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DIAGNOSIS OF FREEMARTINISM IN HEIFERS BORN CO-TWIN TO A BULL

B. L. Larson and G. H. Kiracofe

Summary

The wide variance in anatomical characteristics among heifers born co-twin to a bull make diagnosis of freemartinism by measurements of vaginal length or the distance between the anus and vulva unreliable. Change in serum luteinizing hormone (LH) in response to injection of estrogen or gonadotropin releasing hormone (GnRH) is different in freemartins compared to normal heifers but is also an unreliable predictor of freemartinism. Presently, the most conclusive way to establish freemartinism in a heifer born co-twin to a bull is to measure the percentage of XX and XY chromosomes in cells from whole blood, which is a specialized and expensive procedure.

(Key Words: Freemartin, Twins, XY Chromosome, Chimer.)

Introduction

Approximately 2% of all cattle pregnancies result in twins, half of which are male-female pairs. Most females born co-twin to a bull are sterile freemartins, but up to 10% can be fertile. Freemartinism occurs when vascular connections occur between the male and female fetal membranes. These connections, or chorionic anastomoses, allow hormones from the male to pass to the female fetus and, in turn, adversely affect the development of the female reproductive tract and hormonal regulatory centers in the hypothalamus and/or the pituitary gland. Producers would benefit if a simple procedure could be developed to test for freemartinism in heifers born co-twin to a bull. Historically, measuring vaginal length and the distance between the anus and vulva have been used to determine freemartinism. We tested these methods of measurement, assayed LH response to an injection of estrogen or GnRH, and also tested for the percentage of XX and XY chromosomes (chimerism) in blood cells as predictors of freemartinism.

Experimental Procedures

Four heifers born co-twin to a bull and four single heifers from the same ranches were measured six times between 4 and 20 mo of age. Vaginal length was measured by inserting a lubricated probe as far as possible into the vagina with minimal force. The distance between the anus and vulva was measured from the bottom of the anus to the top of the vulva. The same heifers were used to study serum LH response to intramuscular injections of estrogen and GnRH both before and after puberty. Concentrations of LH was measured each 2 to 4 hr from 8 hr before to 32 hr after treatment with estrogen. Luteinizing hormone was also measured

4 hr before treatment; at the time of treatment; and at 1, 3, and 5 hr after treatment with GnRH.

Results and Discussion

Vaginal length and distance between the anus and vulva in normal heifers were quite uniform. Analysis of multiple measurements of freemartins showed that some individuals had smaller than normal genital measurements, some had longer than normal measurements, and others had vaginal length and anal-vulva distance measurements similar to normal heifers. This discrepancy makes a simple external measurement an inaccurate diagnosis of freemartinism in most cases.

Normal heifers exhibit a surge in LH about 16 hr after estrogen and 1 hr after GnRH injection. Other researchers have shown that freemartins respond differently to estrogen injections. Our data agree; we saw no consistency among freemartins in their responses. Some heifers showed a normal LH surge, and others showed a delayed surge or sometimes no surge in LH following estrogen. Similar results were seen following injections of GnRH. These results suggest that freemartins have different degrees of "female responses" ranging from almost normal female to almost normal male. Thus, LH responses are poor predictors of freemartinism.

The most accurate predictor of freemartinism is to examine tissue or blood chromosomes. Females should have 100% XX sex chromosomes, and males should have 100% XY sex chromosomes. If a female has even one XY chromosome, the condition is called chimerism and is an accurate predictor of clinical freemartinism. The disadvantages to this method are that only a few laboratories can run the test and the cost is at least \$50 per sample. All four heifers born co-twin to a bull in our experiment were blood chimeras. The percent chimerism ranged from 30 to 80% of cells having XY chromosomes. The percent chimerism did not correlate to abnormal external measurements nor to hormonal differences from normal heifers. When reproductive tracts were examined at slaughter, all four heifers born co-twin to a bull had abnormalities that would have prevented conception.

Although some heifers born co-twin to a bull are easily identified as freemartins because of gross abnormalities of external genitalia, many freemartins do not have noticeable external changes. The lack of consistent physical and hormonal differences from normal heifers means that an accurate, quick, and inexpensive diagnostic procedure is unavailable at this time. If a heifer born co-twin to a bull does not have noticeable genital abnormalities, the most accurate test is for blood cell chimerism.