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Studies on the Production of Twins in Cattle

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Abstract

Induction of twinning was attempted by transfer of two whole- or demi-embryos in Holstein cows and heifers.

Cows were superovulated with follicle stimulating hormone(FSH) administered twice daily in intramuscular injection. On day of 6.5 to 7 post-estrus, embryos were collected nonsurgically, Normal morulae and early balstocysts were obtained from superovulated cows. The embryos were bisected with a micro-blade made from a razor. Twenty seven pairs of half embryos were successfully produced and 23 pairs of half embryos were transferred to recipients(Group A). Twenty cows were treated with low unit of FSH (3mg×2, 2mg×2; Group B1, 2mg×2, 1mg×2, 1mg×2; Group B2) to induce double or triple ovulations. The cows of Group B1 and B2 were inseminated artificially(A1) at following estrus. Twenty four heifers were bred by AI and received an additional embryo into the uterine horn contralateral to the corpus luteum(CL) 6 days later(Group C). One embryo was transferred into each uterine horn of 16 heifers 6 days after estrus(Group D). Fourteen heifers were received two embryos into the uterine horn ipsilateral to the CL at day 6 of estrous cycle(Group E).

Pregnancy rates at 60 days in Goup A, B1, B2, C, D and E were 34.8%, 70.0%, 60.0%, 66.7%, 62.5% and 57.1%, respectively. Twinning rates were 8.7%, 20.0%, 10.0%, 16.7%, 18.7% and 21.4%, respectively.

The present experiments demonstrate that FSH treatement(Group B1) and ipsilateral transfer(Group E) of two whole-embryos are more useful methods to produce twins than the others.

Introduction

The rate of natural twin births in cattle has been reported to be generally between 1 and 5 percent and varies according to breed, age and environment(Arthur, 1964; Meadows and Lush, 1957). Many attempts to increase this twinning rate have been carried out in mammals.

Induction of twinning by breeding(Mechling and Carter, 1964) or induction of multiple ovulation (McGowan *et al.*, 1985) has been performed but the results of these experiments have not been satisfactory.

Production of twin calves by embryo transfer following AI and transfer of two embryos were reported by many researchers(Anderson *et al.*, 1979; Rowson *et al.*, 1971; Tervit *et al.*, 1972; Sreenan *et al.*, 1976; Bon Durant *et al.*, 1982) and these techniques have been shown to be effective for inducing twin pregnancies in cattle. However,

the rates of twinning were between 28% and 70% with researchers and transfer techniques.

Recently, the artificial production of monozygotic twin by bisection of embryos has been extensively investigated in farm animal. Although the pregnancy and twinning rates were high following transfer of demi-embryos(Ozil *et al.*, 1982), there are difficulties in embryo bisection and culture of demi-embryos.

The objective of present studies was to compare several methods of inducing twinning and to evaluate the practical applicability of these methods in cattle.

Materials and Methods

Total of 113 Holstein cows and heifers were used in these experiments.

Ten mature donor cows were superovulated with twice-daily intramuscular injections(12hours apart) of 5mg of follicle stimulating hormone (FSH;FSH-P*, Burns Biotec, USA) for 4days starting on day 10 to 13 of the estrous cycle (estrus=day 0). A 25mg-dose of prostaglandin $F_2\alpha$ ($PGF_2\alpha$;Lutalyse*, Upjohn Co., USA) was administered twice intramuscularly 48 hours and 60 hours after the first FSH injection to induce luteolysis. Donor cows were inseminated artificially with one unit of frozen semen from one Holstein bull twice at 12-hour intervals, starting 12 hours after the onset of standing estrus.

At 6.5 to 7 post-estrus, minimun essential medium(MEM;MEM*, Flow laboratories, USA), containing 2% heat-treated fetal calf serum(FCS) and an antibiotic solution(100units of penicillin and 50μg of streptomycin/mℓ of medium), was used to collect embryos non-surgically from each donor female. Uterine horns were flushed using an 3-way Foley catheter according to procedures previously described(Newcomb *et al.*, 1978).

Immediately after collection, ova were morphologically evaluated and assigned a quality grade under an Olympus stereomicroscope(60×).

Morphologically normal morulae and early blastocysts were bisected with a micro-blade by the method of Yoshida(1985). During the microsurgery, embryos were held at room temperature (20℃) in a sterile plastic petri dish containing PBS+20% FCS medium. After bisection of embryos, one half embryo was relocated in a parent zona pellucida and another was located in an additional zona pellucida obtained from degenerated egg.

The basic culture procedure was similar to that of Wright and Bondioli(1981). Twenty seven pairs of demi-embryos were put into microdrops(100 $\mu\ell$) of BMOC-3 medium under paraffin oil and then incubated at 37℃ for 6 to 12 hours under an atmosphere of 5% CO_2 and 95% air. Re-formation of the blastocoele was observed under an inverted microscope(American Optical Co., USA).

Transfer of demi-embryos(Group A) : Twenty seven pairs of half embryos were successfully produced and twenty three pairs were transferred to recipients. Each pair of demi-embryos was loaded in a 0.5mℓ French straw and transferred to the tip of uterine horn ipsilateral to the corpus luteum using a Cassou gun by the method of Rowe *et al.* (1980).

Induction of double or triple ovulations with low unit of FSH : Twenty Holstein cows were treated with twice-daily intramuscular injections(12hours apart) of FSH(3mg×2, 2mg×2;Group B1, 2mg×2, 2mg×2, 1mg×2;Group B2) on day of 10 to 13 of the estrous cycle. A 25mg of $PGF_2\alpha$ was administered intramuscularly 24 hours after the first FSH injection to induce luteolysis. After the onset of standing estrus, cows were inseminated artificially with one unit of frozen semen twice at 12-hour intervals. The number of corpus luteum was estimated by rectal palpation seven days later.

AI and transfer of an additional embryo(Group C) : Twenty four Holstein heifers were bred at natural esturs by AI with frozen semen. AI was carried out 2 times at the interval of 12hours after estrus. Six days after estrus an additional embryo

was transferred into the uterine horn contralateral to the CL.

Bilateral transfer of two embryos(Group D) : One embryo was transferred to each uterine horn of 16 Holstein recipient heifers. Each embryo was respectively located in a 0.5㎖ French straw and transferred to recipients using a Cassou gun.

Table 1. Induction of Superovulation with FSH and $PGF_2\alpha$ in Holstein Cows

No. of donor cows	No. of corpus lutea	No. of embryos recovered(%)	No. of transferable embryos(%)
18	287	196(68.3)	127(64.8)

Table 2. Embryos non-surgically Collected for Micromanipulation and Subsequent non-surgical Transfer to Holstein Recipients

Item(Group A)	Number	Percent
No. of embryos selected for micromanipulation	33	100
No. of embryos bisected sucessfully	27	81.8
No. of recipients available	23	100
No. of pregnant at day 60	8	34.8
No. of recipients calved twin	2	8.7

Table 3. Ovarian Response and Pregnancy Rate in Low Unit of FSH Treated Cows

Dose of FSH	No. of cows treated	No. of corpus lutea	Pregnancy rate(%)	Twin birth rate(%)
Group B1				
3mg×2	10	31	70	20
2mg×2				
Group B2				
2mg×2				
1mg×2	10	33	60	10
1mg×2				

Table 4. Reproductive Performances with non-surgical Transfer to Holstein Heifers

Embryos transferred	No. of recipients transferred	No. of recipients pregnant D60	No. of calves delivered	Twin birth (%)
Group C				
A1+1*	24	16(66.7%)	20	4(16.7)
Group D				
1+1**	16	10(62.5%)	13	3(18.7)
Group E				
2ET***	14	8(57.1%)	11	3(21.4)

* : One embryo was transferred into the uterine horn contralateral to the corpus luteum after artificial insemination at estrus.

** : Two embryos were transferred into each uterine horn.

*** : Two embryos were transferred into the uterine horn ipsilateral to the corpus luteum.

Unilateral transfer of two embryos(Group E) : Two whole-embryos were located in a 0.5mℓ French straw and transferred to the uterine horn ipsilateral to the CL in 14 Holstein heifers.

Sexual behavior of recipients was checked twice daily to detect esturs return. An early pregnancy diagnosis by rectal examination was performed on day 60 after embryo transfer and AI. After calving, twinning rates were confirmed by the number of calves born.

Results and Discussion

The result of superovulation with FSH and $PGF_2\alpha$ in Holstein donor cow was shown(Table 1). A total of 287 ovulations(No. of CL) was estimated by rectal palpation, and 196 developing embryos and/or unfertilized ova were recovered from the donors with a recovery rate of 68.3%(196/287×100). Out of the 196 embryos, 127(64.8%) were transferable. The rate of ovulation(overian response) was higher but the rates of recovery and transferable embryos tended to be lower than those of other experiments(Wubishet *et al.*, 1986, Savage *et al.*, 1987). These differences indicated that more defined technique was needed.

Thirty three embryos were selected for micromanipulation. Twenty seven of 33 embryos(84.8%) were bisected successfully using a micro-blade under the micromanipulator. Twenty three pairs of demi-embryos were transferred to Holstein recipients.

Pregnancy diagnosis at day 60 revealed that 8 of 23 recipients were pregnant(34.8%). Out of them, two sets of monozygotic twins were born (twinning rate of 8.7%). In the present study, the pregnancy rate following non-surgical transfer of recipients was 34.8%. This pregnancy rate was lower than 64.2% of pregnancy rate following non-surgical transfer of paired demi-embryos into one uterine horn of paired demi-embryos to recipients by Ozil *et al.*(1982) and 52% by Baker(1985), but higher than the 16.6% pregnancy rate by Williams *et al.*(1982). The twinning rate in the present study was 8.7% and was lower than 50.0% of twinning rate reported by Ozil(1983).

Ovarian response and the rate of pregnancy and twinning in the cows treated with low unit of FSH were presented in Table 3.

The number of CL and pregnancy rate are not different markedly between Group B1 and B2. However, the rate of twin birth in Group B1 was slightly higher than that in Group B2.

From the results, the difference of FSH treatments did not affect on the ovarian response. Therefore, the procedure of Group B1 can be applicated effectively in induction of multiple ovulation.

Pregnancy and twinning rates in Group C, D and E were presented in Table 4. The pregnancy rates were 66.7%, 62.5% and 57.1%. The twinning rates were 16.7%, 18.7% and 21.4%, respectively.

The pregnancy rates among the groups were similar with the reports by Anderson *et al.*(1976, 1979) and Rowson *et al.*(1971), but the rates of twinning were lower than the above reports.

The overall pregnancy rate in the present study is similar with those of previous reports but the twinning rates are low for practical application. It is suggested that more field trial and technical improvements are needed to increase the efficiency in induction of twinning.

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소의 쌍자 생산술 개발에 관한 연구

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초 록

소에 있어서 효율적인 쌍자생산 방법을 검토하기 위하여 이분수정란의 이식, 저단위 FSH에 의한 다배란 유도후의 인공수정, 인공수정후의 수정란이식, 수정란 2개의 양측 및 단측 자궁각에의 이식 등의 방법을 적용하여 본 실험을 수행하였다.

각 실험군의 60일 수태율은 각각 34.8%, 65.0%(평균), 66.7%, 62.5%, 57.1%였으며 쌍태임신율은 8.7%, 15.0%(평균), 16.7%, 18.7%, 21.4%였다.

위의 결과에서 볼때 저단위 FSH투여법(Group B1)과 수정란 2개의 단측 자궁각 이식방법에 있어 쌍태율이 높게 나타나는 경향이 있었으나 실용적 측면에서는 낮은 수준이었다.