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INCREASING TWINNING RATE IN BEEF CATTLE THROUGH EMBRYO TRANSFER

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ABSTRACT

Thirty-nine Limousin X Friesian heifers and forty-five Limousin X Friesian cows were randomly allocated to three treatments in a twin-induction breeding program. Animals were inseminated twice with Limousin semen (treatment A) or inseminated twice also with Hereford semen and each implanted with one embryo 7 days later (treatment B) or implanted with three embryos without prior insemination (treatment C). The pregnancy rates were 0%, 28% and 45% respectively. The twinning rates obtained in the three treatments were 59%, 64% and 56% respectively. There were three abortions in treatment B and six in treatment C. Twins had lower birth weight (38.5 kg versus 29.1 kg) and in the first seven weeks then twins (1.22 kg/day versus 0.87 kg/day). Transfer of three embryos to unbred recipients forms another potential method of inducing twinning in beef cattle.

Key words: Embryo transfer; twinning rates

INTRODUCTION

The combination of single calving and a gestation length of nine months results in an average production of five calves per cow in a lifetime and this puts a limit on the female contribution, both towards productivity output and towards genetic improvement (Diskin and Sreenan, 1984). The main constraint to productivity and profitability in the beef herd is the cow's reproductive rate. The use of embryo transfer technology could exploit significantly the female potential contribution by induction of twinning, thus increasing biological and economic efficiency (Davis, *et. al.*, 1989; Newcomb and Rowson, 1976).

A weaning rate of only 0.95 calves per cow per year has obtained even in well managed herds but increased rate of twinning will increase this rate significantly. Seidel (1981) points out that in North America, approximately 80% of the cows are beef animals whose only purpose is to produce one calf each year. About 70% of the nutrients consumed by each cow goes towards her maintenance whereas only the remaining 30% goes towards growth and maintenance of the calf during pregnancy and lactation. Seidel, therefore argues that in terms of converting feed stuff into beef, the process would be much more efficient with cows bearing twins.

Twinning is often associated with a number of management problems, such as increased frequency of dystocia, retained placenta and re-breeding problems. However even with these drawbacks, Cunningham (1976) points out that with twinning there is a significant net gain in the input of the population. Studies done by Diskin and Sreenan (1984) show that induced twin calving would raise the revenue in beef herd by 60% as compared to 12% in a dairy herd. Furthermore, Cunningham (1976) points out that increasing the twinning rate means that less cows will be needed as dams of both males and females of the next generation and therefore the dams can be selected with greater intensity.

Various methods have been used in attempts to induce twinning in cattle. Exogenous gonadotrophins have been used and found to be very unreliable. Selection for natural twinning has been tried but is very slow because of low repeatability and low heritability – typically less that 5% (Sreenan, 1977; Gordon, 1976, Rowson *et al*, 1975; Morris and Day, 1986). Selection may have a long-term role but it seems unlikely to make an immediate impact on increasing the calf crop (Newcomb and Rowson, 1976). Embryo transfer can offer a practical method of producing a high and reliable rate of twin pregnancy (Diskin and Sreenan, 1985; Gordon 1976).

Embryo transfer techniques have been shown to be effective for twin-birth induction in cattle. Methods for establishing twin-pregnancy include (i) transfer of one embryo to each uterine horn of a recipient, (ii) transfer of one embryo to the uterine horn contralateral to the corpus luteum of a bred recipient. Transfer of three embryos to an unbred recipient is another possible method. There is no published data available on twin-birth induction following transfer of more than two embryos. Numerous studies have been done to compare twin pregnancy rates following embryo transfer to a pre-

viously mated cow and two egg transfers to unmated recipients (Gordon, 1983; Sreenan, 1977; Parson, 1990, Rowson *et al*, 1971, Anderson *et al*, 1979). Results from these studies are not consistent. The main objective of this experiment was therefore to compare success in twin-birth induction following transfer of three embryos and the transfer of one embryo to a bred recipient. The other objective was to study the effects of twinning on gestation length, birth weight and growth rate.

MATERIALS AND METHODS

Animals: Forty-eight Limousin X Friesian heifers and fifty-two Limousin X Friesian cows were used in this experiment. All cows except six were suckling their calves. Heifers were ranked by liveweight and allocated to six groups at random within 8 liveweight classes. The cows were sorted according to calving dates and allocated to the 6 groups in a balanced arrangement with 6 dry cows allocated two to each treatment.

Treatments: Animals were allocated to three treatment groups at random with the classes described above. Under treatment A, thirty-one animals were synchronised and inseminated twice with Limousin semen. Under treatment B, twenty-six animals were synchronised, inseminated twice with Hereford semen and implanted with one embryo each. Twenty-seven animals in treatment C were synchronised and implanted with three embryos each.

All animals were inserted with Progesterone-releasing intravaginal devices (PRIDs) in the afternoon of the first day. Seven days later, the recipients were synchronised with a single injection of prostaglandins (PGF2a) in the morning. The following day, PRIDs were removed. Following PRID removal, oestrus was observed several times daily for the next seventy-two hours. Animals showing signs of oestrus were inseminated twice (treatments A and B) using a single dose of previously frozen semen at 12-hr intervals. Six days after insemination, embryo transfer was carried out non-surgically in animals under treatments B and C. Morphologically normal embryos in the morula through hatched blastocyst stage of development were transferred.

Ultra-sound scanning for detection of pregnancy was done at 36 days from first inseminations. A second ultra-sound scanning was carried out at 64 days from first inseminations. This second scanning was done to detect the cows which were carrying twins.

At late pregnancy, dams were placed in pens and fed according to whether they were carrying twins or single. Condition scoring was used to detect animals that needed an increment in nutrition. Animals were observed closely for signs of parturition. Any complications during calving were recorded. Birth weight, gestation length and growth rate were also recorded.

Statistical analysis: Data was analysed using GLM procedures (SAS, 1995). Pregnancy and twinning rates were compared between treatment groups using the chi-squared test. Gestation length, birth weight and growth rate were analysed with type of birth, age of animal, sex of calf and all possible two-way interactions as fixed effects.

RESULTS AND DISCUSSION

Results of the pregnancy and twinning rates are shown in Table 1. High pregnancy rates were achieved in inseminated cows that received an additional embryo (86%) and in heifers that had AI only (80%). Similar pregnancy rates ($P>0.01$) were observed in cows and heifers receiving three embryos. Low pregnancy rates were observed in cows that were inseminated only (37.5%) and in inseminated heifers receiving an additional embryo (41.6%). With heifers and cows combined, a higher pregnancy rate ($P>0.01$) was obtained in animals bred at oestrus and receiving an additional embryo (65%) in comparison with 58% and 56% respectively for inseminated (AI only) animals and animals receiving 3 embryos.

Low calving rates were observed for both heifers and cows receiving three embryos (33%). The low calving rates were also observed in bred heifers receiving an extra embryo and in cows receiving AI treatment. Cows in the AI treatment carried the pregnancy to term, even though the calving rate was very low.

Cows and heifers in the AI treatment group did not produce any twins, further establishing that natural twinning is very low. The twinning rate was lower ($P<0.01$) in the AI plus one embryo treatment group (B) than in the three-embryo transfer group (C). The twinning rates were similar ($P<0.05$) between heifers and cows in treatments B and C. In the study done by Anderson *et al.* (1979), the twinning rate obtained in cows inseminated prior to the transfer of an additional embryo was 70%. In this study, the twinning rate was only 30% in the AI plus one embryo treatment group. In the experimental studies reported by Sreenan and Diskin (1984) twining rates of 40% to 50% were consistently achieved following transfer of either fresh or frozen-thawed embryos to inseminated cows. In this study, a twinning rate 44% (cows and heifers combined) was achieved following transfer of three embryos. Studies on induced twinning have shown that insemination followed by one embryo transfer can be successfully used to induce twinning (Diskin and Sreenan, 1986; Anderson *et al*, 1979; Gordon, 1983). The results of this experiment suggest that transfer of three embryos may also be a successful means of inducing twinning.

Least number of abortions were in the AI only treatment group while the most number of abortions

Table 1: Pregnancy and twinning rates in cows and heifers

	Treatment					
	Cows			Heifers		
	AI only	AI plus embryo	Three embryos	AI only	AI plus embryo	Three embryos
Number bred	16	14	15	15	12	12
Number pregnant @ 60days (%)	6(37.5)^{c}	12(85.7)^{a}	8(53.3)^{b}	12(80.0)^{a}	5(41.6)^{c}	7(58.3)^{b}
Aborted*	0	2	3	1	1	3
Calved (% of those bred)	6(37.5)^{b}	10(71.4)^{a}	5(33.3)^{b}	11(73.3)^{a}	4(33.3)^{b}	4(33.3)^{b}
Produced twins (% of those calving)	0(0)^{a}	3(30)^{b}	2(40)^{c}	0(0)^{a}	1(25)^{b}	2(50)^{c}
Calves born/cow calving	1.0	1.3	1.4	1.0	1.25	1.5

^{abc} Means with different superscripts within a row (within group) are different at P<0.05
Figures in parentheses are percentages

*Leptospirosis confirmed in two out of six aborted foetuses examined

were in the embryos treatment group. These abortions which occurred in late pregnancy were confirmed in some cases to have been due to leptospirosis. The overall calving rates as a proportion of cows bred were therefore disappointing. However, other studies show calving rates of about 50%. Diskin and Sreenan (1984) for-instance obtained 58% and 50% respectively in AI animals only and AI plus one embryo animals.

Retained foetal membranes were observed in 6 cows and heifers. Most of the animals that had retained foetal membranes had aborted twins. One set of triplets was produced in the 3 embryo treatment (Group C) but were born dead. Eight heifers and 3 cows required assistance calving.

Table 2: Mean birth weights, gestation lengths and growth rates of singles and twins

	Heifers		Cows		All	
	Single	Twin	Single	Twin	Single	Twin
Calf birth Weight (KG)	34.0	25.9	42.0	31.0	38.5	29.1
	±0.8^{a}	±1.3^{b}	±1.7^{a}	±1.5^{b}	±1.2^{a}	±1.2^{b}
Calf weight per cow calving	34.0	51.8	42.0	62.0	38.5	58.2
	±0.9^{a}	±2.0^{b}	±1.1^{a}	±2.1^{b}	±1.2^{a}	±2.1^{b}
Gestation Length (days)	289.0	276.0	290.0	276.6	289.2	278.0
	±5.0^{a}	±4.8^{b}	±3.8^{a}	±4.6^{b}	±4.2^{a}	±3.0^{b}
No. cows with RFM*	1	5	2	4	6	6
Growth rate (kg/day)					1.22	0.87
0-50 days					±0.05^{a}	±0.04^{b}

ab Means with different superscripts within a row (within group) are different at (P<0.05)
*RFM – Retained foetal membranes

Table 2 shows mean birth weights, gestation lengths and growth rates for the twin and single calves. Twins had lower birth weight than singles ($P<0.01$) regardless of whether they were produced by heifers or cows. Twins born to heifers had lower ($P<0.05$) birth weight than twins born to cows. Also, the single produced by heifers had a mean birth weight lower ($P<0.05$) than single calves regardless of whether they were produced by heifer or cows. Twins born to heifers and cows had similar ($P<0.05$) gestation lengths. Twins had lower ($P<0.01$) growth rates than singles. There was no difference in growth rates between twins born to heifers and cows or between singles born to heifers and cows.

Calves born per cow calving was higher in recipients getting three embryos than cows in other treatment groups. In a number of twinning studies, the calf production per cow is on average. 1.6. In this study, the calf production per cow could have been lowered by the high incidence of abortion caused in some cases by leptospirosis. Results show that even though calf loss at birth was higher in twins than singles, the calf production per cow calving was greater for dams producing twins. Numerous studies aimed at inducing twinning have shown that lower birth weights, shorter gestation periods and increased incidence of retained foetal membranes can be expected with twin birth (Guerra-Martinez *et al*, 1987; Turman *et al*, 1971; Reid *et al*, 1986; Echternkamp and Gregory, 1999, Gregory *et al*, 1996, Hunter, 1980). This was confirmed in this study (Table 2).

The study indicates that twin-birth induction can be achieved with relative success following transfer of three embryos to unbred recipients. The study further indicates that the transfer of three embryos to recipients

could give an improved calf production per cow of about 1.5. It should be expected that transferring three embryos will result in increased costs. However, as the reproductive technologies are further improved and refined, it would be possible to produce large quantities of embryos at low cost. Improvement in in-vitro follicular maturation and in-vitro fertilization, sexing of embryo, embryo splitting, cloning and cryopreservation technology will increase the supply of embryos and add flexibility to embryo transfer techniques. This would in turn make viable the transfer of three embryos.

The results of this study are clear but tentative since they could have been affected by low pregnancy rates and increased number of abortion due to leptospirosis. Concrete conclusions therefore may not be made with regards to which method would give greater success in twin-birth induction. Further investigation is necessary to establish which method would be more effective for inducing twin-birth.

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