

CHAPTER 4

RESULTS

Experiment 1. Effect of FSH treatment on retrieved bovine oocytes and in vitro maturation

In this experiment, we obtained a total of 289 oocytes from 32 aspiration sessions of 10 cows before and after crossover, they had performed twice weekly collection by n-OPU technique in non-FSH treatment. Total 282 oocytes from 8 aspiration sessions of 10 cows had FSH treatment and were performed every two weeks by n-OPU technique. Table 2 show that significantly higher retrieved oocytes per session from the n-OPU with FSH treatment (35.25 ± 13.73) than from the n-OPU without FSH treatment (10.32 ± 4.48) ($P < 0.05$). Comparing the effect of FSH-treatment (Group II) and non-FSH treatment (Group I), it was found that Group II had significantly higher of oocyte per cow per session than Group I (7.05 ± 3.88 vs 2.06 ± 0.99) ($P < 0.05$).

The oocytes quality were classified according to their morphology into 4 categories in Table 3 and Figure 10. Considering, the effect of FSH treatment on the quality of retrieved oocytes, it was found that they were no statistically significant differences in the percentage of category A (39.1% vs 42.5%) and category B (36.3% vs 41.5%) between two groups. The percentage of category C had significant lower in Group II as compared to Group I (11.0% vs 22.5%) ($P < 0.05$). While the percentage of category D trended to be higher in Group II (5.0%) as compared to Group I (2.1%).

Table 2. Effect of FSH treatment on retrieved oocytes (mean \pm SEM)

Group	Number of donors	Treatment	OPU sessions	Total number of oocytes	Oocytes per session	Oocytes per cow per session
I	10	Non-FSH	32	289	10.32 \pm 4.48 ^a	2.06 \pm 0.99 ^a
II	10	FSH	8	282	35.25 \pm 13.73 ^b	7.05 \pm 3.88 ^b

^{a,b} Values with different superscripts in the same column differ (P < 0.05)

Table 3. Effect of FSH treatment on quality of retrieved oocytes

Group	Treatment	Oocyte quality, number (%) ^{**}			
		A	B	C	D
I	Non-FSH	113 (39.1)	105 (36.3)	65 (22.5) ^a	6 (2.1)
II	FSH	120 (42.5)	117 (41.5)	31 (11.0) ^b	14 (5.0)

^{**} Category A = compacted cumulus oocyte or cumulus oocyte complexes;

B = partially denuded cumulus oocyte; C = completely denuded cumulus oocyte and

D = expanded cumulus oocyte.

^{a,b} Values with different superscripts in the same column differ (P > 0.05)

The effect of FSH treatment on maturation rate of retrieved oocytes were depicted in Table 4. There were no significantly difference between non-FSH treated and FSH treated cows on MII stage (63.3% vs 69.6%) and MI stage (18.8% vs 23.2%, respectively). Comparing the degenerated oocytes after in vitro maturation had statistically higher in non-FSH treatment (gr. I; 17.9%) than FSH treatment (gr. II; 7.2%) were significantly differ ($P < 0.05$).

Table 4. Effect of FSH treatment on maturation rate retrieved oocytes

Group	Treatment	Usable oocytes	Chromosomal stage of oocytes after IVM (%)**		
			MI	MII	DEG
I	Non-FSH	218	41 (18.8)	138 (63.3)	39 (17.9)
II	FSH	237	55 (23.2)	165 (69.6)	17 (7.2)

**MI = Metaphase I, MII= Metaphase II and DEG= degenerated oocytes

^{a,b} Values with different superscripts in the same column differ ($P > 0.05$)

Experiment 2. Effect of FSH treatment on developmental competence of bovine oocytes

Following IVF (Table 5), the percentage of cleavage embryos (figure 13) in FSH treated cows (49.4%) were statistically significant differ from non-treated cows (32.2%) ($P < 0.05$). The developmental competence in morulae and blastocysts (figure 14) of the FSH treated cows trended to be higher than those of the untreated cows (11.1% vs 10.2% and 8.6% vs 6.8%, respectively).

Table 5. Effect of treatment on developmental competence of retrieved oocytes

Treatment	Number of oocytes	Embryonic stage (%)		
		2-4 cell	Morulae	Blastocysts
Non-FSH	59	19 (32.2) ^a	6 (10.2)	4 (6.8)
FSH	81	40 (49.4) ^b	9 (11.1)	7 (8.6)

^{a,b} Values within row with different superscripts differ significantly ($P < 0.05$)

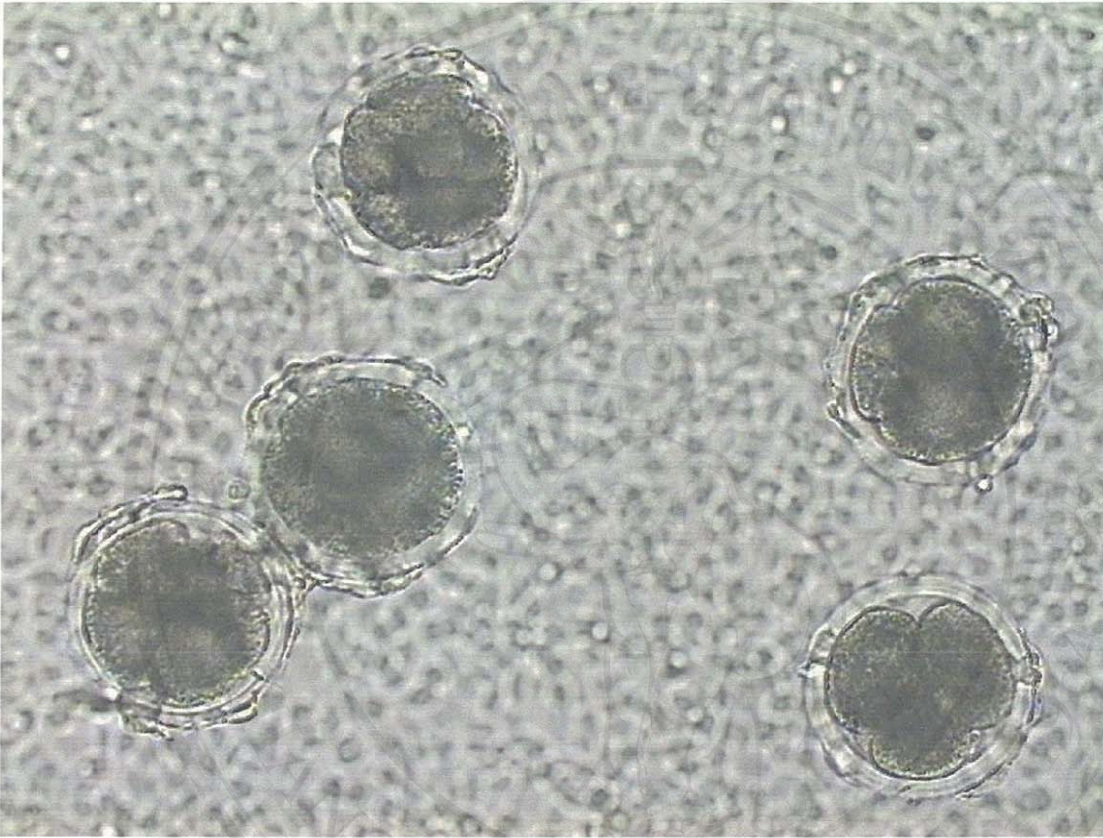


Figure 13. Cleavage stage embryos co-culture with vero cells (200X).

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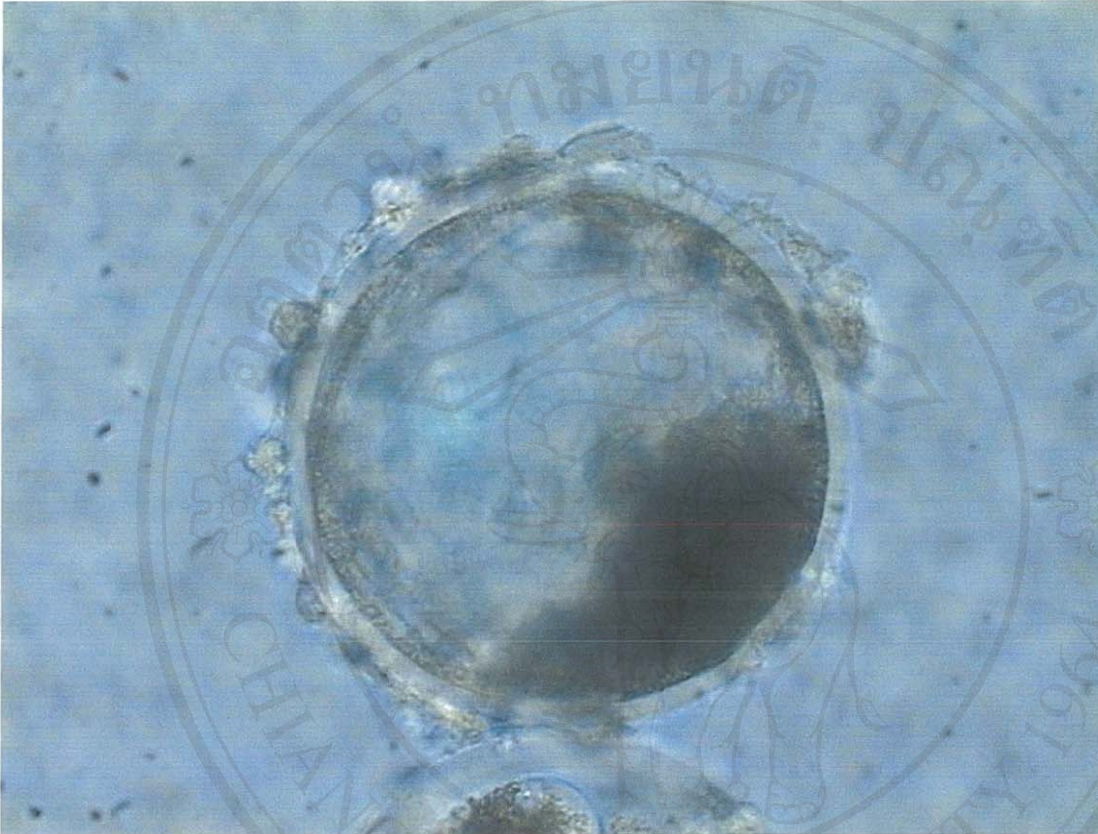


Figure 14. Blastocyst formation of in vitro embryo production in the cow (400X).

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