

Research

## hCG Priming Before Ovary Collection Increasing The Oocyte Quality In The Domestic Cat

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Submitted April 14, 2021 Accepted May 11, 2021

### ABSTRACT

Oocyte competence is a determining factor that influences the embryo development. Embryos produced in vitro have a reduced developmental competence than embryos produced in vivo. Therefore, human Chorionic Gonadotropin (hCG) injection was carried out to improve the quality of the oocytes. The objective of this study was to evaluate the effect of ovarian stimulation with hCG before ovary collection on oocyte quality in the domestic cat. Oocyte donors were either 1) treated with a single dose of 200 IU hCG four days before ovary collection (hCG group), or, 2) no treatment before ovary collection (control group). The oocytes were collected by the slicing method. Immature cumulus oophorus complexes (COCs) from both groups were pooled and matured in vitro for 24-26 hours. Then mature oocytes were fertilized with epididymal sperm and cultured in vitro for seven days. The results study showed that the number of the dominant follicle (DF) and the number of COCs in the hCG group was higher than the control group in right and left ovaries ( $p < 0.05$ ). The morulae and blastocyst rates from cleavage embryos were 88% and 75%, respectively. These results demonstrate that hCG priming of oocytes donors before ovary collection improve oocyte quality.

**Keywords:** hCG, dominant follicle, oocyte quality, embryo, domestic cat

### ABSTRAK

Kualitas oosit merupakan faktor penentu yang memengaruhi perkembangan embrio. Embrio yang diproduksi secara in vitro memiliki perkembangan yang lebih rendah daripada embrio yang diproduksi secara in vivo. Oleh karena itu, injeksi hormon *human Chorionic Gonadotropin* (hCG) dilakukan untuk meningkatkan kualitas oosit. Tujuan penelitian ini adalah mengevaluasi pengaruh pemberian hCG sebelum koleksi ovarium terhadap kualitas oosit pada kucing domestik. Hewan donor dibagi menjadi dua kelompok, yaitu 1) hewan diberikan 200 IU hCG dosis tunggal empat hari sebelum koleksi ovarium (kelompok hCG), dan, 2) hewan tanpa diberikan hCG sebelum koleksi ovarium (kelompok kontrol). Koleksi oosit dilakukan dengan metode *slicing*. Kompleks kumulus ooforus (COCs) yang belum matang dari kedua kelompok dikumpulkan dan dimatangkan secara in vitro selama 24-26 jam. Kemudian oosit yang matang difertilisasi dengan sperma epididimis dan dikultur secara in vitro selama tujuh hari. Hasil penelitian menunjukkan bahwa jumlah folikel dominan (DF) dan jumlah COC pada kelompok hCG lebih banyak dibandingkan kelompok kontrol baik pada ovarium kanan maupun kiri ( $p < 0,05$ ). Tingkat perkembangan morula dan blastosis dari embrio *cleavage* masing-masing adalah 88% dan 75%. Hasil ini menunjukkan bahwa pemberian hCG pada kucing domestik sebelum koleksi ovarium mampu meningkatkan kualitas oosit.

**Kata kunci:** hCG, folikel dominan, kualitas oosit, embrio, kucing domestik

## INTRODUCTION

Felidae is a family of mammals in the order Carnivora which consist of at least 36 wild felid species. Human activities reduce forest area where they live in which cause in a decline in number of all worldwide felid species. Based on *The International Union for the Conservation of Nature* (IUCN) Red List, over half of all wild felids are listed as vulnerable, critically endanger, or endanger, and many have decreasing wild populations. Conservation strategies that can do to maintain population are captive breeding, habitat management and protection, translocation, and reproductive technology. The first breeding program to maintain the existence of wild felid is natural breeding, but many of them cannot reproduce well because of some reasons, for example behavioral incompatibility, infertility, and male aggression (Thongphakdee et al., 2020). Therefore, reproductive biotechnology is an option as conservation strategies and has been explored to increase the number of wild felid populations by saving the genetic materials.

The domestic cat is suitable as animal model for some research about wild felids because of the similarity in anatomy and reproductive characteristic (Lamberski, 2015; Terio et al., 2018). Reproduction technology like in vitro maturation (IVM) of oocyte and in vitro embryo production system are useful technique to save genetic material of domestic cat and several endanger felid species (Pope, 2014; Veraguas et al., 2017). The domestic cat embryo produced in vitro have a reduced developmental competence and arrest at morulae stage than embryo produced in vivo (Roth et al., 1994). This is because embryo development in vitro has incomplete cytoplasmic maturation (Gomez et al., 2000; Bogliolo et al., 2004). Based on that problem, increasing oocyte quality is needed to improve embryo development. In this present study, we want to conduct ovarian stimulation using single dose of 200 IU hCG four days before ovary collection in the domestic cat.

## MATERIAL AND METHODS

Animal in the present study were 16 adult female cats with healthy condition. The animals were kept indoors with room temperature and natural light. The animals fed twice a day and water was available ad libitum. All the experiments in this research and the animal manipulation procedures were approved by the Ethics Committee of the Faculty of Veterinary Medicine, IPB University (Ethical approval number : 022/KEH/SKE/XII/2020).

The present study divided into two groups; 1) females were treated with single dose of 200 IU hCG four days before OVH (hCG group) and 2) untreated females before OVH (control group). The bodies of the domestic cats were weighted before OVH and the ovaries weighted after OVH. The number of corpus luteum (CL) and DF in the ovaries from both groups was counted. The oocytes were collected by the slicing the cortex of ovaries and classified into COCs, degenerated, and spontaneous cleavage oocytes. The Immature COCs from both groups were pooled and matured in vitro for 24-26 hours.

Healthy male cats > 6 months of age were subjected to orchidectomy. The testis and epididymis were refrigerated at 4 °C for 24 hours. For IVF, the sperm were collected from caudal portion of epididymis which refrigerated before. The COCs were placed in fertilization medium and co-incubated with sperm for 18 hours. After that, the presumptive zygotes were cultured in vitro for seven days. The rates of cleavage, morulae, and blastocyst development rate were evaluated on day 3, day 5, and day 7, respectively.

The independent t-test was used to evaluate body weight, ovary weight, number of CL, number of DF and quality of oocyte from both groups. Pearson correlation was used to evaluate the correlation of body and ovary weight. All analyses were done using the software Minitab 18.  $P < 0.05$  indicated statistical differences between groups.

## RESULT AND DISCUSSION

As shown in Table 1, the body weight in the hCG group was similar to the control group. Significant difference was not found in the mean of ovarian weight between both of groups. There were no correlation between body weight and ovarian weight. Based on analysis of control animal data from Bailey et al. (2004), ovary weights are best compared using organ-to-brain weight ratios than using organ-to-body weight ratios. Understanding the relationship between absolute organ weight, body weight, and brain weight as well as which parameter best predicts a true effect on organ weight will lead to improved organ weight interpretation (Bailey et al., 2004).

The number of CL and DF in the hCG group and control group are shown in Table 2. The number of CL is not significantly different between two groups in the left and right ovaries. In contrast, the number of DF in the hCG group was higher in the left and the right ovaries than control group ( $p < 0.05$ ).

Table 1 Body weight and ovarian weight in the hCG group and control group in the domestic cat

	N	Body Weight (kg) (mean ± SD)	Ovarium Weight (g) (mean ± SD)	Correlation	
				Pearson Correlation	P-value
hCG	8	2.79 ± 0.55 <sup>a</sup>	0.41 ± 0.12 <sup>a</sup>	-0.338	0.413
Control	8	2.84 ± 0.37 <sup>a</sup>	0.31 ± 12 <sup>a</sup>	-0.563	0.146

Table 2 The number of corpus luteum and dominant follicle in hCG group and control group in the domestic cat

	N	Number of corpus luteum		Number of dominant follicle	
		Right	Left	Right	Left
		(mean ± SD)	(mean ± SD)	(mean ± SD)	(mean ± SD)
hCG	8	0.88 ± 1.13 <sup>a</sup>	0.75 ± 0.89 <sup>a</sup>	6.13 ± 2.90 <sup>a</sup>	4.25 ± 3.81 <sup>a</sup>
Control	8	1.13 ± 1.89 <sup>a</sup>	0.13 ± 0.35 <sup>a</sup>	0.25 ± 0.71 <sup>b</sup>	0.13 ± 0.35 <sup>b</sup>

COCs from hCG and control group were matured for 24 hours. Mature oocyte in both of group had expanded cumulus cells (CCs), but CCs in the hCG group was better and had more layers than in the control group. Mature oocyte from both groups were pooled then fertilized with epididymal sperm. The morulae and blastocyst rates from cleavage embryos were 88% and 75%, respectively. It indicates that administration single dose of hCG could improve oocyte competence in domestic cat. hCG is well known causing oocyte maturation and ovulation in felids (Wildt *et al.*, 1998; Howard, 1999), but it also can cause secondary follicular growth (Swanson *et al.*, 1997; Wildt *et al.*, 1978).

CCs that surrounded oocyte have positive impact in oocyte competence. There is bi-directional communication exist between oocyte and CCs which is essential for the production of oocyte competence (Huang & Wells, 2010). CCs are biologically distinct from other follicular cells and perform specialized roles, transmitting signals within the ovary and supporting oocyte growth and maturation during the later stages of follicular development through metabolites produced and secreted through the gap junction mechanism to the oocyte cells (Macaulay *et al.*, 2016; Huang & Wells, 2010).

According to Bilodeau-Goeseels & Panich (2002), oocyte with CCs more than five layer reaches a higher number of cell division than oocytes with less than five CCs layers. Furthermore, Kakkassery *et al.*

(2010), stated that greater the number of CCs, greater was the maturation rate. Oocytes with more than three layers of CCs exhibited significantly higher maturation rate than the denuded oocytes and oocytes with partial cumulus layers.

*“All authors declare that there are no conflicts of interest”.*

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