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## Collection And *In Vitro* Maturation Of Cat Oocytes Obtained From Different Oestrous Cycles

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### ABSTRACT

As a feline, cat has a similarity in biological reproduction with the tiger. To conserve the tiger (especially Sumatran tiger), cat could be used as a model to study the *in vitro* embryo production technology. The purpose of this study was to investigate the quality and quantity of the oocytes collected from different status of the ovaries and maturation *in vitro*. Oocytes were collected from ovaries by aspiration. Ovaries were removed by laparotomy surgery from 15 local female cats, age 1-3 years, weight 2.5-3.5 kg. The oocytes were matured *in vitro* in maturation medium (TCM-199 supplemented with 0.01 mg/ $\mu$ l follicle stimulating hormone and 5% female cat's serum) during 8, 22, 26 and 30 hours of incubation at 37°C in humidified atmosphere of 5% CO<sub>2</sub> in air. From the ovaries in follicular phase (n=8), 686 oocytes were collected, consisted of 14 (21%) oocytes with homogenous cytoplasm and complete cumulus cell (grade A), 117 (17%) oocytes with homogenous cytoplasm and incomplete cumulus cell (grade B), 134 (20%) oocytes with homogenous cytoplasm without cumulus cell (grade C) and 288 (42%) degenerated oocytes (grade D). From the ovaries in luteal phase (n=7), 407 oocytes were collected, consisted of 109 (17%) oocytes of grade A, 98 (24%) oocytes grade B, 77 (19%) oocytes grade C and 123 (30%) oocytes grade D. The maturation rate of cat oocytes were 14%, 4/29; 27%, 7/26; 38%, 8/21; and 10%, 10/31 for 18, 22, 26 and 30 hours of incubation, respectively. These results showed that cat oocytes could be collected from both ovaries in follicular phase or luteal phase, and the oocytes were matured after more than 22 hours of incubation *in vitro*.

**Key words:** ovary cycles, *in vitro* maturation, oocytes, domestic cat

### Introduction

The population of tiger (especially Sumatran Tiger) as endangered animal is becoming decreased. In the 70-th the tiger population is one thousand and decreasing up to about 660 in 80-th and remain 45 females and 20 males in 90-th (Smith and Charles, 1991). One of the alternative approaches for the tiger conservation is through *in vitro* embryo production technology, which include gamete collection, *in vitro* maturation and fertilization and embryo culture. The availability of good quality gametes, both male and female is the main key for the application of this technology. Cat is classified as carnivore ordo and felidae family; and as a feline, cat has a similarity in biological reproduction with the tiger such as polyestrous and non-spontaneous (inductive) ovulation. Therefore, cat can be used as a model to study the *in vitro* embryo production technology. Not only as model for the tiger conservation purposes, this technique can also be used to increase the cat population especially those species that have difficulty in reproduction.

The successful of *in vitro* fertilization technique depends on several factors such as the quality of oocytes and the timing of oocytes to reached metaphase-II, indicating the oocytes a ready to be fertilized by sperm (Goodrowe *et al.*, 1988; Miller *et al.*, 1990; Karen *et al.*, 1989). Oocytes can be obtained by aspiration of ovaries from follicular or luteal phase. Miller *et al.* (1990) obtained 140 oocytes from 145 follicles collected from 7 females puma (*Felis concolor*), with the average of  $20 \pm 5.9$  oocytes/female using aspiration method of ovaries after PMSG and hCG treatment.

Several researchers showed that oocytes could be collected by aspiration or mincing methods. The quantity and quality depends on the timing and method of the oocytes collection. From previous reports, most the oocytes collected were from treated females with gonadotropin. Treatments with gonadotropin not only increased the number of oocytes, but also increased the number of immature oocytes collected. However, these immature oocytes still have the capability to matured *in vitro* (Miller *et al.*, 1990).

The purpose of this study was to investigate the quality and quantity of the oocytes collected from different status of the ovaries (follicular and luteal phase) and matures *in vitro*.

Table 1. Number of cat oocytes collected at different estrous cycles.

Phase	No. of Ovaries	Number of Oocytes (%)				
		Grade A	Grade B	Grade C	Grade D	Total
Follicular	16	147 (21) <sup>a</sup>	117 (17) <sup>a</sup>	134 (20) <sup>a</sup>	288 (42) <sup>a</sup>	688 (100)
Luteal	14	109 (17) <sup>a</sup>	98 (24) <sup>a</sup>	77 (19) <sup>a</sup>	123 (30) <sup>a</sup>	407 (100)

Values within column with different superscripts are significantly different (P<0.05)

The successful of *in vitro* fertilization technique is depend on the development of the oocyte into metaphase II stage (matured oocyte). Sperm could fertilize only oocyte that reaches the metaphase II. The results of this study showed that most of oocytes in the time of aspiration were in germinal vesicle (GV, 63%) and germinal vesicle break down (GVBD, 9%) (Table 2). Oocytes reached metaphase II stage after 22 h of maturation (27%, 38% and 32% for 22, 26 and 30 hours of maturation periods, respectively).

Table 2. Maturation rate of cat oocytes at 0, 18, 22, 26, and 30 hours after *in vitro* maturation

Time of Maturation (Hours)	No. of Oocytes	Maturation stage (%)				
		GV	GVBD	MT-I	MT-II	Deg
0	46	29 (63)	4 (9)	-	-	13 (28)
18	29	2 (7)	19 (65)	4 (14)	-	4 (14)
22	26	-	2 (8)	11 (42)	7 (27)	6 (23)
26	21	-	-	2 (10)	8 (38)	11 (52)
30	31	-	-	-	10 (32)	21 (68)

### Conclusion

The results showed that cat oocytes could be collected from both ovaries in follicular or luteal phase with no significantly different, and oocytes were matured after more than 22 hours of incubation *in vitro*.

### References

- Boediono, A., Takagi, M., Saha, S. and Suzuki T. 1994. The influence of day 0 and day 7 superovulated cow serum during *in vitro* development of bovine oocytes. *Reprod. Fertil. Dev.*, 6:261-264.
- Boediono A, Rajamahendran A, Saha S, Sumantri C and Suzuki T. 1995. Effect of the presence of a CL in the ovary on oocyte number, cleavage rate and blastocyst production *in vitro* in cattle. *Theriogenology*, 43:169 (Abst)
- Donoghue, A.M., Johnston, L.A., Goodrowe, K.L., O'Brien, S.J., and Wildt, D.E. 1993. Influence of day of estrus on egg viability and comparative efficiency of *in vitro* fertilization in domestic cats in natural or gonadotrophin-induced estrous. *J. Reprod. Fert.* 98:85-90.
- Goodrowe, K.L., Wall R.J., O'Brien, S.J., Schmidt, P.M., Wildt, D.E. 1988. Developmental competence of domestic cat follicular oocytes after fertilization *in vitro*. *Biol. Reprod.*39:355-372.
- Karen, L., Goodrowe, K.L., Miller, A.M., Wildt, D.E. 1989. *In vitro* fertilization of gonadotropin stimulated leopard cat (*Felis bengalensis*) follicular oocytes. *J. Exp.Zoo.*252:89-95.
- Miller, A.M., Roelke, M.E., Goodrowe, K.L., Howard, J.G., Wildt, D.E. 1990. Oocytes recovery, maturation and fertilization *in vitro* in the puma (*Felis concolor*). *J. Reprod. Fert.* 88:249-258.
- Smith, J.L.D., and Charles, M. 1991. The contribution of variance in lifetime reproduction to effective population size in tiger. *Conservation Biology* 5(4).