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EFFECT OF GONADOTROPINS ADDITION TO SMART MEDIUM ON HUMAN SPERM PARAMETERS AND CHROMATIN STRUCTURE DURING *IN VITRO* SPERM ACTIVATION

[a]Rasha M. Mohammed-Ali, [a]Sabah N. Alwachi* & [b]Muhammad-Baqir M-R Fakhrildin
[a]Department of Biology, College of Science, University of Baghdad.
[b]Department of Clinical Reproductive Physiology, High Institute of Infertility Diagnosis and ART.

ABSTRACT
A study was conducted to investigate the effect Gonadotropins supplied to culture medium on human sperm parameters and sperm DNA structure during *in vitro* sperm activation. Thirty infertile patients were included. Semen sample was divided into 3 aliquots. One mL of SMART medium either alone (control group) or supplied with one concentration of Gn (0.25 IU or 0.5 IU) was over layered the pellet, and the three tubes were incubated at 37 ^{o}C for 30 min in air incubator. Sperm concentration, motility, grades activity, progressive motility, normal morphology and agglutination were assessed pre- and post-activation *in vitro*. Results of the present study revealed that, percentage, sperm motility, progressive motility, normal sperm morphology and sperm DNA fragmentation index (DFI) were significantly enhanced ($P<0.05$) for both treated groups as compared to the control group post- sperm activation *in vitro.* Using 0.5IU hormones Gn within SMART medium (G3) was significantly improved ($P<0.05$) sperm parameters post- sperm activation *in vitro* as compared to G2 group (0.25IU hormones). Post-sperm activation in vitro significant increment ($P<0.05$) in the sperm DNA fragmentation index (DFI) was assessed when using SMART medium enrich with 0.5IU hormones as compared to 0.25IU. The result showed that addition of hormones (GN) to the culture medium enhance sperm motility (%), percentage of progressive sperm activity and normal sperm morphology (%) using centrifugation techniques.

KEYWORDS: Gonadotropins human sperm parameters sperm DNA structure

INTRODUCTION

The general definition of infertility is a lesser capacity to conceive than the mean capacity of the general population (ESHRE, 2000). Primary infertility is the term used in reproductive medicine for a couple who failed to achieve a pregnancy for one year of marriage and who was never pregnant before, while secondary infertility is the term applied to couple who meet criteria for primary infertility but at some time in the past have been pregnant (Lunenfeld and Steirteghem, 2004). Male infertility patient are often classified as oligozoospermic, asthenozoospermic, or teratozoospermic on the basis of concentration, motility, and morphology or any of these combination (Agarwal *et al*., 2003). Semen analysis is the first tool a medical practitioner uses to assess the male factor in an infertility workup (WHO, 1999; Agarwal and Sharma, 2007). Semen analysis is routinely used to predict fertility, the standard measurements of sperm concentration, percentage motility and morphology may not reveal sperm defects affecting the integrity of the male genome. It is clear that abnormalities in the male genome characterized by damaged Deoxy ribonucleic acid (DNA) may be indication of male subfertility regardless of the routine semen parameters (Aitken and Krausz, 2001). The synthesis and secretion of the gonadotropic hormones involves coordination of signal transduction, gene expression, protein translation, post translation folding and modification and finally secretion (Bousfield and Dias, 2011). Luteinizing hormone (LH) stimulates the interstitial cells of the testes to secrete testosterone which, in turn, acts as an inhibitor of LRF release and therefore of further LH release. Follicle stimulating hormone (FSH) primarily stimulates the growth and development of spermatogenic tissue which in turn is thought to secrete an unknown factor which inhibits FSH release (Odedl and Swerdloff, 1968). For all of that the objective of this study areTo investigate the effect of (Gn) supplied to culture medium on sperm parameters during *in vitro* activation and To investigate the effect of (Gn) enriched to culture medium on sperm DNA structure during *in vitro* activation.

MATERIALS & METHODS

This study includes thirty infertile males with age mean (35.25±1.93) year with history of infertility mean was (6.3±0.71) years. Sperm parameters were assessed involving concentration, motility, and normal morphology, according to the latest issue for WHO standard criteria (WHO, 1999). Acridine orange test was functioned to assess spermatozoa with fragmented DNA as described by Tejada *et al*. Briefly, after washing with Tyrode's solution composed of a small amount of warm distilled water added to 0.24g/L (Mg Cl2),followed by adding all the components (NaCl 7.054g/L, KCl 0.439g/L, CaCl2.2H2O 0.24g/L, NaH2PO4.2H2O 0.187, NaHCo3 1.302g/L) together, then, the volume was completed to (1L) and pH was adjusted to 7.3,medium-thick smears on cleaned slides were air dried, fixed overnight in freshly prepared Carnoy'ssolution (3 parts methanol/1 part glacial acetic acid), air dried again and stained with acid AO (Sigma, Deisenhofen, Germany) solution. All slides were read the

same day on a fluorescencemicroscope. Sperm heads were subdivided into those showing a green color and those with colors ranged from yellow to red as recommended. A total of 300 cells were counted on each slide. The ratio of colors other than green/ colors other than green + green yields the percentage of DNA fragmentation, referred to DNA fragmentation index (DFI%).

Statistical analysis

The data were statistically analyzed using SPSS/PC software (version 18) (SPSS, Chicago).Sperm parameters were analyzed using complete randomized design (CRD) of one way (ANOVA). The mathematical model was

$Yij = \mu + Ti + eij.$

Where

Yij= dependent variables (sperm parameters).

μ= overall mean.

Ti= effect of treatments with the gonadotropine hormones.

eij= error term.

Differences among means were computed using the Duncan multiple ranges test (Duncan, 1955).

RESULTS

In the third study, SMART medium was used and enriched with low concentration 0.25 IU and high concentration 0.5 IU of gonadotropins (both LH and FSH). This study involved 30 semen samples for infertile males with the mean age of (35.25±1.93) years with range (22-54) years. The mean duration of infertility was (6.3±0.71) years with range of (2-14) years. The results of sperm concentration using ISACT were presented in the figure (1). Significant difference (P<0.05) was assessed in the sperm concentration between pre-activation group and post-activation group. In contrast, non significant differences (P>0.05) were noticed in the sperm concentration among all control and both treated groups post- activation. Table (1) shows the percentages of sperm motility and sperm grade activity. Significant differences (P<0.05) were observed in the percentages of sperm motility and non progressive motility between pre-activation group and post-activation groups. While, non significant differences (P>0.05) were assessed among post- activation groups. However, the group of high gonadotropins concentration showed the highest percentages for both parameters when compared to other two groups of post-activation.For progressive sperm motility (%), a significant differences (P<0.05) were assessed between pre-activation group and both treated groups post-activation. However, non significant differences (P>0.05) were appeared among control and treated groups. Also, non significant differences (P>0.05) were observed between pre-activation group and control group post-activation (Table 3-3). From the same table, immotile sperm (%) showed a significant differences (P<0.05) between pre-activation group and post-activation groups. While, non significant differences (P>0.05) were assessed among all treated groups. The percentages of normal sperm morphology were presented in figure (2). Significant differences (P<0.05) were appeared between pre-activation group and post-activation groups. On the other hand, non significant differences (P>0.05) were observed among control and treated groups post activation. However, high concentration group has the best percentage for normal sperm morphology as compared to other groups post-activation. Figure (3) shows effect of gonadotropins supplement within SMART medium on sperm DNA fragmentation. Significant differences (P<0.05) were observed between pre-activation group and post-activation groups. Whereas, non significant differences (P<0.05) were noticed between low gonadotropins concentration and both control and groups with high gonadotropins concentration group. On the other hand, high gonadotropins concentration group has the best percentage for sperm DNA fragmentation when compared to the other groups of pre- and post-activation. Image (1) shows sperms displaying green, yellow, and orange as fragmentation DNA of sperms heads.

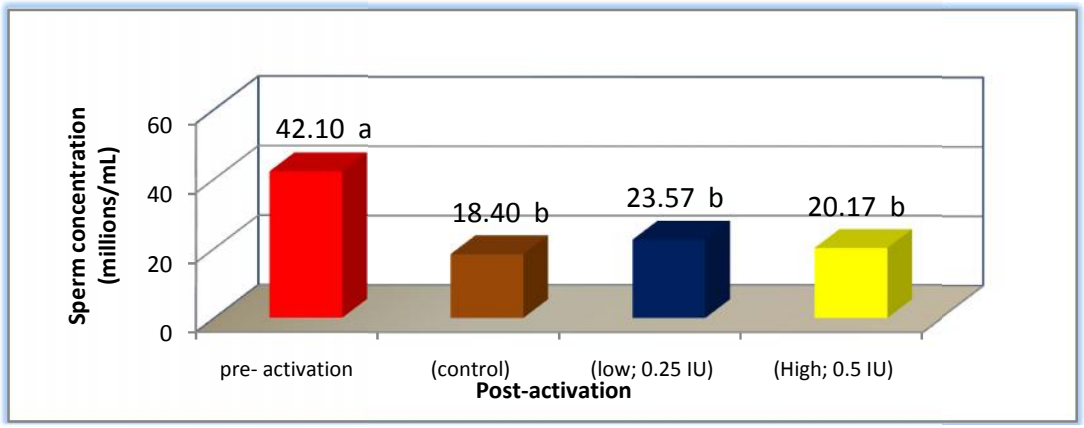

FIGURE 1: sperm concentration pre- and post- activation using SMART medium enriched with two concentrations of gonadotropins.

- Number of infertile patients =30
- Means with different superscripts within each columns are significantly different (P<0.05).
- Means with similar superscripts within each columns are non significantly different (P>0.05).
- Data are the means ±SEM.

TABLE 1: Percentage of sperm motility pre- and post-activation using SMART medium enriched with two concentrations of gonadotropins

Sperm Motility Parameters		Pre- treat. group	Post- activation groups		
			G1:Control	G2:Low concentration	G3:High concentration
Sperm motility (%)		58.46 ±1.90b	79.16 ±3.78a	82.53±3.97a	84.23±3.71a
Sperm grade activity (%).	Progressive sperm motility	29.66 ±1.67b	34.76±3.58ab	39.90±4.80a	40.73±4.63a
	Non progressive sperm motility	28.80 ±1.24b	44.06 ±3.46a	42.80±3.60a	43.46±3.56a
	Immotile sperm	41.70 ±1.92a	20.50 ±3.64b	17.46±3.97b	15.76±3.71b

- Number of infertile patients =30
- Means with different superscripts within each columns are significantly different (P<0.05).
- Means with similar superscripts within each columns are non significantly different (P>0.05).
- Data are the means ±SEM.

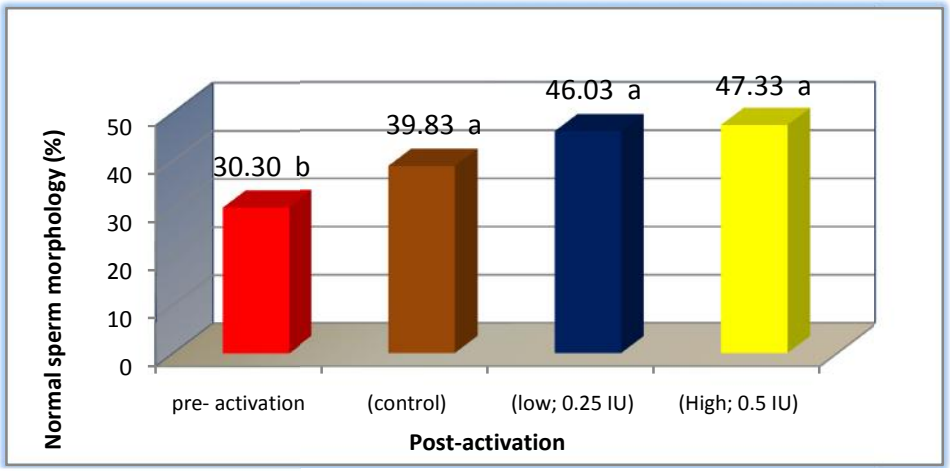

FIGURE 2: Percentage of sperm morphology pre- and post- activation using SMART medium enriched with two concentrations of gonadotropins.

- Number of infertile patients =30
- Means with different superscripts within each columns are significantly different (P<0.05).
- Means with similar superscripts within each columns are non significantly different (P>0.05).
- Data are the means ±SEM.

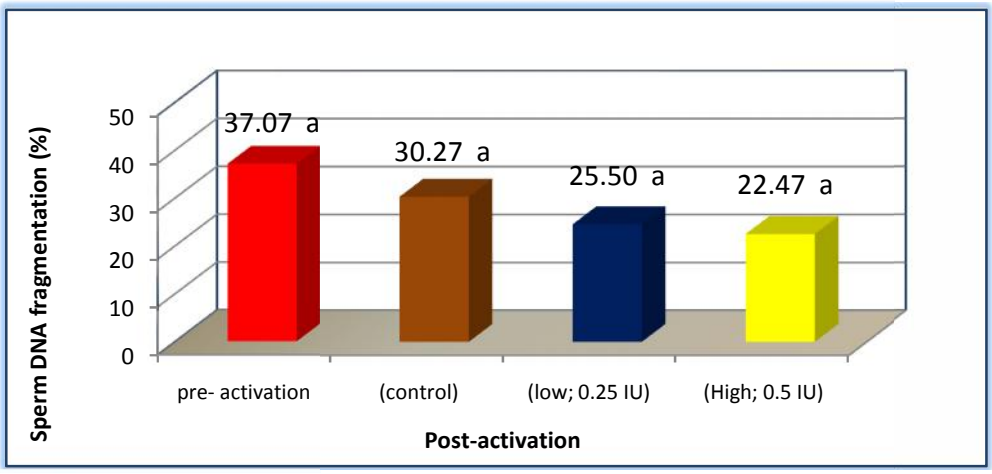

FIGURE 3: Percentage of sperm DNA fragmentation pre- and post- activation using SMART medium enriched with two concentrations of gonadotropins.

- Number of infertile patients =30
- Means with different superscripts within each columns are significantly different (P<0.05)
- Means with similar superscripts within each columns are non significantly different (P>0.05).
- Data are the means ±SEM.

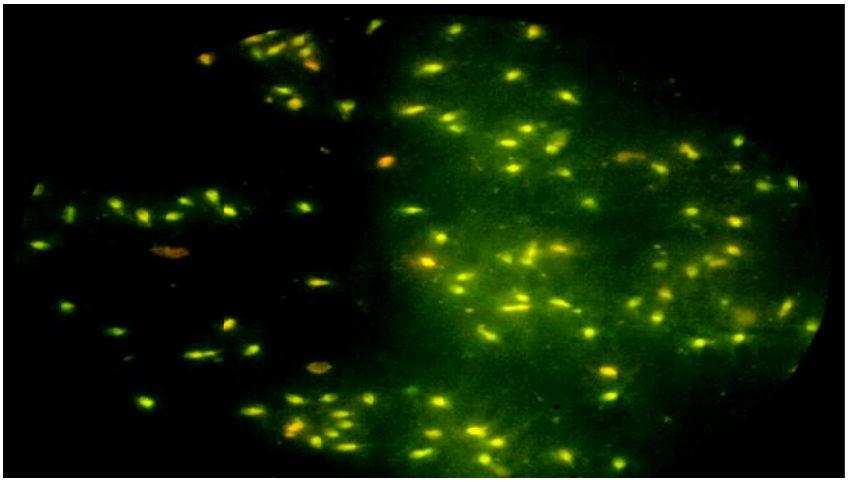

IMAGE 1: Sperms head under (x40) HPF displaying intact and abnormal fragmentation DNA of human sperm

DISCUSSION

In vitro sperm activation is very important step in the laboratory technique that plays an important role in determination the outcome of assisted reproductive technologies like IUI. In addition, the sperm preparation technique used culture media and dependent according to properties of the semen parameters (Vande vort, 2004). In the present study, sperm centrifugation was selected as a method for *in vitro* sperm activation depending of the results of the Shaaban (2007) for different purposes involving removal of effects for morphologically abnormal spermatozoa, immature sperm cells, epithelial cells, and lastly seminal leukocytes. Consequently, ROS and sperm damage are reduced. For the same period, is to collect normal morphologically normal spermatozoa with normal sperm physiology (Shajer, 2013). From the results of this study, sperm concentration for all groups post-activation was significantly reduced ($P<0.05$) compared to pre-activation groups. Similar results were presented by Shaaban (2007). As mentioned previously, centrifugation swim-up technique was used in the present work to remove most sperm with low motility and immotile sperm, with abnormal morphology, and agglutinated spermatozoa (Harrison, 1976). In addition to remove round cells and epithelial cell (WHO, 1999), and reduced bacterial infection, these results here considered normal during *in vitro* activation of human spermatozoa (Harrison, 1976). In the current study, sperm motility (%) and progressive sperm motilty (%) were significantly increased for Gn treated groups post-activation as compared to the control group and pre-activation. Really, enhancement sperm parameters may be considered as normal response for sperm physiology after the removal of seminal plasma, pus cells and agglutinated spermatozoa using sperm preparation techniques. Furthermore, it was reported that only the active motile sperms will swim-up to the upper layer of culture medium *in vitro* human sperm activation (Mortimer, 2000; Henkel and Schill, 2003). The results were obtained using a high concentration of the gonadotropin hormone significantly enhanced in the progressive motility of sperm compared with the results obtained use low concentration. Perhaps the reason for that, the high concentration of hormone which help to move the sperm higher concentration of hormones moves sperm that seemed quite immotile sperm when microscopic examination exceeded the progressive motility of sperm. The low concentration for each of the used hormones can affect the movement speed of motile sperms without having little impact in moving the immotile sperm, when the post-activation groups were compared to per-activation groups; the normal sperm morphology was significantly enhanced for the three groups of post-activation. However, high concentration of Gn groups produces the best improvement for sperm morphology (%) as compared to other groups post-activation. In the present study, sperm activation was done using centrifugation technique which lead to precipitate most cells, pus cells and debris within seminal plasma (WHO, 1999). Consequently, most spermatozoa with normal morphology and progressive motility swim-up into upper layer of culture medium post-activation (Ismail and Al-Zaidi, 1998). Therefore, this procedure will select live motile sperm with mature condense chromatin. Since, there is correlation between sperm condensation and morphology, thus this procedure, also selects sperm with normal morphology (Henkel *et al.*,1994).In the present study, the fragmentation of sperm DNA was considered an important procedure for male fertility and infertility diagnosis (Bungum, 2011). SCSA, first described by Evenson *et al.*(1980) is show to be an independent marker of fertility *in vivo* and may also help in selection of the most effective ART treatment in each in individual couple (Bungum *et al.*, 2007). Moreover, poor semen quality has been associated with an increase in the proportion of sperm with DNA fragmentation (Irvine *et al*., 2000).

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