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Investigation of Feline Ovulation Time after LH Surge Induced by hCG Injection in Superovulation

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ABSTRACT

Feline ovulation time after LH surge have not been defined because its LH surge is occurred by several times of coital vaginal induction and cat has relatively longer time between LH surge and ovulation compared with other mammalian species. This study was performed to investigate the feline ovulation time after LH surge that was induced by hCG injection for superovulation with PMSG. For superovulation, all cats were received an initial injection of PMSG (200 IU, i.m.) followed 80 hrs later with an injection of hCG (200 IU, i.m.). And then, sampling of both ovaries was surgically performed at each 6 different times (10, 18, 22, 26, 29, and 32 hrs) after hCG injection. Cumulus-oocyte-complexes (COCs) were collected from 2 sides of oviducts and ovaries were fixed for ovarian histology. Total 38 COCs were collected only at hCG 32 hrs and no COCs were shown at earlier 5 times. However, in the ovarian histology, corpus haemorrhagicum or corpus luteum was not shown in all groups including ovary at hCG 32 hrs that COCs were collected. In conclusion, it was suggested that feline ovulation was occurred at 29～32 hrs after LH surge and taken relatively long time for CL formation after ovulation.

(Key words: feline, ovulation, superovulation, LH surge)

INTRODUCTION

The feline estrous characteristics were defined as seasonal polyestrous with coital induced ovulation (Jemmett & Evans, 1977; Johnston *et al.,* 1996; Pope 2000; Root *et al.,* 1995). Cats are induced ovulators; that is, the ovulation occurs only after coitus or similar vaginal stimulation, which induces the release of the gonadotropin releasing hormone (GnRH) in the hypothalamus that leads to a luteinizing hormone (LH) surge for Graafian follicular rupture, which occurs 24 to 50 hours after the coitus (Goodrowe *et al.,* 1989; Johnson & Gay, 1981a; Wildt *et al.,* 1980). Feline ovarian follicular development was regulated by endocrine, autocrine and paracrine factors mainly, and follicles were exposed to 2 gonadotropins including follicle stimulating hormone (FSH) and LH at different concentrations according to the estrous cycle (Alves *et al.,* 2012).

In normal feline estrous cycle, LH release from the pituitary gland and subsequent ovulation is induced by copulation and the magnitude of LH release has been demonstrated to increase with increase in number of copulation (Concannon *et al.,* 1980; Johnson & Gay, 1981b). Serum LH concentrations are low before coital contact, however it increases sharply and induce ovulation 29 to 40 hrs that including GnRH secretion and LH surge (Shille *et al.,* 1983; Wildt *et al.,* 1981). LH secretion in response to coitus is dependent on exposure time to estrogen of the hypothalamus and pituitary gland (Banks & Stabenfeldt, 1982). Therefore, the feline superovulation regime must be performed with consideration of these particular mechanisms for ovulation. Administration protocol of exogenous gonadotropin, apart time of 2 gonadotropin for enough estrogen exposure time, and ovulation time after LH or hCG hormone for LH surge are very essential for successful ovulation after superovulation treatment (Shille *et al.,* 1983; Wildt *et al.,* 1981).

After first cloned cat was reported at 2002 (Shin *et al.,* 2002), studies of feline reproductive biotechnology including *in vitro* production, cloning have been reported in many groups in the past decade (Cho *et al.,* 2010; Herrick *et al.,* 2010; Spinaci *et al.,* 2007; Yin *et al.,* 2005). Feline oocytes could be used as

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recipient oocytes in the interspecies somatic cell nuclear transfer to preserve the endangered felidae (Yin *et al.,* 2006a; Yin *et al.,* 2006b). Therefore, collection of feline matured oocytes with good viability is needed for reproductive biotechnology. For this oocytes collection, surgical procedure with laparotomy is needed for *in vivo* oocytes that have better viability than *in vitro* oocytes.

Therefore, this study was performed to investigate when ovulation is occurred after LH surge that was induced by 2 gonadotropin injection with 80 hrs apart in the cat. Our results will be beneficial to understand the feline reproductive mechanisms and accurate ovulation time to collect the oocytes for feline biotechnology.

MATERIAL AND METHOD

1. Animals Care and Superovulation

This experiment was approved by the Animal Care and Use Committee of Chungnam National University. Six domestic female cats without defined breed characteristics were used in the experiment. Age of all cats was less than 1 year old and housed in stainless steel cages, with commercial dry food and water ad libitum. Superovulation was performed in the 6 female domestic cats by administration of pregnant mare serum gonadotropin (PMSG; 200 IU, I.M.) to induced follicle development and then 80 hrs later with human chorionic gonadotropin (hCG; 200 IU, I.M.) to induce the ovulation.

2. Ovarian Sampling

Ovarian sampling for confirmation of the ovulation and ovarian histology was performed with each 6 different time (10, 18, 22, 26, 29 and 32 hrs) after hCG injection that was conducted to induce the LH surge. For ovarian sampling, cats were anesthetized with zolazepam (Zoletil®, Virbac) at each times and both ovaries with oviducts were collected after routine ovariohystorectomy. Collected ovaries were washed in the PBS (Life Technologies) with penicillin/streptomycin (Life Technologies) for 3 times. Before ovarian fixation, oviducts were flushed with PBS to collect the cumulus-oocyte-complexes (COCs) and counted the ovulated COCs under stereomicroscope.

3. Ovarian Histology

For ovarian histology, collected ovaries were fixed in the 10% formalin and processed by conventional histologic methods. Ovaries were embedded in paraffin, and whole ovarian section including the cortex and medulla at 5 um were cut by microtome, mounted on slides, and stained with hematoxylin/eosin. Whole ovarian section were observed under microscope (Motic, China) and slide scan was applied to take the picture of whole ovarian section.

RESULTS

1. Collection of COCs at Each Different Times

COCs were collected at each six different times after hCG injection for LH surge, and then COCs were collected only at hCG 32 hrs (Fig. 1). In the other five groups with shorter time after hCG injection, many of Graafian follicles were shown but COCs were not collected after flushing of uterine oviduct. Total thirty nine COCs were collected at hCG, however most of oocytes were immature oocytes without first polar body after denuding.

2. Ovarian Histology

Graafian follicles were shown in all groups in ovarian histology and these were also shown in the hCG 32 hrs group which COCs were collected (Fig. 2). In the ovary at hCG 10 hrs, many of antral follicles were shown with differentiated granulosa cells and theca externa cells. One large corpus luteum was shown that was presumed to be form in previous estrous cycle. Ovary at hCG 18 hrs also shown similar histology with that of hCG 10 hrs and 2 large corpus luteum. At hCG 22 hrs, follicular wall in the superficial layer became weaken and separation of membrana granulosa and theca were started. We founded interesting structure in the ovary of hCG 26 hrs that many of non-ovulated follicles were shown and these were surrounded by luteal cells (Fig. 2 d). One follicle was showed the follicular rupture with slight hemorrhage (Fig. 2 d). Many of Graafian follicles were shown in the ovary of hCG 29 hrs, however no COCs was collected (Fig. 2e). In the ovary of hCG 32 hrs, many of Graafian follicles were shown with complete separation of membrana granulosa and theca. However, total 39 COCs were shown in the oocytes collection.

DISCUSSION

This study was conducted to examine the exact ovulation time after LH surge in the cat. Ovarian sampling was conducted

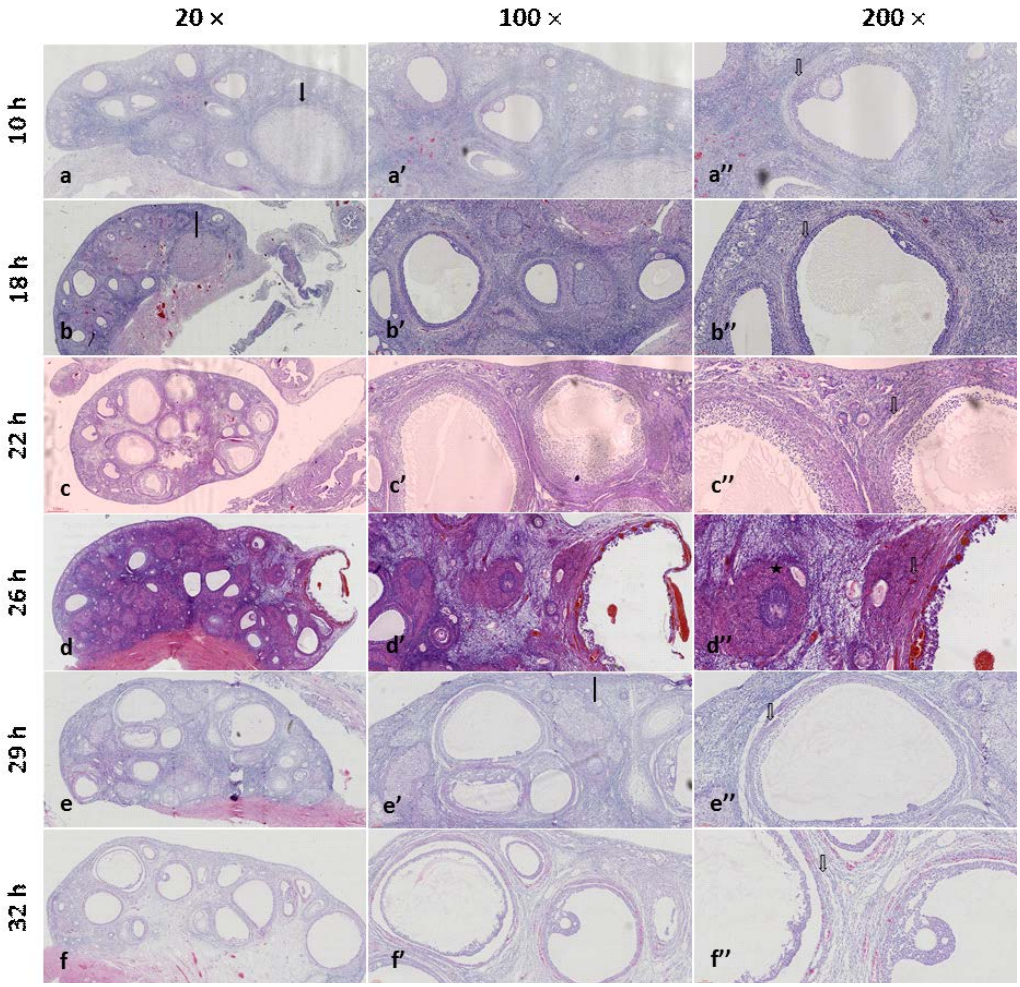

Fig. 1. Feline ovarian histology at each 6 different times after hCG injection for superovulation. Ovaries were collected from less 1 year old female cats after superovulation with PMSG and hCG with 80 hrs apart and stained with hematoxylin and eosin. All ovaries have Graafian follicles (↓) and some ovaries have corpus lutea (★) presumed to be formed in the previous estrous cycle. Differentiated cells of granulosa, theca interna, and externa were shown in all Graafian follicles. In the ovaries at hCG 26 hrs (d''), many of arrested follicles surrounded with luteal cells (★) were shown.

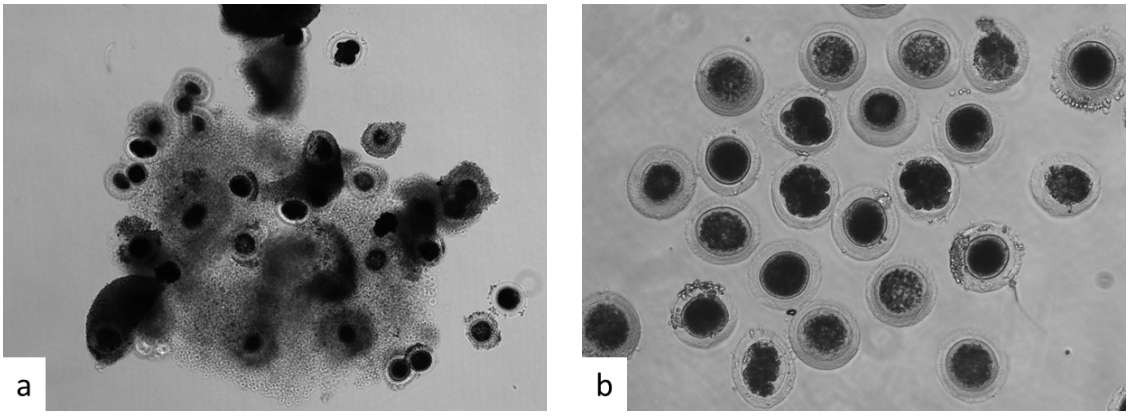

Fig. 2. Collected cumulus-oocyte-complexes (COCs) and denuded oocytes at hCG 32 hrs. COCs were firstly collected from oviducts at 32 hrs after hCG injection and matured oocytes were not shown.

Table 1. Numbers of oocytes retrieved at each different times after hCG injection for superovulation in female cats

Time (hrs) after hCG injection	10	18	22	26	29	32
No. of oocytes	0	0	0	0	0	39

at six different times and COCs were collected only at 32 hrs after hCG injection. Therefore, it could be concluded that ovulation in the female cat would be occurred between 29 hrs and 32 hrs in this study. However, ruptured follicle or corpus hemorrhagicum was not shown in the ovarian histology at hCG 32 hrs. Follicular rupture was shown in the ovary of 26 hrs only in the 1 follicle.

Several different regime of feline superovulation have been reported to induce the follicular rupture for ovulation to collect the *in vivo* matured COCs or embryos after coitus using the gonadotropin including the PMSG, FSH, hCG (Howard *et al.,* 1992; Kanda *et al.,* 1995; Platz *et al.,* 1978; Verstegen *et al.,* 1993; Wildt & Seager, 1978). Out of these previous gonadotropins, FSH had a short half-life and it needed daily administration to induce the follicular stimulation in the study of Wildt *et al.,* (1978). However, the use of PMSG have showed the enough hyperstimulation only with a single administration (Donoghue *et al.,* 1992; Goodrowe *et al.,* 1988; Pope *et al.,* 1998; Tsutsui *et al.,* 2000). In our study, 200 IU PMSG was injected and this concentration was effective to induce the follicular development in all cats. However, LH surge induced by hCG injection was not effective in follicular rupture for ovulation. In our study, 200 IU hCG used to induce the LH surge and its dosage was enough to compared with previous studies (Pope *et al.,* 1998; Tsutsui *et al.,* 2000). It might be thought that these phenomenons might be caused by several reasons. In the natural cycling, feline ovarian follicles were grown by FSH and then, estrous behavior is shown to attract the male cats. LH surge was induced by vaginal stimulation with several times of coitus. Therefore, feline superovulation regime must have similar with the hormone profile of natural cycling. Out of this profile, duration of two gonadotropins is essential for enough development of follicles, weakening of follicular walls and increase of LH receptor in the follicles. We injected the hCG at 80 hrs after PMSG injection and it was not regarded as enough time for follicular rupture because ovulation was occurred at hCG 32 hrs compared with other studies and most of ovulated COCs were not matured. Therefore, more than 80 hrs after PMSG injection was needed in enough follicular development for ovulation in the cats. Feline estrous cycle is seasonally polyestrous that require 12 hrs or more light to maintain the normal cycle. However, our study was conducted in the non-estrous season and it could be one of factor for delayed ovulation although animals were raise under 14 hrs bright per day. It also can be thought that body condition of female cats were not good in this study.

In the ovarian histology, only antral pre-ovulatory follicles were shown in all animals without any ruptured follicles and corpus hemorrhagicum. However, in this histology, we found the two interesting phenomenon that antral follicle surrounded by luteal cells at hCG 26 hrs and COCs were collected in the oviducts at hCG 32 hrs that only antral follicles were shown. It could be though that antral follicles surrounded by luteal cells at hCG 26 hrs were formed by conversion of granulosa cells and theca cells in the follicle into luteal cells by injected hCG. However, these injected hCG regime were not appropriate for follicular rupture.

In this study, it can be concluded that ovulation was occurred at 29～32 hrs after LH surge in the female cats. These results will be very useful information for collection of *in vivo* matured oocytes in the feline biotechnology. However, this study has limitation for clear conclusion that we used only one cat in each groups and this was conducted in the non-breeding season. In the future, study using the more female cats at appropriate season must be conducted to overcome these limitations.

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