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Induction of Twin Pregnancy Using Frozen-Thawed Embryo Transfer in Cattle

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동결 우수정란의 융해후 쌍태유기

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초 록

공란우 89두와 수란우 94두를 사용하여 호르몬 투여에 대한 난소반응, glycerol의 평형과 제거방법에 따른 융해란자의 형태적 정상성과 동결 융해란의 이식후 임신율과 쌍태유기율을 조사하였다.

1. 호르몬처리에 대한 난소반응 : 1) 과배란 처리후 배란된 난자수는 A. B. C군에서 각각 8.3, 7.8, 9.5개로서 C군이 전체평균의 8.3개보다 증가되었다. 2) 난자의 회수율은 42.1%, 두당회수된 난자수는 3.5개였으며, 회수란자중 정상발육한 상실배와 배반포기의 난자는 63.3%, 36.7%(114/311)가 형태적 이상란자로 확인되었다.

2. 수정란의 동결 융해후 형태적 정상성 : 1)동결융해후 회수된 난자 중 정상란자의 비율은 60.4%였으며, 배반포(62.3%)가 상실배(58.3%) 보다 우수하였다. 2) 1단계 glycerol의 제거시에는 동결전 glycerol의 평형을 3단계로 실시한 군(C. G)이 1단계 평형군(A. E)보다 우수하였다.

3. 동결융해란의 이식후 쌍태유기 : 1) 수정란의 이식후 임신율은 단태(대조)구에서 30.0%, 쌍태유기군인 A(AI+단태), B(편각쌍태) 및 C(쌍각쌍태)구에서 각각 55.6, 44.4, 37.5%로서 대조구보다 증가되었다. 2) 쌍태유기에 공용된 26두의 수란우에 착상된 수정란수는 17(65.4%)개로서 단태구(30.0%) 보다 배가되었다.

Introduction

Bovine embryos have been successfully frozen by a variety of treatments (Trounson et al., 1978; Bilton et al., 1979; Lehn-Jensen et al., 1981) since the first live calf was produced from a frozen-thawed embryo in (Wilmut et al., 1973). However, one impediment to the practical use of freezing as an adjunct to embryo transfer in cattle has been the method used to remove the cryoprotectants from the thawed embryos. This multi-step dilution method required several hours and is performed under a microscope and laboratory apparutus.

The main cost of production in the beef cow herd is what associated with cow maintenance. Because of this, cows producing twin calves would be more effecient than cows producing single calves. In the dairy herd, twin calving also have relevance, particularly to the portion of the herd not required for replacement breeding.

The present work was carried out to investigate the feasibility of step dilution of cryoprotectant by a surcrose solution followed by direct transfer of embryos frozen-thawed, and the twin-induction by the transfer with embryos frozen-thawed.

Materials and Methods

Donor cows were injected on day 9 to 14 of the estrus cycle with FSH (group A) or PMSG(group B) following $PGF_{2\alpha}$. Of donors treated with PMSG and $PGF_{2\alpha}$, a part was given hCG injection at the onset of estrus(group C). Donors were inseminated 3 times after the onset of estrus. Ovarian responses were determined via rectal palpation. Embryos were recovered on 7-8 days of estrus cycle by non-surgical flushing from the uterine horn with modified Dulbecco's PBS medium using Foley catheter (Rush, Germany).

Embryos were frozen in PBS solution plus 10 %

Table 1. Methods for Equilibration and Elimination of Glycerol before and after Freezing Embryos

Group	Embryo stage	Equilibration of glycerol	De-glycerol
A	Modula	1 step	1 step
B	"	"	3 step
C	"	3 step	1 step
D	"	"	3 step
E	Blastocyst	1 step	1 step
F	"	"	3 step
G	"	3 step	1 step
H	"	"	3 step

Table 2. Effect of Hormone Treatment on the Ovarian Response

Treatment	No. of animals	No. of C.L. (No./cow)	No. of ova recovered (No./cow)	Recovery rate (%)
A	19	158 (8.3)	67 (3.5)	42.4
B	48	372 (7.8)	164 (3.4)	44.1
C	22	209 (9.5)	80 (3.6)	38.3
Total	89	739 (8.3)	311 (3.5)	42.1

A: FSH 28AU + $PGF_{2\alpha}$ 35mg B: PMSG 2,000-3,000IU + $PGF_{2\alpha}$ 25mg
C: PMSG 2,000-3,000IU + $PGF_{2\alpha}$ 25mg + hCG 2,000 IU

glycerol. Gycerol was added in two ways (1 and 3 step) to the embryo before freezing and removed in two ways (1 and 3 step) after thawing embryos (table 1). The embryos were cooled from room temperature to -70°C at 1°C/min, seeded at -7°C after 5 minutes, further cooled to -35°C at 0.3°C/min, and to -40°C at 0.1°C/min and then plunged into LN_2(-196°C). Embryos recovered after thawing were assessed by morphological normality with the dissecting microscope.

Embryos frozen-thawed were unilaterally transferred on Day 7-8 (estrus: Day 0) to the tip of the uterine horn ipsilateral to the C.L.(contrl), contralateral to C.L. of recipients inseminated (group A-, and bilaterally transferred to the uterine horn ipsilateral to the C.L. (group B), or to the uterine horn ipsilateral and contralateral to the C.L. (group C). Recipients were used synchronized by $PGF_{2\alpha}$ or natural estrus. All recipients were palpated per rectum on Day 40-60 to assess pregnancy.

Results and Discussion

Effect of hormone treatments on the ovarian response was summarized in Table 2. The best result obtained in numbers of corpus luteum and ova recoved was showed in PMSG 2,000-3,000IU + $PGF_{2\alpha}$ 25mg + hCG 2,000IU (group C). However the recovery rate of group B (PMSG 2,000-3,000IU + $PGF_{2\alpha}$ 25mg) was more than those of group A (FSH 28AU + $PGF_{2\alpha}$ 35 mg) and C. The results obtained from non-surgical recovery in these experiments were slightly lower than that of Hasler et al. (1983), but similar to Gu and Chung (1982) generally. The differences of recovery rate might be reflected on the condition of donor, the method of hormone treatment and the skill of operator.

Developmental stage and morphology of ova re-

Table 3. Developmental Stage and Morphology of Ova Recovered on Day 7-8 of Estrus

Treatment	No. of ova recovered	Embryo stage and morphology			
		Normal		Abnormal	
		Morulae(%)	Blast(%)	1-8cell(%)	degen.(%)
A	67	24 (35.8)	28 (41.8)	5 (7.8)	10 (14.9)
B	164	56 (34.1)	44 (26.8)	30 (18.3)	34 (20.7)
C	80	19 (23.8)	26 (32.5)	14 (17.5)	21 (26.3)
Total	311	99 (31.8)	98 (31.5)	49 (15.8)	65 (20.9)

Table 4. Morphology of Frozen and Thawed Bovine Embryos

Group	Embryo stage	No. of eggs frozen	No. of eggs recovered after thawing	Morphology of embryos thawed			
				Intact(%)	Damaged(%)		
					Partial	Complete	Total(%)
A	Morula	13	11	5 (45.5)	3	3	6 (54.5)
B	Morula	13	12	7 (58.3)	1	4	5 (41.7)
C	Morula	11	11	7 (63.6)	2	2	4 (36.4)
D	Morula	14	14	9 (64.3)	3	2	5 (35.7)
Sub-total		54	48	28 (58.3)	9	11	20 (41.7)
E	Blast.	13	13	7 (53.8)	2	4	6 (46.2)
F	Blast.	13	12	8 (66.7)	2	2	4 (33.3)
G	Blast.	15	14	8 (57.1)	2	4	6 (42.9)
H	Blast.	14	14	10 (71.4)	1	3	4 (28.6)
Sub-total		53	53	33 (62.3)	7	13	20 (37.7)
Total		109	101	61 (60.4)	16	24	40 (39.6)

Table 5. Twin-Pregnancy Rate Following Frozen and Thawed Embryo Transfer

Group	No. of embryo and site of transfer		No. of recipient	No. of pregnant (%)	No. twinning / pregnant recipient(%)	Total fetuses / recipients (%)
	+ CL	− CL				
Control	1	—	10	3 (30.0)	—	3/10 (30.0)
A	AI	1	9	5 (55.6)	2/5 (40.0)	7/9 (77.8)
B	2	—	9	4 (44.4)	2/4 (50.0)*	6/9 (66.7)*
C	1	1	8	3 (37.5)	1/3 (33.3)	4/8 (50.0)
Total			26	12 (46.2)	5/12 (41.7)	17/26(65.4)

* Estimated number: pregnant rate = 50%
+ CL: Uterine horn ipsilateral to corpus luteum
−CL: Uterine horn contralateral to corpus luteum
AI: Artificial insemination before embryo transfer

covered on 7-8 days of estrus induced by hormone treatments were summarized in Table 3. Of 311 ova recovered, the numbers of morulae and blastocysts were 99 and 98 respectively. These were 6.3 % of total embryos recovered. In hormonal treatments, normal rate of embryos recovered in group A was 77.6 %, and higher than those of group B (61.0 %) and C(56.3 %). The percentage of embryos judged as abnormal was 36.7 %. This result was slightly higher than those of Elsden et al. (1978) and Newcomb et al. (1976), who reported that the percentage of ova judged as abnormal were 16 to 31.1.

Morphology of frozen and thawed embryos was showed in Table 4. Of 101 embryos recovered after thawing, 61(60.4 %) were showed normal morphologically. The normal rate of blastocyst (62.3 %) was higher than that of morula (58.3 %). The result in this experiment was in accord with Sugie et al. (1979). The normal rate among groups removed glycerol by 1 step groups (C.G) was superior to the groups (A, E) equilibrated with glycerol by 1 step, although 3 step method (group B, D, F, H) was superior to the method which removed glycerol directly by the 1 step (group A, C, E, G).

Twin-pregnancy rate following frozen and thawed embryo transfer was summarized in Table 5. Pregnancy rate in single (control), twin A(AI + single), Twin B(+CL) and Twin C (+CL, −CL) was 30.0, 55.6, 44.4 and 37.5 %, respectively. Pregnancy rate in twin-induction was better than in single. This result was accord with Renard et al. (1977), who reported that fresh blastocyst was transferred non-surgically. Total number of survival fetuses following embryo transfer to 26 recipients for twin-induction was 19(65.0 %), and this result was approximately a double increase in survival rate compared with control group (30.0 %).

Summary

The aim of this experiment was to investigate the effects of hormone treatment, the morphology of embryos recovered by non-surgical method, the feasibility of dilution of glycerol by a sucrose solution followed by direct transfer of embryos frozen-thawed, and the twin-induction by the transfer with embryos frozen-thawed. The best number of ova recovered among the hormone treatment was showed in group C (PMSG 2,000–3,000 IU + PGF_2 and 25mg + hCG 2,000IU) and recovery rate of group B(PMSG 2,000–3,000IU+ $PGF_{2\alpha}$ 25mg) was better than those of group A (FSH 29AU$PGF_{2\alpha}$ 35mg) and group C. Normal cleavage stage of ova recovered on 7-8 days following estrus were mostly morulae (31.8 %) and blastocyst (31.5 %) and the abnormal rate of ova recovered was 20.9 %. In the morphology of embryo frozen-thawed, the normal rate of blastocyst (62.3 %) was higher than that of morula (58.3 %). The normal rate among groups removed glycerol by 1 step, 3 step group (C, G) were superior to the groups (A, E) equilibrated with glycerol by 1 step. The pregnancy rate in single, twin A (AI + single), B (+CL) and C (+CL, -CL) was 30.0, 55.6, 44.4 and 37.5 % respectively. Total number of survival fetuses following embryo transfer to 26 recipients for twin-induction was 19 (65 %) and this result was approximately a double increase in survival rate compared with single group (30.0 %).

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