	3.1[b]	3.4[b]
(NG/ML)	45	2.6[a]	3.0[b]	3.8[b]
	65	2.4[a]	3.4[b]	3.8[b]
	85	3.2	4.4	4.3
ESTRADIOL-17ß	25	4.0[ab]	3.6[a]	4.3[b]
(PG/ML)	45	4.1[a]	3.6[a]	5.0[b]
	65	4.4[a]	4.5[a]	5.5[b]
	85	9.1[c]	5.2[d]	5.5[cd]
VEGF	25	1.2[ab]	1.4[a]	1.0[b]
(NG/ML)	45	1.4[ab]	1.5[a]	1.0[b]
	65	1.1[ab]	1.2[a]	0.9[b]

[a,b] Superscripts that differ within each row are different ($P < 0.05$)

[c,d] Superscripts that differ within each row tend to be different ($P = 0.06$)

Progesterone

Complete Loss

Logistic Regression Progesterone on day 25 was of predictive value, whereas concentrations on other days were not. From day 25 to 65 of gestation and from day 25 to term, complete loss decreased as concentrations of progesterone on day 25 of gestation increased ($P < 0.05$; Figure 5).

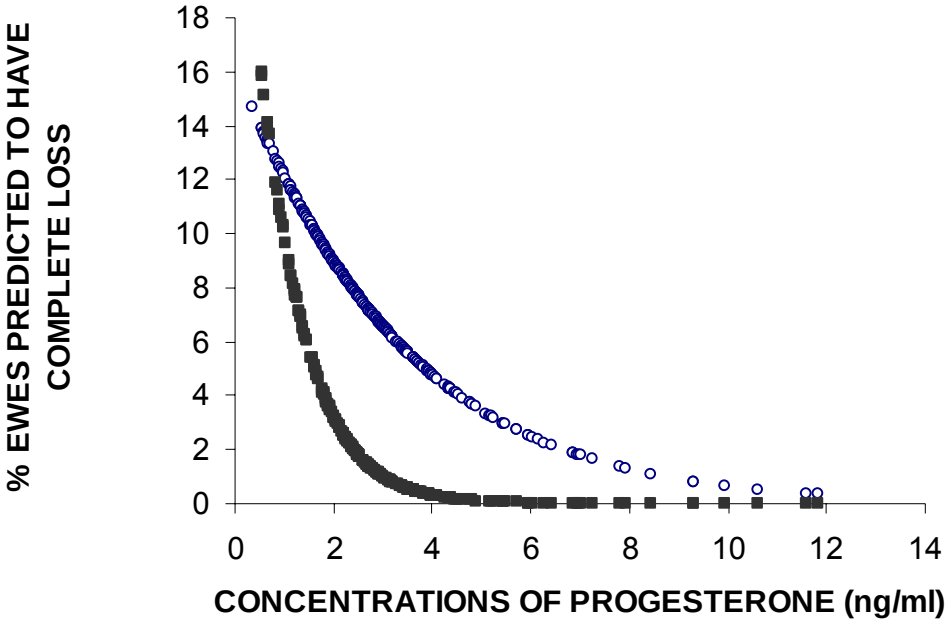

Figure 5. Logistic regression of complete pregnancy loss on concentrations of progesterone on day 25 for losses from day 25 to 65 (■) and from day 25 to term (○).

Classification Complete losses were higher between day 25 and 65 ($P < 0.05$) in ewes with low (8.1%) than with medium (1.3%) or high (1.4%) concentrations of progesterone on day 25 (Table 13). Complete losses from day 25 to term were greater ($P < 0.05$) in ewes with medium (9.0%) or low (8.4%) than with high (1.2%) concentrations of progesterone on day 25 of gestation (Table 13). However, between day 65 and term, complete loss was higher in ewes with medium (6.2%) than those with low (0%) or high (1.4%) concentrations of progesterone on day 25 ($P < 0.05$; Table 13). Ewes with medium or high

concentrations of progesterone on day 45 had no losses to day 65 while ewes with low concentrations lost 3.3% of pregnancies by day 65 of gestation ($P < 0.05$; Table 14).

Partial Loss

Logistic Regression The relationship of partial losses to concentrations of progesterone on day 25 of gestation varied with stage of pregnancy. Partial loss from day 25 to 45 increased as concentration of progesterone on day 25 increased ($P < 0.05$; Figure 6), whereas partial loss from day 65 to term decreased as concentration of progesterone on day 25 increased ($P < 0.05$; Figure 6). No other significant associations were detected.

Classification Partial losses between day 25 and 45 were greater ($P < 0.05$) in ewes with high (12.2%) than medium (5.2%) or low (2.7%) concentrations of progesterone on day 25 (Table 13). From day 25 to 85 of gestation, partial losses were greater in ewes with medium (28.8%) than low (8.8%) or high (5.9%) concentrations of progesterone on day 25 of gestation ($P < 0.01$; Table 13).

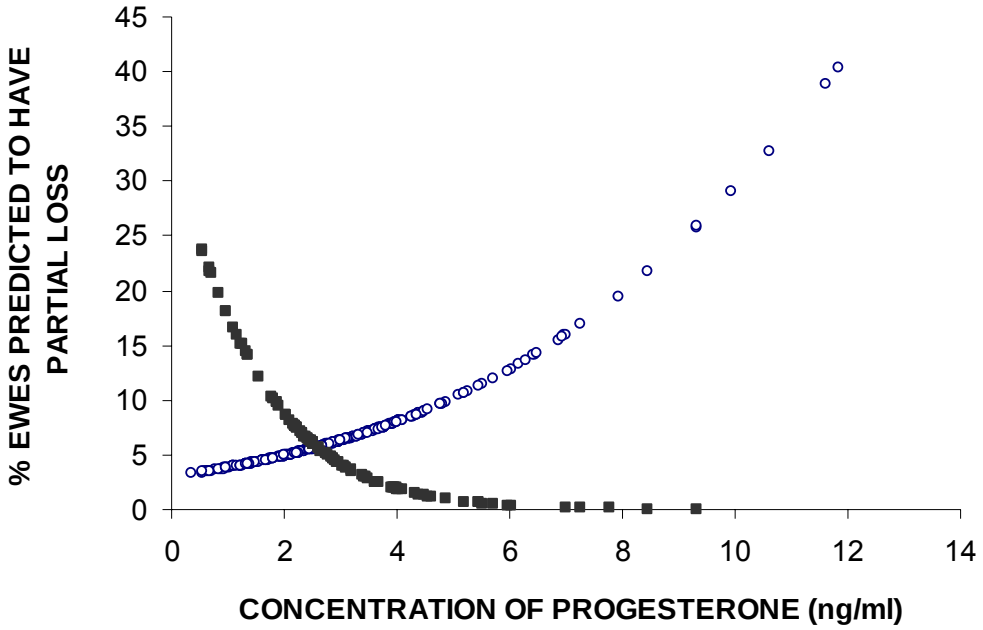

Figure 6. Logistic regression of partial pregnancy loss on concentrations of progesterone on day 25 for losses from day 25 to 45 (○) and from day 65 to term (■).

Table 13. **ASSOCIATION OF PERCENTAGES OF EWES IN GROUPS 1 AND 3 WITH COMPLETE (CL) OR PARTIAL (PL) LOSSES OF PREGNANCY AND CONCENTRATIONS OF PROGESTERONE ON DAY 25 DURING SEVERAL INTERVALS OF GESTATION***

		%EWES WITH		RANGE OF
INTERVAL	CLASS.	CL	PL	P4 D25 (NG/ML)
D25-45	LOW	2.7	2.7[b]	0.3-1.9
(N =300)	MED	0.6	5.2[ab]	2.0-3.8
	HIGH	0.0	12.2[a]	3.0-11.9
D25-65	LOW	8.1[a]	9.4	0.3-1.9
(N = 300)	MED	1.3[b]	17.2	2.0-3.6
	HIGH	1.4[b]	13.0	3.6-11.9
D25-85	LOW	8.8	8.8[b]	0.3-2.2
(N=136)	MED	4.6	28.8[a]	2.3-3.9
	HIGH	0.0	5.9[b]	3.9-9.3
D25-TERM	LOW	8.4[a]	32.5	0.3-1.8
(N=333)	MED	9.0[a]	30.1	1.9-3.6
	HIGH	1.2[b]	33.7	3.7-11.9
D45-65	LOW	1.7	6.7	0.3-1.9
(N=240)	MED	0.8	6.6	2.0-3.8
	HIGH	0.0	6.7	3.8-11.9
D45-85	LOW	0.0	5.0	0.3-1.9
(N=79)	MED	2.6	12.8	2.0-3.9
	HIGH	0.0	5.0	3.9-9.3
D45-TERM	LOW	4.4	30.9	0.3-1.8
(N=272)	MED	5.1	23.0	1.9-3.7
	HIGH	1.4	25.0	3.8-11.9
D65-85	LOW	0.0	3.0	0.3-2.1
(N=134)	MED	3.0	5.9	2.0-3.9
	HIGH	0.0	3.0	4.0-9.3
D65-TERM	LOW	0.0[a]	20.5	0.3-1.9
(N=292)	MED	6.2[b]	15.1	2.0-3.6
	HIGH	1.4[a]	16.4	3.7-11.9
D85-TERM	LOW	0.0	14.7	0.3-2.1
(N=136)	MED	6.0	5.9	2.0-3.9
	HIGH	0.0	2.9	4.0-9.3

* Data shown are only for animals from which blood samples were available. Classification was based on the lower 25%, middle 50%, and upper 25% of ranked values for concentrations of progesterone.

[a,b] Values in the same column with different letters differ ($P < 0.05$)

Table 14. **ASSOCIATION OF THE PERCENTAGES OF EWES IN GROUPS 1 AND 3 WITH COMPLETE (CL) OR PARTIAL (PL) LOSSES OF PREGNANCY AND CONCENTRATIONS OF PROGESTERONE ON DAY 45 DURING SEVERAL INTERVALS OF GESTATION***

		%EWES WITH		RANGE OF
	CLASS.	CL	PL	P4 D45 (NG/ML)
D45-65	LOW	3.3[a]	8.3	0.4-2.0
(N=240)	MED	0.0[b]	7.4	2.1-3.8
	HIGH	0.0[b]	6.7	3.8-12.4
D45-85	LOW	5.9	11.8	0.8-2.3
(N=68)	MED	0.0	11.8	2.4-3.5
	HIGH	0.0	5.9	3.5-5.6
D45-TERM	LOW	5.5	28.8	0.4-2.0
(N=291)	MED	2.1	28.3	2.1-3.6
	HIGH	2.7	23.3	3.7-14.4
D65-85	LOW	5.9	5.9	0.8-2.4
(N=65)	MED	0.0	8.9	2.4-3.5
	HIGH	0.0	5.9	3.6-5.6
D65-TERM	LOW	1.7	20.0	0.4-2.1
(N=240)	MED	0.8	19.2	2.1-3.8
	HIGH	3.3	18.3	3.8-12.4
D85-TERM	LOW	0.0	0.0	0.8-2.3
(N=67)	MED	0.0	8.3	2.3-3.5
	HIGH	0.0	5.9	3.5-5.6

* Data shown are only for animals from which blood samples were available. Classification was based on the lower 25%, middle 50%, and upper 25% of ranked values for concentrations of progesterone.

[a,b] Values in the same column with different letters differ ($P < 0.05$)

Estradiol

Complete Loss

Logistic Regression Concentrations of estradiol had no predictive value for either complete or partial losses of pregnancy.

Classification Complete losses to day 65 of gestation did not differ with concentrations of estradiol on day 25 or 45 of gestation. Complete losses from day 65 to 85 ($P < 0.05$), day 65 to term ($P < 0.01$), and day 85 to term ($P = 0.05$) were greater in ewes with low (6.2%, 8.8%, 9.7%, respectively) than medium (0%, 1.5%, 1.6%, respectively) or high (0%, 1.9%, 0%, respectively) concentrations of estradiol on day 65 of gestation (Table 16).

Partial Loss

Partial losses from 25 to 45 were greater ($P < 0.05$) in ewes with high (10.1%) than with medium (3.2%) concentrations of estradiol on day 25 of gestation (Table 15). Similarly, partial losses from day 25 to term were greater in ewes with high (35%) than with medium (23.9%) or low (21.3%) concentrations of estradiol on day 25 of gestation ($P < 0.05$; Table 15). In contrast, partial losses from day 85 to term were greater ($P < 0.05$) in ewes with low (16.1%) or medium (17.5%) than with high (0%) concentrations of estradiol on day 65 of gestation (Table 16).

Table 15. **ASSOCIATION OF THE PERCENTAGES OF EWES IN GROUPS 1 AND 3 WITH COMPLETE (CL) OR PARTIAL (PL) LOSSES OF PREGNANCY AND CONCENTRATIONS OF ESTRADIOL ON DAY 25 DURING SEVERAL INTERVALS OF GESTATION***

		%EWES WITH		RANGE OF
INTERVAL	CLASS.	CL	PL	E2 D25 (PG/ML)
D25-45	LOW	0.93	6.5[ab]	1.3-2.7
(N=434)	MED	1.4	3.2[b]	2.8-4.6
	HIGH	0.90	10.1[a]	4.7-23.5
D25-65	LOW	4.9	9.9	0.7-2.7
(N=400)	MED	3.0	9.5	2.8-5.4
	HIGH	3.0	13.9	5.4-23.5
D25-85	LOW	11.1	7.4	0.7-2.1
(N=108)	MED	5.0	13.0	2.2-4.3
	HIGH	0.0	18.5	4.3-11.7
D25-TERM	LOW	9.0	21.3[a]	1.0-2.7
(N=492)	MED	4.9	23.9[a]	2.8-4.3
	HIGH	4.9	35.0[b]	4.3-11.7
D45-65	LOW	0.0	3.1	1.3-2.8
(N=263)	MED	1.5	6.1	2.9-4.9
	HIGH	1.5	6.1	5.0-23.5
D045-85	LOW	0.0	12.5	1.3-2.8
(N=31)	MED	0.0	0.0	2.9-3.9
	HIGH	0.0	12.5	4.3-8.8
D45-TERM	LOW	2.1	13.8	1.3-2.8
(N=373)	MED	3.8	18.9	2.9-4.6
	HIGH	4.3	22.3	4.70-23.5
D65-85	LOW	4.0	0.0	0.7-2.0
(N=107)	MED	0.0	1.8	2.1-4.2
	HIGH	0.0	0.0	4.3-11.7
D65-TERM	LOW	4.6	13.8	0.7-2.7
(N=347)	MED	1.7	16.9	2.8-5.4
	HIGH	3.4	18.2	5.5-23.5
D85-TERM	LOW	7.4	18.5	0.7-2.1
(N=108)	MED	1.9	13.0	2.2-4.3
	HIGH	3.7	14.8	4.3-11.7

* Data shown are only for animals from which blood samples were available. Classification was based on the lower 25%, middle 50%, and upper 25% of ranked values for concentrations of estradiol.

[a,b] Values in the same column with different letters differ ($P < 0.05$)

Table 16. **ASSOCIATION OF THE PERCENTAGES OF EWES IN GROUPS 1 AND 3 WITH COMPLETE (CL) OR PARTIAL (PL) LOSSES OF PREGNANCY AND CONCENTRATIONS OF ESTRADIOL ON DAY 65 DURING SEVERAL INTERVALS OF GESTATION***

		%EWES WITH		RANGE OF
INTERVAL	CLASS.	CL	PL	E2 D45 (PG/ML)
D65-85	LOW	6.2[a]	3.1	1.7-3.1
(N=126)	MED	0.0[b]	3.2	3.2-5.7
	HIGH	0.0[b]	3.1	5.8-12.9
D65-TERM	LOW	8.8[a]	18.6	1.4-3.1
(N=408)	MED	1.5[b]	16.3	3.20-5.9
	HIGH	1.9[b]	14.5	6.0-15.5
D85-TERM	LOW	9.7[a]	16.1[a]	1.7-3.2
(N=125)	MED	1.6[b]	17.5[a]	3.3-5.7
	HIGH	0.0[b]	0.0[b]	5.8-12.9

* Data shown are only for animals from which blood samples were available. Classification was based on the lower 25%, middle 50%, and upper 25% of ranked values for concentrations of estradiol.

[a,b] Values in the same column with different letters differ ($P < 0.05$)

VEGF

Complete Loss

There was no relationship of complete loss to concentrations of VEGF using logistic regression, and complete losses did not vary with classes of concentrations of VEGF on day 25, 45, or 65, as tested by ANOVA.

Partial Loss

Logistic Regression There was no relationship of partial loss to concentrations of VEGF on days 25 and 65. Partial losses from day 65 to term decreased as concentration of VEGF on day 45 increased ($P < 0.05$; Figure 7).

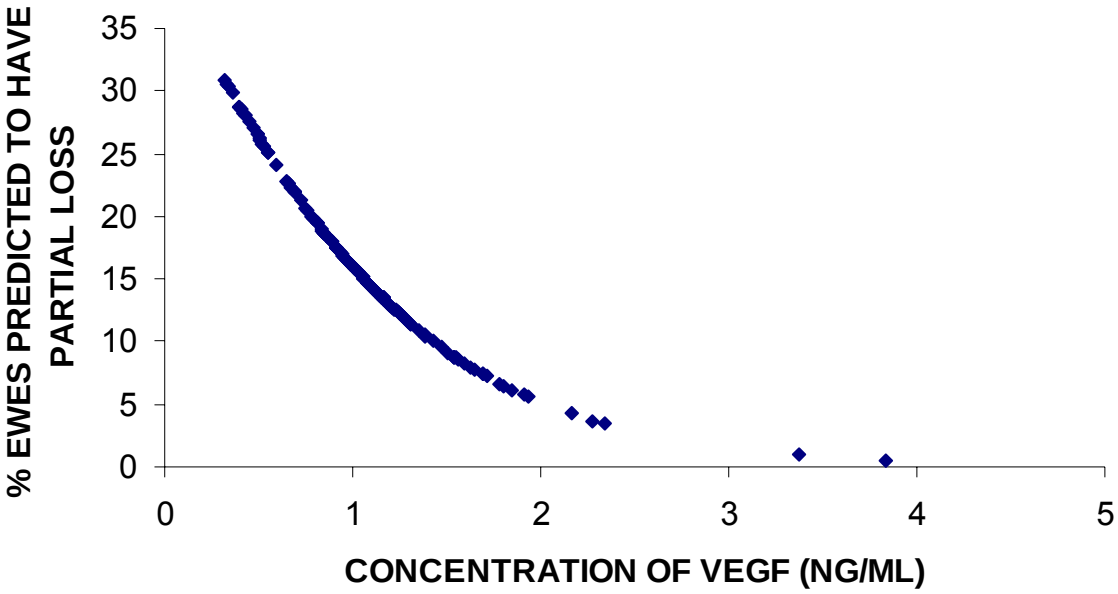

Figure 7. Logistic regression of percentages of ewes in group 1 that partially lost a pregnancy from day 65 to term on concentrations of VEGF on day 45 of gestation.

Classification From day 25 to 65, partial losses were greater ($P < 0.05$) in ewes with high (16.7%) and low (14.3%) than with medium (5.5%) concentrations of VEGF on day 25 (Table 17). Specifically, between days 45 and 65, partial losses were greater ($P < 0.05$) in ewes with high (12.5%) than medium (2.7%) concentrations of VEGF on day 25 of gestation (Table 17). In contrast, partial losses between day 65 and term were greater ($P < 0.05$) in ewes with low (22%) and medium (17.5%) than with high (6.0%) concentrations of VEGF on day 45 of gestation (Table 18). Partial losses did not vary with concentrations of VEGF on day 65 of gestation.

Table 17. **ASSOCIATION OF THE PERCENTAGES OF EWES IN GROUP 1 WITH COMPLETE (CL) OR PARTIAL (PL) LOSSES OF PREGNANCY AND CONCENTRATIONS OF VEGF ON DAY 25 DURING SEVERAL INTERVALS OF GESTATION***

		%EWES WITH		RANGE OF
INTERVAL	CLASS.	CL	PL	VEGF D25 (NG/ML)
D25-45	LOW	3.7	7.1	0.3-0.7
(N=221)	MED	0.9	3.7	0.8-1.3
	HIGH	0.0	3.6	1.4-4.7
D25-65	LOW	1.8	14.3[a]	0.3-0.7
(N=221)	MED	1.8	5.5[b]	0.8-1.3
	HIGH	0.0	16.7[a]	1.4-4.7
D25-TERM	LOW	5.3	32.0	0.3-0.7
(N=221)	MED	2.8	22.9	0.8-1.3
	HIGH	1.8	23.2	1.4-4.7
D45-65	LOW	0.0	5.4[ab]	0.3-0.7
(N=221)	MED	2.8	2.7[a]	0.8-1.3
	HIGH	0.0	12.5[b]	1.4-4.7
D45-TERM	LOW	1.7	3.6	0.3-0.7
(N=221)	MED	1.8	0.9	0.8-1.3
	HIGH	0.0	3.6	1.4-4.7
D65-TERM	LOW	0.0	16.0	0.3-0.7
(N=221)	MED	0.9	19.0	0.8-1.3
	HIGH	2.0	8.0	1.4-4.7

* Data shown are only for animals from which blood samples were available. Classification was based on the lower 25%, middle 50%, and upper 25% of ranked values for concentrations of VEGF.

[a,b] Values in the same column with different letters differ ($P < 0.05$)

Table 18. **ASSOCIATION OF THE PERCENTAGES OF EWES IN GROUP 1 WITH COMPLETE (CL) OR PARTIAL (PL) LOSSES OF PREGNANCY AND CONCENTRATIONS OF VEGF ON DAY 45 DURING SEVERAL INTERVALS OF GESTATION***

		%EWES WITH		RANGE OF
INTERVAL	CLASS.	CL	PL	VEGF D45 (NG/ML)
D45-65	LOW	0.0	1.9	0.3-0.7
(N=220)	MED	2.7	7.1	0.8-1.3
	HIGH	0.0	7.4	1.4-11.5
D45-TERM	LOW	1.8	3.7	0.3-0.8
(N=220)	MED	1.8	0.8	0.8-1.3
	HIGH	0.0	3.7	1.4-11.5
D65-TERM	LOW	2.0	22.0[a]	0.3-0.7
(N=204)	MED	0.0	17.5[a]	0.8-1.3
	HIGH	2.0	6.0[b]	1.4-4.3

* Data shown are only for animals from which blood samples were available. Classification was based on the lower 25%, middle 50%, and upper 25% of ranked values for concentrations of VEGF.

[a,b] Values in the same column with different letters differ ($P < 0.05$)

DISCUSSION

The most important findings in the present study were that late embryonic and fetal losses occurred throughout gestation and that partial losses were more frequent than complete losses. A total of 19.9% of ewes had late embryonic or fetal losses from day 25 to term and 21.6% of all embryos or fetuses were lost. Estimated loss of potential offspring from determination of ovulation rate based on day 8 to lambing for ewes pregnant on day 26 to 30 was 22.4%, based upon the average calculated from the data of Knights et al. (2001a,b) obtained in many of the same flocks in prior years, and from the ewes treated with CIDR-G and FSH in the present study. By difference, an estimated 0.8% of all potential offspring were lost from ovulation to day 25 of gestation in ewes that were pregnant at day 25 (72% of ewes exposed). Combining these two values, loss of all potential offspring in all ewes, including pregnant ewes and non-pregnant ewes was estimated to be 28.8% from ovulation to day 25 (fertilization failure included). Thus, the estimated proportion of total loss of potential offspring from ovulation to term in all ewes was 50.4% (table 19). That value is greater than calculated by Bolet (1986), who estimated total embryonic and fetal loss in all ewes from breeding to term as approximately 30%.

Losses were continuous from day 25 to term, with no single interval having the majority on a per day basis. Approximately 4% of the embryos present at day 25 were lost during each 20-day interval of pregnancy beyond that point. In contrast, Moor and Rowson (1966) suggested that the majority of loss occurring after day 18 of gestation directly preceded attachment of the embryo to

the maternal interface. Most frequently, ewes with a multiple pregnancy lost one, but not all embryos or fetuses. The majority of ewes that had partial loss were pregnant with two embryos or fetuses on an observed day with one being born at term. Complete loss of a single pregnancy occurred more often than complete loss of a multiple pregnancy in agreement with Kelly and Allison (1979).

Several authors observed the loss of individual embryos without the total loss of pregnancy (Rhind et al., 1980; Schrick and Inskeep, 1993). A greater proportion of ewes experienced partial with twin ovulations than complete loss in ewes with single ovulations when number of embryos present at day 18 (Quinlivan et al., 1966) or day 22 to 25 (Kelly and Allison, 1979) of gestation were compared to the numbers of CL (Quinlivan et al., 1966). "Uterine efficiency", described by Meyer (1985) as the marginal response in litter size due to ovulation of an additional egg, decreased as ovulation rate increased.

Loss did not differ between ewes bred during anestrus or the transitional period. Previous authors noted seasonal differences in embryonic loss. Dutt (1954) observed higher rates of fertilization failure and embryonic mortality to day 18 during the early breeding season than during mid-season. Others noted that a high percentage of ewes did not lamb when bred before September and that fertility improved as the breeding season advanced (Hulet et al., 1956). Effects were thought to be due to high ambient temperature exposure during the anestrous period. In fact, percentages of ewes experiencing loss during the late breeding season were numerically higher than during the anestrous or transitional periods. Thus, losses in the current study were not explained by

seasonal differences in temperature. Most studies in which a high percentage of embryonic mortality was observed during anestrus were conducted with ewes that were exposed to temperatures in the upper 80's to 90's on a consistent basis. Ewes were never exposed to such temperatures on a steady basis and a THI of 2 was calculated for very few farms or intervals.

Service period did not affect late embryonic or fetal loss. In contrast, Lunstra and Christenson (1981) observed a lower percentage of ewes lambing and Knights et al. (2001a, b) observed higher percentages of complete pregnancy loss after day 26 in ewes that conceived during second service period of the spring/summer breeding season.

Breed-type differences were apparent in the number of embryos or fetuses lost during several extended intervals of pregnancy. Black-faced ewes in this study, mainly of Suffolk breeding, experienced the most loss, regardless of stage of pregnancy. White and mottled-faced ewes had similar proportions of embryos and fetuses lost from day 25 or day 45 to term. From day 85 to term, white-faced ewes had very little fetal loss compared to black-faced and mottled-faced ewes. Effect of breed on reproductive performance in sheep has been studied extensively. However, studies on the effect of breed on late embryonic or fetal mortality are limited. Cumming et al. (1975) noted that embryonic survival from breeding to day 26 to 30 in twin-ovulating ewes was significantly higher in crossbred ewes than in twin-ovulating Merino ewes. Differences in survival were not seen across breeds in single-ovulating ewes. Foote et al.

(1959) found that Columbia ewes lost fewer embryos to day 18 than Hampshire ewes.

On average, Dorsets have a longer breeding season than other breeds of sheep (Hafez, 1952) and are often used in flocks interested in out-of-season breeding (Notter, 1992). Dorset rams are less seasonal and have been shown to have a higher libido, both of which are thought to affect the induction of ovulation in anestrous ewes. Dorset rams were more effective in inducing ovulation than Suffolks (Nugent et al., 1988) or Hampshires (Barr et al., 1968).

Concentrations of progesterone were lowest in black-faced ewes, whereas white-faced ewes had the highest concentrations of progesterone on days 25, 45, and 65. Concentrations of estradiol on day 45 and 65 were greater whereas concentrations of VEGF were lower in mottle-faced ewes than in black- or white-faced ewes. Concentrations of estradiol and VEGF are thought to be involved in placental development and might explain the low proportions of fetuses lost from day 45 to term and day 85 to term in mottled-faced ewes.

The lower concentrations of progesterone and higher percentages of loss observed in black-faced ewes might be due to the fact that the Suffolk breed has a shorter breeding season and thought to go into a “deeper anestrus” than white or mottled-faced ewes (DeBaca et al., 1954). Black-faced ewes generally are not selected for breeding during the summer months as compared to white or mottled-faced ewes. The end of breeding for Suffolk ewes often occurs in February when days are relatively short (Robinson and Karsch, 1984). Black-faced ewes might have more trouble retaining pregnancies or embryos when

induced to ovulate during anestrus. Higher embryonic or fetal survival rates as well as high and low concentrations of estradiol and VEGF, respectively, observed in the mottled-faced ewes might be due to heterosis, or "hybrid vigor" from crossbreeding black- and white-faced ewes.

Losses and their association with steroids and VEGF

Late embryonic development Complete losses of single or multiple pregnancies were associated with decreased concentrations of progesterone on days 25 and 45 of gestation. This association is logical, as progesterone is required for maintenance of pregnancy (Casida and Warwick, 1945). Late embryonic mortality between day 30 and 60 of gestation was associated with low concentrations of progesterone in lactating dairy cows sampled once on days 28 to 37 of gestation (Starbuck, 2001). In beef cows in which pregnancies were maintained to day 36 in the absence of a primary corpus luteum by exogenous progesterone, induced corpora lutea on the ovary adjacent to the uterine horn containing the embryo were able to maintain pregnancy beyond that point in 100% of cows (Bridges et al., 2000). However, if new corpora lutea were induced before day 36, only 50% of pregnancies were maintained (Bridges et al., 2000).

During late embryonic development from day 25 to 45, the embryo is establishing and expanding upon its own cardiovascular system (Table 19). Vasculature allowing exchange between the mother and embryos is also

Table 19. **EVENTS DURING GESTATION AND ESTIMATED LOSSES OF POTENTIAL OFFSPRING IN PREGNANT AND NON-PREGNANT EWES**

DAY	ASSOCIATED EVENTS		DAY OF COUNT	ESTIMATED LOSS TO DATE FROM PREVIOUS DATE PREG EWES	ALL EWES
0	Fertilization				
4-8	Spherical blastocyst				
8-11	Filiform blastocyst / Invades opposite horn				
15	Initial attachment of chorion to uterine epithelium	EMBRYONIC DEVELOPMENT			
18	Initial cardiovascular development / Interdigitation of microvilli with caruncular epithelium				
22-23	Initial placentome development / 2-fold increase in increase in vasculature (gravid horn)		25	0.8%*	28.8%**
30	2-fold increase of vasculature (non-gravid horn)	PLACENTATION			
42	Chorionic villi interlocked with caruncular epithelium Branching of villi		45	4.1%	
			65	4.1%	
70	Proliferation of blood vessels at site of attachment				
			85	3.2%	
110	Vascular density of cotelydon begins to increase	FETAL DEVELOPMENT			
	Growth: 5 to 6 mm per day				
120 to 130	Growth: 4 to 5 mm per day				
	Growth: < 6 mm per day				
140 to 150	Parturition		TERM	10.2%	
			TOTAL	22.4%	50.4%

*Estimate based on the difference of number of corpora lutea and number of lambs born (22.4%; Knights et al., 2001) and embryos lost from day 25 to term in the current study (21.6%).

**Estimate based on 28% of ewes that failed to become pregnant in the current study + the difference of number of corpora lutea and number of lambs born (22.4%; Knights et al., 2001) and embryos lost from day 25 to term in the current study (21.6%).

increasing, establishing placental function. On approximately day 22 of gestation, placentome development is apparent. The chorionic villi have interdigitated with the caruncular epithelium, initiating the development of the placenta. As chorionic villi expand within the caruncular tissue to form cotelydons, the vascular growth of the gravid uterine horn begins to increase.

Partial losses of multiple pregnancies during late embryonic development were associated with increasing concentrations of progesterone on day 25, whereas losses during fetal development were associated with decreasing concentrations of progesterone on day 25. Most partial losses by day 45 occurred in ewes with high concentrations of progesterone on day 25. This association might reflect the greater loss of embryos with higher ovulation rates seen by Rhind et al., 1980a, because ewes with more than one CL would be expected to have higher progesterone (Stormshak et al., 1963). Alternatively, high concentrations of progesterone on day 25 might reflect concentrations during earlier periods of development. Concentrations of progesterone during early development or on day 25 could alter gene expression of certain functions and affect subsequent late embryonic or fetal development. Genes of the mouse that were regulated by progesterone during implantation included growth and transcription factors, enzymes, cell adhesion molecules, protease inhibitors, and molecules involved in immune response (Bagchi et al., 2003).

More partial losses from day 25 to 45 and from day 25 to term occurred in ewes with high concentrations of estrogen on day 25 of gestation, whereas ewes with low or medium concentrations did not differ. This association is consonant

with the effect of low concentrations of progesterone discussed above. Exposure to high concentrations of estradiol during early pregnancy might affect subsequent development of the embryo or fetus. High estrogen:progesterone ratios on days three to six were associated with abnormally-developed embryos in cattle (Maurer and Echternkamp, 1982). Thatcher (2001) suggested that elevated concentrations of estrogen in ovarian follicles during maternal recognition of pregnancy in cattle (days 14 to 17) might be detrimental to maintenance of pregnancy. Decreased pregnancy rates in cattle were associated linearly with increased concentrations of estradiol during this period (Pritchard et al., 1994). Effects of delayed ovulation on increased embryonic mortality and congenital abnormalities in the rat were shown to be due to prolonged exposure of the oocyte to increased concentrations of estrogen during preovulatory follicular development (Butcher et al., 1973; Butcher and Pope, 1979; Butcher and Page, 1981; Page and Butcher, 1982). Concentrations of estradiol on day 25 are thought to originate from the conceptus and might be involved in vascular growth of the gravid uterine horn. One embryo from a multiple pregnancy might have problems associated with placentation due to the high concentrations of estradiol on day 25 and as a result, does not survive beyond placental development. The high concentrations on day 25 could simply be a result of other events associated with late embryonic mortality.

Partial losses to day 65 varied with concentrations of VEGF on day 25 of gestation. Partial losses from day 25 to 65 were greatest in ewes with low and high concentrations of VEGF, whereas partial losses from day 45 to 65 were

greatest in ewes with high concentrations only. Ewes with medium concentrations of VEGF on day 25 experienced minimal partial losses to day 65. Certain concentrations of VEGF might be necessary during embryonic cardiovascular development and initial placentation. Specific thresholds of VEGF were required to inihibit apoptosis of endothelial cells during angiogenesis and were essential for the stabilization of newly-formed blood vessels (Newfeld et al., 1999). When tissues were exposed to high concentrations of VEGF, hyperproliferation of blood vessels and other abnormalities occurred. Exposure of quail embryos to high concentrations of VEGF resulted in excessive fusion of vessels and formation of vessels with abnormally large lumens (Drake and Little, 1995). However, if concentrations of VEGF were reduced or completely inhibited, angiogenesis was impaired, leading to abnormal or inhibited organ development (Newfeld et al., 1999).

Partial loss to day 85 was associated with medium concentrations of progesterone on day 25. Fetuses that were capable of surviving beyond placental development might have been altered by medium concentrations of progesterone prior to or on day 25 and experienced alterations in certain gene function, affecting survival to day 85 of gestation.

Fetal development. From day 65 to term, proliferation of blood vessels at the maternal-fetal attachment site is complete, but placental growth will continue on the microvascular level (Table 19). Angiogenesis will continue to provide a large amount of microvascular growth within the cotelydon, increasing vascular density

to meet the demands of the growing fetus. The fetus will grow at an increasing rate until day 110, when growth begins to decline as the fetus approaches the end of gestation (Table 19).

Complete losses to day 65 were more frequent in ewes having low concentrations of progesterone on day 25 of gestation, whereas complete losses after day 65 were more frequent in ewes with medium concentrations of progesterone. Complete losses were minimal in ewes with high concentrations of progesterone on day 25. Progesterone has been shown to affect growth of the conceptus during embryonic development. Increasing peripheral concentrations of progesterone resulted in a higher rate of growth of bovine embryos (Fox et al., 1988; Garrett et al., 1988) and uterine protein secretory activity (Nephew et al., 1991). Concentrations of progesterone on day 25 might be typical of concentrations of progesterone during early embryonic development.

An optimum concentration of progesterone might be necessary during embryonic development to ensure proper fetal growth and development. Kleeman et al. (1994) observed the greatest amount of fetal growth on day 74 when ewes were treated with progesterone on days 1 to 3 or 1 to 6 after mating. In a separate experiment, embryos exposed to progesterone on days 1 to 3 were transferred to recipient ewes that had or had not received progesterone. An increase in fetal mass on day 76 was observed in the recipient group of ewes that received progesterone (Kleeman et al., 1994). Kleeman et al. (1994) suggested that progesterone supplementation during the first three days of pregnancy might enhance the growth of surviving fetuses.

Low concentrations of progesterone might occur with increases in metabolism as a result of increased feeding, frequency of feeding, or diet composition (Parr et al., 1982; Rabiee, 2000), because blood flow to the liver of ewes increased with feeding (Bensadoun and Reid, 1967). Therefore, variations in peripheral blood progesterone might be due to differences in metabolism of individual sheep.

The only hint that concentrations of estradiol might affect pregnancy loss beyond day 25 was that more complete fetal losses occurred in ewes with low concentrations of estradiol on day 65 of gestation. Placental estrogens may be involved in angiogenesis. Estrogen binding to its receptors within the vascular smooth muscle in endometrial arterioles and glandular epithelium might regulate VEGF and bFGF activity (Culinan-Bove and Koos, 1993), affecting subsequent angiogenesis in the placenta during gestation. Low concentrations of estradiol on day 65 could be associated with placental function. On approximately day 70 of gestation, proliferation of blood vessels at the maternal-fetal attachment site is nearly complete. If concentrations of estradiol are low on day 65, then placental function might be decreased and not capable of meeting the demands for the growing fetus.

Partial losses from day 65 to term decreased with increasing concentrations of VEGF on day 45. Proper thresholds of VEGF would allow sufficient angiogenesis during fetal development. When concentrations of VEGF increase, the concentration gradient of VEGF stimulates growth of new blood vessels for tissues producing VEGF (Newfeld et al., 1999). Low concentrations

might result in reduced angiogenesis, possibly affecting the growth and development of one fetus of a multiple pregnancy.

Partial losses were lowest in ewes with high concentrations of estradiol on day 65 of gestation, whereas the majority of partial losses occurred in ewes with medium and low concentrations of estradiol. Ford (1995) observed a decrease in estradiol from day 46 to 55 followed by an increase around mid-gestation. Given estradiol's indirect role in angiogenesis, lower concentrations of estradiol on day 65 might result in limited angiogenesis, affecting placental function, and result in the death of one fetus of a multiple pregnancy. Alternatively, low concentrations of estradiol might simply reflect some event associated with partial loss.

Many factors play a role in embryonic and fetal loss in the ewe. Bolet (1986) suggested these losses are due to one of three components: a) the male by the "quality" of semen, b) the female by the "quality" of the ova and uterine environment, or c) the embryo itself. Losses from breeding to term include fertilization failure and early embryonic mortality. Many factors contributing to early embryonic losses are due to an abnormal embryo, abnormal uterine environment, or asynchrony between the uterine environment and embryo (Wilmut et al., 1986). According to Wilmut et al. (1986) "completion of a successful pregnancy depends upon a large number of mechanisms operating successfully ". Thus is the case with losses occurring during late embryonic and fetal development.

Using the same approach as McMillan et al. (1998) to estimate embryo the roles of the embryo and recipient in embryo survival in embryo transfer

studies in cattle, the ewe is predicted to have a far lesser role in contributing to mortality after day 25 of gestation as compared to the conceptus (Table 20).

Table 20. **Expected numbers of ewes with either 0 or 1 lambs born in ewes with a single pregnancy or 0, 1, or 2 lambs born in ewes with a multiple pregnancy.**

Number of lambs born/ewe	Number of lambs born / ewe: Single Pregnancy observed	Single Pregnancy expected	Multiple Pregnancy observed	Multiple Pregnancy expected
0	38	$(1-c*d)N_1 = 48$	19	$(1-2*c*d + c^2*d)N_2 = 41$
1	204	$c*d*N_1 = 242$	163	$2*c(1-c)d*N_2 = 185$
2	-----	-----	415	$c^2*d*N_2 = 370$
total	242	236	597	596

c = 0.81 (potential conceptus survival rate = number of lambs born / number of conceptuses present on day 25)

d =0 .97 (potential pregnancy rate = potential number of dams with 0 or 1 lambs born / number of dams pregnant on day 25)

$N_1 = 242$ or $N_2 = 597$ (actual number of dams pregnant with one or two conceptuses on day 25)

The variables *c* and *d* represented the intrinsic potential of the conceptus to survive and the dam to lamb, respectively. According to the model, 81% of conceptuses have the potential to survive from day 25 to term, while 97% of all ewes have the potential to carry a pregnancy to term.

In summary, losses of embryos and fetuses occurred throughout pregnancy and overall rate varied with type of loss. Late embryonic and fetal losses occurred at similar rates in the anestrous, transitional, and late breeding

seasons and were not associated with heat stress. Proportion of embryos r fetuses lost varied with breed-type of ewe. Concentrations of progesterone on day 25 and 45 might be predictive of complete losses. Concentrations of estradiol were not of predictive value and variation in concentrations might be a result of other factors associated with late embryonic and fetal loss rather than a direct cause. The survival of individuals within a litter might be related to a role of VEGF in placentation. The conceptus seems to be playing a larger role in late embryonic and fetal losses from day 25 to term and might explain the lack of predictability of the concentrations of steroids and VEGF. The interplay of these factors in late embryonic and fetal losses is worthy of further investigation.

EFFECTS OF ALLANTOIS EXPANSION ON DAY 18 OF GESATION ON LATE EMBRYONIC AND FETAL MORTALITY AND EFFECTS OF LOCATION OF CORPORA LUTEA ON ALLANTOIS EXPANSION ON DAY 18 OF GESTATION

INTRODUCTION

Embryonic and fetal losses are a major cause of economic loss in the sheep industry. Bolet (1986) estimated embryonic and fetal losses at approximately 30% comparing the number of offspring born at term to fertilized ova. Quinlivan et al. (1966) observed that the majority of pregnancy loss in the ewe occured before day 18 of gestation. Embryonic mortality after day 18 was thought to occur during the early post-implantation period, on approximately day 20 (Moor and Rowson, 1966), while losses after this period were minimal. Factors associated with late embryonic and fetal losses are currently unknown.

Embryonic development in the sheep leads to a growing filiform trophoblastic vessel by day 11 of gestation (Rowson and Moor, 1966), which fills one-half of the length of the uterine horn by day 17 of gestation (Green and Winters, 1946; Chang and Rowson, 1965; Rowson and Moor, 1966; Bindon, 1971). Once elongation of the trophoblast has begun, the mesodermal layer begins to develop from endoderm of the embryonic disc to form a thin sheet of tissue between the ectoderm and the underlying endoderm (Perry, 1981). The mesoderm eventually splits, forming two layers, one overlying the endoderm (somatopleure), and the other underlying the ectoderm of the embryonic disc (splanchnopleure). The somatopleure and the outer trophoblastic layer eventually fuse to form the outermost extra-embryonic layer, the chorion. The inner sphlanchnopleure, along with the yolk-sac, form blood islands that eventually differentiate into a rudimentary heart and blood vessels by approximately day 18 of gestation (Perry, 1981). It is at this stage that the

allantois begins to expand and fuses with the chorion by approximately day 20. Dr. James Peterson (personal communication) observed that the degree of expansion of the allantois varied in embryos flushed from the uterus on day 18. He proposed that if expansion of the allantois does not occur on day 18, then development of the chorio-allantoic placenta might be compromised, leading to subsequent mortality.

There is evidence that side of ovulation might affect embryonic survival. In the cow, the right ovary ovulates more frequently and has been termed more functional (Reece and Turner, 1938). The first ovulation in post-partum cows occurred on the ovary ipsilateral to the previously gravid uterine horn in approximately 40% of cows (Foote and Peterson, 1968; Graves, 1968). Bridges et al. (2000) noted that conception rates were not reduced when ovulation occurred from the ovary ipsilateral to the previously gravid uterine horn and that conception rates tended to be greater after ovulation from the ovary on the ipsilateral side. Conception rate tended to be greater when post-partum cows ovulated from the right ovary (Bridges et al., 2000). Single pregnancies were more likely to fail when embryos were transferred to the uterine horn opposite the CL (Newcomb and Rowson, 1976; Sreenan, 1976; Christie et al., 1979; Newcomb et al., 1980). Likewise, when bovine embryos were transferred into separate uterine horns, embryonic death was greater in the horn opposite the CL (Del Campo and Ginther, 1973; Ginther, 1974; Ford et al., 1976). In the ewe, Casida et al. (1966) noted that the majority of corpora lutea were located in the

right ovary, regardless of single or multiple pregnancies. Effect of the location of the CL on allantois expansion on day 18 or 19 is unknown.

The objectives of the current study were to determine the effect of timing of allantois expansion on late embryonic and fetal mortality and if the location of CL in single or multiple pregnancies affected allantois expansion on day 18 of gestation.

MATERIALS AND METHODS

In September 2001 (N = 45) and October 2002 (N = 38), non-lactating ewes of mixed breeding (including Suffolk, Border Leicester, and Dorset) were assigned at random into two breeding groups, with a ewe:ram ratio not exceeding 1:45 for the first 60 days of breeding. Rams wore harnesses with painted crayons to determine date of breeding.

Pregnancy diagnosis, counts of embryos, and observation for expansion of the allantoic membrane were done with ultrasonography using an Aloka 500 (Corometrics Medical Systems, Wallingford, CT) with a 7.5 mHz linear transrectal probe on days 18 and 19 after mating. Lambing dates and numbers of lambs born were recorded. A total of 39 ewes were diagnosed pregnant to the first service on day 18. First service ewes were observed only in the current study.

Statistical Analysis

Ewes studied were pregnant with one (single pregnancy) or multiple embryos (multiple pregnancy) on day 18. In single pregnancies, the allantois was or was not expanded on day 18 (by day 19 the allantois was expanded in all but one case). In multiple pregnancies, one, two, or three or more of the embryos had an expanded allantois by day 18. They were classified in to two groups, all expanded or (at least) one not expanded. Expansion was underway on day 19 in all embryos. One-way analysis of variance was conducted using GLM procedures of SAS (SAS Inst. Inc. Cary, NC) to determine the effects of allantois expansion on day 18 on the loss of embryos from day 18 to term, the

percentage of ewes pregnant on day 18 that lambed, number of embryos per ewe pregnant on day 18, lambs born per ewe pregnant on day 18, and the number of lambs born per ewe lambing. Effects of location of the corpus luteum of pregnancy (right, left, or both ovaries) on embryos with expanded or non-expanded allantois were determined within ewes pregnant with single or multiple embryos.

RESULTS

In ewes pregnant to the first service, 58% of embryos were retained from day 18 to term. The number of embryos per ewe and lambs born per ewe pregnant on day 18 were 1.67 ± 0.02 and 1.14 ± 0.04, respectively, for those containing embryos with an expanded allantois (Figure 8) and 1.73 ± 0.04 and 1.21± 0.08, respectively, and for those with one embryo that had a non-expanded allantois (Table 21, Figure 9). Number of lambs born per ewe lambing tended to be greater (P = 0.06) in ewes with one embryo with non-expanded allantois (2.13 ± 0.04) than those with embryos with expanded allantois (1.67 ± 0.04) on day 18 of gestation (Table 21). There were no ewes with a multiple pregnancy in which both embryos had failed to undergo allantoic expansion on day 18. One embryo in a single pregnancy failed to undergo allantois expansion by day 19. All others had embryos with expanded allantois on day 19 (Figure 10). The ewe pregnant with this single embryo was observed on day 20 and the embryo was absent.

Table 21. **CHARACTERIZATION OF EWES WITH EXPANDED ALLANTOIS IN ALL EMBRYOS OR AT LEAST ONE EMBRYO WITH NON-EXPANDED ALLANTOIS**

Response variables	All embryos expanded d18	At least one embryo non-expanded d18
Total ewes (N)	61.5% (24/39)	38.5% (15/39)
% ewes lambing preg d18*	68.7% (15/22)	57.1% (8/14)
% embryos retained d18 to term	59.0%	57.2%
No. embryos per ewe preg d18	1.67 ± 0.02	1.73 ± 0.04
No. lambs born per ewe preg d18	1.14 ± 0.04	1.21 ± 0.08
No. lambs born per ewe lambing	1.67 ± 0.04[a]	2.13 ± 0.04[b]

* 3 ewes were missing lambing data
[a,b] Values with different superscripts within a row tended to differ (P = 0.06).

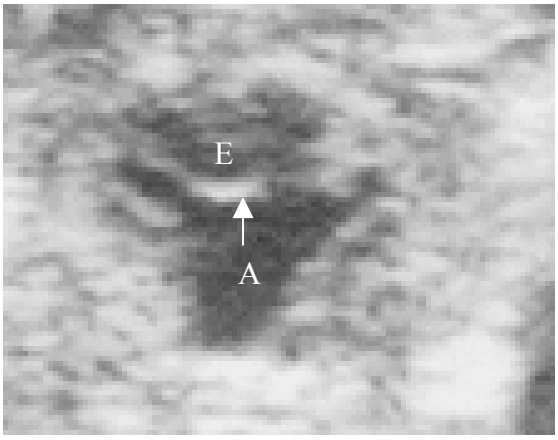

Figure 8. Expanded allantois (A) of an embryo (E) on day 18 of gestation.

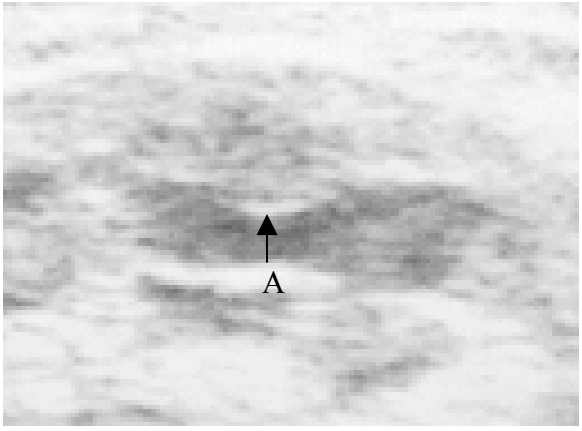

Figure 9. Non-expanded allantois (A) on day 18 of gestation.

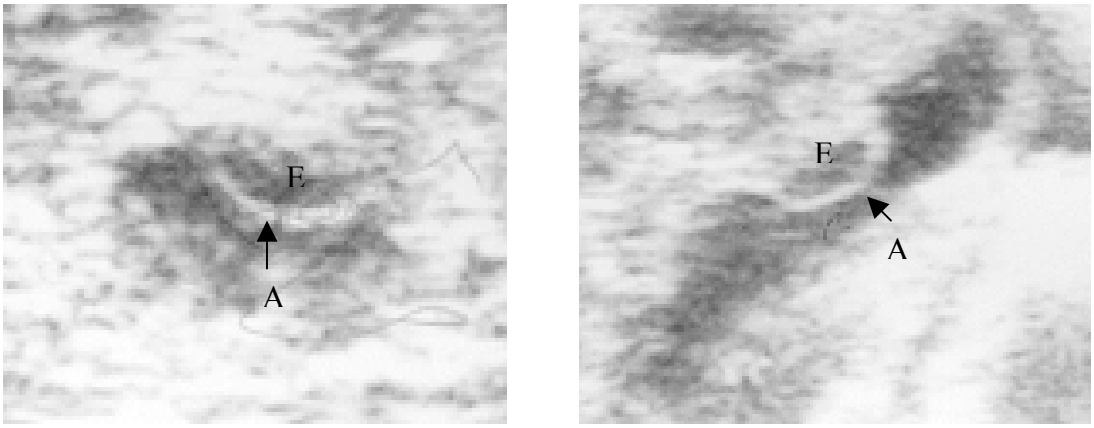

Figure 10. Expanded allantoic membrane (A) of an embryo (E) on day 19 of gestation.

A greater proportion of ewes pregnant with single embryos failed to lamb (84.5%) than ewes with multiple offspring (13%; $P < 0.05$; Table 22). A higher proportion of embryos were as single embryos (84.6%) than as embryos within a multiple pregnancy (20%; $P<0.05$; Table 22). A greater number of pregnancies were lost when ewes had an embryo that failed to undergo allantoic expansion (50%) than when all embryos within a ewe had an expanded allantois on day 18 of gestation (31.8%; $P <0.05$; Table 22). Numbers of embryos that were lost from day 18 to term averaged 34.5% and did not differ between ewes in which all embryos had an expanded allantois and those in which one embryo had a non-expanded allantois (Table 22).

Location of corpora lutea

In ewes that had CL located in the right ovary only, the proportion of pregnancies (32.4%) and embryos (34.0%) lost from day 18 to term did not differ. In ewes with CL in the left ovary only, 60% of ewes had lost the entire pregnancy, while 71% of all embryos were lost from day 18 to term. In ewes with CL in both ovaries, 21.4% of pregnancies failed and 16.7% of all embryos were lost from day 18 to term (Table 22). These values did not differ by location group for either ewes or embryos. When all ewes with at least one CL in the right ovary (31% of pregnancies and 29% of embryos lost) were compared to all ewes with no CL in the right ovary (71% of pregnancies and 60% of embryos lost), survival was improved by the presence of CL on the right ovary. However, due to small numbers of ewes and embryos, these differences were not significant.

Single pregnancies When corpora lutea were located in the right ovary, approximately 67% of ewes had embryos with expanded allantois, while 33% had an embryo with a non-expanded allantois (Table 22). In contrast, when CL were located in the left ovary, 25% of pregnant ewes had embryos with an expanded allantois and 75% of ewes had an embryo with a non-expanded allantois (Table 22). Regardless of allantoic expansion, ewes with CL on the right ovaries (78%) had similar percentages of pregnancy loss to those with CL located on the left ovary (100%).

Multiple pregnancies Approximately 71% of ewes had all embryos with an expanded allantois when CL were located in the right ovaries of ewes compared to 33% when CL were in the left ovary and 67% when there were CL in both ovaries (Table 22). Regardless of allantoic expansion, ewes with multiple CL located on the left ovary only had the greatest amount of pregnancy loss (33%), while ewes with CL located on the right side only had the least amount of pregnancy loss (7%). Similarly, ewes with CL located on the left ovary only had the greatest amount of loss (33%), while those with CL on the right ovary only or both ovaries averaged 20%. With the small number of animals per subgroup, differences were not significantly significant.

Table 22. **EFFECTS OF SINGLE OR MULTIPLE PREGNANCY AND LOCATION OF CORPORA LUTEA ON EXPANSION OF THE ALLANTOIS ON DAY 18 AND PREGNANCY LOSS TO TERM ***

Type of Pregnancy	Location of CL	Proportion (%) Expanded D18	Proportion (%) Non-Expanded D18	No. Embryos with expanded allantois	No. Ewes	Proportion Pregnancies Lost D18-Term	Proportion Embryos Lost D18-term
Single Pregnancy	RO	67	33**	1	6	4/6	4/6
				0**	3	3/3	3/3
	LO	25	75	1	1	1/1	1/1
				0	3	3/3	3/3
				Total	*13*	*11/13*	*11/13*
Multiple Pregnancy	RO	71	29	2	10	1/10	5/20
				1	4	0/4	0/8
	LO	33	67	2	1	0/1	0/2
				1	2	1/2	2/4
	Both	67	33	2-3	4	1/4	3/9
				1	2	0/2	0/5
				Total	*23*	*3/23*	*10/48*
				All Expanded on d18	22	7/22	13/38
				One Non-Expanded on d18 (expanded d19)	13	6/13	7/22
				One Non-Expanded d18 and 19	1	1/1	1/1
				Total	*36*	*14/36*	*21/61*

SUMMARY

	Pregnancies Lost	Embryos Lost
Single Preg	84.5[a]	84.6[a]
Multiple Preg	13.0[b]	20.0[b]
Expanded	31.8[a]	34.2
Non-Expanded	50.0[b]	34.7
Right Ovary	32.4	34.0
Left Ovary	60.0	71.0
Both Ovaries	21.4	16.7

[a,b] Different letters in each column differ ($P < 0.05$)

* Data were calculated for ewes with lambing data only

** In one ewe, the allantois failed to expand on day 19. In all other ewes in all groups, allantoic expansion had occurred for all embryos by day 19.

DISCUSSION

In the current study, a greater proportion of pregnancies was lost in ewes with embryos that failed to undergo allantoic expansion on day 18. Even though more ewes had embryos that had undergone allantoic expansion on day 18, the number of embryos on day 18 per ewe did not differ between groups. In contrast, the number of lambs born per ewe lambing was greater in ewes with embryos that failed to expand on day 18. This was due to the fact that all single pregnancies with non-expanded embryos were lost before term. Similar percentages of embryos with an expanded allantois were lost in single pregnancies, thus, allantois expansion might not influence an embryo's ability to survive, if a member of a single pregnancy.

In the current study, a greater number of offspring were lost in ewes carrying single embryos. Losses of all embryos or fetuses within a multiple pregnancy were minimal. This is in contrast with observations made by Dixon et al. (2002), in which the majority of losses occurred in ewes losing one, but not all embryos or fetuses, followed by the complete loss of a single pregnancy. In that study, late embryonic and fetal losses in the ewe from day 25 to term were continuous throughout gestation (Dixon et al., 2002), with approximately 3% of embryos present on day 25 lost during each 20-day interval of pregnancy beyond that point to term. About 21% of ewes had complete loss of a single pregnancy from day 25 to term. The high rate of losses of single pregnancies in the current study might be due to the fact that ewes in the current study were members of a flock that has been selected for high twinning rates.

In the present study, more corpora lutea were located in the right ovary of ewes with a single ovulation and those with multiple ovulations, which agrees with those observed by Casida et al., (1966). In both single and multiple pregnancies, ewes with CL located in the left ovary had a numerically greater proportion of pregnancies that were lost, as well as embryos that failed to survive to term. However, with small numbers of animals observed, differences were not significant. Ewes with the CL located in the right ovary only had the greatest percentage of embryos that were expanded on day 18, as compared to ewes with CL on the left only, or both ovaries.

Several authors have suggested that the location of the CL of pregnancy effected embryonic mortality. In the cow, the right ovary ovulates more frequently and has been termed more functional (Reece and Turner, 1938). Gunn et al. (1972) and Doney et al. (1973) suggested that embryonic mortality to day 18 was less when ovulation occurred from both ovaries rather than from one ovary only. When comparing location of the CL to lambs born, Kelly and Allison (1979) observed results similar to the current study. Ewes with a single CL in the right or left ovary had a similar proportion that lambed. Ewes with two CL in the right ovary had a tendency to have a higher percentage that lambed than those with CL in the left ovary (Kelly and Allison, 1979).

Location of the CL in relation to the location of the embryo within the uterus influenced embryonic survival. Studies in which embryos were transferred either ipsilateral or contralateral to the CL of pregnancy, single pregnancies were more likely to fail when embryos were transferred to the uterine horn opposite the

CL (Newcomb and Rowson, 1976; Sreenan, 1976; Christie et al., 1979; Newcomb et al., 1980). Likewise, when bovine embryos were transferred into separate uterine horns, embryonic death was greater in the horn opposite the CL (Del Campo and Ginther, 1973; Ginther, 1974; Ford et al., 1976). Location of the embryo within the right or the left uterine horn in comparison to the location of the CL was not determined in the present study.

Even though the numbers were low, all ewes with a single pregnancy in which the embryo had a non-expanded allantois on day 18 failed to lamb. There might be an advantage for a non-expanded embryo to be within a multiple pregnancy, in that other embryos might "protect" the one embryo that failed to expand. Asynchrony between the uterus and embryo might be the result of late ovulating ova, a delay in fertilization, or slow cleavage (Gates, 1965; McLaren, 1982) and might result in the retardation of growth of one embryo in a multiple pregnancy.

Interferon-tau is secreted by the ovine conceptus beginning on approximately day 10 of pregnancy and secretion increases as morphological changes occur on day 12 and 13 (Bazer et al., 1997) and must produce sufficient quantities by day 16 to prevent luteolysis. Secretion of the protein continues until day 21 in the ewe (Godkin et al., 1984).

If one embryo within a multiple pregnancy is growing at a faster rate than the other, then the faster embryo might produce enough IFN-tau to sustain development of the slower growing embryo throughout maternal recognition of pregnancy, attachment, or initial placental development. Administration of

interferons during maternal recognition of pregnancy improved pregnancy rate at day 45 and 55 of gestation and there was a tendency for interferon treatment to increase the number of ewes with a single CL that carried a lamb to term (Schalue-Francis et al., 1991).

In conclusion, allantois expansion on day 18 might be a contributing factor to pregnancy loss. Regardless of type of pregnancy, most corpora lutea were located on the right ovary. How allantois expansion on day 18 of gestation is affected by the location of CL warrants further study.

GENERAL DISCUSSION

In the first study, patterns of late embryonic and fetal loss were consistent from day 25 to term. More ewes experienced the loss of one, but not all embryos or fetuses, followed by the complete loss of a single pregnancy, and a minimal number of ewes experienced multiple complete losses. In the second study, more ewes lost a single pregnancy. The proportion of embryos or fetuses lost from day 18 to term was much higher in ewes with single embryos than in those with multiple embryos or fetuses. These results were surprising, given the fact that in the first study, more ewes experienced multiple partial than complete loss. Ewes in the second study were members of flock that was selected for 38 years for high twinning rates, whereas ewes in the first study were in flocks that might have had a variety of selection criteria and often included purchased ewes.

Observations in the first study might represent a pattern of loss that is seen for a general population of ewes. The number of ewes that were pregnant at initial pregnancy diagnosis in the first study was large and represented 12 different flocks of sheep in 4 different states. Ewes were exposed to a variety of weather patterns, living conditions, etc. and consisted of 3 main breed types. Ewes in the second study were from one flock and exposed to similar living conditions, weather patterns, etc. They were selected to remain in the flock because they were members of a multiple pregnancy or produced multiple lambs in past pregnancies. Thus, the patterns of loss observed in the second study might be unique to the particular flock of ewes. Patterns of loss for each

individual farm in the first study varied considerably. One farm might have a larger percentage of ewes with one type of loss than ewes from other farms.

Ewes in the second study that were pregnant with multiple embryos on day 18 had a smaller amount of partial loss than those from the first study (21% vs 36%). Ewes in the second flock might be more capable of carrying multiple embryos or fetuses than those of the first study due to genetic selection.

The loss of single pregnancies of ewes in the second study might be an example of how genetics might play a role in late embryonic or fetal losses. Black-faced ewes of the first study had the greatest amount of embryos and fetuses lost from day 25 to term compared to white- or mottled-faced ewes. Mottled-faced ewes, especially from day 85 to term, had the lowest proportion of losses compared to black-or white-faced ewes. The conceptus was thought to play a larger role in affecting mortality from day 25 to term rather than the dam. Embryos or fetuses of different breeds might differ in their competency to survive due to differences in genetics. Black-faced ewes in the first study had lower concentrations of progesterone on all days examined compared to white- and mottled-faced ewes. This might be an example where one breed might be at a disadvantage over another to produce viable numbers of offspring, especially during out-of-season breeding.

Selection for twinning might affect the competency of the conceptus to survive. Multiple pregnancies in the first study were more likely to survive than single pregnancies, suggesting that a multiple pregnancy has a “protective” effect on survival. Embryos within ewes that are selected for twinning might require

more interferon-tau than single embryos. The presence of multiple embryos might produce greater concentrations of progesterone that aid in conceptus survival.

In conclusion, patterns of loss within a general population of ewes seem to suggest that most ewes experience the loss of one, but not all, embryos or fetuses. Breed differences in proportions of embryos and fetuses lost and concentrations of steroids and VEGF as well as differences in patterns of loss between the first and second study might mean that genetics plays a large role in late embryonic and fetal losses. How breed differences and selection of ewes within breed for certain traits affect the roles of the dam and conceptus in contributing to late embryonic and fetal losses remains unknown.

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VITA

Name:	Alison Brown Dixon
Parents:	Jerry B. Brown Gail R. Brown
Date of Birth:	April 7, 1974
Place of Birth:	Kingsport, Tennessee

Schools Attended:

Dobyns-Bennett High School Kingsport, Tennessee	1988-1992
University of Tennessee Knoxville, Tennessee B.S. in Animal Science/ Minor in Biology	1992-1996
University of Tennessee Knoxville, Tennessee M.S. in Curriculum and Instruction	1996-1997
University of Tennessee Knoxville, Tennessee M.S. in Reproductive Physiology	1998-2000