Maturation of Bovine Oocytes by Co-culture with Rabbit Preovulatory Follicle Cells

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(Received May 12, 1989)

Key words : bovine, rabbit follicle cells, blastocyst stage

Since the first birth of a calf by a technique of *in vitro* maturation and fertilization was reported by HANADA *et al*¹⁾, many attempts have been made to produce bovine embryos derived from *in vitro* matured and fertilized oocytes²⁻⁴⁾. Establishment of this technique would lead to mass production of bovine embryos for beef and preparation of early fertilized eggs for biotechnological purposes. However, developmental ability to the morula or blastocyst stage of oocytes matured *in vitro* was lower⁵⁾ than that matured *in vivo*⁶⁾. CRITSER *et al*⁷⁾ reported that the addition of follicle cells from bovine preovulatory follicles to the medium was very effective to obtain bovine morulae or blastocysts after *in vitro* fertilization (0% and 36% without and with the cells, respectively).

In this report, effects of inter-species (rabbit) follicle cells to maturation of bovine oocytes *in vitro* and their subsequent development to blastocysts were described.

Materials and Methods

Each rabbit was superovulated with FSH⁸⁾ or hMG⁹⁾. Three hours after the hCG injection, follicle cells were collected and diluted¹⁰⁾ with a

maturation medium¹¹⁾ at a concentration of $1-2 \times 10^6$ cells/ml.

Bovine oocytes collected from slaughterhouse ovaries were cultured in 50 μ l of the maturation medium (10 oocytes/50 μ l)⁴⁾ under paraffin oil with or without the follicle cells for 26-28 h in a 39°C CO₂ incubator (5% CO₂ in air, 100% humidity).

Bovine *in vitro* fertilization was carried out by the method described previously by IRTANI *et al*¹¹⁾. Five hours after insemination, the oocytes were denuded by vortex agitation⁴⁾ and cultured in 50 μ l of TCM-199 supplemented with 10% heat-inactivated fetal calf serum (FCS) (10%-FCS-TCM-199)¹¹⁾ for 12-15 h.

Some of the oocytes were fixed and stained to assess the fertilization¹²⁾.

The rest of the oocytes were transferred into ligated oviducts of female New Zealand White rabbits caged individually (3.4-3.7 kg body-weight, nullipara) and incubated for 6-7 days for blastulation^{6,11)}. The recovered eggs were fixed¹³⁾ immediately or 1 day after cultivation in 10% FCS-TCM-199, stained with 0.05% acridine-orange and their nuclei were counted under a fluorescent microscopy.

ウサギ卵細胞との共培養で成熟させたウシ卵細胞の体外受精後の発育能：佐伯和弘・長尾慶和・宇高健二・石森久雄（雪印乳業㈱受精卵移植研究所 苫小牧市植苗 119）

Table 1. *In vitro* fertilization and development of bovine oocytes co-cultured with rabbit preovulatory follicle cells*

Co-culture with follicle cells	Fertilization			Development in rabbit oviducts			
	No. of oocytes examined	No. (%) ^a of oocytes fertilized	No. (%) ^a of oocytes normally fertilized ^b	No. of oocytes transferred	No. (%) of embryos recovered	No. (%) ^c of blasto-cysts ^d	Mean number of nuclei in blastocysts $\pm$ SD
+	35	28(80)	23(66)	117	55 (47)	17(31)	121 $\pm$ 30
— (control)	42	38(90)	30(71)	100	69 (69)	9(13)	114 $\pm$ 46

* : Data were pooled over four replicates

^a : Percentages of the total number of oocytes examined

^b : Monospermic oocytes with male and female pronuclei

^c : Percentages of the total number of embryos recovered

^d : Significant difference ($P < 0.05$) between treatments

Results

The results were summarized in Table 1. There were no significant differences in total fertilization as well as normal. However, the rate of blastocysts increased significantly ($P < 0.05$) by an addition of rabbit follicle cells. No significant difference of number of nuclei in blastocysts were observed.

Discussion

To our knowledge, this is the first demonstration that the co-culture with the inter-species (rabbit) follicle cells at bovine oocyte maturation enhanced the developmental ability of the oocytes after *in vitro* fertilization. However, the proportion of developed embryos in the present study (31%) are lower than those reported by LU *et al*²⁾ (78%) and XU *et al*³⁾ (38%), who used the co-culture system with bovine follicle cells collected from the slaughterhouse ovaries and 20% serum from estrus cows as protein supplement, and did not add hormones to the maturation medium. The success rate presented here are also slightly lower than that reported by CRITSER *et al*⁷⁾ (36%) using the co-culture system with the follicle cells from bovine preovulatory follicle.

Since the mean number of nuclei of the blastocysts in the present study was almost same as that of day 7 bovine blastocysts (135

± 42 , unpublished data), it seems that the blastocysts derived from the oocytes matured and fertilized *in vitro* were normal.

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