

Effect of Additive Tamoxifen Citrate to SMART Medium on Human Sperm Parameters during *In vitro* Sperm Activation

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Abstract: This study was aimed to investigate the effect of addition of tamoxifen citrate on the outcome of *in vitro* human sperm activation. One hundred semen samples for fertile and infertile men were randomly collected from the subjects at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University. The mean age was (32.21 ± 0.83) years and mean duration of infertility was (5.26 ± 0.41) years. Before using centrifugation swim-up technique, the washed samples were divided into three groups: control group (G1) without tamoxifen citrate, while in the second(G2) and third(G3) group, two concentrations of tamoxifen citrate ($20\mu\text{g/mL}$ and $40\mu\text{g/mL}$) respectively were added post-activation enriched with SMART medium. Macroscopic examination of semen included: Semen liquefaction time, semen pH, semen viscosity and semen volume, while microscopic examination of semen included: sperm concentration, motility, morphology, agglutination and cells other than spermatozoa. The results showed significant ($P < 0.05$) increased in sperm motility, progressive sperm motility and normal sperm morphology of post-activation as compared with pre-activation. Among treated groups, a significant ($P < 0.05$) decreased was found in sperm concentration, immotile sperm and total progressive sperm, while a significant ($P < 0.05$) increased was observed in progressive sperm motility and normal sperm morphology in (G3) as compared with others groups. Based on the results of this study, it can be concluded that the addition of $40\mu\text{g/mL}$ of tamoxifen citrate to washed sperms can improve sperm motility.

Keywords: Tamoxifen citrate, SMART medium, *in vitro* sperm activation.

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I. Introduction

Infertility is a lesser capacity of a couple to achieve a pregnancy after one year of regular and unprotected sexual intercourse [1]. Meanwhile, most men seen for infertility have either normal or reduced sperm counts, motility or morphology alone or in combination [2]. Male infertile patients are often classified as oligozoospermic, asthenozoospermic, or teratozoospermic on the basis of concentration, motility, and morphology or any of this combination [3]. Infertility may be managed by *in vitro* sperm activation (ISA) using different types of stimulators. Basically, the culture medium used for Assisted Reproductive Technologies (ARTs) are modification of balanced salt solution, and it is apparent that spermatozoa of mammalian species including human can acquire the ability to fertilize after a short incubation in defined culture media [4]. Tamoxifen citrate is a non-steroidal compound with antioxidant action and antiestrogenic action on the mammary tissue but estrogenic action on plasma lipids, endometrium and bone. In man, it acts primarily as antiestrogens, increasing the effects of androgen, probably by binding with estrogen receptors [5]. Antiestrogens have been one of the oldest and most commonly prescribed forms of therapy to inhibit the negative feedback effect of estrogen on the hypothalamus and pituitary, increasing endogenous gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion directly from the pituitary, increasing their levels, there by stimulating spermatogenesis when administrated orally. Antiestrogen stimulates the proliferation of the spermatogonia and formation of the primary spermatocytes, while androgens are involved in stimulating the meiosis division of the primary spermatocyte and their final conversion into the spermatids [6]. Tamoxifen citrate was introduced three decades ago as an empiric treatment for idiopathic oligozoospermia because of its stimulatory action on gonadotropin secretion, and its postulated direct effect on Leydig cell function, and 5α -dihydrotestosterone production in seminiferous tubules and epididymis. The overall effect of tamoxifen on spermatogenesis is stimulatory, resulting in two folded increases in spermatozoa concentration but no marked change in motility and morphology [7].

II. Materials And Methods

Subjects and semen samples collection

One hundred subjects were involved in this study during their attendance at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al- Nahrain University. The mean age was (32.21± 0.83) years old with the range (20-53) years. Semen samples were collected after 3-5 days of sexually abstinence by masturbation directly into a clean, dry and sterile disposable Petri-dish and the required information was recorded for all subjects assessed in this study, the specimens were incubated at 37°C for 30 minutes to allow liquefaction [8]. Semen analysis was performed before activation according to WHO criteria [1] and each sample was divided into three groups, after being centrifuged once with 2500 rpm for 6 minutes. The supernatant was discarded and then gently (1mL) of SMART medium was added only for the first group (G1), (1mL) of SMART + (20µg/mL) of tamoxifen citrate for the second group (G2) and (1mL) of SMART + (40µg/mL) of tamoxifen citrate for the third group (G3). The samples placed in the incubator an inclined position and sperm parameters were read after 30 minutes. Simple medium for ART (SMART) was prepared according to [9].

Statistical analysis

The data were statistically analyzed using statistical package for social science (SPSS/PC) software (version 18) (SPSS, Chicago). Sperm parameters were analyzed using complete randomized design (CRD) of one way analysis of variance (ANOVA) [10].

III. Results

Descriptive characteristics

Macroscopic and microscopic parameters of semen analysis for subjects were assessed in the table (1). In this table, the semen volume, semen liquefaction time, semen pH, sperm concentration, sperm motility (%) and normal sperm morphology (%) were within normal ranges according to the criteria of WHO (1), except sperm agglutination (%) and round cell count were more than the WHO (1) recommended criteria. Depending on WHO (1) recommended criteria, all examined samples were classified into several male infertility factors (MIFs). In addition to normozoospermic subjects, in this study, the MIFs including, asthenozoospermia, teratozoospermia, oligoteratozoospermia, asthenoteratozoospermia. Figure (1) shows the percentage among the infertility factors.

Table (1): Semen parameters for subjects involved in this study

Semen Parameters		Subjects mean± S.E	WHO (2010) criteria
Semen volume (ml)		2.767 ±0.08	1.5 mL
Semen liquefaction time (minute)		48.180 ±1.08	≤ 60 minute
Semen pH		7.763 ±0.03	≥ 7.2
Sperm concentration (million/mL)		39.860 ±2.08	≥ 15
Sperm motility (%)		68.310 ±1.40	≥ 40%
Sperm grade Activity (%)	Progressive motility (%)	40.010 ±1.61	≥ 32%
	Non progressive motility (%)	28.300 ±0.93	
	Immotile sperm (%)	31.690 ±1.40	
Total Progressive sperm (million/ejaculate)		47.207 ±4.15	
Normal sperm morphology (%)		38.494 ±1.55	≥ 30% (WHO,1992)
Sperm agglutination (%)		20.170 ±1.34	< 10%
Round cells count (HPF)		7.439 ±0.49	< 5 cells/HPF

- Number of subjects= 100

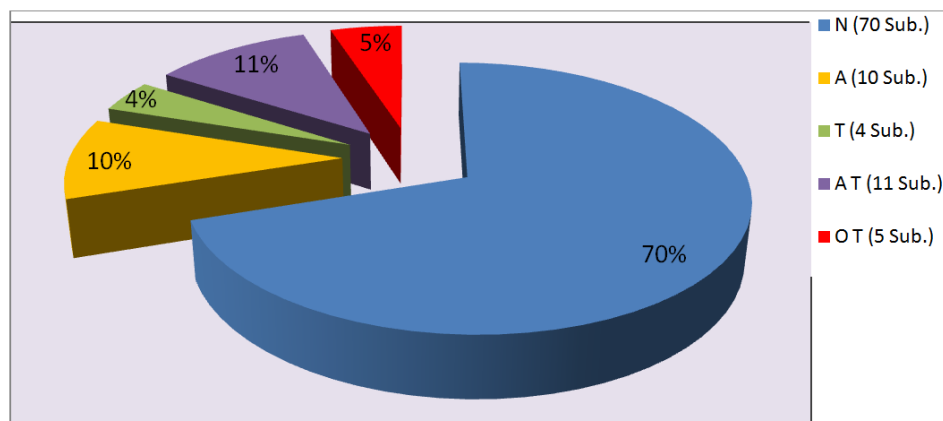


Figure (1): Frequency and distribution of infertility factors groups of patients in this study (Sub.: No. of subjects).

- N: Normozoospermia, A: Asthenozoospermia, T: Teratozoospermia,
- AT: Asthenoteratozoospermia, OT: Oligoteratozoospermia

Comparison of the sperm parameters between pre and post *in vitro* sperm activation

The results of semen analysis for pre and post *in vitro* sperm activation were presented in table (2). It was showed significant ($P < 0.05$) increased in the percentage of sperm motility, progressive sperm motility and normal sperm morphology of post-activation as compared with pre-activation. Among treated groups there are non-significant differences in sperm concentration. While, a significant ($P < 0.05$) decreased in non-progressive motility and immotile sperm. However, significant ($P < 0.05$) increased in progressive sperm motility, total progressive sperm and normal sperm morphology in high concentration (G3) as compared with other groups.

Table (2): Sperm parameters of pre and post *in vitro* sperm activation using SMART medium enriched with two concentration of tamoxifen citrate for all subjects involved in the study (mean \pm S.E)

Sperm Parameters	Pre - activation	Post- activation			
		Control (G1)	20 μ g/mL of TC (G2)	40 μ g/mL Of TC (G3)	
Sperm concentration (millions/mL)	40.050 a ± 2.06	23.130 b ± 1.63	21.960 b ± 1.59	22.090 b ± 1.75	
Sperm motility (%)	68.970 c ± 1.37	89.010 b ± 1.08	93.980 b ± 1.02	97.990 a ± 0.52	
Sperm grade activity (%)	Progressive motility (%)	40.670 d ± 1.61	61.440 c ± 2.05	73.860 b ± 2.35	89.640 a ± 1.47
	Non progressive motility (%)	28.300 a ± 0.93	27.870 a ± 1.62	20.020 b ± 1.75	8.250 c ± 1.14
	Immotile (%)	31.130 a ± 1.37	10.870 b ± 1.09	6.220 c ± 1.02	2.210 d ± 0.52
Total progressive sperm (millions/mL)	47.994 a ± 4.14	14.514 c ± 1.24	16.295 b c ± 1.32	19.469 b ± 1.47	
Normal sperm morphology (%)	38.494 d ± 1.55	56.650 c ± 1.26	60.170 b ± 1.26	72.000 a ± 1.08	

- Number of subjects= 100
- Means with different superscripts within each row are significantly different ($P < 0.05$).
- Means with similar superscripts within each row are non-significantly different ($P > 0.05$).

IV. Discussion

In the present study, sperm centrifugation was selected as a method for *in vitro* sperm activation, it is one of the common sperm preparation techniques for both experimental and practical programs [11], characterized with simplicity, rapid and cost effectiveness [12]. The results of this study are agreement with [13] and [14], for different purposes involving removal of effects for morphologically abnormal spermatozoa, immature sperm cells, epithelial cells, and lastly seminal leukocytes, sperms isolated with the swim up are clean and motile, but damaged by the Reactive Oxygen Species (ROS) and with higher DNA integrity [15].

In the present study, the centrifugation force was 2500 rpm for 6 min to avoid oxidative damages of sperm plasma membrane via produce very high levels of ROS by pelleting of the semen with the impairment of sperm functions and

decrease in the percentage of normally chromatin-condensed spermatozoa [16, 17]. From the results of this study, sperm concentration for all groups post-activation was significantly reduced. Similar results were presented by [(18)]. 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, agglutinated spermatozoa and remove round cells and epithelial cell, and reduced bacterial infection[19]. In this study SMART medium was used, as a culture medium, SMART medium contains certain components like NaHCO_3 which play important regulatory roles in promoting capacitation and hyper activation according to [20].

Serum albumin used in SMART medium acts as a powerful antioxidant that prevents oxidative stress-induced damage and play a protective role to mature spermatozoa decreasing their sensitivity to membrane oxidizing agents[21]. It was noticed in the current study that the use of *in vitro* culture media causes a significant increases in the sperm motility and the reason may be that, the seminal fluid with high viscosity obstructs sperm progressive motility so that the uses of *in vitro* media with aqueous nature lead to decrease the viscosity of the seminal fluid and as a result sperms move more freely because of their aqueous nature with lower viscosity than of seminal plasma resulted in making spermatozoa move more freely ,also the presence of calcium in the incubation medium is very important because extracellular calcium acts as limitless reservoir of the ion [22].

Furthermore, calcium plays important role in all modification of sperm cell properties occurring after the ejaculation, such as motility, capacitation, and the acrosome reaction, which causes increase in sperm activity, may be through activation of sperm mitochondria [23]. Tamoxifen citrate is antiestrogen drug have antioxidant action which inhibited ROS production by break the oxidative chain reaction, thereby, reduce the oxidative stress. Also, prevention of lipid peroxidation and increased fluidity and flexibility which help the sperm to engage in membrane, fusion events associated with the fertilization [24].

The results also showed that percentage of sperm motility and progressive sperm motility were significantly increased in tamoxifen citrate treated-groups post-activation and this may be due to the using of tamoxifen citrate enhances sperm motility, viability, capacitation, acrosome reaction by enhances the level of cAMP by stimulating Ca^{2+} or Mg^{2+} ATPase which leads to activation of calcium channel opening, thereby depositing more Ca_i^{2+} [25]. 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 [26]. The results were obtained using (40 $\mu\text{g}/\text{mL}$) of tamoxifen citrate significantly enhanced progressive motility and morphology of sperm. Since the membrane wall and the various compartments of the organelle are high in lipid content, addition of tamoxifen citrate may be protect these structures from the ever-increasing free radical species [27].

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