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Studies on the Sexing of Bovine Embryo by the Chromosomal Analysis and H-Y Antibody

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염색체 분석 및 H-Y 항체처리에 의한 우수정란의 성판별에 관한 연구

고광두 · 양부근 · 정희태 · 박연수 · 김정익
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초 록

우수정란의 이식전 성판별에 관한 연구를 수행하기 위하여 GTH와 $PGF_{2\alpha}$ 투여에 대한 난소반응과 회수난자의 발육단계별 동결융해후 생존성을 조사하였으며, 이식전 수정란의 성판별을 위하여 H-Y항체 처리한 정상발육 난자의 염색체를 분석하여 다음과 같은 결과를 얻었다.

웅성 비장세포(male, spleen cells)를 면역원으로 mouse와 rat에 투여, 항혈청의 항체를 확인한 결과 mouse에서는 C_{57} BL계통과 rat에서는 DonRyu 계통이 항체생산능력이 우수하였다. 공란우 87두에 hormone (2500IU PMSG, 25mg PGF_2 alpha) 처리하여 평균 57.8%의 채란율과 두당 4.9개의 난자가 회수되었으며, 전체 회수난자(427개)중 morula(162개)와 blastocyst(190개)의 정상발육단계는 82.4%였다.

동결융해후 회수된 난자(312개)중, 형태적으로 정상인 난자(241개)의 비율은 77.2% 발육단계별의 성적은 blastocyst(83.4%)가 morula(71.0%)보다 우수하였다. 항체와 보체(Guinea pig serum)로 처리된 82개의 morula 중 15개(18.3%)가 blastocyst로 발육되어 이중 5개(33.3%)가 성이 판별되었으며, 모두 xx형 성염색체를 갖는 자성수정란으로 판명되었다.

Introduction

Histocompatibility-Y(H-Y) specific antigen was first observed when, in a certain strain of mice, skin grafts were rejected by female, whereas skin grafts exchanged among the other sex combinations were accepted (Eichwald & Slimer, 1955). Most of data indicates that H-Y(male) antigen is postulated to be the products of sex determinating genes (Wachtel, 1975; Ohno, 1976).

Cell lysis was observed in a approximately half of preimplantation mouse embryos exposed to mouse H-Y antiserum plus guinea pig complement (Krco et al., 1976; Epstein et al., 1980; White et al., 1982). Moreover, the embryos unaffected by the above culture were found to be mostly female (92 %) through chromosomal analysis (Epstein et al., 1980). Utsumi et al. (1984) also found the fact that rat H-Y antibody can identify the embryo sexuality in goat and cow.

Therefore, this study was performed to examine the possibility whether rat antibody could distinguish the sex of frozen-thawed bovine embryos before the transplantion. To obtain a additional basic information on the embryo transfer, the ovarian response to the hormone treatment and the morphology of both fresh and frozen-thawed embryos also were examined with cows of 87 donors.

Materials and Methods

1. Preparation of Antiserum

Spleen cells ($1 \times 10^{7-8}$) of Don Ryu strain male rat were intraperitoneally injected six times a week. The titer of antisera was assessed by the cytotoxcity of epididymal spermatozoa. The sperm cells were stained by trypan blue and assayed for dead cells by staining with trypan blue. The cytotoxicity index (C.I.) was determined by the formula: C.I. = Dead cells/Total cells x 100.

2. Embryo Collection

Superovulation was induced by the injection of 2,500 IU PMSG on the days of 7-13 of estrus and the following injection of 25mg $PGF_{2\alpha}$ at 48hrs. Donor

cows were inseminated at the onset of estrus induced with PMSG and $PGF_{2\alpha}$, and the embryos were nonsurgically collected by uterine flushing using baloon catheter between Day 6 and 7 after artificial insemination.

3. Freezing of Embryos

Recovered normal embryos were frozen in PBS solution plus 10% glycerol. Embryos were cooled from room temperature to -7°C with the rate of 1°C/min and to-35°C by cooling 0.3°C/min, and then plunged into LN (-196°C). Morphological normality of embryo, recovered after thawing, were assessed using microscope.

4. Embryo Culture

Frozen-thawed bovine embryos in morula stage were incubated with H-Y antiserum (10%, V/V) and complement (20%, V/V) in BSA free modified Whitten's medium (m-WM; Krco et al., 1976) under the gas phase of 5% CO_2 in air at 37°C for 12 to 24hrs. After incubation, the embryos were washed 3 times in fresh medium (m-WM) containing BSA, and were observed under microscope to classify embryos as either blastolated or disposed.

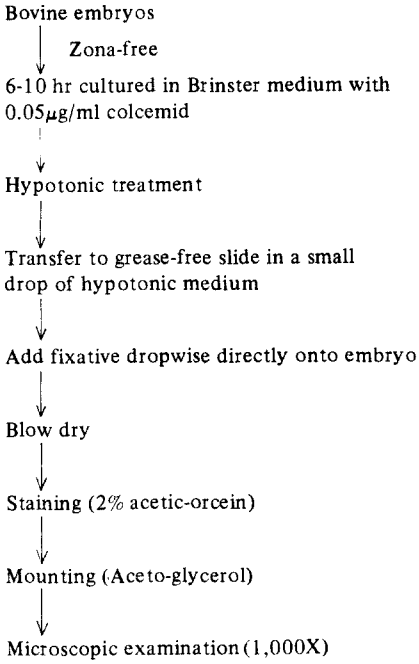

Fig. 1. Method for sexing preimplantation of embryos by chromosomal analysis

5. Chromosomal Analysis of Embryos after Treatment with H-Y antibody

For the detection of sex of preimplanted embryos, sex chromosome of bovine embryos, which were shown normal development after culture in m-WM containing H-Y antiserum and complement, was conducted by chromosomal analysis method (Fig. 1).

Results and Discussion

Effect of hormone treatment on the ovarian response was summarized in Table 1. The average number of corpus luteum,formed in ovaries which were stimulated with administration of PMSG and PGF_2 alpha, was 8.5. The embryo recovery rate among the formed copora lutea averaged 57.8 % and the number of recovered embryos per cow was 4.9. The average number of corpora lutea formed following administration of hormone in this experiment were lower than that of 17 ± 3 by Sreenan (1983), but slightly higher than that of 3.8-7.8 by several workers in Korea (Gu and Chung, 1982; Kim, et al., 1983; Im, et al., 1983). On the other hand, Oliver-Angel (1984) reported that the mean embryo recovery rate based on the number of corpora lutea was only 14.8 % in case of heifers and that of the adult cows was 39.5 %. The differences of recovery rate might be affected by the condition of donor, method of hormone treatment and technical skill of operator.

Table 1. Ovarian Response and Recovery of Following Hormone

No. of cow	87
No. of ovulation	739
Ovulation point per cow	8.5
No. of recovered ova	427
No. of recovered ova per cow	4.9
Recovery rate of ova	57.8

Developmental stage and morphology of ova recovered on 6-7 days of estrus induced by hormone treatments were summarized in Table 2. Of 427 ova recovered, the number of morula and blastocysts were 162 and 190, respectively, indicating 82.4 % normality out of total

embryos. This result was lower than those of Newcomb et al. (1976), Greve et al. (1979) and Schilling et al. (1980), who reported that approximately 25 % of all the embryos recovered were generated.

Morphology of frozen-thawed embryos was shown in Table 3. Of 312 embryos recovered after thawing, 241 (77.2 %) were morphologically normal. The rate of normality in blastocyst (83.4 %) was higher than that of morula (71.0 %). The result in this experiment was in accordance with Sugie et al. (1979), who reported that the normality of frozen-thawed blastocysts (73.3 %) was superior to that of morula stage (22.2 %).

Results on the development of bovine embryos treated with H-Y antibody were summarized in Table 4. When frozen-thawed bovine morula were exposed to H-Y antibody derived from rat male antispleen serum, 18.3 %(15/82) of bovine morula were developed to blastocysts and remained morula were arrested or destroyed. On the other hand, 44.0 % (11/25) of frozen-thawed bovine morula which were exposed to H-Y antibody free medium were developed to blastocysts. The above results were lower than that of Utsumi et al.(1984), who reported that 4(50 %) of 8 bovine morula, treated with rat H-Y antibody, were developed to blastocysts. This difference could be originated from the utilization of frozen-thawed morula in this experiment, compare to the fresh embryo by Utsumi et al. (1984).

The results of sex chromosomal analysis from bovine embryos, which were shown normal development in H-Y antibody free medium, the number of embryos identified to have XX and XY chromosome was 2(18.2 %) and 3 (27.3 %), respectively and 6 (54.5 %) embryos were not identified. The result in this experiment was in accordance with a study by Han et al. (1986), who reported that the number of embryos identified to have XX and XY chromosome was 25 % and 28 %, respectively and 47 % of embryos was not

Table 2. Developmental Stage of Ova Recovered 6-7 Day after Estrus

No. of animals	No. of ova recovered (No./cow)	Stage of embryos			
		Normal		Abnormal	
		Morula(%)	Blastocyst(%)	1-8cell(%)	Degenerated(%)
87	427 (4.9)	162 (37.9)	190 (44.5)	51 (12.0)	24 (5.6)

Table 3. Morphology of Frozen and Thawed Bovine Embryos

Stage of embryo	No. of eggs frozen	No. of eggs recovered after thawing	Morphology of embryos thawed	
			Intact (%)	Damaged (%)
Morula	159	155	110 (71.0)	45 (29.0)
Blastocyst	163	157	131 (83.4)	26 (16.6)
Total	322	312	241 (77.2)	71 (22.8)

Table 4. Development of Bovine Morula in Various Media

Culture media	No. of* embryos	No. of embryos developed (%)	No. of embryos arrested or destroyed (%)
WM+BSA	25	11 (44.0)	14 (56.0)
WM+NGPS+H-Y antiserum	82	15 (18.3)	67 (81.7)

*Frozen/thawed embryos of morula stage
BSA: Bovine Serum Albumin
WM: Whitten's medium
NGPS: Normal Guinea Pig Serum

Table 5. Sex Chromosomes of Bovine Blastocysts Shown Normal Development after Culture in Various Media

Culture media	No. of embryos	Distribution of sex chromosome		
		XX	XY	unidentified
WM+BSA	11	2	3	6
WM+NGPS+H-Y antiserum	15	5	0	10
Total	26	8 (38.5%)	3	16 (61.5%)

identified. On the other hand, of 15 blastocysts cultured in m-WM containing H-Y antiserum and complement, 5 (33.3 %) embryos analysed their sex chromosomes were identified to be XX bearing embryos.

Summary

Immunological means and sex chromosomal analysis were used to determine the sex of frozen-thawed bovine embryos prior to embryo transfer. Antisera to histocompability-Y(H-Y) antigen were prepared in adult DonRyu female rat by repeated intraperitoneal injections of spleen cells from male of the same strain. Frozen-thawed bovine morula were incubated with antiserum (10 %, V/V) and complement (20 %, V/V) in BSA free m-WM. After 12 to 24hrs of culture, embryos were classified as either affected or unaffected. Finally, sex chromosome of bovine embryos shown normal development after culture in medium containing H-Y antibody was confirmed by chromosomal analysis.

The average rate of recovery was 57.8 % (427/739) and the number of ova per cow was 4.9 of 427 ova recovered, the number of normal morula and blastocyst was 162 and 190 respectively, and 17.6 % (75/427) of total recovered embryos were morphologically abnormal. Among 312 embryo (155 morula, 157 blastocysts) thawed after deep freezing (-196°C), 241 (77.2 %) appeared to be morphologically normal. The normality of blastocyst (83.4 %; 131/157) was higher than that of morula (71.0 %; 110/155). Of 82 morula treated with H-Y antiserum and complement, 15 (18.3 %) embryos were developed to blastocysts and 67 (81.7 %) were arrested or destroyed. Among 15 blastocysts cultured in medium containing H-Y antiserum and complement, 5 (33.3 %) embryos analysed their sex chromosomes were identified to be XX bearing embryos. On the other hand, normal development was observed in 11 (44.0 %) of 25 embryos cultured in medium free H-Y antibody. Among 11 blastocysts, the number of embryos identified to have XX and XY chromosome was 2 (18.2 %) and 3 (27.3 %), respectively, and 6 (54.5 %) were not identified.

References

1. Eichwald, E.J. and Slimer, C.R. (1955). Untitled Publication. Transplantation. Bulletin, 2: 148-149.
2. Epstein, C.J., Smith, S. and Travis, B. (1980). Expression of H-Y antigen on preimplantation mouse embryos. Tissue Antigens, 15: 63-68.
3. Kroco, C.J. and Goldberg, E.H. (1976). H-Y(male) antigen; Detection on eight-cell mouse embryos. Science, 193: 1134-1135.
4. Newcomb, R., Rowsons, L.E.A. and Trounson, A.O. (1976). The entry of super-ovulated eggs into the uterus. In: Egg transfer in Cattle. (Eds.) L.E.A. Rowson, pp.1-15.
5. Sreenan, J.M. (1983). Methods of consistent supply recovered and transfer of embryo in the cattle. In: Strategics for the most efficient beef production. Proc. Intern. Sym. Beef Prod. Kyoto, Japan, pp.197-212.
6. Utsumi, K., Satoh, E. and Yuhara, M. (1984). Sexing of goat and cow embryos by rat H-Y antibody. Proc. 10th Int. Congr. Anim. Reprod. and A.I.,pp.234-235.

7. Wachtel, S.S. (1984). H-Y antigen in the study of sex determination and control of sex ratio. Theriogenology, 21: 18-28.
8. Wachtel, S.S., Ohno, S., Koo, G.C. and Boyse, E.A. (1975). Possible role H-Y antigen in the primary determination of sex. Nature, 257: 235-236.
9. 구자홍, 정창국(1982). 젖소의 비수술적 수정란 회수 및 이식시험, 대한수의사회지 18 : 45-82.
10. 백청순, 한용만, 이경광, 성김생, 김종배, 고대환(1986). 염색체 분석에 의한 생쥐분할란의 성감별. 한국축산학회지, 28 : 708-713.
11. 임경순, 이용빈, 성구민(1983). 소에 있어서 비외과적 방법에 의한 수정란의 채란기술에 관한 연구. 한국축산학회지, 25 : 244-254.