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Evaluation of Relaxin as Biomarker for the Assessment Quality of Selected Sperm During Glass Wool and Sephadex Filtration Techniques in Infertile Men

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Abstract

Background: Relaxin is a circulating hormone. It is secreted from the prostate gland into the seminal fluid; however, the role of Relaxin in male reproduction is debated. Studies conducted in the past have suggested possible actions on human spermatozoa, but the data were contrasting**. Objectives:** The aim of the current study is to study some sperm characteristics in asthenozoospermia men in comparison with normozoospermia men before and after glass wool and Sephadex activation in correlation seminal fluid Relaxin concentration. **Subjects, Materials and Methods:** This study involved 60 males; the recruited individuals were divided into 2 groups, (40 asthenozoospermic and 20 normozoospermic subjects) during the period of attendance to the infertility clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. The collected semen samples were obtained, and seminal fluid analysis was assessed. Semen samples were divided into 3 parts. The first part prepared was *in vitro* sperm characterization before activation, the second part using Glass wool filteration (GWF) technique, while the last part was prepared using Sephadex and measure Relaxin level in all 3 parts. **Results:** Mean Relaxin level was significantly higher in normozoospemia group than in asthenozoospermia group before activation ($P<0.001$), while treatment with glass wool makes the level of Relaxin between both groups ($P = 0.087$) and becomes even significantly higher following treatment with Sephadex ($P = 0.010$).In groups, normozoospemia or asthenozoospermia, there was no significant correlation between Relaxin level and any sperm characteristics whether before or after activation. However, when all cases of studied groups; normozoospermia and asthenozoospermia; were taken together, there was significant positive correlation between Relaxin level and Sperm Motility Grade A % and negative significant correlation between Relaxin and grade C and D % indicating that higher semen Relaxin is correlated with higher Sperm Motility Grade A sperms and lower Sperm Motility Grade B and C %. This was in case before activation. Following glass wool activation there was significant positive correlation between Relaxin level and sperm concentration. Following Sephadex treatment, there was a significant positive correlation between Relaxin level and Sperm Motility Grade A and B % and also with morphologically normal sperm %. **Conclusion:** Both Sephadex and glass wool techniques has been proved effective to improve semen quality and semen concentration of Relaxin showed positive correlation to good quality sperms.

Keywords: Relaxin, Sephadex, Seminal plasma, asthenozoospermia, Infertile men.

Introduction

Infertility has been classified as one of the master issue in medical science, it has defined as the inability to fulfill pregnancy after 12 months of regular sex intercourse with no contraceptive taken, which affects 15% of reproductive-aged couples [1]. Approximately 20% of cases of infertility were caused entirely by male factor, with an additional (30-40) % of cases involving both male and female factors; therefore, a male factor is present in half of infertile couples [2]. Relaxin (RLN)is a structural homologue of insulin and other growth factors; the function of Relaxin in male reproduction is still ambiguous [3]. Initially, it is thought that Relaxin was mainly produced by the prostate and released into the seminal fluid to influence on sperm motility [4]. Recently, it has been found that Relaxin receptor is expressed in human spermatozoa and that Relaxin catalyze sperm motility, apoptosis, mitochondrial function, capacitation and acrosome reaction, and providing extra evidence that RLN is important for fertilizing ability and preservation of sperm functionality [5].Many of sperm preparation techniques have been developed in ART's, these technique can be categorized into three major groups: sperm migration method (swim-up), density gradient centrifugation method and Adherence column method (Sephadex column filtration and glass wool column filtration)[6].These groups have been developed to separate viable sperm from the seminal fluid and to achieve the largest number of morphologically normal and free from for seminal plasma, bacteria, leukocytes, agglutination and aggregation [7].The principle of glass wool filtration (GWF) technique is rested on the self-propelled movement of the spermatozoa and filtration effect of the glass wool fibers [8]. A major advantage of this approach is the selection of normally chromatin-condensed spermatozoa, a parameter considered as predictive for fertilization ability *in vitro*. The GWF technique is very simple, but it is a more expensive procedure [9]. Some debris was usually still present in the sample after the GWF [10].While, filtration through Sephadex is based on the ability of the sperm to move in addition to their interaction with the filter substrates, Sephadex beads or membrane pores [11] .It has considered that non- viable sperm tend to adhere to the matrix more than motile cells. Sephadex either allows immotile and dead sperm to agglomerate because of changes in surface charges, or a protein present on damaged spermatozoa binds to the Sephadex particles [12].

Subjects, Materials and Methods

Total 60 infertile males were involved in this study, individuals were divided into 2 groups, (40: asthenozoospermic and 20: normozoospermic subjects) during their attendance to the infertility clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies; Al- Nahrain University. The seminal fluid analysis was assessed, and each semen sample was divided into 3 parts. The first part was prepared for sperm characterization and assessment of RLN before activation, the second part using GWF technique and assessment of RLN after activation, while the last part was prepared using Sephadex with an assessment of RLN.

Glass wool filtration (GWF) technique

Glass wool gently inserting inside one mL syringe and compressed to a final thickness of 3mm, was commercially available [13]. Prior to GWF technique, 1mL semen was diluted with one mL of Ferticult Flushing medium and mixed gently. Following dilution, the semen suspension was centrifuged for 10 min at 2500 rpm. Supernatant was removed and 1mL of Ferticult medium was added then left for 15-20 min after that the solution was aspirated. The glass wool was then rinsed with 1mL of medium, then washed sperm suspension was placed gently over the wet glass wool and allowed to filter by gravity. A drop of 10µL was aspirated and put on a slide with cover slip and examined under the microscope at 400X objective to assess the sperm parameters.

Sephadex G – 25 filtration technique

Glass wool gently inserting inside 5 mL syringe and compressed to adopter of syringe and then add one ml of Sephadex to disposable syringe. Prior to Sephadex filtration technique, 1mL semen was diluted with one mL of Ferticult Flushing medium and mixed gently. Following dilution, the semen suspension was centrifuged for 10 min at 2500 rpm. Supernatant was removed and 1mL of Ferticult medium was added then left for 15-20 min after that the solution was aspirated. The Sephadex column was rinsed with 1mL of medium, then washed sperm suspension was placed gently over the Sephadex column and allowed to filter by gravity. A drop of 10µL was aspirated and put on a slide with cover slip and examined under the microscope at 400X objective to assess the sperm parameters.

Enzyme - Linked Immunosorbent Assay for Relaxin Evaluation

All study semen plasma samples were carried out for the measurement of RLN (before and after activation), with the aid of a commercially available ELISA kit, YH Biosearch Laboratory, China. According to the manufacture leaflet, the procedure was performed.

Results

Mean Relaxin level in normozoospermia and asthenozoospermia groups before and after activation

Mean Relaxin level was significantly higher in normozoospermia group than in asthenozoospermia group before activation (P <0.001), while treatment with glass wool makes the level of Relaxin between both groups (P = 0.087) and becomes even significantly higher following treatment with Sephadex (P = 0.010), Table 1.

In normozoospermia group, treatment with glass wool caused significant rise in the level of Relaxin (P < 0.05) however, treatment with Sephadex resulted in significant decline in Relaxin level (P < 0.05). Whereas, in asthenozoospermia group, treatment with both glass wool and Sephadex caused significant rise in the level of Relaxin (P< 0.05), treatment with glass wool resulted in more significant rise in Relaxin level (P < 0.05), Table 1.

Table 1: Mean Relaxin level in normozoospermia and asthenozoospermia groups before and after activation.

Activation	Groups		*P*†
	Normozoospermia *n* = 20	Asthenozoospermia *n* = 40	
Before	121.61 ±26.78 B	56.73 ±31.06 C	< 0.001 HS
Glass wool	150.34 ±26.76 A	131.27 ±45.14 A	0.087 S
Sephadex	98.01 ±18.88 C	114.35 ±23.98 B	0.010 HS

n: number of cases; †: independent samples t-test; S: significant at $P \leq 0.05$; HS: highly significant at $P \leq 0.01$; Data were expressed as mean ± standard deviation; Comparison was carried out using one way ANOVA followed by post hoc LSD test; Capital letters were used to indicate level of significance; similar letters indicate no significant difference at $P \leq 0.05$ whereas, different letters indicate significant difference at $P \leq 0.05$; letter A is the highest value.

Correlation of Relaxin level to sperm characteristics

In groups, normozoospermia or asthenozoospermia, there was no significant correlation between Relaxin level and any of sperm characteristics whether before or after activation, as illustrated in Tables 2 and 3. However, when all cases (normozoospermia and asthenozoospermia) were taken together, there was significant positive correlation between Relaxin level and Sperm Motility Grade A % and negative significant correlation between Relaxin and Sperm Motility Grade C and D % indicating that higher semen Relaxin is correlated with higher Sperm Motility Grade A sperms and lower Sperm Motility Grade B and C %. This was in case before activation. Following glass wool activation, there was significant positive correlation between Relaxin level and sperm concentration. Following Sephadex treatment, there was significant positive correlation between Relaxin level and Sperm Motility Grade A and B % and also with morphologically normal sperm %, as shown in Table 4.

Table 2: Correlation of Relaxin level to sperm characteristics in normozoospermia.

Characteristic	Before		Glass wool		Sephadex	
	r	*P*	*r*	*P*	*R*	*P*
Sperm concentration (m/ml)	-0.156	0.510 NS	0.216	0.361 NS	-0.235	0.318 NS
Sperm Motility Grade A%			-0.036	0.881 NS	-0.121	0.611 NS
Sperm Motility Grade B%	-0.397	0.083 NS	-0.008	0.974 NS	0.175	0.461 NS
Sperm Motility Grade C%	0.111	0.640 NS	0.075	0.753 NS	-0.035	0.883 NS
Sperm Motility Grade D%	0.324	0.163 NS	0.119	0.616 NS	-0.240	0.307 NS
Morphologically normal sperm %	-0.448	0.057 NS	0.302	0.196 NS	-0.302	0.053 NS
Round cells	0.346	0.135 NS	---	---	---	---
Sperm agglutination%	-0.388	0.091 NS	---	---	---	---

Table 3: Correlation of Relaxin level to sperm characteristics in asthenozoospermia.

Characteristic	Before		Glass wool		Sephadex	
	r	*P*	*r*	*P*	*R*	*P*
Sperm concentration (m/ml)	-0.109	0.504 NS	0.251	0.119 NS	-0.024	0.885 NS
Sperm Motility Grade A%	---	---	-0.047	0.774 NS	-0.175	0.281 NS
Sperm Motility Grade B%	-0.177	0.275 NS	0.261	0.104 NS	0.302	0.058 NS
Sperm Motility Grade C%	-0.012	0.940 NS	-0.228	0.156 NS	-0.054	0.742 NS
Sperm Motility Grade D%	0.175	0.280 NS	0.059	0.716 NS	-0.089	0.584 NS
Morphologically normal sperm %	0.099	0.542 NS	-0.121	0.456 NS	-0.051	0.754 NS
Round cells	-0.061	0.708 NS	---	---	---	---
Sperm agglutination%	-0.096	0.557 NS	---	---	---	---

Table 4: Correlation of Relaxin level to sperm characteristics in all studied groups men.

Characteristic	Before		Glass wool		Sephadex	
	r	*P*	*r*	*P*	*R*	*P*
Sperm concentration (m/ml)	0.045	0.734 NS	0.288	0.026 S	-0.199	0.127 NS
Sperm Motility Grade A%	0.785	<0.001 HS	0.165	0.208 NS	0.398	0.002 HS
Sperm Motility Grade B%	0.083	0.528 NS	0.084	0.525 NS	0.337	0.009 HS
Sperm Motility Grade C%	-0.654	<0.001 HS	-0.226	0.083 NS	0.169	0.197 NS
Sperm Motility Grade D%	-0.564	<0.001 HS	0.033	0.804 NS	-0.153	0.243 NS
Morphologically normal sperm %	0.165	0.207 NS	0.076	0.563 NS	0.289	0.025 S
Round cells	-0.104	0.431 NS	---	---	---	---
Sperm agglutination%	-0.107	0.418 NS	---	---	---	---

Discussion

Relaxin is a circulating hormone with functions in pregnancy, parturition, and other aspects of female reproduction. It is also secreted from the prostate gland into the seminal fluid; however, the role of Relaxin in male reproduction is debated. Studies conducted in the past have suggested possible actions on human spermatozoa, but the data were contrasting[14, 15].Prostate Relaxin is a main source of this peptide in the seminal plasma. The Relaxin effects on sperm motility and fertilization have been reported[16].Several studies showed that Relaxin not only increased sperm motility but also increased the rate of sperm capacitation and acrosome reaction[16].
In the current study, it was shown that mean Relaxin level was significantly higher in normozoospermia than in asthenozoospermia before activation; however, treatment with glass wool makes the difference in the level of Relaxin between both groups insignificant and the level becomes even significantly higher following treatment with Sephadex. Moreover, it has been observed in the current study that in normozoospermia, treatment with glass wool caused significant rise in the level of Relaxin; however, treatment with Sephadex resulted in significant decline in Relaxin level. Whereas, in study group, treatment with both glass wool and Sephadex caused significant rise in the level of Relaxin; on the other hand, treatment with glass wool resulted in more significant rise in Relaxin level. To the best of our knowledge, this is the first study that compared the level of seminal Relaxin between two groups, normozoospermia and asthenozoospermia before and after treatment with Sephadex and glass wool. The results showed that glass wool was superior in increasing Relaxin level in asthenozoospermia group. Experimental studies have shown that the addition of exogenous Relaxin resulted in significant improvement of spermatozoa motility and this improvement in motility was attributed to suggested receptor ligand interaction between Relaxin and receptors of the surface of spermatozoa (Relaxin receptors RXFP1 and RXFP2)[17].Indeed, an experimental study was done on frozen semen sample using Relaxin to study its effect on sperm motility and it was found that adding Relaxin before freezing resulted in no improvement in sperm motility, while adding it at time of thawing resulted in significant improvement of sperm motility suggesting that freezing adversely affected the biological activity of the hormone Relaxin[18].Actually some experimental studies on animals also showed that Relaxin could be a valuable motility booster of stored- or aged-spermatozoa for assisted reproduction techniques[17].However, experimental studies on animals have shown that the concentration of immunoreactive Relaxin level in semen was directly proportional in a significant manner to the sperm characteristics such as curvilinear motility and motile sperm percentage [19,20].

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