FERTILIZATION RATE AND EARLY EMBRYONIC LOSS IN BRAHMAN CROSSBRED HEIFERS

M. F. Smith, K. J. Nix, D. C. Kraemer, M. S. Amoss, M. A. Herron and J. N. Wiltbank

Texas Agricultural Experiment Station, Beeville 78102 and College Station 77843

Summary

The fertilization rate and incidence of embryonic loss in virgin Brahman crossbred heifers between d 3 and 35 postinsemination (d 0 = first day of estrus) were determined. One hundred fifty-five virgin heifers, maintained in a dry lot, received approximately 22 Mcal head⁻¹ d⁻¹ and were allotted to one of three groups (d 3, 16 or 35) according to body weight at the time of estrous detection.

Seven empty ruptured zona pellucidae (ERZP) were recovered from five heifers in the d 3 group. It was impossible to determine whether the ERZP originated from fertilized or unfertilized ova and whether they were ovulated at the estrus immediately preceding or during a previous cycle. Consequently, the fertilization rate (d 3) was calculated to be 80 or 93% depending upon whether the ERZP were included or deleted from the calculations, respectively. Because of the large number of ERZP recovered, a second study was conducted with an additional 21 virgin Brahman crossbred heifers from which ova were recovered on d 3 postinsemination. The results of the two studies were similar. The percentage of heifers with an embryo on d 16 was 78 (10% had degenerating embryos and 12% no embryos), and the percentage pregnant at d 35 was 72. The conclusions suggested from this study depend upon the classification of the ERZP. If the ERZP are designated as ova ovulated during a previous cycle or ova damaged in the collection process and are deleted from the calculations, the fertilization rate is high (93%), and embryonic loss apparently occurs between d 3 and 35 (P<.05). However, if the ERZP are classified as ova ovulated at the immediately preceding estrus, unavailable for further embryonic development, and are included in the calculations, the fertilization rate is comparatively low (80%). In the latter case, the primary loss of potential embryos occurs before d 3, and the loss after d 3 is negligible (P>.05).

(Key Words: Fertilization Rate, Embryonic Loss, Zona Pellucida, Brahman Crossbred Heifers.)

Introduction

Prenatal mortality has been reported in cows, mares, ewes, sows, rabbits, squirrels, mice, and numerous other mammals (see reviews by Brambell, 1948; Hanly, 1961; Ayalon, 1978). Kidder et al. (1954) reported a 40% loss of potential embryos in dairy cattle attributable to genital abnormalities (3%), defective ova (9%), fertilization failure (12%) and embryonic mortality (16%). Tanabe and Casida (1949) divided 104 infertile dairy cows into three categories based on reproductive performance: fertilization failure, 39.7%; embryonic abnormalities and mortality (<34 days), 39.2%; and normal embryos at 34 days, 21.1%. The preceding reports indicate that early embryonic mortality, in fertile and infertile dairy cattle, is an important factor affecting pregnancy rates.

Although several investigators have reported early embryonic mortality in cattle (Ayalon, 1978), the time at which the loss occurs is unclear. In fertile cattle, Ayalon (1972) reported a significant reduction in embryonic survival

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after d 16 postbreeding; whereas other studies report embryonic mortality occurring prior to d 16 (Boyd et al., 1969; Diskin and Steenan, 1980).

The objective of the present study was to determine the incidence and time of embryonic loss through d 35 postinsemination (d 0 = estrus) among virgin Brahman crossbred heifers maintained on a high nutritional level and artificially inseminated to a bull of proven high fertility.

**Materials and Methods**

One hundred and fifty-five virgin Brahman crossbred heifers were allotted to one of three experimental groups (d 3, 16 or 35) according to body weight at the time of estrous detection. The number of heifers in each group was 47, 51, and 57, respectively.

Heifers were maintained in a dry lot and fed to gain approximately 34 kg·head⁻¹·d⁻¹ until slaughter or pregnancy diagnosis. The diet consisted of sorghum-sudan grass hay (IFN 1-04-480; fed ad libitum), 2.12 kg of ground corn (IFN 4-02-931) and .14 kg cottonseed meal (IFN 5-01-615).

The heifers were checked for estrous behavior once every 4 h and only animals that demonstrated standing homosexual behavior were considered in estrus. Heifers were artificially inseminated by an experienced technician approximately 12 h after estrous detection; frozen semen from a Holstein bull of proven high fertility was used. Rectal temperature was recorded at the time of insemination as a measure of heat stress.

The time of ovulation was recorded as the interval from the onset of estrus (d 0 = first day of estrus). Randel (1976) concluded that there is no difference in ovulation time in relation to onset of estrus between Hereford and Brahman × Hereford heifers. Therefore, in the present study, we assumed that ovulation occurred approximately 30 h after the onset of estrus (Wiltbank et al., 1967; Massey, 1974).

Ova or embryos from heifers assigned to the d 3 or the d 16 group, respectively, were collected as follows. The reproductive tracts were obtained within 15 to 45 min after slaughter and refrigerated until the time of ova or embryo collection (1 to 3 h after slaughter). In the d 3 group, the oviduct adjacent to the ovary containing the ovulation site was carefully dissected from the uterotubal junction and the mesovarian ligament so that the oviduct was straight instead of convoluted. A 13-gauge blunt-end needle was inserted into the infundibulum until approximately 1 cm had entered the ampulla. Four milliliters of Nutrient Mix-

<table>
<thead>
<tr>
<th>Category</th>
<th>No. heifers</th>
<th>% of total heifers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of heifers</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>Genital abnormalitiesb</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Ovulation failurec</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Recovery failured</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Total recovered ova</td>
<td>35</td>
<td>75</td>
</tr>
<tr>
<td>Fertilized ova</td>
<td>28</td>
<td>60</td>
</tr>
<tr>
<td>Unfertilized ova</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Empty ruptured zona pellucida</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

aD 0 = first day of estrus.

bOne heifer had a double os cervix, one had a hydrosalpinx in the right oviduct and a pyosalpinx in the left oviduct and one had a cyst in the left oviduct.

cNo ovulation site was detected on either ovary.

dThree unsuccessful flushing attempts of the oviduct adjacent to the ovary containing the ovulation site constituted recovery failure.

eOva were designated as fertilized if cleavage had occurred.
ture F-10\textsuperscript{7} were gently infused into the oviduct. The uterotubal junction of the oviduct was held securely and pointed directly into a watch glass. The ovum was located in the watch glass with a dissecting microscope and transferred to a hanging drop slide for more detailed observation. Ova were designated as fertilized if cleavage had occurred. Three unsuccessful flushing attempts constituted recovery failure.

In the d 16 group, the uterine horn ipsilateral to the corpus luteum was clamped at the uterotubal junction and dissected from the tract up to the internal bifurcation. Another clamp was placed at the anterior end of the cervix and the uterine horn was completely dissected from the reproductive tract. The first clamp was released from the uterotubal junction and a blunt-end needle was inserted into the uterine lumen. The second clamp was released, and the uterine lumen at the posterior end of the uterine horn was pointed directly into a petri dish. Five milliliters of Nutrient Mixture F-10 were gently infused into the uterine horn. The embryo normally appeared in the first few drops of fluid flushed into the petri dish. Three unsuccessful flushing attempts of each uterine horn constituted recovery failure. After embryo recovery, the membranes were teased apart and the length was recorded.

<table>
<thead>
<tr>
<th>Category</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Fertilized ova</td>
<td>28</td>
<td>93</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>Unfertilized ova</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>ERZP\textsuperscript{d}</td>
<td>5</td>
<td>14</td>
<td>2</td>
<td>11.8</td>
</tr>
</tbody>
</table>

\textsuperscript{d}ERZP = empty ruptured zona pellucidae.

Heifers in the d 35 group were observed for a return estrus and bled via puncture of a tail vessel on d 21 for progesterone analysis by radioimmunoassay (Atkins et al., 1976). Pregnancy diagnosis by palpation per rectum was conducted at 35 d postbreeding and later verified using calving records.

The data were analyzed by chi-square procedures for differences in the number of zygotes or embryos detected on d 3, 16 and 35 postinsemination (Steel and Torrie, 1960). Within the d 16 group, difference in extraembryonic membrane length relative to embryonic age were analyzed by one-way analysis of variance and Duncan's multiple range test (Steel and Torrie, 1960).

Results

In the d 3 group, ova were recovered from 35 (81%) of the 43 heifers that ovulated and had an anatomically normal reproductive tract (table 1). The fertilization rate (study 1) reported in table 2 was based on the 35 recovered ova.

From five heifers in the d 3 group, a total of seven empty ruptured zona pellucidae (ERZP) were recovered. It was impossible to determine whether the ERZP were from fertilized or unfertilized ova and whether they were ovulated at the immediately preceding estrus or during a previous cycle.

The fertilization rate was calculated two ways, depending upon the classification of the ERZP (table 2). When the ERZP were considered as ova ovulated at a previous cycle or as damaged in the collection process and were
deleted from the calculation of fertilization rate, the percentage of heifers with a fertilized ovum was 93%. However, when the ERZP were classified as ova ovulated at the immediately preceding estrus, unavailable for further embryonic development, and were included in the calculation of fertilization rate, the percentages of heifers with a fertilized ovum, unfertilized ovum, or an ERZP were 80, 6, and 14, respectively.

Because of the large number of ERZP recovered, a second experiment was conducted with 21 virgin Brahman crossbred heifers (artificially inseminated to the same bull) to replicate the d 3 results. In this study, rectal temperature at the time of insemination was not recorded. Ova were recovered from 81% of the heifers, and ERZPs from two heifers (11.8%). The fertilization rate (study 2) reported in table 2 was based on the 17 recovered ova and calculated two ways, depending upon the classification of the ERZP. When the ERZP were not included in the calculations, the percentage of heifers having a fertilized ovum was 87%, which was similar to the 93% reported in study 1. When the ERZP were included, the percentage was 77%, which was also similar to the 80% reported for study 1. Hence, the results of the two studies are similar.

The ova recovered in study 1 were collected during April and May (average high temperature = 29.4 C; average low = 17.8 C), whereas ova recovered in study 2 were collected from December through February (average high temperature = 16.9 C; average low = 4.6 C). The rectal temperature at insemination in study 1 was similar for all heifers from which an ERZP, unfertilized or fertilized ovum was recovered on d 3 (table 3).

In the d 16 group, the percentages of heifers (with a normal reproductive tract) having an embryo, embryonic membrane fragments or no embryo were 78, 10, and 12%, respectively.

Considerable variation in the length of the extraembryonic membranes at d 16 was noted. The mean extraembryonic membrane length was 133.1 ± 86.4 mm and the range was 4 to 375 mm. The percentages of heifers in which the extraembryonic membrane length was <50 mm, 51 to 100 mm, 101 to 200 mm or >201 mm were 18, 25, 39, and 18%, respectively. Differences in ovulation time relative to embryo collection may account for some of the variation in membrane length (table 4).

The percentage of heifers pregnant at d 35 was 68, and assuming that approximately 5% of the heifers had a genital abnormality as in the d 3 and 16 groups, this total was adjusted to 72%. Reproductive status (pregnant or non-pregnant) was correctly diagnosed in 90% of the heifers from serum progesterone levels (>2.0 ng/ml) at d 21.

The percentages of nonpregnant heifers not returning to estrus, having a short estrous cycle (<9 d), returning to estrus approximately 21 d later or having a prolonged estrous cycle (>32 d) were 7, 6, 12, and 7%, respectively. The proportion of potential embryos at d 3, 16 and 35 is shown in table 5. Interpretation of the data depends on classification of ERZP.

Discussion

The origin of the ERZP recovered from animals in the d 3 group is unclear. One might think these ERZP are the remains of hatched blastocysts; however, this explanation was discounted because the heifers were not exposed to a bull or artificially inseminated before the

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**TABLE 3. RELATIONSHIP BETWEEN DAY 3 RESULTS AND RECTAL TEMPERATURE**

<table>
<thead>
<tr>
<th>Day 3 group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. heifers</th>
<th>Rectal temperature (C)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Fertilized ova</td>
<td>28</td>
<td>39.0 ± .5</td>
</tr>
<tr>
<td>Unfertilized ova</td>
<td>2</td>
<td>39.5 ± .2</td>
</tr>
<tr>
<td>ERZP&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>39.3 ± .7</td>
</tr>
</tbody>
</table>

<sup>a</sup>D 0 = first day of estrus.

<sup>b</sup>Rectal temperature taken at the time of insemination.

<sup>c</sup>ERZP = empty ruptured zona pellucidae.
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TABLE 4. RELATIONSHIP BETWEEN EMBRYO AGE AND EXTRAEMBRYONIC MEMBRANE LENGTH

<table>
<thead>
<tr>
<th>Embryonic age, d&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. embryos</th>
<th>Membrane length</th>
<th>Mean ± SE, mm</th>
<th>Range, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;14.6</td>
<td>16</td>
<td>102.1 ± 14.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18 — 242</td>
<td></td>
</tr>
<tr>
<td>14.7 — 14.8</td>
<td>11</td>
<td>116.8 ± 19.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37 — 252</td>
<td></td>
</tr>
<tr>
<td>14.9 — 15.0</td>
<td>9</td>
<td>208.2 ± 37.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4 — 375</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Embryonic age = (H from onset of estrus to slaughter) — (30 h from estrus to ovulation) — 24 h/d
<sup>b</sup>,<sup>c</sup>Means in the same column with different superscripts are different (P<.01).

study, and the ova were recovered from the oviduct rather than the uterus.

Other investigators report empty and(or) ruptured zona pellucidae in cattle and sheep (Tanabe and Casida, 1949; Dutt, 1954; Kidder et al., 1954; Dutt et al., 1959; Amann and Griel, 1974). In studies of dairy cattle, empty zona pellucidae were recovered from fertile (Kidder et al., 1954; Bearden et al., 1956; Amann and Griel, 1974), and infertile (Tanabe and Casida, 1949) animals and deleted from the calculation of fertilization rate or embryonic mortality. The fertilization rate in this study, as previously reported, was calculated with and without the ERZP. Dutt (1954) reported that seven of 57 ewes had ruptured zona pellucidae after recovery when first examined with a dissecting microscope. Consequently, the microscopic examination technique under a higher power was probably not responsible for the broken zona pellucidae. He also reported the collection of partially collapsed ova containing very little cytoplasm. Dutt (1954) suggested that some of these ova may have been ovulated at a previous cycle, because two normal ova and one ovum with little cytoplasm were recovered

TABLE 5. POTENTIAL EMBRYOS AT DAY 3, 16 AND 35 IN BRAHMAN CROSSBRED HEIFERS

<table>
<thead>
<tr>
<th>Group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ERZP not included&lt;sup&gt;bd&lt;/sup&gt;</th>
<th>ERZP included&lt;sup&gt;cd&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>D 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>28/30</td>
<td>93&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>D 16&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos recovered</td>
<td>38/49</td>
<td>78&lt;sup&gt;gh&lt;/sup&gt;</td>
</tr>
<tr>
<td>D 35&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>39/54</td>
<td>72&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>D 0 = first day of estrus.
<sup>b</sup>Fertilization rate = No. fertilized ova / (No. fertilized ova + No. unfertilized ova) × 100.
<sup>c</sup>Fertilization rate = No. fertilized ova / (No. fertilized ova + No. unfertilized ova + No. ERZP) × 100.
<sup>d</sup>ERZP = empty ruptured zona pellucidae.
<sup>e</sup>Two heifers with genital abnormalities were detected and were not included in the calculations.
<sup>f</sup>Five percent genital abnormalities assumed on the basis of findings for the d 3 and 16 groups.
<sup>g</sup>,<sup>h</sup>Values in the same column with different superscripts are different (P<.05).
from an oviduct adjacent to an ovary with only two ovulation sites.

In the present study, two ERZP were recovered from each of two heifers in the d 3 group, however, only one ovulation site was found on the ovary. A normal ovum (fertilized or unfertilized) and an ERZP were not recovered from any heifers, consequently, the origin of the ERZP remains unclear. Van Niekerk and Gerneke (1966) reported that in the mare unfertilized ova are retained in the oviduct, whereas fertilized ova are transported to the uterus for further embryonic development. Steffenhagen et al. (1972) observed ova retention in the oviducts of pregnant and nonpregnant mares. The mechanism(s) involved in ova retention in the mare are presently unclear. To our knowledge there is no evidence indicating that ova are retained in the oviduct of the bovine, however, that is one possible explanation for the large number of ERZP recovered.

There is evidence in the ewe that heat stress increases the percentage of abnormal ova (broken zona pellucida, empty zona pellucida, ruptured vitelline membrane, etc.) by approximately 3 d postbreeding (Dutt et al., 1959; Alliston et al., 1961; Dutt, 1963). Dutt (1963) concluded that the ovine zygote is most susceptible to heat stress while undergoing cleavage in the oviduct. The relationship between high ambient temperature and the incidence of abnormal ova (i.e., ERZP) in cattle is not known. Stott and Williams (1962), however, concluded that the poorer reproductive performance of dairy cattle during periods of high temperature and humidity is due to a lower fertilization rate and higher embryonic mortality.

It is conceivable that some of the heifers in study 1 were heat-stressed, since high ambient temperatures and humidity are not uncommon in South Texas during April and May. Conversely, the heifers in study 2 were not heat-stressed and the incidence of ERZP was similar to that observed in study 1. This finding does not eliminate the possibility of heat stress, however, because body temperature at the time of insemination may be unrelated to the mechanisms involved in gamete transport, fertilization, and cleavage.

Possibly, rectal temperature at another time or even a different measurement of heat stress may provide greater insight into the high incidence of ERZP.

In the d 16 group, a large variation in the length of bovine extraembryonic membranes was observed. Other researchers also have found a wide variation in the length of bovine extraembryonic membranes on d 16 and 17. Hawk et al. (1955) and Greenstein and Foley (1958) reported average extraembryonic membrane lengths in dairy cattle (d 16) at 95 mm (range 2 to 255 mm) and 43.6 mm (range 1.5 to 90 mm), respectively, however, the embryonic disc diameter remained fairly constant during this period. The d 16 embryos are reported to be undergoing gastrulation and rapid elongation of the extraembryonic membranes. Therefore, the variation in membrane length noted above is not surprising.

The data in table 4 indicate that some of the variation in extraembryonic membrane length may be accounted for by differences in embryonic age. Ovulation was assumed to occur approximately 30 h after the onset of estrus, and embryonic age was calculated as the time from ovulation to slaughter. As embryonic age decreased, so did the extraembryonic membrane length, although considerable variation was noted within age groups.

In summary, the conclusions drawn from this study depend upon the classification of the ERZP (table 5). When the ERZP are classified as ova ovulated at a previous cycle or ova damaged in the collection process and are deleted from calculations, the fertilization rate is high (93%). The difference of 21 percentage units between the d 3 and 35 groups was significant, suggesting that embryonic loss occurs in Brahman crossbred heifers between d 3 and 35 postinsemination. Since most of this loss (15 percentage units) occurred by d 16 (P = .07), these results support the previous studies of Boyd et al. (1969) and Diskin and Sreenan (1980).

If the ERZP are classified as ova ovulated at the immediately preceding estrus, unavailable for further embryonic development, and are included in the calculations, the fertilization rate is relatively low (80%). The difference of 8 percentage units between the d 3 and 35 groups is negligible (P>.05), leading one to conclude in this case that the major loss of potential embryos occurs before d 3 postinsemination.

Literature Cited


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