Original Article

Recombinant Human Follicle Stimulating Hormone and Growth Factors Improve the Meiotic and Developmental Competence of Cat Oocytes

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Abstract

In vitro embryo production has proven as potential tool for understanding factors involving developmental biology of embryos. This study aimed at examining the effects of recombinant human follicle stimulating hormone (rhFSH) on meiotic resumption and also on developmental competence of cat oocytes. Experiment 1 studied mRNA expression of luteinizing hormone (LH), follicle stimulating hormone (FSH), epidermal growth factor (EGF) and insulin like growth factor-1 (IGF-1) receptors (R) in cumulus cells. mRNA expression of LH receptor in cumulus cells after oocyte maturation was additionally examined. Experiment 2 examined the effect of different rhFSH concentrations (0.01, 0.05, 0.1 and 1 IU/ml) on meiotic competence. *In vitro* oocyte maturation without rhFSH served as the control group. The effects of 'chosen-dose' rhFSH in combination with rhLH and growth factors (IGF-1 and EGF) on developmental competence were tested in Experiment 3. Cumulus cells surrounding immature oocytes expressed mRNA of *FSHR*, *EGFR* and *IGF1R*, but not *LHR*. *LHR* was only expressed after *in vitro* maturation. All rhFSH concentrations significantly increased maturation rates of cat oocytes in a dose-dependent manner when compared with the control group ($p<0.05$). The 0.1 IU/ml rhFSH was selected for Experiment 3 due to its efficacy. The developmental competence of cat oocytes was not affected by rhLH supplementation ($p>0.05$), but IGF-1 and EGF significantly promoted cleavage and blastocyst formation rates compared with rhFSH and rhFSH plus rhLH ($p<0.05$). It is concluded that cumulus cells express *FSHR*, *LHR*, *EGFR* and *IGF1R*. However, *LHR* mRNA was only expressed after *in vitro* maturation. Efficacy of FSH in meiotic resumption is in a dose-dependent manner. The combination of rhFSH and growth factors (IGF-1 or EGF) best improves cytoplasmic maturation of feline oocytes in terms of cleavage and blastocyst rates.

Keywords: cat, embryo development, growth factor, oocyte, recombinant follicle stimulating hormone

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Introduction

Fundamental and application study of reproductive biotechnologies including *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture of embryo (IVC) in domestic cat has become an increasingly important prospects for conservation of valuable/endangered felid species (Swanson, 2006) and also for biomedical research (O'Brien et al., 1999). However, these techniques have met only limited success, especially when developmental competence between *in vivo* and *in vitro* derived oocytes is compared (Roth et al., 1994; Swanson et al., 1994). Generally, *in vivo* follicle development, meiotic resumption and subsequent ovulation are centrally governed by luteinizing hormone (LH) and follicle stimulating hormone (FSH) as a part of gonadal-pituitary axis. The luteinizing hormone (LH) receptors express in correspondence with increased size of follicles (Xu et al., 1995; Bao et al., 1997). These receptors bind specifically to circulating LH and meiotic resumption is then initiated (Mehlmann, 2005; Tsafriri et al., 2005). As a result, an immature oocyte within a dominant follicle undergoes a series of nuclear and cytoplasmic changes in order to complete its competence. In contrast to *in vivo* situation, the LH as meiotic resumption inducer plays a lesser role in this process*in vitro* when compared with follicle stimulating hormone (FSH) (Eppig, 1991; Su et al., 1998). The FSH binds to the FSH receptors located at the membrane of cumulus-corona radiata cells and subsequently stimulates signals, via gap junctions between oocyte and its companion cells, to induce meiosis resumption (Matzuk et al., 2002).

Currently, a number of strategies have been developed, aiming specifically at improving the cytoplasmic competence of mammalian oocytes during oocyte maturation (Gilchrist and Thompson, 2007). These strategies revealed mechanisms insight the process of oocyte maturation, and also found potential markers that could reflect oocyte's developmental competence. Indeed, the study on the effects of individual gonadotropin (LH and FSH) have been restricted due primarily to the fact that most gonadotropins used for IVM are frequently contaminated with LH or other by-products (Törnell et al., 1995). It is therefore important to test the role of FSH without LH contamination. Recombinant FSH has been shown to stimulate oocyte maturation and subsequent embryo development in several species, including domestic cat (Sananmuang et al., 2010; Sananmuang et al., 2011; Tharasanit et al., 2011). However, optimal concentration and interaction with LH and other growth factors have not been reported.

While the FSH is known to stimulate both nuclear and cytoplasmic maturation of the oocytes, several studies also indicated that some growth factors such as epidermal growth factor (EGF) and insulin like growth factor-1 (IGF-1) improved the fertilization rate and embryo development in pig (Chen et al., 2008; Uhmet al., 2010), bovine (Lorenzo et al., 1994; Lonergan et al., 1996) and cat (Merloet al., 2005).EGF and IGF-1 play a central role in cell proliferation and differentiation during embryogenesis (Lonergan et al., 1996). In domestic cat, the effects of these growth factors on oocyte maturation and embryo development have been obscured. This study aimed at examining the effects of 'LH-free' gonadotropin (recombinant FSH) on nuclear and cytoplasmic maturation of cat oocytes. Furthermore, the effects of epidermal growth factor and insulin like growth factor-1 on developmental competence of *in vitro* matured oocytes were also studied.

Materials and Methods

All chemicals were purchased from Sigma-Aldrich, St. Louis. USA, unless otherwise specified.

Oocyte collection and in vitro maturation of cumulus oocyte complexes (COCs): Feline ovaries from mixed breeds and various stages of estrous cycle were collected after routine ovariohysterectomy (OVH) from the Veterinary Public Health, Division of the Bangkok Metropolitan Administration and the Fertility and Neutering Clinic, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The ovaries were maintained and transported at room temperature (approximately 26-30°C) in a saline solution (0.9% (w/v) NaCl, Thai Otsuka Pharmaceutical Co, Ltd, Bangkok, Thailand) supplemented with 100 IU/ml penicillin and 100 µg/ml streptomycin (M&H Manufacturing Co, Ltd, Bangkok, Thailand). Within 6hours after OVH, the ovaries were dissected from connective tissue and then washed twice in a holding medium (HM) (Hepes-buffered M199, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin and 4 mg/ml bovine serum albumin). Cumulus oocyte complexes (COCs) were isolated from the follicles by repeatedly slicing the ovaries using a surgical blade. The COCs were morphologically classified grading at a magnification x 40 under a stereomicroscope (SMZ645 Nikon, Tokyo, Japan). The COC quality was determined by the appearance of the ooplasm and the cumulus-corona radiata cells surrounding the oocytes. Oocytes with pale ooplasm as classified as non-fully grown immature oocytes were discarded from the study. The COCs with uniform size with darkened homogenous ooplasm and completely surrounded by at least 5 layers of cumulus cells were selected and randomly allocated according to the experimental design.

For *in vitro* maturation, groups of 35-40 COCs were cultured for 24 h in $NaHCO_3$ buffered M199 supplemented with 2 mM L-glutamine, 1 mM pyruvate, 100 IU/ml penicillin, 4 µg/ml gentamycin and 4 mg/ml BSA (embryo tested grade). The culture was performed at 38.5°C in a humidified condition with 5% CO_2 in air. Depending on experiment, this IVM medium was supplemented with different concentrations of recombinant human follicle stimulating hormone (rhFSH, Organon, The Netherlands), recombinant human luteinizing hormone (rhLH, Lutropin alpha, Bangkok, Thailand), insulin like growth factor-1 (IGF-1) and epidermal growth factor (EGF).

In vitro fertilization and embryo culture: After 24 h of *in vitro* maturation, *in vitro* fertilization was performed. In brief, groups of 10 *in vitro* matured oocytes were transferred to 50 µl droplets of IVF medium (Tyrode's balanced salt solution containing 1% (v/v) MEM non-essential amino acids (NEAA), 6 mg/ml BSA, 100 IU/ml penicillin, 30 µg/ml heparin, 1 mM L-glutamine, 0.36 mM sodium pyruvate and 0.11 mM calcium lactate). Frozen-thawed semen used in this study was collected from a proven-fertility tom cat using an electro-ejaculation as previously described by Wildt et al. (1986). The semen was cryopreserved by horizontally placing the straws at 4 cm above liquid nitrogen vapors for 10 min and then plunged into liquid nitrogen. The semen was thawed at 37°C for 30 sec and examined for progressive motility. Sperm suspension with more than 50% progressive motility were diluted with IVF medium. The sperm were co-incubated with oocytes at a final concentration of 0.5×10^6 sperm/ml.

After IVF for 18-24 h, cumulus cells were gently removed. Five to ten presumptive zygotes were then washed and cultured for 24 hours in 50 µl droplets of Tyrode's medium containing 4 mg/mL BSA, 100 µg/ml streptomycin and 100 IU/ml penicillin. On day 2 after IVF (IVF = day 0), cleaved embryos (4 to 8 cell stage) were further cultured in Tyrode's medium supplemented with 10% (v/v) FCS (Gibco®, Invitrogen, CA, USA). The culture medium was changed every second days. In all cases, *in vitro* culture was performed at 38.5°C in a humidified atmosphere with 5% CO_2.

Assessments of nuclear stages of in vitro matured oocytes and embryo development: To evaluate stage of nuclear maturation of cultured oocytes, cumulus cells were mechanically removed and the denuded oocytes were then fixed in 4% (w/v) paraformaldehyde (PF) overnight. The fixed oocytes were washed and then maintained in phosphate buffered saline (PBS) supplemented with 0.1% (w/v) BSA (PBS-BSA) until analysis. Fluorescent DNA probe (4',6-Diamidino-2-phenylindole: DAPI, 0.1 µg/ml) was used to stain the oocyte nucleus. The fluorescently labelled oocytes were then mounted on a glass microscopic slide and then visualized with an epifluorescent microscope (BX51 Olympus, Shinjuku, Japan). The nuclear status of IVM oocytes was classified according to chromatin morphology including germinal vesicle stage (GV), metaphase I (MI), metaphase II (MII) and degenerate oocytes.

Cytoplasmic maturation of the oocytes was determined by the capability of particular oocytes to develop to blastocysts. Percentages of cleavage and blastocyst (>60 blastomeres with blastocoelic formation) were evaluated under an inverted light microscope on days 2 and 7 of embryo culture, respectively (day 0=IVF). In order to examine embryonic quality, the embryos were fixed in 4% PF for 10 min and then maintained in PBS-BSA for immunolabelling against octamer-binding transcription factor 4 (Oct-4) and DNA in order to count cells within inner cell mass (ICM) and total cell number. This Oct-4 expression reflects the pluripotency of the ICM (Kirchhof et al., 2000). The embryos were first incubated at room temperature (26°C) for 15 min in a blocking solution containing 0.38% (w/v) glycine, 0.1% (v/v) Triton-X 100 and 3% (v/v) donkey serum. After washing twice, the embryos were then incubated at 37°C for 1 hour with goat polyclonal Oct-4 antibody (1: 100, Santa Cruz Biotechnology), followed by incubation for 1 h with donkey Alexa-488 conjugated anti-goat secondary antibody (1: 100, Santa Cruz Biotechnology, Dallas, USA). The embryos were finally labelled with DAPI and mounted on a slide with antifade mounting medium (Vectashield®, Vector laboratories, Burlingame, USA) to mitigate photo bleaching during examination with an epifluorescent microscope. The examination was carried out using 488 and 380 nm fluorescent filters for Oct-4 and DNA, respectively.

Reverse transcriptase polymerase chain reaction (RT-PCR): RNA was extracted from pooled of cumulus cells isolated before and after oocyte maturation (20 COCs for each maturation stage) using an Absolutely RNA Nanoprep Kit (Stratagene, San Diego, CA, USA) following the manufacturer's instructions.

Table 1 Description of forward (FP) and reverse (RP) primers used to assess expression of target genes in cumulus cells obtained from COCs before and after maturation

Gene	Sequence (5'-3'orientation)	Fragment length (bp)	Reference/NCBI Accession number
GAPDH	FP: GGAGAAAGCTGCCAAATATG RP: AGGAAATGAGCTTGACAAAGTGG	191	Sano et al., 2005
FSHR	FP: CATGCTGCTAGGCTGGATCTT RP: CTTGGCGATCTTGGTGTCACT	250	AY521181.1
LHR	FP: CTAATGCCTTTGACAACCTCCTC RP: CCCATTGAATGCATGACTTTGTA	342	KF040100.1
EGFR	FP: AGATTGCGAAGGGCATGAAC RP: GGCGTCTTCACCAGGACATT	118	HQ185236.1
IGF1R	FP: GCACAAGGAGCAGATGACATT RP: CAGGTTCCGGCCACTTTAAA	255	Waurich et al., 2010

The quality and quantity of extracted RNA was determined using a spectrophotometer (Nanodrop ND-2000, Wilmington, Delaware, USA) and immediately stored at -80°C until further use. First-Strand cDNA Synthesis Kit (SuperScript III Kit, Invitrogen, Carlsbad, CA, USA) was used for reverse transcription (RT). The extracted RNA was incubated with random hexamers (50 ng/µl) and 10 mM dNTP mix at 65°C for 5 min prior to being placed on ice for 1 min. The mastermix containing 2 µl of 10xRT buffer, 4 µl of 25 mM $MgCl_2$, 2 µl of 0.1 M DTT, 1 µl of RNaseOut (40U/µl) and 1 µl of SuperScript III RT (200 U/µl) was added. The mixture was further incubated at 50°C for 50 min followed by 5 min incubation at 85°C. RNA template from the cDNA:RNA hybrid molecule was removed by digestion with 1 µl of RNase H (2U/µl) after first-strand synthesis at 37°C for 20 min. The products were finally stored at -20°C for further use in polymerase chain reaction (PCR).

Polymerase chain reaction (PCR) was performed using a thermal-cycler. The primers used in this study are shown in Table 1. Each PCR reaction was consisted of reverse transcription product (1000 ng/µl of cDNA) and reaction mixture containing GoTaq® Green Master Mix (Promega, USA), nuclease free water and primers (Table 1). Nuclease free water was used as a negative control. The thermal cycling conditions were as follows: 2 min at 95°C to activate Tag DNA polymerase, 45 cycles of 30 sec at 95°C for denaturing, 30 sec at 55°C for annealing and 30 sec at 72°C for extension. At the end of program, the amplified products were run in 2% agarose gel (Bio-Rad, CA, USA) prepared in 1xTBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8) containing 0.4 mg/ml ethidium bromide (Promega, WI, USA). The separated products in agarose gel were visualized under UV light using a gel documentation (Syngene, CB, UK). *EGFR* and *FSHR* primers were designed from *Felis catus* complete cds using NCBI primer designing tool. *LHR* primer was designed based on homologous sequences of *Bos taurus* luteinizing hormone choriogonadotropin receptor (*LHCGR*) mRNA (Accession number: NM_174381.1), *Homo sapiens* luteinizing hormone choriogonadotropin receptor (*LHCGR*) mRNA (Accession number: NM_000233.3), *Mus musculus* luteinizing hormone choriogonadotropin receptor (*LHCGR*) mRNA (Accession number: NM_013582.2), and *Rattus norvegicus* luteinizing hormone choriogonadotropin receptor (*LHCGR*) mRNA (Accession number: NM_012978.1). To confirm the amplified *LHR*, the amplicon was extracted using Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, CA, USA) and then was sequenced. The sequences were blasted in NCBI GenBank to determine nucleotide identity to other species using NCBI BLAST. The resulted primers (*LHR*) were submitted to NCBI (Accession number: KF040100.1)

Experimental design

Experiment 1: mRNA expressions of LH, FSH, EGF and IGF-1 receptors in cumulus oocyte complexes (COCs): mRNA expressions of FSH, LH, epidermal growth factor and insulin like growth factor-1 receptors were performed. The cumulus cells of immature oocytes (n=10-20) were collected and RNA was extracted for RT-PCR. *GAPDH* gene expression was used as an internal control. For LH, cumulus cells collected after maturation were additionally analysed. The transcript product of *LHR* was further sequenced and the nucleotide identity of the sequence was identified.

Experiment 2: effect of different rhFSH concentrations on meiotic competence of cat oocytes: A total of 292 COCs were used to test the effect of different rhFSH concentrations as follows: 1) 0.01 IU/ml (n=82), 2) 0.05 IU/ml (n=72), 3) 0.1IU/ml (n=68) and 1 IU/ml (n=70) on meiotic competence (nuclear maturation). COCs (n=103) cultured in IVM medium without rhFSH served as the control group.

Experiment 3: effect of rhFSH, LH and growth factors (IGF-1 and EGF) on developmental competence of cat oocytes: This study was designated to examine the effects of 'chosen dose' of rhFSH during IVM on developmental competence of oocytes in terms of cleavage, morula and blastocyst formation rates. Effect of growth factors (insulin like growth factor-1(n=105) and epidermal growth factor (n=164) and luteinizing hormone (n=100) during IVM was additionally examined.

Statistical analysis

Data were pooled from 4 independent replicates. SPSS program (version 17, IBM Corporation, Armonk, New York, USA) was used for statistical analysis. Differences among oocyte maturation, cleavage and blastocyst formation rates were compared using a Chi-Square statistical test. Total cell number and Oct-4 positive cells within blastocysts were compared by one-way ANOVA. In all cases, differences were considered significant when $p<0.05$.

Results

Experiment 1 demonstrated that cumulus cells surrounding immature oocytes expressed mRNA of several genes including *FSHR*, *EGFR* and *IGF1R*. At immature stage, the cumulus cells did not express *LHR* gene (Fig 1A) butthis receptor subsequently expressed after 24 hours *in vitro* maturation (Fig 1B). The *LHR* was fairly conserved among domestic species with nucleotide identity of 94%, 93%, 89% and 87% for bovine, human, rat and mouse, respectively. The expression of FSHR coincided with the findings that rhFSH supplementation in the oocyte maturation medium significantly increased the numbers of oocytes reaching metaphase II (MII) stage compared with the non-rhFSH control (MII rates: ~60-70 % vs. 36.9, $p<0.05$; Table 2). Although the MII rates were not different among the rhFSH concentrations, the 0.01 IU rhFSH was inferior to the other rhFSH concentrations, in terms of the remaining GV stage oocytes after maturation (% GV: 34% vs. 13-14%, respectively; Table 2). According to results of Experiment 2, the 0.1 IU/ml rhFSH was selected to demonstrate the supportive ability of rhFSH on developmental competence by means of cleavage, morula and blastocyst rates (Experiment 3). Indeed, oocytes that

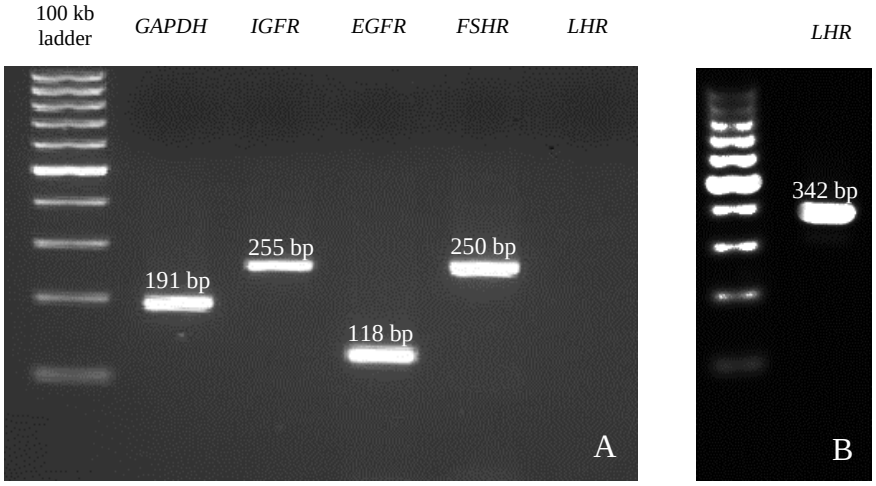

Figure 1 RT-PCR analysis of target genes in COCs. A: PCR products (*GAPDH*, *IGFR*, *EGFR*, *FSHR* and *LHR*) of cumulus cells surrounded immature oocytes. B: *LHR* mRNA expression was observed only in cumulus cells of *in vitro* matured oocytes**.**

were matured in the maturation medium supplemented with only rhFSH had significantly lower developmental capability compared with the oocytes matured with rhFSH and growth factors (EGF or IGF-1, Table 3). In contrast, the addition of LH into the maturation medium did not improve the developmental competence (Table 3). In all cases, the embryonic quality in terms of cell number and Oct-4 positive cells was not significantly different among the groups (p>0.05).

Discussion

This study demonstrates that follicle stimulating hormone, insulin like growth factor-1 and epidermal growth factor synergistically exerted the meiotic and developmental competence of cat oocytes, while LH failed to stimulate embryo development. Experiment 1 indicated that cumulus cells expressed several genes including *FSHR, LHR*, *EGFR* and *IGF1R* but the expression was stage-of-maturation specificity. These genes play a central role in controlling meiotic and cytoplasmic maturation of mammalian oocytes. An action of a particular gene is mediated via its receptors located on the somatic cells surrounding the oocytes rather than directly to the oocytes since the oocytes seem to lack gonadotropin receptors (Amsterdam et al., 1975). The oocytes acquire their meiotic signals via transzonal process and gap junction communication between oocyte and cumulus-radiata cell complex (Matzuk et al., 2002). Although the mechanism by which the gonadotropin stimulates the meiotic resumption is entirely unclear, these gonadotropins are mediated through cyclic adenocine 3′,5′-monophosphate (cAMP) (Downs et al., 1988; Eppig, 1989) and subsequent activation of mitogen activated protein kinase (MAPK). Our study did not observe *LHR* expression in cumulus cells of immature cat oocytes similar to other studies (Van Tol et al., 2006). It is postulated that the LHR expression in cat oocytes is controlled by some factors such as FSH and maybe size of follicles. For example, *LHR* did not expressed in medium-size (2-6 mm) bovine follicles, but was highly expressed only in FSH-primed dominant follicle (Ireland and Roche, 1982). This study also found that the LH receptors were only expressed after *in vitro* maturation. This finding is coin contrast to a report indicating that *LHR* mRNA could not be detected in cumulus cells before or after IVM (Nuttinck et al., 2004). It is unclear about factors within IVM medium that stimulated the *LHR* expression in cumulus cells surrounding cat oocytes but it is possible that FSH can stimulate the LHR expression via increase in intracellular cyclic AMP (Downs et al., 1988; Eppig, 1989). Indeed, specific pathways for FSH primed LHR expression during *in vitro* maturation remain to be studied. The reasons mentioned above support our finding and other studies that adding recombinant LH during IVM did not improve cleavage and blastocyst formation rates (Choi et al., 2001; Ali and Sirard, 2002). In Experiment 2, rhFSH without any LH contamination significantly induced meiotic resumption in a dose- dependent manner. This finding indicated convincingly that FSH alone could stimulate meiosis *in vitro* as similar to other species. When FSH binds to its receptors, intracellular cAMP increases (Downs et al., 1988) along with the activation of mitogen-activated protein kinase (MAPK) (Shimada and Terada, 2002; Su et al., 2002). The activation of FSH coupled receptors can also be downstream via protein kinase A (PKA) (Su et al., 2002; Liang et al., 2005) and kinase C (PKC) (Fan et al., 2004; Jin et al., 2006). Indeed, FSH activates both PKAI and PKAII to increase levels of cAMP in cumulus cells (Downs and Hunzicker-Dunn, 1995). While activation of PKAI prevent meiosis resumption, PKAII is preferable for initiation of oocyte maturation (Newhall et al., 2006). Although FSH is known to stimulate both nuclear and cytoplasmic maturation (Table 2 and 3), we clearly demonstrated that the potential action of FSH on embryo development

Table 2 Meiotic competence of cat oocytes after *in vitro* oocyte maturation for 24 hours in a presence of different concentrations of recombinant human follicle stimulating hormone (rhFSH)

Group	Total oocyte	Oocytes that developed to			
		GV (%)	MI (%)	MII (%)	Degenerate (%)
Control (no rhFSH)	103	55 (53.4)[a]	3 (2.9)[a]	38 (36.9)[a]	7 (6.8)[a]
0.01 IU rhFSH	82	28 (34.1)[b]	2 (2.8)[a]	49 (59.8)[b]	3 (3.7)[a]
0.05 IU rhFSH	72	10 (13.9)[c]	6 (8.3)[a]	49 (68.1)[b]	7 (9.7)[a]
0.1 IU rhFSH	68	9 (13.2)[c]	3 (4.4)[a]	49 (72.1)[b]	7 (10.3)[a]
1.0 IU rhFSH	70	9 (12.9)[c]	6 (8.6)[a]	50 (71.4)[b]	5 (7.1)[a]

[a, b and c] Within a column, different superscripts denote values that differ significantly.

Table 3 Developmental competence and embryonic quality of cat oocytes matured *in vitro* in maturation medium supplemented with rhFSH (0.1 IU/ml), rhLH, EGF and IGF-1

Group	No. oocyte	Embryo development		Cell number (mean±SD)	Oct-4 positive cells
		Cleavage (%)	Blastocyst* (%)		
rhFSH	160	65 (40.6)[a]	20 (30.8)[a]	80.7±16.7[a]	8.3±7.2[a]
rhFSH/rhLH	100	41 (41)[a]	11 (26.8)[a]	76.9±13.1[a]	6.9±6.2[a]
rhFSH/EGF	164	102 (62.2)[b]	38 (37.2)[b]	89±22.5[a]	11±8.5[a]
rhFSH/IGF-1	105	58 (55.2)[b]	23 (39.6)[b]	90.2±16.3[a]	9.7±6.2[a]

* blastocyst development in relation to cleaved embryos

[a, b] within a column, different superscripts denote values that differ significantly

(cytoplasmic maturation) was limited. However, when IGF-1 and EGF were added into FSH containing IVM medium, the two growth factors synergistically improved the developmental competence of cat oocytes (Lonergan et al., 1996; Merlo et al., 2005). As similar to several reports, these two growth factors bind to its receptors and, in turn, activation PI3K and ERK pathways. While the mechanisms underlining the cytoplasmic maturation have yet to be further examined, the PI3K and ERK-MAPK pathways are essential for organelle remodeling, cell survival and proliferation (Li et al., 2008). This cellular activation during cytoplasmic maturation is critical for capability of embryos to develop to blastocyst and also to term because maternal mRNA stored within ooplasm will vanish during early development (2-16 cell stage) depending on species. Thus, premature reduction in the maternal mRNA will adversely affect maternal zygotic genome transition, resulting in embryonic block and death (Wang et al., 2006; Schier, 2007).

It is concluded that cumulus cells express several genes (*FSHR*, *LHR*, *EGFR* and *IGF1R*) necessary for oocyte maturation and embryo development. These receptor genes were present at an immature stage except that of *LHR* which was later on expressed after *in vitro* maturation. FSH without other gonadotropins contamination can be used to improve nuclear and cytoplasmic maturation of cat oocytes in a dose- dependent manner. rhFSH at 0.1 IU/ml was the most appropriate dose for *in vitro* maturation and the effects in promoting embryo development is synergistic with IGF-1 and EGF.

Acknowledgements

This study was financially supported by Thailand Research Fund (RSA5680028). We would like to thank the Veterinary Public Health Division of the Bangkok Metropolitan Administration and the Fertility and Neutering Clinic, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University for sample collection.

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บทคัดย่อ

ฮอร์โมนรีคอมบิแน้นต์ ฟอลลิเคิล สติมูเลติ้งและโกรทแฟคเตอร์เพิ่มประสิทธิภาพการเจริญพร้อมปฏิสนธิของโอโอไซต์และตัวอ่อนแมว

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การผลิตตัวอ่อนภายนอกร่างกายเป็นเทคนิคที่มีศักยภาพในด้านการศึกษาเพื่อให้เข้าใจปัจจัยที่เกี่ยวข้องกับชีววิทยาของตัวอ่อน การศึกษานี้มีวัตถุประสงค์เพื่อตรวจผลของการเสริมฮอร์โมนรีคอมบิแน้นต์ ฟอลลิเคิลสติมูเลติ้ง (เอฟ เอส เอช) ต่อการพัฒนาพร้อมปฏิสนธิและการเจริญของตัวอ่อนแมว การทดลองที่ 1 เป็นการศึกษาการแสดงออกยีนตัวรับฮอร์โมน ลูทีไนซิ่ง (แอล เอช) เอฟ เอส เอช และ โกรท-แฟคเตอร์ชนิด อิพิเดอร์มอล (อี จี เอฟ) และ คล้ายอินซูลินที่ 1 (ไอจีเอฟที่ 1) ในเซลล์คิวมูลัสของโอโอไซต์ การทดลองที่ 2 เป็นการศึกษาผลของ เอฟ เอส เอช ในระดับความเข้มข้นต่างๆ กัน (0.01, 0.05, 0.1 และ 1 ไอยูต่อมล.) ต่อการเจริญพร้อมปฏิสนธิของโอโอไซต์ โดยใช้การเลี้ยงโอโอไซต์ในน้ำยาที่ไม่มีการเสริม เอฟ เอส เอช เป็นกลุ่มควบคุม การทดลองที่ 3 เป็นการศึกษาผลของ เอฟ เอส เอช ร่วมกับ แอล เอช หรือ ร่วมกับโกรทแฟคเตอร์ต่ออัตราการพัฒนาของตัวอ่อนการศึกษาครั้งนี้พบว่าเซลล์คิวมูลัส แสดงออกยีนของตัวรับ เอฟ เอส เอช อี จี เอฟ และไอ จี เอฟ ที่ 1 แต่ไม่พบการแสดงออกยีนตัวรับของฮอร์โมน แอล เอช เมื่อทำการตรวจในโอโอไซต์ระยะก่อนการเลี้ยงพร้อมปฏิสนธิ ตัวรับของฮอร์โมนนี้จะแสดงออกภายหลังการเลี้ยงโอโอไซต์ให้พร้อมปฏิสนธิ การเสริม เอฟ เอส เอช มีผลกระตุ้นการพัฒนาของโอโอไซต์ให้อยู่ในระยะพร้อมปฏิสนธิในลักษณะแปรผันตามปริมาณที่ใช้อย่างมีนัยสำคัญ ($p<0.05$) เมื่อเทียบกับกลุ่มควบคุม ระดับ เอฟ เอส เอช ที่ความเข้มข้น 0.1 ไอยูต่อมล. ถูกนำมาใช้ในการศึกษาที่ 3 เนื่องจากให้ผลดีที่สุด โดยพบว่าเมื่อทำการเสริม เอฟ เอส เอช อย่างเดียว หรือใช้ร่วมกับ แอล เอช ให้ผลต่อร้อยละการพัฒนาของตัวอ่อนแมวระยะคลีเวจและบลาสโตซีสต์ ด้อยกว่ากลุ่มทดลองที่ใช้ เอฟ เอส เอช ร่วมกับ โกรทแฟคเตอร์ อย่างมีนัยสำคัญ ($p<0.05$) การศึกษานี้สรุปได้ว่า เซล์คิวมูลัสที่อยู่ล้อมรอบโอโอไซต์แสดงออกยีนตัวรับฮอร์โมน เอฟ เอส เอช แอล เอช และโกรทแฟคเตอร์ชนิด อี จี เอฟ และไอ จี เอฟ ที่ 1 ผลการเสริม ฮอร์โมน เอฟ เอส เอช ต่ออัตราการเจริญพร้อมปฏิสนธิเป็นไปในลักษณะที่แปรผันตามปริมาณฮอร์โมนที่ใช้ การเสริมฮอร์โมน เอฟ เอส เอช ร่วมกับโกรทแฟคเตอร์ให้ผลดีที่สุดในการเจริญพัฒนาระดับไซโตพลาสซึมของโอโอไซต์ ที่ซึ่งเกี่ยวข้องกับการพัฒนาของตัวอ่อนระยะคลีเวจและบลาสโตซีส

คำสำคัญ: แมว การพัฒนาตัวอ่อน โกรทแฟคเตอร์ โอโอไซต์ ฮอร์โมนรีคอมบิแน้นต์ ฟอลลิเคิล สติมูเลติ้ง

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