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Study on inducing twin calves in beef cattle through embryo transfer technology

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Abstract
The low reproduction rate of beef cattle constrains the development of the beef industry. Twin calving has long been a focal point of research due to its potential to enhance reproductive efficiency and increase beef production. In Hohhot, Inner Mongolia, two groups of Simmental cattle were evaluated to assess the effectiveness of twin calving induction through embryo transfer technology and artificial insemination. Group I involved the simultaneous transfer of two embryos, while Group II employed a combined strategy integrating single embryo transfer with artificial insemination. The results showed that 18 out of 22 cows in Group I exhibited estrus, resulting in an 82% estrus detection rate. Subsequently, 14 cows conceived (77.8% conception rate), and 10 delivered twins, resulting in a 71.4% twin rate. In Group II, 45 out of 50 cows exhibited estrus (90% estrus detection rate), 36 conceived (80% conception rate), and 22 gave birth to twins, resulting in a 62% twin birth rate. This study significantly enhances reproductive efficiency in cows and provides valuable technical support for implementing twin calving in beef cattle production.

Keywords: Beef cattle, twin calves, embryo transfer technology, artificial insemination

Introduction
As living standards improve, beef demand has risen, leading to price surges due to reliance on imported beef. The heritability of twinning in cattle is notably low, with heritabilities of 0.07 and 0.03 for twin ovulation and birth rates, respectively[1]. Furthermore, the lengthy generational intervals in cattle hinder the increase of twinning rates through genetic selection. Consequently, the limited reproductive efficiency of cattle is a significant obstacle to the advancement of China's beef industry.

Since the 1940s, researchers worldwide have employed a variety of techniques, including genetic selection, reproductive hormones, and embryo transfer (ET), to investigate the artificial induction of twinning in cattle, with positive results. Renard et al. (1977) reported a 50% twinning rate by transferring two in vivo embryos. Subsequent studies have confirmed this finding [2]. Additionally, synchronized estrus, supernumerary ovulation, and the establishment of a favorable uterine environment following artificial insemination (AI) and ET can improve twin production rates [3-7]. Therefore, reproductive biotechnologies for inducing twinning in beef cattle are an effective strategy for enhancing reproductive efficiency in the beef industry.

This study aimed to investigate the advantages and disadvantages of two biotechnological methods: double ET and single ET combined with AI, through a comparative analysis. In the double ET method, the probability of obtaining twins is increased by transferring two embryos into the same cow, but this also increases the risk of multiple pregnancies. In contrast, the single ET plus AI method allows more precise control over the number of embryos, thereby improving genetic quality and reproductive efficiency. Unlike previous studies, this study focused on combining these two methods in a specific population of Simmental cattle in the region. The aim was to optimize reproductive efficiency and reduce physiological burdens, providing a more practical breeding strategy for the regional beef industry. This approach combines the advantages of double ET with the precise control of in vitro fertilization, providing greater flexibility in adjusting strategies during reproduction to achieve more efficient breeding outcomes.

Materials and Methods

Selection of Donor Cows:

Twenty high-quality Simmental cattle were selected as donors. These cattle were 14 months old, exhibited healthy physiques, had well-developed ovaries, and tested negative for Brucella and tuberculosis. They also possessed complete pedigrees and were free from reproductive ailments[8]. In vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC) of Simmental cattle embryos were carried out following the method described in the 'In Vitro Production of Bovine Embryos' published by the P.J. Hansen Laboratory, Department of Animal Sciences, University of Florida.

Selection of Recipient Cows:

Simmental beef cattle were selected based on the following criteria: they tested negative for Brucella and tuberculosis, demonstrated above-average nutritional status, and exhibited robust reproductive health. The cattle were aged between 3 and 5 years, had 2 to 4 prior calving experiences, and possessed normal reproductive organs with no history of uterine inflammation, dystocia, or miscarriage. Eligible candidates exhibited a normal estrous cycle, well-developed ovaries with active corpora lutea or follicles, and a body size indicative of good overall health and reproductive potential [9, 10]

Grouping of Experimental Cows:

The cows were allocated into two groups. Group I consisted of 22 recipient cows; each implanted with two embryos. Group II comprised 50 recipient cows, each undergoing ET coupled with AI.

Experimental Drugs:

The study utilized an intravaginal progesterone insert (CIDR-1380), 5 mL of cloprostenol sodium injection (PG) from NINGBO SANSHENG Company, 2 mL of gonadorelin (100 μg X 10) from NINGBO SANSHENG Company, and 20 mL of Folltropin (FSH, 700 IU) from Canada (REF: 21000/0004).

Experimental Procedure:

Group I: Estrus in recipient cows was synchronized (Table 1), with the onset of estrus designated as day 0. On day 7, the position of the corpus luteum in recipient cows was examined, and two fresh embryos were transplanted into the uterine horn adjacent to it [11, 12].

Group II: Estrus in recipient cows was synchronized (Table 1), with estrus onset marked as day 0. Insemination was performed once at the conclusion of estrus and again the following morning. On day 7, after checking the position of the corpus luteum in recipient cows, one fresh embryo was transplanted into the uterine horn adjacent to it.

Table 1.

The Schedule of Superovulation of Donor Cattle and Synchroestrus AI, ET of Recipient

Day time		0	9	10	11		12		13	14	21
Donor	a.m. 6:00	Handls CIDR	FSH 1.8 mL	FSH 1.4 mL	FSH 1.0 mL	PG 5.0 mL	FSH 0.5 mL	OUT CIDR		AI	Collection of eggs
	p.m. 5:00		FSH 1.8 mL	FSH 1.4 mL	FSH 1.0 mL	PG 5.0 mL	FSH 0.5 mL		AI		
Group I		PG 5.0 mL			PG 5.0 mL						ET (2 embryos)
Group II	a.m. 7:00 p.m. 6:00	PG 5.0 mL			PG 5.0 mL				AI	AI	ET (1 embryo)

Results

The experimental outcomes, detailed in Table 2, show that both Groups I and II successfully induced twin calves. In Group I, recipient cattle had an average estrus rate of 82% and a conception rate of 77.8%, leading to the birth of 24 calves and a twin rate of 71%. In Group II, recipient cows had an average estrus rate of 90% and a conception rate of 80%, resulting in the birth of 58 calves and a twin rate of 62%. Notably, Group I had a higher twin rate of 71%. With similar pregnancy rates, we hypothesize that the difference in twinning rates may be attributed to the use of double embryos, individual variability, and differences in ET procedures.

Table 2.

The experiment results in producing twins in every group Recipient

Group	Recepto r	Oestrous symptom	Oestrous rate (%)	Pregnanc y symptom	Pregnancy rate (%)	Calving situation		
						Calving number	Twins	Twin rate (%)
I	22	18	82	14	77.8	24	20	71
II	50	45	90	36	80	58	44	62

Discussion

Cattle are naturally uniparous, with a cow typically producing one calf every three years under natural conditions, resulting in an average lifetime production of 7~8 calves. The reproductive cycle of cattle is prolonged, and the heritability rate for twinning in cows is extremely low, at just 0.04%[13]. The results of this study show that the twin rate reaches 71% through the induction of twinning via the transplantation of two embryos. Moreover, combining AI with ET to induce twinning results in a twin rate of 62%, significantly improving reproductive efficiency in cattle.

The conception rates of 77.8% for Group I and 80% for Group II emphasize the importance of careful selection and management of recipient cows to maximize the success of embryo transplantation, pregnancy, and calving. This underscores the importance of recipient cow selection and management in improving the efficacy of ET procedures, consistent with the findings of Men Hongsheng et al. (1998) [14].

The study suggests selecting recipient cows with 2~4 prior calvings for transplantation, as this maximizes twin rates up to 71%, supporting previous research. While some scholars have suggested younger cows are ideal due to better manageability, enhanced fertility, fewer reproductive diseases, and lower fat content[15], this study supports the preference for cows with 2~4 calvings as the optimal candidates for embryo transplantation. Regarding post-pregnancy outcomes, twinning of different sexes occurs when the fetuses are male and female. In such cases, each twin has its placenta, but the chorionic membranes merge, allowing vascular anastomosis and shared blood circulation. Approximately 95% of female calves born in male-female twin pregnancies are sterile. This is attributed to the earlier development of the male fetuses' testes, which suppress the hormonal secretion of the female fetus, resulting in a hormonal imbalance. Prolonged exposure to male hormones hinders the development of the female calf's reproductive system during fetal life. This disrupts the regulation and secretion of hormones within the hypothalamic-pituitary-gonadal axis in adulthood. As a result, poorly developed and malformed reproductive organs form, characterized by a narrow vulva, elongated clitoris, slender uterus, and small, underdeveloped ovaries. These abnormalities render the female calf’s incapable of producing mature follicles or undergoing ovulation. Although sex control technology can mitigate these breeding issues, they do not pose a concern for the meat-focused beef industry, making this experimental approach viable for beef production.

The use of double ET and single ET plus AI in beef production can significantly improve economic outcomes and scalability. Double ET increases success rates by producing more twins, enabling rapid growth in the number of high-quality breeding cows and improving production efficiency. This method is particularly suitable for large-scale breeding, rapidly increasing the number of breeding stock and improving meat production. However, double ET is more costly, requires additional technical support and resources, and may increase the physiological burden on the cow.

On the other hand, single ET plus AI transfers fewer embryos each time but reduces production costs by precisely controlling mating timing and

improving sperm quality. It is more economical and sustainable, making it especially suitable for small- and medium-scale breeding. In addition to these promising results, challenges in implementing these methods and study design limitations must be addressed. First, a major challenge in implementing these methods is the high management demands for recipient cattle, as success relies on selecting suitable cows with a history of giving birth to ET. These management demands require significant resources and expertise, which may be difficult to maintain in large-scale farms or resource-limited areas. A limitation of this study was the lack of a control group for direct comparison due to cost constraints, making it difficult to determine whether the observed results were solely attributable to the double ET method or influenced by other uncontrolled factors. Future studies should include control groups and explore the scalability of these techniques across various farm sizes and regions. This will help better understand their practical application in beef production and optimize their dissemination.

Conclusions

In this study, two embryos were successfully transplanted to induce twinning in cows, significantly improving reproductive efficiency. The experimental results demonstrate that careful selection and management of recipient cattle led to a high pregnancy rate, with the highest twin rate reaching 71%. Selecting recipient cattle with 2–4 prior calvings is key to achieving a high twin rate. This study demonstrates the efficacy of ET, combined with AI, in enhancing twin rates in Simmental beef cattle, with a maximum twin rate of 71% observed with double ET. The findings emphasize the importance of careful selection and management of recipient cows to achieve optimal reproductive outcomes. However, it is worth noting that about 95% of female calves in male-female twin pregnancies experience infertility due to the hormonal influence of male fetuses. While sex control technology can alleviate these breeding issues, it does not directly affect beef production, making these methods viable for the beef industry. This study provides an effective twin breeding technology for beef cattle in southwest mountainous areas and offers a new approach to improving breeding efficiency. Future research should focus on scaling these methods, addressing postnatal health issues in calves, and improving sex-control technologies to mitigate reproductive challenges.

Conflict of interests

The authors declare no conflict of interests.

Authors' contribution

L S. writing original draft preparation, performed reseacrch, O. S. contributed to the experiments and E. P. supervision, review and editing. All authors have read and approved the final manuscript.

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