Patterns of late embryonic and fetal mortality and association with several factors in sheep

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ABSTRACT: Embryonic and fetal mortality reduce lambing rates and litter sizes, thus contributing to economic losses in the sheep industry. In the current study, the timing of late embryonic and fetal loss in ewes and the factors with which these losses were associated were examined. Ewes lambing and lambs born were compared with pregnancy diagnosis and counts of embryos by ultrasonography near d 25, 45, 65, or 85 of gestation. Approximately 19.9% of the ewes experienced late embryonic loss, fetal loss, or both; and 21.2% of the embryos or fetuses were lost from d 25 to term. Potential offspring were lost throughout gestation; 3.7% of embryos from d 25 to 45, 4.3% of fetuses from d 45 to 65, 3.3% from d 65 to 85, and 11.5% from d 85 to parturition; thus, approximately 3 to 4% of the potential offspring were lost for each 20-d period of pregnancy beyond d 25. A greater proportion of ewes lost one (36.7%) rather than all (20.5% single; 3.8% multiple) embryos or fetuses. The patterns of loss were similar in ewes mated during the anestrous season and the transitional period and did not vary with service period within breeding season or method of synchronization of estrus. Late embryonic or fetal losses were not related to the temperature-humidity index. Maternal serum collected near d 25, 45, 65, or 85 of gestation was assayed for concentrations of progesterone, estradiol-17β, and vascular endothelial growth factor (VEGF). The proportions of embryos or fetuses lost were associated with breed type (P < 0.05), as were concentrations of progesterone (P < 0.01), estradiol (P < 0.05), and VEGF (P < 0.01). The relationships of loss or retention of pregnancy to hormonal variables at the 4 stages studied were limited. Complete and partial losses increased rapidly as maternal progesterone at d 25 decreased below 2 ng/mL (P < 0.05). Survival of fetuses within a litter from d 25 to 65 was greater for ewes with medium concentrations of VEGF near d 25 and from d 65 to parturition was greater for ewes with high concentrations of VEGF near d 45 (P < 0.05). In summary, late embryonic or fetal losses occurred from d 25 throughout gestation and varied with breed type and with concentrations of progesterone in maternal serum on d 25.

Key words: breed type, embryo, ewe, fetus, mortality, pregnancy

INTRODUCTION

In sheep, embryonic and fetal mortality contribute to a large economic loss. Estimates of embryonic and fetal loss have averaged approximately 30% (Bolet, 1986). Most embryonic loss has been reported to occur before d 18 (Hulet et al., 1956; Moore et al., 1960; Quinlivan, 1966). Complete losses from d 18 to lambing were estimated at 9.4% (Hulet et al., 1956), and late embryonic or fetal losses from d 30 to term were only 1 to 5% (Quinlivan, 1966). Losses increased with increasing ovulation rate (Quinlivan, 1966; Knights et al., 2003; Kleemann and Walker, 2005b).

In pregnancies with multiple embryos or fetuses, individual potential offspring can be lost without a total loss of the pregnancy (Henning, 1939; Rhind et al., 1980; Schrick and Inskeep, 1993). Of ewes with twin ovulations that did not return to estrus before d 18, 47 to 50% retained 2 live embryos, 43 to 47% had only 1 live embryo, and 2 to 6% had no live embryos when

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slaughtered at 18, 30, or 140 d of pregnancy (Quinlivan, 1966). Embryonic mortality early in the breeding season (Dutt, 1954; Hulet et al., 1956) was attributed to high ambient temperatures (90°F) within 3 d after the onset of estrus (Alliston and Ulberg, 1961). Lunstra and Christensen (1981) observed fewer ewes lambing, Knights et al. (2001a,b) observed a greater loss of pregnancy after d 26, and Quinlivan et al. (1996) saw more CL not represented by live lambs born (48 vs. 27%) in ewes that conceived from second compared with first service during an induced spring-summer breeding season.

Based on work in cattle, the concentrations of progesterone or estradiol during placentation might affect late embryonic and fetal survival (Bridges et al., 2000; Lopez-Gatius et al., 2004; Starbuck et al., 2004), perhaps affecting angiogenesis in the developing embryo or placenta, or both, by altering the levels of vascular endothelial growth factor (VEGF; Cullinan-Bove and Koos, 1993).

The objectives of the current study were to characterize the timing of late embryonic and fetal losses and to identify the factors with which these losses were associated in ewes.

**MATERIALS AND METHODS**

**Animals and Induction and Synchronization of Estrus**

All procedures with animals were approved by the West Virginia Animal Care and Use Committee.

**Group 1.** A total of 916 nonlactating ewes of mixed breeding (mainly Dorset and Suffolk) from 7 commercial farms were bred in early May and June (anestrus) or late July, August, and September (transition) of 2000. During anestrus, estrus was induced and synchronized for 4 flocks by controlled internal drug releasing devices (CIDR-G, containing 300 mg of progesterone, InterAg, Hamilton, New Zealand). A CIDR-G was inserted intravaginally for 5 d 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 h before CIDR withdrawal/ram introduction. In the transition season, ewes in 3 flocks (n = 292) received 25 mg of progesterone i.m. (Aldrich Chemical Company, Milwaukee, WI) at ram introduction, followed by 20 mg of PGF2α, (Lutalyse, Pfizer Animal Health, New York, NY) i.m. 14 d later. Ewes in 1 flock (n = 150) received a CIDR-G for 5 d, followed by an injection of 20 mg PGF2α, at insert removal/ram introduction or PGF2α, only at ram introduction. In all cases, the rams remained with the ewes for 26 to 30 d after ram introduction or, in the transition season, treatment with PGF2α, allowing the ewes 2 opportunities to conceive (during the first 3 to 4 d or 1 cycle later, designated as service periods 1 and 2). A total of 692 ewes (76%) were diagnosed as pregnant on approximately d 25 after the first (n = 220 in anestrus and 232 in transition) or second (n = 132 in anestrus and 108 in transition) service period.

**Group 2.** In May and June of 2001, 459 ewes of mixed breeding (mainly Dorset and Suffolk) in 4 flocks received an i.m. injection of 25 mg of progesterone at ram introduction, followed by an i.m. injection of 20 mg of PGF2α, 14 d later. Rams remained with the ewes for 26 to 30 d after ram introduction (d 0). A total of 264 ewes (58%) were diagnosed as pregnant on approximately d 25 after the first (n = 159) or second (n = 105) service period. The second service period extended into early July in some flocks.

**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 at approximately d 25 to 30 after breeding, as described by Schrick and Inskeep (1993). In most flocks, a second observation was made on approximately d 45 to 50, which allowed diagnosis of pregnancy to a second service at approximately 30 d after that mating. An Oviscan 4 (BCF Technology, Ltd., Livingston, UK) with a 3.5-mHz transabdominal sector probe was used to recheck pregnancy and recount fetuses on d 45 to 50, 65 to 70, or 85 to 90 of gestation. For simplicity, dates of pregnancy diagnosis are hereafter referred to as d 25, 45, 65, and 85. Late embryonic or fetal mortality was determined from these counts and the numbers of lambs born.

**Determination of Temperature-Humidity Indices**

Daily means for ambient temperature and percent relative humidity were collected from the National Weather Service Station nearest each farm in group 1 during the months of May, June, July, August, and September of 2000. Daily values were determined within farm (n = 7) during each of 6 intervals before and during gestation [d −7 to 0 (breeding), 0 to 7, 0 to 25, 25 to 45, 45 to 65, and 65 to 85]. A temperature-humidity index (THI) for each day in each interval was determined for each farm in group 1. The index was determined from the mean ambient temperatures and percent relative humidity per farm using a livestock THI chart (Smith et al., 1998). Based on the range and frequency of values during an interval, each farm was classified into 1 of 3 categories (index scores; THI < 72 = 0, THI 72 to 74 = 1, THI > 74 = 2) for each of the 6 intervals. Thus, an index score of 0 represented no heat stress, and scores of 1 and 2 represented increasing possibilities of exposure to heat stress.

**Blood Collection and Hormone Assays**

A blood sample (5 mL) was collected by jugular venipuncture at each pregnancy diagnosis for ewes on most
of the farms in groups 1 and 2. After collection, the samples were placed on ice, transported to the laboratory, refrigerated at 4°C for 12 to 24 h, and then centrifuged for 20 min at 2,500 × g. Serum was harvested and frozen at −20°C, until concentrations of progesterone were determined by RIA (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 RIA. The intra- and interassay CV were 9.7 and 15.3%, respectively, for the progesterone ELISA, and 8.6 and 13.4%, respectively, for the progesterone RIA, and 7.0 and 14.1%, respectively, for estradiol. The intraassay CV for the single assay for VEGF (group 1 samples in 3 flocks at d 25, 5 flocks at d 45, and 6 flocks at d 65) was 13%. The sensitivities of the assays were 0.10 ng/mL, 0.33 ng/mL, 0.2 pg/mL, and 25 pg/mL for the progesterone RIA, progesterone ELISA, and VEGF RIA, respectively.

**Analyses on a Flock Basis Using Weighted Means of Fetal Losses**

**Percentages of Ewes Experiencing Late Embryonic (d 25 to 45) or Fetal (After d 45) Loss.** Stages of pregnancy, based on each interval and each combination of consecutive intervals among days of pregnancy diagnosis and parturition (e.g., d 25 to 65, d 45 to parturition), were examined for patterns of embryonic (d 25 to 45) and fetal (after d 45) loss. 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 embryo of fetus at examination that subsequently lost the pregnancy were classified as single complete losses. Ewes with multiple embryos or fetuses that lost all of them were classified as multiple complete losses; those that lost at least 1, but not all, embryos or fetuses were termed multiple partial losses; some ewes lost no potential offspring after diagnosis.

The percentages of ewes that experienced each type of loss were determined for each season (anestrus, transitional) and service period (first or second) for all possible intervals based on the combinations of days of pregnancy diagnosis and parturition. The exact numbers of ewes sampled for each interval studied are shown in Table 1. Not all stages were sampled on all farms or in both groups, and once a ewe had lost a complete pregnancy, she was not sampled on subsequent occasions. Therefore, estimates of embryonic loss from d 25 to 45 were actual values, but the cumulative proportions of ewes that lost fetuses during each stage beyond d 45 and the total number of ewes examined per stage were used to construct weighted means representing loss of pregnancy up to each stage of pregnancy diagnosis (d 65, d 85, and parturition) on each farm. Weighted means were calculated as illustrated in Table 2. The results were the weighted means of the cumulative percentages of animals that experienced each type of pregnancy loss for each stage between pregnancy diagnoses or between pregnancy diagnosis and parturition. The relationship between the proportions of ewes with loss and the stage of pregnancy was examined by linear regression, and the regressions were compared among types of loss (single complete, multiple partial, multiple complete).

**Effects of Season, Service Period, and Synchronization Regimen.** Analysis of variance using the GLM procedure (SAS Inst. Inc., Cary, NC) was used to determine the effects of season (anestrus or transitional), service period (first or second), synchronization regimen (CIDR + FSH, progesterone + PGF₂α, or CIDR + PGF₂α), and the interaction of these effects on the weighted mean proportions of ewes in groups 1 and 2 that experienced late embryonic or fetal loss. Group was not included as a variable, because group 2 contributed to only 1 season, and group was confounded with synchronization regimen.

**Effect of Temperature-Humidity Index As an Indicator of Heat Stress.** The proportion of pregnant ewes at the beginning of each stage that experienced late embryonic or fetal loss was calculated for each stage of pregnancy (approximately d 25 to 45, d 45 to 65, and each day to parturition) from the cumulative weighted means on each farm in group 1 for each type of loss. Effects of category of THI index score (0, 1, or 2), type of loss (single complete, multiple partial, multiple complete), and their interaction on the proportions of ewes with loss during each stage of pregnancy that followed or included the respective intervals for which the THI index score had been calculated were determined by ANOVA, using the GLM procedure of SAS.

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**Table 1. Ewes examined and embryos or fetuses counted at different stages of pregnancy in groups 1 and 2.**

<table>
<thead>
<tr>
<th>Stage of pregnancy</th>
<th>No. of ewes observed</th>
<th>No. of embryos or fetuses</th>
<th>Embryos or fetuses lost, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 25 to 45</td>
<td>702</td>
<td>1,263, 1,216</td>
<td>3.7</td>
</tr>
<tr>
<td>d 25 to 65</td>
<td>620</td>
<td>1,140, 1,048</td>
<td>8.1</td>
</tr>
<tr>
<td>d 25 to 85</td>
<td>174</td>
<td>318, 282</td>
<td>11.3</td>
</tr>
<tr>
<td>d 45 to 65</td>
<td>464</td>
<td>854, 817</td>
<td>4.3</td>
</tr>
<tr>
<td>d 45 to 85</td>
<td>84</td>
<td>161, 151</td>
<td>6.2</td>
</tr>
<tr>
<td>d 65 to 85</td>
<td>156</td>
<td>276, 267</td>
<td>3.3</td>
</tr>
<tr>
<td>d 85 to birth</td>
<td>173</td>
<td>295, 261</td>
<td>11.5</td>
</tr>
<tr>
<td>d 65 to birth</td>
<td>564</td>
<td>992, 860</td>
<td>13.3</td>
</tr>
<tr>
<td>d 45 to birth</td>
<td>643</td>
<td>1,136, 960</td>
<td>15.5</td>
</tr>
<tr>
<td>d 25 to birth</td>
<td>783</td>
<td>1,399, 1,125</td>
<td>19.6</td>
</tr>
</tbody>
</table>

1Group 1 consisted of 916 nonlactating ewes of mixed breeding (mainly Dorset and Suffolk) from 7 commercial farms and that were bred in early May and June (anestrus) or in late July, August, and September (transition) of 2000. Group 2 included 459 ewes of mixed breeding (mainly Dorset and Suffolk) in 4 flocks and that were bred in May and June of 2001.

2Days of initial and subsequent observations.

3Numbers of ewes pregnant at the initial day and observed at the subsequent day.
Late embryonic and fetal mortality in ewes

Table 2. Calculation of weighted means for cumulative losses for each type of fetal loss

<table>
<thead>
<tr>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>D 25 = 0% loss</td>
</tr>
<tr>
<td>D 45 value = actual embryonic loss from d 25 to 45</td>
</tr>
<tr>
<td>Cumulative embryonic and fetal loss to d 65 =</td>
</tr>
<tr>
<td>1. D 25 value + loss from d 25 to 65 x number animals for the interval = value 1</td>
</tr>
<tr>
<td>2. D 45 value + loss from d 45 to 65 x number animals for the interval = value 2</td>
</tr>
<tr>
<td>3. Sum of values 1 and 2/average number of animals</td>
</tr>
<tr>
<td>Cumulative embryonic and fetal loss to d 85 =</td>
</tr>
<tr>
<td>1. D 25 value + loss from d 25 to 85 x number animals for the interval = value 1</td>
</tr>
<tr>
<td>2. D 45 value + loss from d 45 to 85 x number animals for the interval = value 2</td>
</tr>
<tr>
<td>3. D 65 value + loss from d 65 to 85 x number animals for the interval = value 3</td>
</tr>
<tr>
<td>4. Sum of values 1, 2, and 3/average number of animals</td>
</tr>
<tr>
<td>Cumulative embryonic and fetal loss to term =</td>
</tr>
<tr>
<td>1. D 25 value + loss from d 25 to term x number animals for the interval = value 1</td>
</tr>
<tr>
<td>2. D 45 value + loss from d 45 to term x number animals for the interval = value 2</td>
</tr>
<tr>
<td>3. D 65 value + loss from d 65 to term x number animals for the interval = value 3</td>
</tr>
<tr>
<td>4. D 85 value + loss from d 85 to term x number animals for the interval = value 4</td>
</tr>
<tr>
<td>5. Sum of values 1, 2, 3, and 4/average number of animals</td>
</tr>
</tbody>
</table>

Analyses on an Individual Ewe or Embryo/Fetus Basis

Percentages of Late Embryonic (d 25 to 45) or Fetal (After d 45) Losses and Effects of Season, Service Period, Farm, and Synchronization Regimen. The data (Table 1) for the proportions of embryos or fetuses lost during each stage (e.g., d 25 to 45 or d 45 to 65) and each combination of stages (e.g., d 25 to 65 or d 45 to parturition) were examined by ANOVA using PROC GLM of SAS. The model included the effects of season, service period, synchronization regimen, and farm, and their interactions.

Effects of Face Color (Breed Type). Face color was recorded in 10 flocks in groups 1 and 2, as an indication of breed type. Analysis of variance using PROC GLM of SAS was used to determine the effects of face color of the ewe on the number of embryos at d 25 or fetuses at d 45, 65, or 85, and the proportions of embryos or fetuses lost during each stage of pregnancy and each possible combination of stages. Losses of embryos and fetuses were determined for ewes in groups 1 and 2 with face colors of black (mainly Suffolk), white (mainly Dorset), or mottled (crossbred). In separate analyses, ANOVA was used to test the effects of face color on the concentrations of progesterone and estradiol-17β on d 25, 45, 65, and 85 (10 flocks), and VEGF on d 25, 45, and 65 (3, 5, and 6 flocks, respectively; these flocks had very few black-faced ewes, so only white- and mottled-faced ewes were considered for VEGF). In each of these analyses, the model included the number of embryos or fetuses on the date of sampling.

Effects of Hormones. 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 single samples from each ewe in groups 1 and 2 (progesterone and estradiol-17β on d 25, 45, 65, or 85) or in group 1 (VEGF on d 25, 45, and 65). In one analysis, logistic regression (PROC LOG REG of SAS) was used to predict the percentages of ewes experiencing complete or partial loss during a particular stage of pregnancy, based on concentrations of hormones at an earlier day of pregnancy (d 25, 45, 65, or 85). In separate analyses, for each day of pregnancy sampled, the concentrations of progesterone, estradiol-17β, and VEGF were ranked from least to greatest, then divided into 3 classifications (low quartile, middle half, high quartile) and the effects of this classification on complete and partial losses of pregnancy beyond that interval of pregnancy were evaluated for each hormone by ANOVA using PROC GLM of SAS.

RESULTS

Timing of Late Embryonic and Fetal Losses During Anestrous and Transitional Seasons

Losses occurred at each stage of gestation evaluated; 3.8% of ewes lost 1 or more embryos from d 25 to 45, 6.2% lost 1 or more fetuses from d 45 to 65, 0.5% from d 65 to 85; and 9.4% from d 85 to parturition (Figure 1). Based on the regression of cumulative weighted means on stage of pregnancy, losses occurred at a linear rate (Figure 2). Cumulatively, a greater percentage of ewes lost 1 or more, but not all, embryos or fetuses from a multiple pregnancy during d 25 to parturition (36.7%) than completely lost a single pregnancy (20.5%) or a multiple pregnancy (3.8%; P < 0.05; Figure 2). Mean losses of embryos or fetuses averaged 3.7% of embryos from d 25 to 45, 4.3% of fetuses from d 45 to 65, 3.3% from d 65 to 85, and 11.5% from d 85 to parturition (Figure 1), thus approximately 3 to 4% for each 20 d of pregnancy beyond d 25.
Effects of Season, Service Period, Synchronization Regimen, and THI

There were no effects of season, service period, or method of synchronization or their interactions on proportions of ewes in groups 1 and 2 that experienced late embryonic or fetal mortality. Similarly, proportions of loss of embryos or fetuses did not differ with season, service period, synchronization regimen, or farm, or interactions among these factors.

Factors Associated with Face Color (Breed Type) in Relation to Numbers of Embryos or Fetuses

Average number of embryos present per pregnant ewe at d 25 was greater \((P < 0.05)\) in mottled-faced (1.91; \(n = 192\)) than in black-faced (1.73; \(n = 62\)) or white-faced ewes (1.79; \(n = 146\)). In ewes examined at d 45, mottled-faced (1.83; \(n = 150\)) and white-faced (1.81; \(n = 113\)) ewes had more fetuses than black-faced ewes (1.56; \(n = 82\)). At d 65, numbers of fetuses (1.56 in 32 black-faced ewes, 1.69 in 118 white-faced ewes, and 1.74 in 130 mottled-faced ewes) did not differ. In 80 ewes examined at d 85, there was an average of 1.72 fetuses and there was no effect of face color.

Losses of embryos from d 25, or fetuses from d 45 or 85 of pregnancy to parturition, were related to face color. Black-faced ewes had greater \((P < 0.05)\) loss of embryos or fetuses from d 25 to parturition (29.0% of 88 original embryos) than white-faced ewes (17.7% of 165) or black-faced ewes (19.2% of 305; pooled SEM ± 1.3) ewes. From d 45 to parturition, black-faced ewes had greater proportions of fetal loss (21.6% of 104 fetuses) than mottled-faced (13.3% of 275) or white-faced ewes (15% of 116; \(P < 0.05\); pooled SEM ± 1.3). Ewes with black face color lost a greater proportion of fetuses between d 85 and parturition (31.3% of 16) than white-faced (4.6% of 38) or mottled-faced ewes (15.8% of 46; \(P < 0.01\); pooled SEM ± 1.9).

Black-faced ewes had lower \((P < 0.01)\) concentrations of progesterone (ng/mL) on d 25 (2.2) and 45 (2.7; \(n = 34\) and 68, respectively) than those of white- (3.0 and
3.0, respectively; n = 75 and 76) or mottled-faced (3.3 and 3.8, respectively; n = 131 and 111) ewes (pooled SEM ± 0.1). Values on d 65 (overall mean 3.6; n = 198) and 85 (overall mean 4.2; n = 66) did not differ, but only 9 and 2 black-faced ewes were sampled on those days. Progesterone did not vary with number of embryos/fetuses or the interaction of number and face color at any day sampled. Values for 45 to 72 ewes with singles ranged from 3.0 to 3.8 and for 140 to 195 ewes with multiples from 3.1 to 3.5 ng/mL.

Concentrations of estradiol-17β (pg/mL) on d 25 were lower (P < 0.05) in white-faced ewes (3.6; n = 146) than in black-faced (4.0; n = 62) or mottled-faced ewes (4.3; n = 192), respectively (pooled SEM ± 0.2). On d 45 and 65, 151 and 128 mottled-faced ewes had greater (P < 0.01) concentrations of estradiol-17β (5.0 and 5.5, respectively) than 117 and 115 white-faced ewes (3.6 and 4.4, respectively, pooled SEM ± 0.2). Concentrations of estradiol-17β did not differ with face color on d 85 (n = 75; overall mean 6.1 pg/mL; pooled SEM ± 0.3). Estradiol-17β did not differ with number of embryos at d 25 or fetuses at d 45, 65, or 85, or the interaction of numbers with face color.

Concentrations of VEGF (ng/mL) were lower (P < 0.01) in mottled-faced ewes on d 25 (0.9, n = 78), 45 (1.0, n = 98), and 65 (0.9, n = 90) than in white-faced ewes (1.4, n = 42; 1.5, n = 59; 1.2, n = 59, respectively; pooled SEM ± 0.1). There were no differences between ewes carrying singles (1.0, n = 18 at d 25; 1.0, n = 28 at d 45; 1.1, n = 35 at d 65) and ewes carrying twins (1.1, n = 102; 1.2, n = 137; and 1.0, n = 122, respectively) and no interactions of number of embryos or fetuses with face color.

**Association of Losses with Concentrations of Steroids and VEGF**

**Progesterone: Complete Loss.** Concentrations of progesterone on d 25 were of predictive value in the logistic regression analysis, whereas concentrations on other days were not. From d 25 to 65 of gestation and from d 25 to parturition, complete loss decreased as concentrations of progesterone on d 25 of gestation increased (P < 0.05; Figure 3A). Complete losses were greater between d 25 and 65 in ewes with low (8.1%) than with medium (1.3%) or high (1.4%) concentrations of progesterone on d 25 (P < 0.05; pooled SEM ± 1.0). Complete losses from d 25 to parturition were greater in ewes with medium (9.0%) or low (8.4%) than in ewes with high (12.2%) concentrations of progesterone on d 25 of gestation (P < 0.05; pooled SEM ± 1.4). However, between d 65 and parturition, complete losses were greater in ewes with medium (6.2%) than in those with low (0%) or high (1.4%) concentrations of progesterone on d 25 (P < 0.05; pooled SEM ± 1.2). Ewes with medium or high concentrations of progesterone on d 45 had no losses to d 65, whereas ewes with low concentrations lost 3.3% of pregnancies by d 65 (P < 0.05; pooled SEM ± 0.1).

**Progesterone: Partial Loss.** The relationship of partial losses to concentrations of progesterone on d 25 of gestation varied with stage of pregnancy. Partial loss from d 25 to 45 increased as concentrations of progesterone on d 25 increased (P < 0.05; Figure 3B), whereas partial loss from d 65 to parturition decreased as concentration of progesterone on d 25 increased (P < 0.05; Figure 3B), whereas partial loss from d 25 to term (P < 0.05) of complete pregnancy loss on concentrations of progesterone on d 25 for losses from d 25 to 65 (■) and from d 25 to term (♦).

**Estradiol-17β.** Concentrations of estradiol-17β had no predictive value for complete or partial losses of pregnancy in the logistic regression analyses. Complete losses to d 65 did not differ with concentrations of estradiol-17β on d 25 or 45 of gestation. Complete losses from d 65 to 85 (P < 0.05), d 65 to parturition (P < 0.01), and d 85 to parturition (P = 0.05) were greater in ewes
Figure 4. Logistic regression of percentages of ewes mated during the anestrous season in group 1 that partially lost a pregnancy from d 65 to term on concentrations of vascular endothelial growth factor (VEGF) on d 45 of gestation ($P < 0.05$).

with low (mean 2.6 pg/mL; 6.2, 8.8, and 9.7% losses, respectively) than medium (4.3 pg/mL; 0, 1.5, and 1.6, respectively) or high (7.8 pg/mL; 0, 1.9, and 0, respectively) concentrations of estradiol-17$\beta$ on d 65 (pooled SEM $\pm$ 1.2).

Partial losses from d 25 to 45 were greater ($P < 0.05$) in ewes with high (mean 7.3 pg/mL; 10.1%) than with medium (3.6 pg/mL; 3.2%) or low (1.6 pg/mL; 16.1%) or medium (3.3 pg/mL; 17.5%) concentrations of estradiol-17$\beta$ on d 25 ($P < 0.05$; pooled SEM $\pm$ 2.0). In contrast, partial losses from d 85 to parturition were greater ($P < 0.05$) in ewes with low (1.9 ng/mL; 16.7%) or low (0.6 ng/mL; 14.3%) than in ewes with medium (1.1 ng/mL; 5.5%) concentrations of VEGF on d 25 (pooled SEM $\pm$ 2.0). Specifically, between d 45 and 65, partial losses were greater ($P < 0.05$) in ewes with high (12.5%) than in ewes with medium (2.7%) concentrations of VEGF on d 25 (pooled SEM $\pm$ 1.6). In contrast, partial losses between d 65 and parturition were greater ($P < 0.05$) in ewes with low (22%) and medium (17.5%) than in ewes with high (6.0%) concentrations of VEGF on d 45 (pooled SEM $\pm$ 2.5). Partial losses did not vary with concentrations of VEGF on d 65.

**DISCUSSION**

The most important findings in the current study were that late embryonic and fetal losses after d 25 occurred throughout the remainder of 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 d 25 to parturition. The direct estimate of all embryos or fetuses lost was 19.6% for ewes observed at d 25 and term, and the sum of losses estimated for each stage was 22.8%. Using the mean of these 2 values, 21.2% of the 72% of ewes that were pregnant lost 15.3% of all potential offspring, whereas the 28% that were nonpregnant lost 28% of all potential offspring (fertilization failure included). Thus the estimated total loss of potential offspring from ovulation to parturition in all ewes was 43.3%, which is somewhat greater than the 30% total embryonic and fetal loss from breeding to parturition calculated by Bolet (1986).

Losses occurred from d 25 to parturition, with no single interval having the majority on a per day basis. Approximately 3 to 4% of the embryos present at d 25 were lost during each 20-d interval of pregnancy beyond that point. Most frequently, ewes with a multiple pregnancy lost one, but not all embryos or fetuses; usually 2 embryos or fetuses were observed, but only one was present later or born at parturition. Complete loss of a single pregnancy occurred more often than complete loss of a multiple pregnancy, which agrees with Kelly and Allison (1979).
Several authors have observed loss of individual embryos without total loss of pregnancy (Rhind et al., 1980; Schrick and Inskeep, 1993). In early work, Hammond (1921) and Henning (1939) reported that reproductive wastage was greater in ewes with greater ovulation rates. In Henning’s study of slaughterhouse material, percentages of dead or missing fetuses in ewes with 1, 2, or 3 CL were 8, 26, and 43, respectively. More ewes experienced partial loss with twin ovulations than complete loss in ewes with single ovulations, when numbers of embryos present at d 18 (Quinlivan, 1966) or d 22 to 25 (Kelly and Allison, 1979) of gestation were compared with numbers of CL. Survival to term was estimated at 0.95 for single embryos, 0.85 for 2 and 0.70 for 3 embryos by Geisler et al. (1977). White et al. (1981) concluded that embryo survival was greater in single-ovulating than in bilateral twin-ovulating ewes, which in turn were superior to unilateral twin-ovulating ewes. They further observed that survival was lower for oocytes that migrated to the opposite uterine horn, confirming the earlier observation by Casida et al. (1966). In a recent study in a Merino flock, 20.4% of potential lambs were lost when number of live offspring was compared with number of CL at midgestation (Kleemann and Walker, 2005b). This value is quite similar to the 22.8% estimated in the current study. Meyer (1985) described the marginal response in litter size due to ovulation of an additional egg as uterine efficiency, and showed that it decreased as ovulation rate increased.

Late embryonic and fetal losses did not differ between ewes bred during anestrus or the transitional period. Temperature and humidity may have less effect on the portion of pregnancy studied here than on early gestation. Dutt (1954) observed greater rates of fertilization failure and embryonic mortality to d 18 during the early breeding season than during midseason. Number of ewes pregnant at d 100 of gestation decreased as days of exposure to high ambient temperatures during mating increased (Kleemann and Walker, 2005a). Hulet et al. (1956) noted that a high percentage of ewes did not lamb when bred before September and that fertility improved as the breeding season advanced. Effects were thought to be due to exposure of rams and ewes to high ambient temperatures during anestrus. North Carolina workers showed that exposure of ewes to 90°F during the first 3 d after estrus was detrimental (Alliston and Ulberg, 1961). In the current study, losses were not explained by potential for heat stress, as indicated by THI, although the degree of elevation of THI was limited on these farms.

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

Breed-type differences were apparent in the percentages of embryos or fetuses lost during several extended stages of pregnancy and were not accounted for by differences in numbers of embryos or fetuses present at the beginning of the stage. Black-faced ewes, mainly of Suffolk breeding, experienced the greatest loss, regardless of stage of pregnancy. White and mottled-faced ewes lost similar proportions of embryos and fetuses from d 25 or 45 to parturition. White-faced ewes had very little fetal loss from d 85 to parturition compared with black-faced ewes, and losses were intermediate in mottled-faced ewes. Previous studies on effects of breed on late embryonic or fetal mortality are limited. Cumming et al. (1975) noted that embryonic survival from breeding to d 26 to 30 was greater in crossbred than in Merino twin-ovulating ewes, but did not differ with breed in single-ovulating ewes. Foote et al. (1959) found that Columbia ewes lost fewer embryos up to d 18, 25, or 140 than Hampshire ewes.

Dorset sheep have longer breeding seasons (Hafez, 1952) and often are used by producers interested in out-of-season breeding (Notter, 1992). Dorset rams are less seasonal, have greater libido, and are more effective in inducing ovulation in anestrous ewes than Suffolks (Nugent et al., 1988) or Hampshire ewes (Barr et al., 1968). These factors might have contributed to greater efficiency of white-faced ewes in this study, which had predominantly Dorset breeding and often were mated to Dorset rams.

The lower concentrations of progesterone and greater percentages of loss observed in black-faced ewes might be related to a shorter breeding season and deeper anestrus in Suffolks than in white or mottled-faced ewes (De Bacca et al., 1954). Black-faced ewes generally are not selected for ability to breed during summer, and anestrus in Suffolk ewes often begins in February (Robinson and Karsch, 1984). Black-faced ewes might have more trouble retaining pregnancies or embryos when induced to ovulate during anestrus, but they represent an opportunity for improvement in embryonic/fetal survival.

Concentrations of estradiol on d 45 and 65 were greater, whereas concentrations of VEGF were lower in mottled-faced ewes than in black- or white-faced ewes. Estradiol and VEGF are thought to be involved in placental development. Greaterestradiol at d 45 and 65 and lower VEGF on d 25 through 65 might help to explain the lower losses from d 45 or 85 to parturition in mottled-faced ewes. Greater embryonic or fetal survival rates as well as high and low concentrations of estradiol and VEGF, respectively, in mottled-faced ewes might be due to heterosis from crossbreeding black- and white-faced ewes.

As might be expected (Casida and Warwick, 1945) complete losses of pregnancy to d 65 were more frequent in ewes with low progesterone on d 25 of gestation, whereas complete losses after d 65 were more frequent in ewes with medium progesterone. Complete losses were minimal in ewes with high concentrations of progesterone on d 25. Thus, the lower the early progesterone, the sooner losses occurred due to inadequate progesterone. Increasing peripheral concentrations of pro-
Gestosterone increased rate of growth of ovine (Lawson et al., 1983) or bovine embryos (Fox et al., 1988; Garrett et al., 1988) and secretion of uterine proteins (Nephew et al., 1991). Kleemann et al. (1994) observed greater fetal mass on d 74 or 76 in ewes treated with progesterone on d 1 to 3 or d 1 to 6 after mating. Late embryonic mortality between d 30 and 60 of gestation was associated with low concentrations of progesterone in lactating dairy cows sampled once on d 28 to 37 of gestation (Starbuck et al., 2004). Pregnant dairy cows treated with a progesterone-releasing intravaginal device during early fetal development had greater retention rates at d 60 (94.7%) than controls (88%; Lopez-Gatius et al., 2004). However, supplemental progesterone therapy from d 6 through 50 was not of value in sheep (Diskin and Niswender, 1989).

Partial losses of multiple pregnancies during late embryonic development (before d 45) increased with increasing concentrations of progesterone on d 25, whereas losses during fetal development were associated with decreasing concentrations of progesterone on d 25. That association might reflect the greater loss of embryos with greater ovulation rates seen by Rhind et al. (1980) because ewes with more than 1 CL would be expected to have greater concentrations of progesterone (Stormshak et al., 1963) before placentation. Replacements of progesterone did not vary with numbers of embryos or fetuses present in the current study. More partial losses from d 25 to 45 or from d 25 to parturition occurred in ewes with high concentrations of estrogen on d 25 of gestation, whereas ewes with low or medium concentrations did not differ. Whereas effects of elevated estrogen on early embryonic mortality have been observed in cattle, effects of estrogen on late embryonic mortality in that species have been equivocal (reviewed by Inskeep and Dailey, 2004) and we are not aware of similar studies in sheep.

More complete fetal losses occurred in ewes with low concentrations of estradiol on d 65 of gestation and partial losses were lowest in ewes with high concentrations of estradiol on d 65. Low concentrations of estradiol on d 65 might have limited subsequent fetal or placentation development, or simply reflect some event associated with losses. Ford (1995) observed a decrease in estradiol from d 46 to 55 followed by an increase around midgestation. Weems et al. (2006) have shown that, at 90 d of gestation, estradiol regulates placental secretion of pregnancy specific protein B, which regulates placental and luteal secretion of PGE, which in turn regulates placental secretion of progesterone. Estrogen binding to its receptors within the vascular smooth muscle in endometrial arterioles and glandular epithelium might regulate VEGF and basic fibroblast growth factor activity (Cullinan-Bove and Koos, 1993), thus affecting subsequent angiogenesis in the placenta.

Partial losses from d 25 to 65 were greater in ewes with low or high than medium concentrations of VEGF, whereas partial losses from d 45 to 65 were greater only in ewes with high VEGF. Partial losses from d 65 to parturition decreased with increasing concentrations of VEGF on d 45. Specific thresholds of VEGF were required to inhibit apoptosis of endothelial cells during angiogenesis and were essential for the stabilization of newly formed blood vessels (Neufeld 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 caused 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 (Neufeld et al., 1999).

In this study, VEGF in jugular plasma did not differ between ewes carrying multiple or single fetuses. Similar results were reported by Echternkamp et al. (2006) in cattle. They measured VEGF in jugular plasma of beef cows gestating single or twin fetuses at d 57, 121, 192, and 234 of pregnancy. No differences were observed at any individual stage, but the overall means (ng/mL) were 1.19 ± 0.08 for singles vs. 1.48 ± 0.09 for twins (P < 0.05).

Many factors play a role in embryonic and fetal loss in the ewe. Bolet (1986) suggested that these losses were due to 1 of 3 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. The greater losses of 1 embryo or fetus in a multiple pregnancy in the current study indicated that the conceptus played a larger role than the dam, which might explain the limited predictive value of maternal concentrations of steroids and VEGF for survival or loss. In the case of embryo transfer in cattle, McMillan (1998) compared a binomial model and an embryo and recipient model and found that the survival data on single or double transfers fit the embryo and recipient model in most cases. McMillan estimated that embryo and recipient played nearly equal roles in outcome of pregnancy. In earlier work in sheep, Restall et al. (1976) found that fertilization rate fit an all or none model, that is fertilization occurred on a ewe basis, not an oocyte basis. In that study, early embryonic survival (to d 25 to 30) for 4 flocks fit a binomial model, each embryo having an independent fate. In a second report, Restall and Grifiths (1976) examined 4 other possible models of embryonic survival but reached no firm conclusions because fertilization rate could influence whether distribution of losses deviated from the binomial distribution. Geisler et al. (1977) utilized data from the literature and concluded that the embryonic survival in sheep generally fit the binomial model.

Data for 796 ewes on 14 farms that were pregnant on d 25 in the current study were examined for goodness of fit with each of the models examined by McMillan (1998). Data for 9 of 14 farms, examined individually, fit each model, but the overall data did not fit either model (P < 0.001). Each model estimated that 79.3% of
embryos had the potential to survive, which was in fact the survival percentage in the data. However, the binomial model assumed, and the embryo and recipient model estimated, that all ewes pregnant at d 25 were competent to complete pregnancy. In fact, only 93.6% of ewes carried pregnancies to parturition. From these data, one might conclude that the ewe appears to be playing a lesser role than the conceptus in prenatal mortality after d 25. Sire factors were not evaluated in the current study and would contribute to viability of the conceptus.

In summary, losses of embryos and fetuses occurred throughout pregnancy and overall rate varied with type of loss, complete or partial. Late embryonic and fetal losses occurred at similar rates in the anestrous and transitional seasons and were not associated with heat stress during several periods before or after mating. Proportion of embryos or fetuses lost was greatest in black-faced ewes. Lower concentrations of progesterone on d 25 or 45 predicted greater complete losses. Concentrations of estradiol-17β were not of predictive value. 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 than the dam in late embryonic and fetal losses from d 25 to parturition, which might explain the limited predictive value of the maternal concentrations of steroids and VEGF. The interplay of breed types, concentrations of progesterone, and placental development in late embryonic and fetal losses are worthy of further investigation.

**IMPLICATIONS**

Reproductive wastage is great in the ewe, averaging 43.3% of potential offspring in the current study. Losses during the late embryonic and fetal periods accounted for 15.3%, whereas complete pregnancy failure before day 25 accounted for 28%. Thus there is a tremendous opportunity to improve reproductive output in sheep. The most important contributing factors identified in this study were the predominance of partial losses, the variation among breed types, and the value of greater concentrations of progesterone in maternal serum. These factors can be used in plans for future research into mechanisms of loss.

**LITERATURE CITED**


