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Possible Improvement of Oocyte Supply by the use of Aged Mice and Different Gonadotrophins

Myungook Lee[1], Jong Il Ahn[2], Hyosook Kwun[1], Dong Woo Ko[1], Jiyeon Ahn[1] and Jeong Mook Lim[1,2,†]

[1]*Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea*
[2]*Research Institutes of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea*

Abstract

This study was conducted to examine the influences of two human chorion gonadotrophins (hCGs) being injected into young or aged (45- to 65-week old) outbred (ICR) mice on developmental capacity of oocytes retrieved. In vitro-culture and parthenogenetic activation of oocytes retrieved were employed for the assessment. Superovulation was determined as being induced when more than 25 oocytes were retrieved. No aged mice were superovulated, while in contrast, 67-100% were superovulated in the 6- to 8-week-old (young) mice. In the aged, hCG injection yielded better retrieval (5 vs. 13 to 14.8 oocytes/mouse). Overall, no significant difference between two hCGs was detected but between the young and aged, significant differences in maturational arrest (0% vs. 39% MI arrest and 46% vs. 15% degeneration) and developmental capacity (24% vs. 46% 8-cell embryo development) were detected. In conclusion, hCG injection contributes to increasing oocyte retrieval from aged outbred mice, but the kinds of gonadotrophin influenced the efficiency of hyperstimulation induction in specific ages.

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† Correspondence: Jeong Mook Lim (ORCID: 0000-0002-2112-442X)
Phone: +82-2-880-4817, Fax: +82-2-874-2555
E-mail: limjm@snu.ac.kr

INTRODUCTION

Superovulation was one of general protocols for artificial reproductive technology (ART) and standard method has been established for superovulation (Legge and Sellens, 1994; Johnson et al., 1996). Different inbred and outbred strains have been employed for superovulation treatment to date, and strong effect of gonadotrophins drives regardless of ages and strains. Numerous reports pointed out the effect of endogenous factors on the effectiveness of hyperstimulation treatment, while different gonadotrophin might yield different results (Zarrow and Wilson, 1961; Gates and Bozarth, 1978; Suzuki et al. 1996; Auerbach et al., 2003; Byers et al., 2006).

In this study, we compared the influence of both age and gonadotrophin on the developmental capacity of oocytes retrieved. We used young (6 to 8 weeks old) or aged (more than 45 weeks old) outbred mice for superovulation and two different hCGs were employed. As the parameters for monitoring developmental competence of oocytes retrieved, in vitro-culture for both maturation and development, and parthenogenetic activation were employed.

MATERIALS AND METHODS

Experimental animals

ICR outbred mice were employed as experimental animals. Mature oocytes were harvested from young (6-8 weeks) or aged (over 45 weeks) female mice after experimental treatments. All animals were maintained under the conditions of controlled lightening (14 h of light/10 h of darkness), temperature (20-22°C) and humidity (40 – 60%). All procedures of animal management, breeding and euthanasia were performed according to the standard protocols of Seoul National University. The experimental protocols were approved by the Institutional Animal Care and Use Committee (approval number SNU-120220-1). Standard operation protocols were employed for managing experimental samples and quality control of the laboratory facility and equipment were regularly conducted.

Collection of mature oocytes

Naturally ovulated oocytes were collected by oviduct flushing of ICR female mice in estrus 16 h after they were mated with a vasectomized male mice. Vaginal smears were performed to check the estrous cycle stage of the female donors. The flushing medium was M2 medium (Sigma-Aldrich, St Louis, MO). For ovarian hyperstimulation, 5 IU of PMSG (Folligon, Intervet International, Boxmeer, the Netherlands) was injected intraperitoneally and ovulation was induced by intraperitoneal injection of 5 IU of different hCGs (hCG A; Folligon, Intervet International, Boxmeer, Netherlands or hCG B; Ovidrel, Fertility lifelines, Darmstadt, Germany) 48 hours later. Oocytes were recovered 16 hours post-hCG. After hormonal treatments females were sacrificed by cervical dislocation. Superovulation was determined as being induced when more than 25 oocytes were retrieved (Byers et al., 2006). Oocyte maturation at the metaphase II stage was verified by the extrusion of the first polar body in the perivitelline space. For this analysis, oocytes were released from cumulus cells through incubation in M2 medium supplemented with hyaluronidase (Sigma-Aldrich; 200 IU/ml) for 5 min at 37°C.

Parthenogenetic activation and IVF of oocytes

To activate oocytes parthenogenetically, oocytes released from cumulus cells were cultured in calcium-free potassium simplex optimized medium (KSOM) supplemented with 10 mM $SrCl_2$ and 5 μg/ml cytochalasin B for 4 h. Oocytes that had been activated parthenogenetically *in vitro* were then cultured in 5 μl droplets of modified Chatot, Ziomek, and Bacister (CZB) medium for a further 120 h at 37°C under an atmosphere of 5% CO2 in air. Pronucleus formation, and development to the two-cell, four-cell, eight-cell, and over stages were monitored under an IX70 inverted microscope (Olympus, Tokyo, Japan) at 6, 24, 48, 72 and 120 h after activation or fertilization.

Statistical analysis

All experiments were replicated more than three times, and the data obtained were subjected to statistical analysis. Not all oocytes retrieved from each experiment were provided because the superovulation treatment yielded different number of oocytes retrieved. To compensate this discrepancy, randomly selected oocytes were allotted to several treatment groups. A generalized linear model (PROC-GLM) created using Statistical Analysis System (SAS) software version 9.1 (SAS Institute, Cary, NC) was used to analyze the data. When a significant model effect was detected, comparisons among groups were subsequently conducted using the least-squares or Duncan methods. A p-value of less than 0.05 indicated a significant difference.

RESULTS

Total 31 mice (17 young, 14 aged) were treated for superovulation, Superovulation was validated only when more than 25 oocytes were retrieved. Under this criteria, no aged mice were superovulated (figure 1), while 67 to 100% of young mice was successfully induced. Significant (p<0.0001) difference was detected between two ages. When aged mice were superovulated (tables 1 and 2), total 217 oocytes were obtained. In the young, remarkable effect of the kinds of gonadotrophin was detected and more oocytes were retrieved after superovulation than after natural ovulation [15 from natural ovulation (5 oocytes/head), 39 from hCG A (13 oocytes/head) and 163 from hCG B (14 oocytes/head) injections; p=0.0105]. No significant effect between two hCGs was observed (table 1).

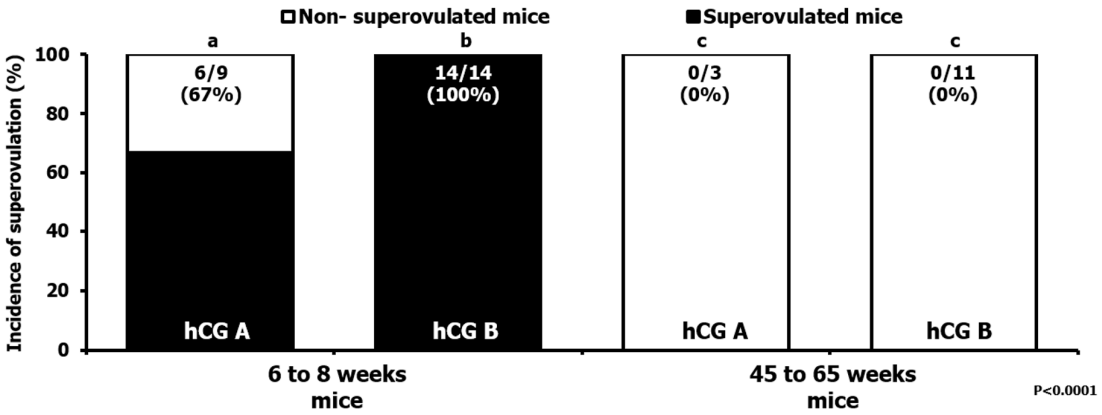

Figure 1. Superovulation of ICR mice with different human chorionic gonadotrophins (hCG). Either 6 to 8 weeks old (young) or more than 45 weeks old (aged) mice were treated with difference hCG. The mouse yielding more than 25 ovulated oocytes were considered as a superovulated mouse. Superovulation outcome of total mice (n=31), the young mice (n=17) and the aged mice (n=14).

Table 1. Maturation outcome after the superovulation of aged mice with different human chorionic gonadotrophins (hCG) injections.

Treat	Outcome	No. of mice treated	No. of oocytes retrieved	Mean (±SE) no. of oocytes retrieved	No.(%)[a] of oocytes developed to		No. of oocytes degenerated
					GVBD-MI	MII	
None	Natural (control)	3	15	5.0±0.6[b]	0 (0)[b]	7 (47)	8 (53)[b]
hCG A	Unsuccess	3	39	13.0±0.6[c]	0 (0)[b]	21 (54)	18 (46)[b]
hCG B	Unsuccess	11	163	14.8±1.5[c]	63 (39)[c]	76 (47)	24 (15)[c]

GVBD=Germinal vesicle breakdown; MI=Metaphase I; MII=Metaphase II

Model effects on the mean number of oocytes retrieved, the number of oocytes developed to the stage of GVBD-MI and MII, and the number of oocytes degenerated, which were indicated as p value, were 0.0105, <0.0001, 0.7191 and <0.0001, respectively.

[a]Percentage of the total number of oocytes retrieved.

[bc]Different superscripts within the same parameter indicate significant differences among the treatment, P<0.05.

In maturational development, significant differences were detected in the number of GVBD-MI (0% vs. 39% of total retrieved oocytes; $p<0.0001$) and degenerated oocytes (46% to 53% vs. 15% of total retrieved oocytes; $p<0.0001$) between superovulation groups and natural ovulation group. No difference in maturation (47% to 54% of total retrieved oocytes; $p=0.7191$) however, were observed among treatments (table 1).

As shown in table 2, the number of oocytes formed pronuclei after parthenogenetic activation (43% vs. 52% vs. 61%; $p=0.5777$) and developed beyond the 2-cell stage embryos (29% vs. 52% vs. 51%; $p=0.5079$) were not significantly different among treatments. However, the number of embryos developed beyond the 8-cell stage were significantly increased after the hCG B injection than any other treatments ($p=0.0180$). No differences were detected between two hCGs. Compared with the young mice, the hCG B significantly stimulated the development beyond the 8-cell stage (24% vs. 46%; $p=0.0680$).

Table 2. Activation of mature oocytes retrieved after different human chorionic gonadotrophin (hCG) injections into aged mice.

Hormones	No. of mature oocytes cultured	No. (%)[a] of oocytes formed pronuclei	No. (%)[a] of oocyte developed	
			To 2-cell embryo	Beyond 8-cell embryo
None	7	3 (43)	2 (29)	0 (0)[b]
hCG A	21	11 (52)	11 (52)	5 (24)[bc]
hCG B	76	46 (61)	39 (51)	35 (46)[c]

Model effects of treatment on the number of oocytes formed pronulcei, and the number of oocytes developed to the 2-cell, and beyond 8-cell stage, which were indicated as p value, were 0.5777, 0.5079, and 0.0164, respectively.
[a]Percentage of the number of MII oocytes retrieved.
[bc]Different superscripts within the same parameter indicate significant differences among the treatment, $p<0.05$

DISCUSSION

Clear evidence was shown in this study, which the kind of gonadotrophin and the state (age) of hCG recipients greatly influenced hyperstimulation outcome. Care should be taken to determine the regime of hyperstimulation (kind, dose and administration subject etc). The use of gonadotrophins is especially beneficial for utilizing aged animal, which increases the number of developmentally competent oocytes retrieved following hyperstimulation treatment. As a matter of facts, aged mice was usually abandoned due to their poor response for gonadotrophins. Our experimental outcome definitely contributes to expanding animal resources.

From different viewpoint, hyperstimulation treatment makes it possible to utilize developmentally competence oocytes in aged ovaries and probably rescues some oocytes arrested their development at meiotic maturation. Superovulation influences embryonic development *in vitro* or *in vivo* (Ertzeid and Storeng, 2001; Van der Auwera and D'Hooghe, 2001), oocyte quality , ovarian follicle number (Choi et al., 2011), oocyte degeneration (Tarin et al., 2001), chromosome abnormality (Velde and Pearson, 2002) and cellular or molecular processes (Miao et al., 2009). Further studies to enhance hyperstimulation regime can improve stem cell establishment, as well as can produce developmentally competent embryos.

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