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Study the effect of different super ovulation hormones (Urinary Human Menopausal Gonadotropin, Urinary Follicular Stimulating hormone or Recombinant Follicular Stimulating Hormone) in combination with Human Chorionic Gonadotropin on mice oocytes yield and quality and embryonic development after *In vitro* fertilization

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Abstract
Advanced reproductive techniques enable greater numbers of oocytes or embryos of high quality to be used in basic reproductive research. Therefore, hormonal control for multiple follicular growth in IVF program was directed toward maximizing the yield of fertilizable oocytes. The present study was conducted to evaluate the efficacy of different types of gonadotropin used for induction of superovulation in mouse. 15 IU human menopausal gonadotropin (hMG), Urinary follicle stimulating hormone (U-FSH) and recombinant follicle-stimulating hormone (rFSH) were injected in mouse followed 48 hours later by injection 15 IU human chorionic gonadotropin (hCG). After 14 hours from hCG injection the oocytes were collected and evaluated according to cumulus oocyte complexes (COCs) expansion and oocyte maturation rate. Matured oocytes were *In vitro* fertilized using mouse epididymal spermatozoa, then IVF oocytes were cultured *In vitro* to the blastocyst stage. Result indicated that hMG was superior than U-FSH and rFSH. hMG. hMG produced a significantly ($P<0.05$) higher oocytes yield and quality than (pure FSH) the worst oocyte yield and quality was obtained by using (rFSH). *In vitro* fertilization, cleavage and blastocyst rates were significantly ($P<0.05$) higher for hMG group than pure FSH. Nevertheless, in U-FSH group, fertilization and cleavage rates were significantly ($P<0.05$) higher than oocytes retrieved from rFSH treated animals. In conclusion, 15 IU hMG followed 48h later by injection of 15 IU hCG is the optimal protocol for induction of superovulation in mouse. Also, the developmental competence of oocytes obtained by this protocol was higher than that of U-FSH or rFSH.

Keywords: mouse, superovulation protocol, types of gonadotropin, *in vitro* fertilization, Blastocyst rate

1. Introduction

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) play a predominant role in ovarian follicular development and ovulation process. Follicle-stimulating hormone induces growth in the cohort of follicles that have reached the early antral stages, stimulates estrogen secretion and LH receptor in granulosa cells [1]. During the final follicular maturation and ovulation, there is a complex interplay of cell-matrix-cytokine interactions for which gonadotropins laid the baseline during differentiation of the follicle [2].

Superovulation is a commonly used technique to obtain a great number of oocytes in the same developmental stages in assisted reproductive technology (ART) and in clinical or experimental animal studies. Mice are the most commonly used animal model in reproductive studies, especially to produce transgenic animals and in embryo assay quality control tests for human *In vitro* fertilization (IVF) programs [3]. Adult mouse normally releases 8-10 oocytes in each cycle, which can be increased to 40-50 using different protocols of superovulation [4]. Different sources for gonadotropin have been used for induction of superovulation in mammals. Among these, pregnant mare serum gonadotropin (eCG) [5], human menopausal gonadotropin (hMG) [6], pure follicle stimulating hormone (FSH) and recombinant follicle stimulating hormone (rFSH) [7] have been used for the induction of superovulation in mice. Higher ovarian response, higher recovery rate of oocytes, higher fertilization rate and embryo quality were achieved by using hMG than U-FSHin mice [8, 9].

Furthermore, there are many other factors affecting the success of the superovulation program. Strain differences have been proven to be crucial components in mouse *In vitro* fertilization (IVF) and superovulation protocols, responses to standardized mouse IVF protocols vary significantly between different strains [10]. Previous studies have convincingly indicated that superovulation alone can cause impaired oocyte maturation, delayed embryo development, decreased implantation rate and increased postimplantation loss. Superovulation with low or high doses of eCG significantly

altered Epab and Pabpc1 mRNA levels in GV oocytes, MII oocytes and 1- and 2-cell embryos compared with their respective controls [11]. In addition hyper stimulation using rFSH was reported to increase the risk of spindle assembly and preimplantation developmental arrest in mouse oocytes [7]. While, high levels of FSH *in vivo* may have an adverse effect on follicular function and oocyte health, possibly in relation to spindle assembly and chromosomal alignment [5]. Today, various commercial preparations of these hormones are available and are being used widely in human therapy. The response of animal ovaries to these preparations and their usage in superovulation have not been adequately investigated. Therefore, this study was under taken to evaluate the effect of different sources of gonadotropin used for induction of superovulation in mice on oocytes maturation rate, and their ability to fertilized and developed *In vitro*.

2. Materials and Methods

This work was reviewed and approved by the Ethical Committees of Faculty of Science, Al-Azhar University and Nuclear Materials Authority.

2.1 Animals

In this study, female BALB/C mice of age 4 - 6 weeks were purchased from Biological and Vaccine Production Holding Co. (VACSERA, Egypt). Female immature mice were kept for two weeks in the animals' lab at standard conditions, i.e., temperature of 23 to 25 °C, humidity of 50 to 55%, a 12-hour light/dark cycle, and easy access to food and water for compatibility with the environment. The mice were divided randomly into six of equal groups: For the induction of oocyte development and reproduction in each mouse, Group 1) 15 IU of human menopausal gonadotropin (hMG) hormone (Merional, IBSA Farmaceutici, Italia) was injected intraperitoneally (i.p.). Group 2) 15 IU of Urinary-FSH (U-FSH, Fostimon, IBSA Farmaceutici, Italia) was injected i.p. Group 3) 15 IU of recombinant human FSH hormone (Gonapure, Minapharm, Egypt) was injected i.p. For the purpose of inducing the ovulation process, 48 h later 15 IU hCG (Chorimon, IBSA Farmaceutici, Italia) was injected in i.p. in all groups.

2.2 Collection of mature oocytes

Twelve hours prior to the collection of matured oocytes, human tubal fluid (HTF) and potassium simplified optimized medium (KSOM) were prepared for *In vitro* fertilization. Fertilization media were dropped and covered with mineral oil and maintained in incubators at 37C at least 2 h before use for equilibration. On the isolation day and 12 hours after hCG hormone injections, the female mice were killed by neck beads' displacement, then the uterus was excised out and transferred to 10 cm Petri dish. The set of eggs and cumulus cells were collected from the ampulla of the Fallopian tubes of both sides and placed into the 35 mm Petri dish containing HTF.

2.3 Sperm collection

In order to collect sperm, the epididymis tail of the mice were dismembered and put into a Petri dish containing HTF *In vitro* previously equilibrated in CO_2 incubator. Then, the tail of the epididymis was split into smaller pieces and placed for 1 hour inside CO_2 incubator (Class 100 Thermo Co., Germany) at 37 °C under 5% CO_2 for induction of capacitation.

2.4 *In vitro* fertilization of mature oocytes

The collected oocytes were washed at least three time in *In vitro* fertilization medium, then 10 to 15 mature oocytes allocated to 100 µl of IVF medium and covered with mineral oil. At this point, sperm number was adjusted to 2 $x10^6$ sperm/ml, spermatozoa were added to drops containing oocytes. Then the Petri dish containing oocyte co-incubated with the sperm was put in the CO_2 incubator for 4 to 5 hr. Then, the oocytes likely of insemination were transferred to a Petri dish containing 5 drops of KSOM *In vitro*. Oocytes were finally transferred to the fifth in the middle of the Petri dish after a wash in four side drops. Twenty-four hours after insemination, the number of two cell (and probably four-cell) embryos were counted and recorded under stereomicroscope (Zeiss Co., Germany). Monitoring the evolution of the embryos continued until the blastocyst stage was reached in the following days. To check the reversibility status of the possible effects of gonadotropin on oocytes and embryos of the mice, all the steps listed above were repeated at least 6 times.

2.5 Embryo grading

The morphology of two blastomer embryos were divided into four Grades of A, B, C, and D (14). Grade A with equal blastomers, round, with no fragmentation, smooth cytoplasm, and bright yellow zona; Grade B with slightly different blastomers in size, up to 10% fragmentation with granules in cytoplasm; Grade C with unequal blastomers, up to 50% fragmentations and large granules and vacuoles in cytoplasm; and Grade D with blastomers of unequal size, extreme fragmentation, with dark and large granules and presence of vacuoles in cytoplasm.

2.6 Statistical analysis

Oocytes yield was analyzed by one-way ANOVA. While COCS expansion, maturation rate, degeneration rate, fertilization rate and embryonic development were tested for significance using the X^2chi-square analysis. A value of $P < 0.05$ was regarded as indicative of statistical significance.

3. Results

3.1 Recovery rate of oocytes

Table 1 showed the effect of three different types of superovulation hormone U-hMG, U-FSH and rFSH on oocytes yield, quality and their effect on embryonic development. There was a highly significant difference between the examined groups. Number of oocytes recovered after induction of superovulation using U-hMG was significantly ($P<0.001$) higher than the group treated with U-FSH or rFSH. Also, number of the recovered oocytes was higher ($P<0.001$) for the group treated with U-FSH than rFSH treated mice. Recovery rate of COCs with full cumulus cell expansion was significantly ($P<0.05$) higher mice treated with U-hMG than that treated with U-FSH or rFSH for induction of superovulation (Fig. 1. Although, oocytes with full cumulus cell expansion was higher ($P<0.05$) in animals treated with U-

FSH than that treated with rFSH (Fig.1 C). The percentage of oocytes reaching the MII stage was significantly (P<0.05) higher in U-hMG and U-FSH groups than in rFSH treated mice (Fig. 1D). Also, number of degenerated oocytes was lower (P<0.05) in U-hMG treated mice than U-FSH or rFSH groups. The highest percentage of degenerated oocytes was obtained in group of mice superovulated with rFSH (P<0.05) (Fig.1 F).

Table 1: Effect of different types of gonadotropin on maturation rate and oocytes quality in superovulated BALB/C mice.

Group	Ovaries No.	No. oocytes recovered	Full Cumulus cell expansion	MII	Degenerated
U-hMG	60	794[a] 132.2 ± 5.93	703 (88.5%)[a]	732 (92.2%)[a]	11 (1.38%)[a]
U-FSH	60	513[b] 85.5 ± 2.66	403 (78%)[b]	433 (84.4%)[a]	23 (4.5%)[b]
r-FSH	60	351[c] 58.5 ± 1.3	209 (59.5%)[c]	261 (74.3%)[b]	56 (15.9%)[c]

a, b within the same column differ significantly at P<0.05.
b, c within the same column differ significantly at P<0.05.

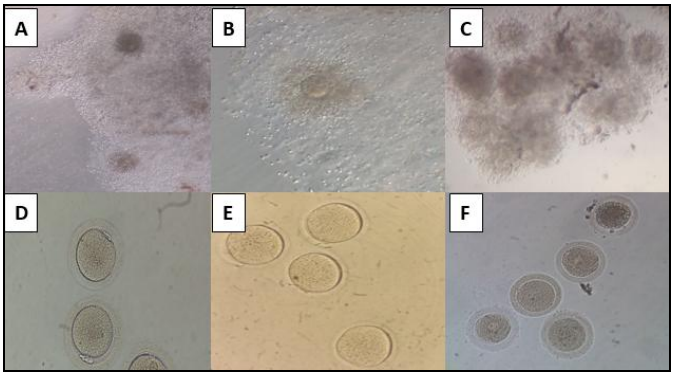

Fig 1: Photograph showing maturation rate of oocytes recovered from super ovulated mice. A and B) Oocytes with full cumulus cell expansion as a good indicator for oocyte maturity 40X and 100X, respectively; C) Oocytes with moderate cumulus cell expansion, 100X. D) Mature oocytes with 1st polar body as an indicator for MII phase, 400 X. E) Immature ooytes with no 1st polar body, as indicator for MI phase, 400 X. F) Degenerated oocytes with very dark and granulated cytoplasm, 400X.

3.2 Embryonic development

The effect of type of gonadotropin used for induction of superovulation in mice and the subsequent ability of mature oocytes to fertilize and develop *In vitro* is presented in Table 2. Results illustrated that cleavage rate was higher (P<0.05) in U_hMG treated group when compared with rFSH treated mice. Developmental rate showed that a significantly (P<0.05) higher percentage of oocytes from rFSH treated mice were arrested at the 2-cell stage when compared with U-hMG treated group (Fig. 2A). The percentage of embryos developed to the morula stage was higher in rFSH group compared with U-hMG treated mice. While, the highest percentage of blastocyst rate was achieved in group of mice treated with U-hMG for induction of superovulation when compared with U-FSH or rFSH groups (Fig. 2B, C). Also, blastocyst rate was higher (P<0.05) in U-FSH than in rFSH injected one.

Table 2: Cleavage rate and embryo development of *In vitro* fertilized matured BALB/C mice oocytes obtained after superovulation using different gonadotropin sources.

Group	Mature oocytes	Cleavage rate (%)	Embryo development (%)			
			2 cell	8 cell	Morula	Blastocyst
hMG	732	623 (85.1%)[a]	63 (10.1%)[b]	54 (8.66%)	62 (9.9%)[b]	444 (71.2%)[a]
U-FSH	433	349 (80.6%)	59 (16.9%)	39 (11.1%)	55 (15.7%)	196 (56.1%)[b]
rFSH	261	197 (75.4%)[b]	45 (22.8%)[a]	29 (14.7%)	36 (18.2%)a	87 (44.1%)[c]

a, b within the same column differ significantly at P<0.05.
b, c within the same column differ significantly at P<0.05.

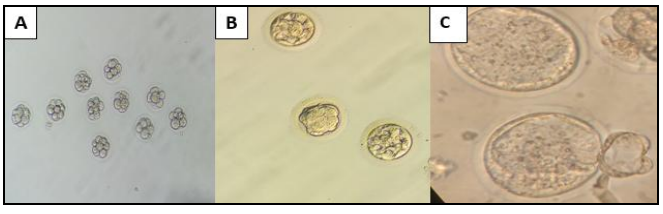

Fig 2: Figure shows different stages of embryonic development. A) Different embryonic stages 4 cell (24-30 hours) and 8 cell (48 hours) stages, 200 X; B) 20*0*X show the Morula and early blastocyst stage (Compacted blastocyst) 72 hours after culture. And C) show the blastocyst stages (expanded and hatched) which obtained after 96 hours from embryo culture some blastocysts started to hatch as a good sign on embryonic development 400X.

4. Discussion

In this study we compared different sources of gonadotropin for the induction of superovulation in BALB/C mice, and to study the effect of superovulation regimen on maturation rate of mice oocytes and their subsequent ability to fertilize and develop *In vitro*. In the present work, superovulation regimen in mice using 15 IU hMG followed 48 h later by injection of 15 IU hCG appear to be better in terms of the number of mature oocytes recovered or the induction of full cumulus cell expansion and decrease the percentage of degenerated oocytes when compared with U-hmG, U-FSH or rFSH. Also, cumulus cell expansion and the percentage of degenerated oocytes were higher (P<0.05) in hMG treated mice than in U-FSH or rFSH group. While, the percentage of oocytes reaching the MII stage was significantly (P<0.05) higher in hMG and U-FSH treated mice than rFSH treated one. Similarly, U-hMG was superior than U-FSH [12] or rFSH [8] for induction of superovulation in mice. The FSH in the HP-hMG preparation

has a longer half-life than the FSH in the rFSH preparation [8]. In contrast, [9] recorded that rFSH was superior than HP-hMG for induction of superovulation in mice. Also, in the human superovulation protocol, more oocytes were retrieved after rFSH treatment compared with HP-hMG treatment [13, 14, 15]. This discrepancy could be attributed to species difference or due to the batch and dose of gonadotropin used. Moreover, the percentages of *in vivo* matured oocytes reaching the MII stage were significantly (P<0.05) higher for groups of mice treated with hMG or U-FSH when compared with rFSH treated group. Concomitant to this result, reported that HP-hMG was found to produced higher synchronous nuclear maturation rate than rFSH [1, 13, 16]. and this was attributed to the higher Tgfb1 mRNA and protein levels, fewer COCs with an increased collagen expression **[1]**. In contrast, **[15]** found no difference in maturation rate of oocytes retrieved from women super ovulated with hMG or rFSH.

Furthermore, in the current work, induction of superovulation in mice using HP-hMG and U-FSH produced significantly (P<0.05) higher cleavage rate than group treated with rFSH. Also, higher (P<0.05) percentage of matured oocytes recovered from animals treated with HP-hMG were able to develop to the blastocyst stage when compared with group of mice super ovulated with U-FSH or rFSH. *In vitro* fertilized oocytes recovered from rFSH group were arrested at the 2-cell stage embryo. These results are concomitant with that previous studies in which fertilization rate and embryo development were better in patients super ovulated with HP-hMG than rFSH. **[17]**. On the other hand, no superiority in terms of *In vitro* fertilization, embryo developmental or pregnancy rates for patients undergoing super stimulation programs using HP-hMG or rFSH [15]. This difference could be due to species difference, dose or source of gonadotropin used.

5. Conclusion

HP-hMG is superior Than U-FSH or rFSH for the induction of superovulation in BALB/c mice. This was reflected by the higher number of recovered mature oocytes and their ability to fertilized and develop to the blastocyst stage *In vitro*.

6. Ethical approval

This study was reviewed and approved by the Ethical Committee of Faculty of Science, Al-Azhar University, and the Ethical Committee of Nuclear Materials Authority.

7. References

1. Adriaenssens T, Mazoyer C, Segers I, Wathlet S, Smitz J. Differences in collagen expression in cumulus cells after exposure to highly purified menotropin or recombinant follicle-stimulating hormone in a mouse follicle culture model. Biology of Reproduction. 2009; 80:1015-1025.
2. Brannstrom M, Wang L, Norman RJ. Effects of cytokines on prostaglandin production and steroidogenesis of incubated preovulatory follicles of the rat. Biology of Reproduction. 1993; 48:165-171.
3. Fakhrildin B, Abdul-Majeed R, Sulaiman K. Effect of Different Superovulation Programs and Culture Media on Quality, *In vitro* Maturation and Fertilization of Mice Oocytes. Pure Sciences, 2006, 33.
4. Luo C, Zuñiga J, Edison E, Palla S, Dong W, Parker-Thornburg J. Superovulation strategies for 6 commonly used mouse strains. Journal of the American Association for Laboratory Animal Science. 2011; 50:471-478.
5. Roberts R, Iatropoulou A, Ciantar D, Stark J, Becker DL, Franks S, Hardy K. Follicle-stimulating hormone affects metaphase I chromosome alignment and increases aneuploidy in mouse oocytes matured *In vitro*. Biology of Reproduction. 2005; 72:107-118.
6. Ozgunen KT, Erdogan S, Mazmanoglu N, Pamuk I, Logoglu G, Ozgunen T. Effect of gonadotrophin dose on oocyte retrieval in superovulated BALB/c mice. Theriogenology. 2001; 56:435-445.
7. Ganesh A, Chattopadhyay R, Narendra Babu K, Chakravarty B, Chaudhury K. Analysis of spindle characteristics and embryo quality in mice stimulated with letrozole using Polscope imaging. Fertility and Sterility. 2010; 93:1477-1481.
8. Smitz J, Andersen AN, Devroey P, Arce JC. Endocrine profile in serum effect on CC gene expression 1023 and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients. Human Reproduction. 2007; 22:676-687.
9. Ziebe S, Lundin K, Janssens R, Helmgaard L, Arce JC. Influence of ovarian stimulation with HP-hMG or recombinant FSH on embryo quality parameters in patients undergoing IVF. Human Reproduction. 2007; 22:2404-2413.
10. Vergara GJ, Irwin MH, Moffatt RJ, Pinkert CA. *In vitro* fertilization in mice: Strain differences in response to superovulation protocols and effect of cumulus cell removal. Theriogenology.1997; 47:1245-1252.
11. Ozturk S, Yaba-Ucar A, Sozen B, Mutlu D, Demir N. Superovulation alters embryonic poly(A)-binding protein (Epab) and poly(A)-binding protein, cytoplasmic 1 (Pabpc1) gene expression in mouse oocytes and early embryos. Reproduction, Fertility and Development. 2016; 28:375-383.
12. Edirisinghe WR, Law HY, Ng SC, Chia CM, Ratnam SS. Superovulation of Mice with Human Menopausal Gonadotropin or Pure Follicle Stimulating Hormone in Combination with Human Chorionic Gonadotropin and the Effects of Oocyte Aging on *In vitro* Fertilization. Journal of *In vitro* Fertilization and Embryo Transfer. 1986; 3:314-318.
13. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Human Reproduction. 2006; 21:3217-27.
14. Celik C, Sofuoğlu K, Selçuk S, Asoğlu MR, Abalı R, Cetingöz E, Baykal B, Uludoğan M. Comparison of ovulation induction and pregnancy outcomes in IVF patients with normal ovarian reserve who underwent long protocol with recombinant-FSH and highly purified-hMG. Journal of the Turkish-German Gynacological Association. 2011; 12:15-20.
15. Khlifi A, Kacem O, Maroueni M, Elgoul L, Hidar S, Fekih M, *et al*. The influence of gonadotropins on clinico-biological ICSI outcome: a retrospective comparative

study rFsH vs HP-hmg. La Tunisie médicale. 2016; 94:167-177.
16. Nyboe A, Devroey P, Arce JC. (Menotrophin vs Recombinant FSH *In vitro* Fertilisation Trial) Group Clinical outcome following stimulation with HP-hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Human Reproduction, 2006.
17. Platteau P, Nyboe Andersen A, Loft A, Smitz J, Danglas P, Devroey P. Highly purified HMG versus recombinant FSH for ovarian stimulation in IVF cycles. Reproductive Biomedicine Online. 2008; 17:190-918.