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Studying the efficiency of the swim up from pellet technique in improving sperm parameters and reducing the levels of antisperm antibody and interleukins for infertile men

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Abstract---Studying the efficiency of the swim up from pellet technique in reducing antisperm antibody and some cytokine levels in seminal plasma. Semen samples were collected from oligozoospermic and Normozoospermic who attended a fertility center. The collected samples of normozoospermia patients were (15) and oligospermia patients were (13). antisperm antibody, interleukin-1, interleukin-6, and zinc levels were evaluated in seminal plasma before and after semen processing for all study groups. The results revealed that there was a statistically significant decrease ($p < 0.05$) in antisperm antibody, interleukin 1B (IL- β), interleukin (IL-6), and zinc. After sperm preparation by swim up from pellet compared with before sperm preparation, The result illustrates there was a statistically significant decrease ($p < 0.05$) in sperm concentration and leucocytes count while there was significant increase ($p > 0.05$) in progressive motility, normal morphology after preparation by swim up from pellet compared with before the preparation. In concluded Processing by swim-up techniques decreases sperm concentration, and also shows an increase in sperm normal morphology and progressive motility in normospermia, oligospermia, and asthenospermia groups. Antisperm antibody, interleukin 1 (IL-), interleukin 6 (IL-), and zinc levels are all reduced.

Keywords---swim up, pellet, sperm, antisperm antibody, interleukins.

Introduction

It is recognized that male infertility causes a decrease in the production of sperm with normal morphology and progressive motility (Shokoohi et al., 2018). Karvolos et al. (2013) describe immunoinfertility as one of the primary causes of male infertility, accounting for 10-15% of all cases of infertility and over 40% of idiopathic cases. Antisperm antibodies (ASA) are a well-known cause of male and female refractory infertility (Shibahara and Koriyama, 2013). Agarwal et al. (2016) say that sperm is processed using "swim-down," "swim-up," "migration sedimentation," "density gradient centrifugation," "magnetic activated cell sorting," and "glass wool filtration." The swim-up method is useful for selecting motile spermatozoa due to its reliance on sperm's ability to swim into the culture medium. The culture media can either be put directly over the sperm or over the pellet generated after centrifuging the sample (WHO, 2010).

Materials and Methods

Participants

This study was conducted between October 2021 and December 2021 in the advanced research laboratory of the Department of Laboratory Analyses, Faculty of Science, University of Kufa, and the laboratory of the Fertility Centers in AL-Saader Medical City. Oligozoospermic and normozoospermic men who attended a fertility facility provided sperm samples. The obtained samples of normozoospermia patients numbered (15), while those of oligospermia patients numbered (13). The age of infertile patients ranged from 20 to 55 years old. The wife was aged between 12 and 38. In all study groups, antisperm antibody, interleukine-1, interleukine-6, and zinc levels were measured in seminal plasma.

Study Design

The study was designed according to the following steps

1. Semen samples were examined microscopically and macroscopically.
2. The samples were divided into oligozoospermia, asthenozoospermia, and normozoospermia as a control group according to the quid lines of World Health Organization WHO 2010
3. The semen samples were placed in a centrifuge to separate the seminal plasma from the sperm pellet.
4. The seminal plasma was placed in an Eppendorf tube and kept in the refrigerator at a temperature of 20 ° C until the appropriate time for examining the studied immunological tests.
5. (0.5) ml of culture medium was added on top of the sperm pellet and incubated in the incubator for 30 minutes. (Modified swim up from pellet)
6. The sperm parameters were examined after 30 minutes by taking a drop from the culture medium near the sperm pellet.
7. The culture medium was transferred into another test tube and placed in a centrifuge to obtain a sperm-free culture medium.

8. The sperm-free culture medium was transferred to an Eppendorf tube and kept in the refrigerator at a temperature of 20 °C until the appropriate time for the examination of the immunological.

Semen Collection and Analysis

After (2–7) days of sexual abstinence, semine samples were obtained directly in a dry, clean, and sterile disposable container by masturbation in a room next to the laboratory for seminal fluid analysis. The container was labeled with the patient's name, age, and sexual abstinence at the time of sample collection. The specimens were placed in an incubator at 37°C for 30 minutes to facilitate liquefaction. The liquid specimens were thoroughly combined for a few seconds before being inspected under a microscope. WHO (2010) describes the analysis and classification of infertile patients as done to get an idea of what seminal fluid analysis would show.

Sperm preparation for in vitro fertilization

The modified centrifugation swim up was used for activation of sperm

1. The whole semen samples were kept for 30 to 45 minutes in an incubator (37°C) before processing to allow the specimen to liquefy completely.
2. Specimens were transferred from a plastic container to a sterile 15ml conical centrifuge tube.
3. The conical tubes were centrifuged at 3000 rpm for 5 minutes.
4. The supernatant was aspirated without disturbing the pellet, and resuspension of the pellet in 0.5ml of media was carried out.
5. The supernatant (sperm free culture medium) was stored at -20°C (refrigerator) prior to biochemical tests in the future.
6. The conical tubes were incubated at 45 degrees for 30 minutes.
7. After the incubation period, the supernatant was aspirated, without disturbing the pellet.
8. A small drop (10 ml) was taken and the sild for analysis was prepared and sperm concentration, progressive motility, normal sperm morphology, and leukocytosis count were examined.
9. The supernatant (sperm free culture medium) was stored at -20°C (refrigerator) prior to biochemical tests in the future.

Biochemical investigation

Seminal plasma ASA and cytokine (IL-1 and IL-6) levels were assessed using the quantitative sandwich enzyme immunoassay technique (Enzyme-Linked-Immuno-Sorbent-Assay), whereas plasma zinc levels were measured using a flame atomic absorption device.

Statistical Analysis

Data of the present study was analyzed by using SPSS Statistics Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. IBM Corp). Comparison of differences between continuous variables was done by using the Paired t-Test

when compared two means and One Way ANOVA test to compare three variables; Multiple Pairwise Comparisons were performed by using least significant differences (L.S.D). Chi squared test was performed to analyze categorized data; Pearson's correlation analysis was used to determine the relationship between variables. P values ≤ 0.05 and ≤ 0.01 considered as significant and highly significant (Alhasnawi and Aljanaby, 2022; Aljanaby et al., 2022; Medhat and Aljanabay, 2022).

Result

The effect of sperm preparation by swimming up from the pellet on sperm parameters in infertile men with normozoospermia

The table 1 below illustrates there was a statistically significant decrease ($p < 0.05$) in sperm concentration and leucocytes count while there was a significant increase ($p > 0.05$) in progressive motility and normal morphology after preparation by swimming up from pellet compared with before the preparation.

Table 1
Sperm parameters before and after the preparation by swimming up from the pellet

Semen parameters	Sperm preparation by swim up from pellet		P-value
	Before(N=15)	After (N= 15)	
Sperm concentration (Million/ml)	64.6 + 25.193	40.53+ 17.724	<0.01**
Progressive motility (%)	55.33+ 14.573	75.33+ 11.412	<0.01**
Normal sperm Morphology (%)	40.33+ 14.201	53.33+ 4.543	<0.01**
Leucocytes (Million/ml)	1.00+0.000	0.00+0.458	<0.01**

**=significant differences at $P \leq 0.01$, ns= Non significant differences at $P \leq 0.05$,
By using Paired t-test

The effect of sperm preparation by swimming up from the pellet on antisperm antibody, cytokine, and zinc levels in infertile men

The results of the current study showed in table 2 that there were statistically significant decreases ($p < 0.05$) in antisperm antibody, interleukin 1 (IL-), interleukin (IL-6), and zinc. After sperm preparation by swimming up from the pellet, compared with before sperm preparation,

Table 2
Shows the levels of antisperm antibody, cytokines, and zinc in seminal plasma before and after sperm preparation by swimming up from the pellet

Antisperm antibody, cytokine and zinc levels	Sperm preparation by swim up from pellet		P-value
	Before(N=15)	After(N=5)	
Antisperm antibody (np/ml)	541.7 ± 144.064	156.5 ± 64.480	<0.001**

Interleukin 1 β (IL- β) (Pg/ml)	644.80 $\pm$ 158.246	289.47 $\pm$ 176.928	<0.001**
Interleukin 6 (IL- β) (Pg/ml)	18.14 $\pm$ 19.604	6.944 $\pm$ 10.207	0.002**
Zinc (ppm)	121.67 $\pm$ 9.832	67.27 $\pm$ 8.900	<0.001**

**=significant differences at $P \leq 0.01$, by using Paired t-test

Semen parameters in infertile oligozoospermic patients

The results of this study revealed a significant increase ($p < 0.01$) in progressive motility and normal morphology, while the results had a significant decrease ($p < 0.01$) in leucocyte concentration and a non-significant decrease ($p > 0.01$) in sperm concentration. These findings were after sperm preparation by swim up from pellet compared with before the sperm preparation (Table 3).

Table 3

Sperm parameters before and after the preparation by swim up from the pellet

Semen parameters	Sperm preparation by swim up from pellet		P-value
	Before (N=15)	After (N=15)	
Sperm concentration (Million/ml)	5.67+ 4.431	4.93+4.773	0.591 ns
Progressive motility (%)	22.53+16.501	49.80+18.358	<0.001**
Normal sperm Morphology (%)	41.00+17.570	57.00+18.879	<0.001**
Leucocytes (Million/ml)	1.47+1.457	0.00+0.00	0.002**

**=significant differences at $P \leq 0.01$, ns= Non significant differences at $P \leq 0.05$,. By using Paired t-test

Interleukin 1, Interleukin 6, and zinc levels in seminal fluid for infertile oligozoospermic patients

The results in table 4 revealed a statistically significant decrease ($p < 0.05$) in antisperm antibody, interleukin 1B (IL- β), interleukin (IL-6), and zinc. After sperm preparation by swim up from pellets prepared before sperm preparation.

Table 4

Antisperm antibody, cytokines and zinc levels in seminal plasma before and after the sperm preparation by swim up from pellet

Antisperm antibody, cytokine and zinc levels	Sperm preparation by swim up from pellet		P-value
	Before (N=15)	After (N=15)	
Antisperm antibody (ng/ml)	609.13+161.248	206.87+77.768	<0.001**
Interleukin 1B (IL- β)(Pg/ml)	637.07+138.085	139.67+93.643	<0.001**
Interleukin 6 (IL- β)(Pg/ml)	47.625+55.825	21.029+24.996	0.016*
Zinc (ppm)	113.13+25.001	62.67+7.907	<0.000**

**=Significant differences at $P \leq 0.01$, *= significant differences at $P \leq 0.05$, by using Paired t-test

Discussion

The result of this finding also found a significant decrease in sperm concentration after sperm processing with swim-up techniques. And also show an increase in sperm normal morphology and progressive motility after sperm processing by swim up techniques in the normozoospermia, oligozoospermia, and asthenozoospermia groups. This may be because one of the procedures that can increase sperm quality is swim-up separation, which selects the most functional spermatozoa for fertilization (erfanian et al., 2009). Our results agreed with the study that showed the swim-up lowered the number of sperm with head and tail ultrastructural defects (Yamanaka et al., 2016). Evaluated post-swim-up sperm parameters, including total motile sperm count and motility. The swim up method was associated with an increase in sperm count, motility, and pregnancy rate (Jameel, 2008). After sperm preparation, lower concentration recovery rates and increased morphological recovery rates were seen in sperm washing followed by swim-up (Fácio et al., 2016). The proportions of sperm motility, progressive sperm motility (grades A and B), total progressive sperm motility (grades A and B), and normal sperm morphology are described. There is a significant rise between pre-activation and post-activation (Hilo et al, 2015). (Lak et al., 2020) showed that the SU method was better at increasing the number and movement of sperm than the DGC-SU method.

The results of this study showed a significant decrease in antisperm antibody, cytokine, and zinc levels in seminal plasma after processing with swim up techniques. This is because the swim-up technique removes the seminal plasma, which has ASA, interleukins, and zinc, and then adds the activation medium, which doesn't have these things. This causes a clear drop in these variables. Our results agree with studies that show Swim-up was used to recover sperm following incubation. Sperm recovered after processing contained fewer antisperm antibody populations (Ryan et al, 1994). According to another study (Shi et al., 2021), DT and SU techniques were used to select sperm cells with higher motility, normal morphology, and reduced levels of ASAs, DNA fragmentation, caspase-3, and RCDs. The results of this study showed a significant increase in IL-6 and antisperm antibodies in smokers compared to nonsmokers. Our results agreed with the studies that showed IL-6, TNF-, and resistin levels were higher in smokers compared to nonsmokers and in cases of leukocytospermia, which is characterized by an increase in necrotic sperm and a decrease in the number of sperm with normal morphology and motility (Moretti et al., 2014). Current and previous smokers exhibited much greater IL-6 levels than nonsmokers (Helmersson et al., 2005). Another study indicated that ASA resistance is considerably higher in smokers. We know that smoking produces a prothrombotic condition during both acute and chronic usage (Ichiki et al., 1996).

The current study showed a significant increase in interleukin 6 in patients with varicocele compared to those without varicocele. Our results agreed with the study that showed Inflammatory cytokines have been detected in the plasma of varicocele patients (Zeinali et al., 2017). Varicocele may trigger an inflammatory response that may be deleterious to spermatogenesis (Moretti et al., 2009).Varicocele generated a considerable rise in serum and testis IL-6 and interferon gamma when compared to control groups and previous varicocele

groups (Habibi et al., 2015). No statistically significant changes were found in zinc ASA levels between patients with and without varicocele. This study result agrees with previous research indicating that there was no statistically significant difference between the control and varicocele groups in seminal levels of zinc and copper; nevertheless, other research also demonstrates that there is no association between zinc and varicocele (Canale et al., 1986). (Verajankorva et al., 2003) Many researchers have found that there is no link between the development of ASA and varicocele. Selenium concentration was lower in the varicocele group (Camejo et al., 2011).

Conclusions

That processing by swim-up techniques decreases sperm concentration and also shows an increase in sperm normal morphology and progressive motility in the normospermia, oligospermia, and asthenospermia groups. Antisperm antibody, interleukin 1 (IL-), interleukin 6 (IL-), and zinc levels are all reduced.

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