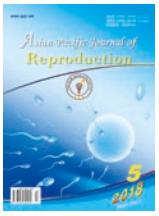

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Human chorionic gonadotropin *in vitro*: Effects on rat sperm motility and fertilization outcome

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ABSTRACT

Objective: To investigate the effect of human chorionic gonadotropin (hCG) on the motility of rat sperm and the fertilization rate following *in vitro* fertilization. **Methods:** hCG concentrations of 25, 50, 75 and 100 ng/mL with incubation time of 1 h and 2 h were used in medium to study sperm motility. Then, 25 and 100 ng/mL of hCG with incubation time of 2 h were applied for *in vitro* fertilization. After 6 h, the number of two pronuclei was counted. Obtained data was subjected to one way ANOVA and Bonferroni-Dun *hoc-post* test. **Results:** Total motility and progressive motility of sperms were increased significantly (P<0.05) in hCG experimental group with concentration of 25 ng/L and incubation time of 2 h compared to control group, however, total motility and progressive motility of sperms were decreased significantly (P<0.05) with increasing dose of hCG. Fertilization rate was decreased significantly (P<0.05) in hCG experimental group with dose of 100 ng/mL compared to 25 ng/mL and control group. **Conclusions:** The effect of hCG on the rat sperm motility and the rate of their fertility is dose-dependent so that hCG with dose of 25 ng/mL leads to an increase in rat sperm motility and to some extent fertilization rate.

1. Introduction

Human chorionic gonadotropin (hCG) is synthesized by syncytiotrophoblast cells of the placenta. hCG hormone is homologue of luteinizing hormone (LH) that is primarily produced by anterior part of pituitary gland. Both hormones bind to the same receptor called LH/choriogonadotropin receptor[1]. hCG is a glycoprotein hormone that consists of two α and β chains. α chain of hCG is similar to three glycoprotein hormones of thyroid-stimulating hormone, LH, and follicle-stimulating hormone while β chain is totally different[2,3]. LH/choriogonadotropin receptors are located on the luteal cells, placenta, endometrium, spiral arteries of the uterus myometrium[4,5] and on the sperms of rat and human[6].

In vitro fertilization (IVF) is a kind of assisted reproductive technology. Success of IVF depends on many factors such as quality of gametes, age of infertile women, the number of oocytes and embryo quality[7]. Sperm parameters are important factors for successful IVF as they are directly associated to embryo

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implantation and normal embryogenesis[8]. After ejaculation, sperms are bound to the ciliated epithelial cells in the isthmus of fallopian tube and they are exposed to secretions of epithelial cells in order to get hyperactivated and capacitated that it is so crucial for fertilization. Following ovulation, the sperms are detached from the epithelial cells and begin to move toward the released oocyte in ampulla for fertilization, so it seems that secretions of fallopian tubal cells are necessary for sperm hyperactivated motility and subsequent fertilization[9]. hCG is secreted by both endometrial cells and fallopian tubal cells that may be important for sperm motility and subsequent fertilization[10] while in IVF process, the sperms do not pass and attach to fallopian tubal cells, therefore they are deprived of secretions of fallopian tube that is necessary for ultimate capacitation.

Since hCG is secreted by fallopian epithelial cells and hCG may play a positive role on the hyperactivated motility of sperms, the present study was designed to investigate whether addition of hCG into T6 medium is able to affect sperm motility and rate of fertilization in rat as an animal model.

2. Materials and methods

All reagents were obtained from Sigma-Aldrich Company (St. Louis, USA) unless otherwise stated. All procedures related to care and use of animals were approved by Ethics committee of Zanjan University of Medical Sciences (ethics code: ZUMS.REC.1394.32). Fertile male wistar rats and female wistar rats of 6-8 weeks old were housed in separate cage for 12 days under conditions of natural day-length and temperature and they had free access to standard diet plate and tap water. For semen analysis, male rats were divided into four groups of five each. Rats were euthanized with a mixture of 75 mg/kg ketamin and 10 mg/kg xylazine, both intraperitoneally (*i.p.*). Caudal epididymides were dissected out and minced; epididymis of the right side was served as experiment and the one on the left side was served as control.

There were four experimental groups and four control groups. For first experimental group, one epididymis of one rat was placed into a tube containing T6 medium (473 mg NaCl, 100 mg KCl, 5 mg NaH_2PO_4, 10 mg $MgCl_2$ $6H_2O$, 26 mg $CaCl_2$ $6H_2O$, 210 mg $NaHCO_3$, 200 μL Na-lactate 100%, 3 mg Na-pyrovate, 100 mg glucose, 6 mg penicilline, 5 mg streptomycine, 1 mg phenol red and 0.6 mg ethylene diamine tetraacetic acid to make 100 mL T6 medium) plus 10% bovine serum albumin (BSA10) plus 25 ng/mL hCG (T6+BSA10+25 ng/mL hCG). For the other experimental groups, one epididymis was placed into a tube with the same T6 and BSA but plus 50 ng/mL hCG (T6+BSA10+50 ng/mL hCG), 75 ng/mL hCG (T6+BSA10+75 ng/mL hCG) and 100 ng/mL hCG (T6+BSA10+100 ng/mL hCG), respectively. For control groups, epidydimis of the other side of each rat placed into a tube containing T6 plus BSA but without hCG (T6+BSA10+0 ng/mL hCG). All tubes were placed in 5% CO_2 incubator (New Brunswick, Galaxy 170 S) at 37 ℃ for 2 h and then sperm motility was checked after 1 and 2 h. All groups of experiments and controls were repeated three times.

After 1 h and 2 h of sperm incubation in the presence or absence of increasing doses of hCG, 10 μL of sperms were collected from the surface of the sperm suspension, then it was placed on a slide glass at 37 ℃. Randomly, five fields from each slide were film-recorded with a video microscope (Olympus, BX51, Germany) at × 200 magnification. Recorded films were analysed to assess sperm motility with counting progressive (movement of sperms in a straight line or in very large circles), non-progressive (movement of sperms as shaking or in very tight circles) and immotile sperms.

Twenty five female rats were received 20 IU pregnant mare serum gonadotropin (Folligon, Intervet, Netherlands, HOR-272) as *i.p.* injection (at 12:00 am), followed by an *i.p.* injection of 20 IU hCG (Bioscience, GmbH, Germany) 48 h later. Next morning following hCG injection (at 8:00 am), superovulated rats were sacrificed by ketamine and xylazine injection and subsequent cervical dislocation and oviducts were bilaterally dissected and placed in T6 medium at 37 ℃. Cumulus-oocyte complexes (COCs) were released from oviducts by tearing ampulla under a stereomicroscope (Motic, SMZ-168, Hong Kong). The collected COCs were washed in T6 and then pipetted to be separated from each other. The COCs with fully expanded cumulus and an evenly granulated cytoplasm were considered for IVF. 75 COCs were used in each group. The number of morphologically normal COCs was counted and then cultured in 50 μL/drop of T6 overlaid by mineral oil and supplemented by 15% BSA. Sperms (1×10^6 per mL) in T6 in the presence of hCG (25 ng/mL or 100 ng/mL) or in the absence of hCG which incubated for 2 h were added to drops containing COCs (3 COCs/drop). Then, COCs and sperms were incubated in 5% CO_2, at 37 ℃. After 6 h incubation, the COCs were washed and the formation of two pronuclei and the second polar body were examined under an invert microscope (Nikon, eclipse Ts2, Japan). IVF was performed in three replicates for both experimental and control groups.

Statistical analysis was performed using SPSS software (Version 22, IBM, Chicago, USA). The mean values were used for statistical analysis. To assess significance of difference within group and between groups, normally distributed data were subjected to one way ANOVA and Bonferroni-Dun *post-hoc* test, respectively. Data were expressed as mean ± standard error (mean ± SE). Differences were considered to be significant if $P<0.05$.

3. Results

Alterations of the total motility (progressive and non-progressive motility) and progressive motility of sperms showed a similar trend

between groups. In experimental group with 25 ng/mL of hCG and 2 h incubation, the total motility and progressive motility of sperms were increased significantly (P<0.05) compared to their controls while there was not any significant changes between groups with 1 h incubation time (Figure 1A).

There were not any significant (P>0.05) changes in the total motility and progressive motility of sperms treated with 50 ng/mL of hCG in both incubation time interval when experiment groups compared to their control groups (Figure 1B).

Motility changes in sperms treated with 75 ng/mL and 100 ng/mL of hCG in both incubation time interval showed a similar trend so that the total motility and progressive motility were decreased significantly (P<0.05) in experimental groups after 2 h in comparison with their control groups (Figure 1C and 1D). Also, by increasing incubation time from 1 h to 2 h, the total motility and progressive motility of sperms in these experimental groups were significantly (P<0.05) decreased (Figure 1C and 1D).

By increasing time of incubation, the percentage of non-progressive and immotile sperms was decreased significantly (P<0.05) in sperms treated with 25 ng/mL of hCG while it was increased significantly (P<0.05) in higher doses of hCG (75 and 100 ng/mL).

The fertilization rate (percentage of male and female pronuclei) in sperms treated with 100 ng/mL of hCG was decreased significantly (P<0.05) in comparison with sperms treated with 25 ng/mL of hCG and control group (Figure 2). The fertilization rate tended to be increased in sperms treated with 25 ng/mL of hCG compared to control group (Figure 2).

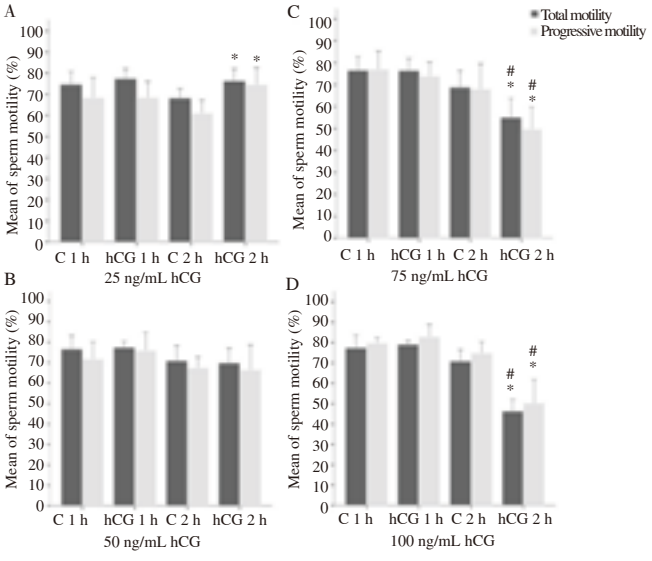

Figure 1. Total motility and progressive motility of sperms in control group and experimental groups treated with hCG in doses of 25 ng/mL (A), 50 ng/mL(B), 75 ng/mL (C) and 100 ng/mL (D).
*Significant difference (P<0.05) compared to control group with 2 h incubation time. # Significant difference (P<0.05) compared to experimental group with 1 h incubation time. C in C 1 h, C 2 h refers to control group.

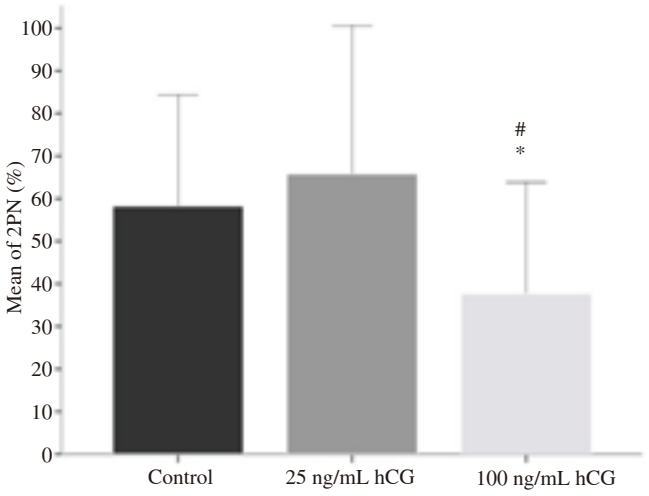

Figure 2. Percentage of two pronuclei formation.
*Significant difference (P<0.05) compared to control group. #Significant difference (P<0.05) compared to experimental group with 25 ng/mL hCG. 2PN: 2 pronuclei.

4. Discussion

Fallopian secreted factors are so crucial for sperm capacitation, however, in IVF the sperms are not exposed to fallopian secretions including hCG that may impact sperm motility and consequence fertilization rate. We, therefore, worked on the *in vitro* effects of hCG on the sperm motility and ability of sperms to fertilize oocytes in rat.

In present study, data showed that hCG in dose of 25 ng/mL and with incubation time of 2 h is able to increase sperm motility and to some extent fertilization rate compared to other doses of hCG and control group. hCG exerts its function through G protein-coupled receptor presence on the sperm membrane and it may affect on sperm motility via elevation of cyclic adenosine monophosphate (cAMP) in sperms. Indeed, hCG can stimulate adenylate cyclase (AC) and in consequence cAMP elevation in sperm. cAMP can activate phosphokinase A (PKA), leading to phosphorylation and activation of elements involving in sperm motility and capacitation[11]. One of the essential factors in sperm motility is polymerization of F actin that can increase by tyrosine phosphorylation following PKA activation[12]. Therefore, hCG in dose of 25 ng/mL is able to create an intracellular cascade signals resulting in activation of AC/cAMP/PKA pathway and finally improvement of sperm motility.

Fallopian epithelial cells and uterine glands are sources of hCG in female genital tract[13-15]. For sperm capacitation, ejaculated sperms bind to ciliated epithelial cells of fallopian tube and are exposed to the fallopian secretions[9]. On the other hand, in IVF, sperms are deprived of fallopian secretions including hCG, however, as shown in our study, hCG with dose of 25 ng/mL could increase sperm motility, therefore optimization of sperm medium with addition of hCG may enhance sperm motility and fertility ability in IVF.

Also, we found that with increasing incubation time to 2 h in dose of 25 ng/mL, the sperm motility can be improved. The reason may be that the level of cAMP in sperms rises with increasing incubation

time[6] that may result in improving sperm motility. hCG in dose of 25 ng/mL with 2 h incubation time was not able to improve the rate of fertilization, although it was slightly increased when compared to control group. It may be because fertilization is a complex process involving many molecules and signals[16], therefore many factors and mechanisms should be considered to improve fertilization rate rather than only one factor such as hCG.

Although hCG in dose of 25 ng/mL improved sperm motility, hCG in doses of 75 and 100 ng/mL had adverse effects on both sperm motility and fertilization rate. In other words, sperm motility and fertilization rate decreases with increasing dose of hCG. A decline in synthesis of hCG receptors or a decrease in sensitization of hCG receptors and/or AC could be occurred through negative feedback mechanism when there is high dose of hCG[17-22]. Also, high concentration of hCG results in increasing synthesis of receptor binding protein and consequent activation of endonucleases, leading to degradation of mRNA relevant to hCG receptor and ultimate downregulation of hCG receptors on the cell membrane[11]. Despite our finding implying that hCG in dose of 100 ng/mL could not improve sperm motility and sperm ability to fertilize oocytes, Eblen *et al* found that treatment of human sperms with 100 ng/mL hCG for 2 h is able to enhance cAMP production and subsequent PKA activation, however, they did not study human sperm motility and fertility ability treated with 100 ng/mL hCG[6].

hCG was applied *in vitro* for the first time to improve rat sperm motility and fertilization rate that could be used to optimize medium of sperms in fertility clinics. We found that the effect of hCG on sperm motility is dose-dependent so that 25 ng/mL hCG could improve sperm motility while 100 ng/mL hCG had adverse effects. However, as we did not get convincing data on the effect of hCG on fertilization rate, further research is required to find out its complexities and underlying mechanisms.

Conflict of interest statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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