FERTILIZATION FAILURE AND EMBRYONIC MORTALITY IN PAROUS AND NONPAROUS BEEF CATTLE

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Summary

One hundred and two nonparous females, 18 to 24 mo old, and 55 parous or multiparous beef females, 3 to 12 yr old, were mated naturally (d 0) and slaughtered on d 2 to 5, 6 to 8 or 14 to 16 of gestation. Each reproductive tract was flushed with phosphate-buffered saline, and the flushings were searched for an oocyte or embryo. Parous females had a higher (P<.05) fertilization rate than nonparous females. In nonparous females, reproductive failure was attributed equally to fertilization failure and embryonic mortality, which had occurred or was occurring by d 8 of gestation. In parous females, reproductive failure was attributed entirely to embryonic death. About 67% of this embryonic mortality had occurred or was occurring by d 8 of gestation; the other 33% occurred between d 8 and 16 of gestation. These findings show that in both nonparous and parous beef females, a large portion of the reproductive failure occurs by d 8 of gestation and would not have any noticeable influence on length of the estrous cycle.

(Key Words: Beef Cattle, Fertilization Failure, Embryonic Mortality, Conception, Heifers, Cows.)

Introduction

The most important factor in the reduction of the net calf crop is the failure of the beef heifer or cow to become pregnant. Factors affecting net calf crop in a disease-free beef herd that was naturally mated over 14 yr were summarized by Bellows et al. (1979). Four factors reducing net calf crop (71.0%) were nonpregnancy of females (17.4%), perinatal calf death (6.4%), calf deaths between birth and weaning (2.9%) and fetal deaths during gestation (2.3%). Therefore, 60% of the reduction in calf crop could be attributed to, failure to mate, fertilization failure and embryonic mortality. This is a very conservative estimate because beef cows could be mated two to three times during the 45 to 60 d exposed to bulls.

Many investigations on reproductive inefficiency have been conducted with dairy cattle, but relatively few studies have involved beef cattle. In two recent reports, Ayalon (1978) and Hawk (1979) reviewed research on embryonic mortality in dairy cattle. Most investigations have been undertaken to determine the incidence of embryonic mortality, but few investigators have attempted to identify when during early gestation conceptuses die. In normal dairy cows, embryonic deaths apparently occur after d 16, whereas in repeat breeder dairy cows, it occurs by d 8 (Ayalon, 1978). However, in estrous-synchronized and artificially inseminated beef heifers, embryonic deaths occurred between d 8 and 16 (Diskin and Sreenan, 1980). Factors that may influence embryonic loss are genetics, nutrition, maternal age, environment, semen quality, time of insemination, hormonal imbalance, disease and biochemical changes within the uterus.

Because of lack of data on beef cattle, this study was undertaken to determine at what stage of embryonic development most embryonic loss occurs in nonparous and parous beef cattle.
Materials and Methods

One hundred and two, two- or three-way crossbred (Black Angus, Red Poll or Simmental sired) nonparous (virgin) females, 18 to 24 mo old, and 55 Black Angus, Limousin × Black Angus or Limousin × Hereford crossbred parous females, 3 to 12 yr old, were studied. All crossbred females were derived from Hereford and Black Angus cows. Females were penned in an open feedlot and fed daily 16 to 18 kg of a diet consisting of 50% corn silage and 50% haylage. All females were observed daily for estrous behavior between 0700 and 0900 h and between 1600 and 1900 h. Estrus was determined by homosexual behavior. When observed in estrus, each female was moved for mating to a pen housing a bull. After a single mating, the female was penned and kept with a second bull of the same breed as the first bull for 10 to 14 h. The same Black Angus and Red Poll bulls, aged 3 to 5 yr, were used for matings of both groups of females. Mated females were placed in another pen and held until slaughtered on d 2 to 5, 6 to 8 or 14 to 16 of gestation (d 0 = day of estrus).

Reproductive tracts were collected at the slaughter plant, placed on ice and returned to the laboratory. The uterine horn and(or) oviduct ipsilateral to the corpus luteum was flushed with 30 ml of phosphate-buffered saline. This procedure was repeated until an embryo or oocyte was found or until the tract had been flushed five times. The flushings were examined with a stereomicroscope (X18).

Oocytes and embryos were further examined with an inverted tissue microscope (X200) for blastomere number and symmetry. At d 2 to 5, fertilization rate and viability were based on the recovery of two- to 12-celled embryos. Embryos with a large proportion of asymmetrical and(or) broken blastomeres were considered degenerate or degenerating. At d 6 to 8, fertilization rate and viability were determined on the basis of recovery of a morula to a blastocyst with a well-formed blastocoele and inner cell mass. At d 14 to 16, viability was determined on the basis of an oblong to elongated blastocyst with an embryonic disc. Blastocysts containing mostly dark, fragile, necrotic tissue were considered degenerate or degenerating.

Chi-square analyses were used to analyze the 4 x 3 x 2 (embryonic state, time and parity), 3 x 2 (time and parity) and 2 x 2 x 4 (ovulation side, parity and embryonic state) factorials.

Results and Discussion

Oocyte and embryo recovery rates, fertilization rates and percentages of degenerating embryos are shown in table 1. Breed of sire (x² = 3.77, 3 df) and breed of dam (x² = 13.94, 9 df nonparous; x² = .04, 3 df parous) effects were nonsignificant (P>.10) and, therefore, the data were pooled for analyses of time, parity and embryonic state effects. The chi-square test of the 4 x 3 x 2 factorial was significant (P<.01; x² = 40.03, 6 dr) indicating the ratio of embryonic states over time and parity differed. The analyses of normal developing embryos

Table 1. Recovery and Fertilization Rates and Percentage of Degenerate and Normally Developing Embryos in Nonparous and Parous Beef Females

<table>
<thead>
<tr>
<th>Day after mating</th>
<th>Parity</th>
<th>No. of females</th>
<th>No. recovery</th>
<th>Unfertilized</th>
<th>Fertilized degenerate</th>
<th>Fertilized normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 to 5</td>
<td>0</td>
<td>39</td>
<td>5 (13)a</td>
<td>3 (7)a</td>
<td>5 (13)a</td>
<td>26 (67)a</td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>19</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (11)</td>
<td>17 (89)</td>
</tr>
<tr>
<td>6 to 8</td>
<td>0</td>
<td>33</td>
<td>1 (3)</td>
<td>8 (24)</td>
<td>6 (18)</td>
<td>18 (55)</td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>17</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (24)</td>
<td>13 (76)</td>
</tr>
<tr>
<td>14 to 16</td>
<td>0</td>
<td>30</td>
<td>8 (27)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>21 (70)</td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>19</td>
<td>2 (11)</td>
<td>0 (0)</td>
<td>3 (16)</td>
<td>14 (73)</td>
</tr>
<tr>
<td>2 to 16</td>
<td>0</td>
<td>102</td>
<td>14 (14)</td>
<td>11 (11)</td>
<td>12 (12)</td>
<td>65 (63)</td>
</tr>
<tr>
<td>(Total)</td>
<td>&gt;1</td>
<td>55</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>9 (16)</td>
<td>44 (80)</td>
</tr>
</tbody>
</table>

aNumbers in parentheses are percentages.
indicated that parous females had a significantly higher percentage (80 vs 64%; \(P<.05\), \(x^2 = 4.46, 1\) df) of normal developing embryos. Gestation time (\(x^2 = 2.00, 2\) df) and the interaction of parity and time (\(x^2 = 1.63, 2\) df) were nonsignificant (\(P>.10\)).

Parity (\(x^2 = .65, 1\) df) and gestation time (\(x^2 = 3.13, 2\) df) were nonsignificant (\(P>.10\)) with regard to the ratio of degenerating embryos. However, a significant interaction (\(P<.05; x^2 = 5.05, 1\) df) between gestation time 2 to 8 and 14 to 16 d of gestation, respectively, whereas nonparous females had decreasing values of 15 to 3% for the same times, respectively.

Unfertilized oocytes recovered were affected by parity and gestation time. Nonparous females had significantly more (\(P<.05, x^2 = 6.37, 1\) df) unfertilized oocytes than parous females (11 vs 0% for nonparous and parous females, respectively). Percentages of unfertilized oocytes from recovered oocytes and embryos were 17 vs 0% for nonparous and parous females, respectively. A significant time of gestation effect (\(P<.01, x^2 = 10.19, 2\) df) was also found. Because the parous females had a 100% fertilization rate the differences in time were differences among nonparous groups. More unfertilized oocytes were found at 6 to 8 than at 2 to 5 d of gestation (16 vs 5%, respectively, \(P<.10, x^2 = 3.44, 1\) df). This reduction was probably the result of random sampling. No unfertilized oocytes were found at 14 to 16 d in either parity group. This resulted in a significant difference (11 vs 0% for the 2 to 8 and 14 to 16 d, respectively \(P<.05, x^2 = 5.36, 1\) df) between the 2 to 8 and 14 to 16 d of gestation. However, this difference is not a real difference biologically because the unfertilized oocytes have either been phagocytized by leukocytes in the uterus or expelled from the uterus by d 14 to 16.

Rates of recovery of oocytes and embryos were influenced by gestation time and parity. More oocytes and embryos were recovered in parous than nonparous females (\(P<.05, x^2 = 4.01, 1\) df; 96 vs 86%, respectively). Recovery rates did not differ between 2 to 5 and 6 to 8 d of gestation, but did differ significantly between 2 to 8 and 14 to 16 d (94 vs 80%, respectively; \(P<.01, x^2 = 13.06, 1\) df). This difference is significant statistically and can be explained biologically in that the unfertilized oocytes and degenerate embryos have been phagocytized by leukocytes found in the uterus or expelled from the uterus by d 14 to 16 leading to the large increase in no recoveries. It is conceivable that in a small proportion of females rupture of the follicle occurred, but the oocyte was not released before the formation of the corpora luteum.

For 28 nonparous and 55 parous females the ovary in which the corpus luteum was found was recorded. All females had only one corpus luteum with 63.8% located on the right ovary and 36.2% on the left ovary (\(P<.05, x^2 = 6.37, 1\) df). The chi-square analysis of the 2 \(\times\) 2 \(\times\) 4 factorial (side of CL \(\times\) parity \(\times\) embryonic state) was significant (\(P<.01, x^2 = 13.41, 3\) df). Upon analyzing each embryonic state it was found that side of corpus luteum was nonsignificant (\(P>.10\) normal \(x^2 = .10, 1\) df; degenerate \(x^2 = .04, 1\) df; unfertilized \(x^2 = .49, 1\) df; no recovery \(x^2 = 2.38, 1\) df). The contribution of parity differences in unfertilized oocytes made the 2 \(\times\) 2 \(\times\) 4 factorial significant (\(x^2 = 8.25, 1\) df, \(P<.01\)).

In nonparous females, embryonic mortality had occurred by d 8 of gestation, and, in parous females, 63% of the embryonic mortality had occurred or was occurring by d 8 and continued to d 16. Both parities showed the same percentage of normal fertilized embryos at d 16 of gestation (table 1). Diskin and Sreenan (1980) reported rates of 10% for no recovery of either an oocyte or embryo, 9% fertilization failure and 4% embryonic mortality in 79 estrous-synchronized and artificially inseminated beef heifers in which embryos were collected on either d 4 or 8 of gestation. The fertilization rate and embryonic survival were higher than those found in this study, but those higher values may have been due to the synchronization of estrus. However, Diskin and Sreenan (1980) reported that embryonic losses had occurred by d 12 in the heifers they studied. Ayalon (1978) reported that embryonic losses occurred by d 12 in the heifers they studied.
8 and found that 100% were fertilized, with an 89% recovery rate in a control group of nine Hereford and Angus heifers. Those recovery rates are comparable with those reported here, but rates of fertilization among those heifers were higher. Other investigators (Wiltbank et al., 1967; Christenson et al., 1975; Spitzer et al., 1978; Bellows et al., 1979; Roche et al., 1979; Smith et al., 1979) reported lower recovery rates for heifers than were reported here. They also reported comparable fertilization rates. We were unable to find similar data in the literature for the mature beef cow.

Several investigators (Wiltbank et al., 1967; Spitzer et al., 1978; Smith et al., 1979) reported collecting empty, ruptured zona pellucidae from heifers. No empty, ruptured zone pellucidae were found in flushings from nonparous females in the present study; however, two empty, ruptured zona pellucidae were found in flushings from parous females. Both zona pellucidae had embedded spermatozoa, and the oocytes were considered to be fertilized, but development was classified as abnormal. Oocytes in nonparous females classified as unfertilized had no accessory spermatozoa embedded in or attached to the zona pellucidae. Therefore, the two empty, ruptured zona pellucidae that did have embedded spermatozoa were classified as fertilized. Recovery of an empty zona pellucida could be the result of abnormal zygote development or the remnants of an oocyte from a previous cycle. Abnormal zygote development would appear to be a more appropriate explanation, because embedded spermatozoa were found in the zona pellucidae, and parous females had not been exposed to a male for several estrous cycles before the current mating.

Rate of embryonic survival was the same for parous and nonparous females at d 14 to 16, but the two parities reached that rate by different means. Among parous females, embryonic mortality accounted entirely for the reduction in reproductive efficiency, whereas among nonparous females, fertilization failure and embryonic mortality accounted equally for this reduction. The causes of fertilization failure and embryonic deaths remain to be found and warrant further investigation.

Literature Cited