

EFFECT OF OXYTOCIN ON SPERM FUNCTION AND SEMINAL BIOCHEMISTRY OF ASTHENOSPERMIC PATIENTS

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Abstract

Semen samples of 90 asthenospermic patients were examined, then they were divided into three main equal groups. In the first group, Oxytocin (OT) addition in three concentrations (2, 4, and 6 IU/ml) with sperm preparation medium (SPM) to the semen samples was investigated. Changes in sperm function tests and seminal biochemistry was used as indicator of effect. Results indicated that a significant increase in sperm grade activity in 2 IU/ml OT with a tendency for a decrease in this motility parameter in higher OT concentration. Concentration 2 IU/ml of OT was applied with SPM and without in the second and third groups, respectively. In presence of SPM, a significant increase in the sperm motility characteristics (the percentage of sperm motility and sperm grade activity) was observed. While when SPM was absent, non-significant change in these parameters was seen. These findings indicate that OT at the concentration of 2 IU/ml has got a stimulatory effect on sperm motility characteristics. Using of SPM potentiated this effect of OT.

Total protein (TP), cholesterol, calcium, creatine kinase (CK), and fructose were measured in the seminal plasma and in the supernatant of sperm suspension of OT-treated and control samples. According to the results, a high significant increase in cholesterol was found in the external medium, this may be attributed to loss of cholesterol from the plasma membrane of the sperm. Also, OT may modify cellular calcium transport across the plasma membrane, resulting in high intracellular calcium concentration and a high significant decrease in level of calcium in the external medium. In addition to a high significant decrease in fructose level of the external medium.

Key words: Oxytocin, Seminal biochemistry, Sperm function, Asthenospermia.

Introduction

Asthenospermia indicates a significant reduction in sperm motility which is an important parameter in evaluating the fertilizing potential of the semen⁽¹⁾. A number of factors may be responsible for reduced sperm motility such as: spermatozoal defects, genital tract infection, partial ductal obstruction, spinal cord injury, sperm agglutination, and antisperm antibodies^(2;3). For several decades attempts have been made to improve the motility of hypokinetic specimens by using various means, one of them is the addition of motility-stimulating agents to the ejaculate prior to its use for artificial insemination. These agents include: hormonal compounds such as progesterone⁽⁴⁾, human chorionic gonadotropin⁽⁵⁾, relaxin⁽⁶⁾; non - hormonal

substances such as pentoxifyllin⁽⁷⁾, caffeine⁽⁸⁾, cAMP⁽⁹⁾.

Oxytocin (OT) is produced, in the male mammal, in similar quantities as in the female. Little is known about its physiology⁽¹⁰⁾. Majority of investigations was performed *in vivo* to study the physiological role of this hormone in the male. For these reasons, we tried in this work to study the effect of OT (as a natural product) on human sperm activation and to explain the proposed mechanisms in which it affects. Such an effect which is probably being a new concept will open the way for helping asthenospermic patients to have their sperm activated using naturally produced material.

In this study we investigate the influence of OT on semen parameters of asthenospermic patients, and exploring the best concentration of

OT that harvests a maximal number of motile spermatozoa.

Materials and Methods

Semen samples were obtained from 90 asthenospermic patients attending the Institute of Embryo Research and Infertility Treatment, University of Al-Nahrain. All samples were collected by masturbation after 3-4 days of sexual abstinence, then they were placed in an incubator at 37 °C, waiting for liquefaction.

The medicult sperm preparation medium (SPM) was used for sperm activation *in vitro*. Oxytocin (OT) used in this study was provided in 50 ml vials (10 IU OT/ml). Normal saline (0.9% NaCl) was used for preparation of three concentrations of OT (2,4, and 6 IU/ml).

Seminal Fluid Analysis (SFA):

Seminal standard parameters following World Health Organization guidelines⁽¹¹⁾ were determined on all samples which including physical parameters (liquefaction time, pH, volume) as well as using light microscopy to determine sperm concentration, sperm motility percent and grade of activity, sperm abnormality percent, leukocytes and phagocytes concentrations, and sperm agglutination.

Oxytocin and Sperm Function Tests:

Semen samples were mixed with OT and SPM⁽¹²⁾. For each concentration of OT (2, 4, and 6 IU/ml), a culture tube was prepared, and 0.4 ml of SPM was mixed with 0.1 ml of each concentration, then 0.5 ml of semen sample was added to each tube. After shaking the contents, the tubes were incubated in 5% CO₂ incubator at 37 °C for 30 minutes, then a drop of the mixture was aspirated by micropipette to be examined under 40X objective lens to study the effect of OT at these concentrations on the sperm function tests. Ten semen samples were studied for each concentration of OT. Each concentration was compared with semen samples treated with normal saline as control (OT=0 IU/ml), so that the total number of patients examined in this experiment was 30 (1st group).

Based on the outcome of this experiment, OT at 2 IU/ml was tried, as shown above, with SPM and without in the 2nd and 3rd groups, respectively. Each group involved 30 semen samples. The results were compared with the control samples in which normal saline was used instead of OT.

The Seminal Biochemical Study:

Seminal plasma was obtained by centrifugation of liquefied semen samples at 3000 rpm for 15 minutes, the sperm-free seminal plasma was then aspirated and kept in a clean tube until time of analysis. Following examination of sperm suspension microscopically, the samples were centrifuged at 3000 rpm for 15 minutes, then the supernatant was isolated and kept in a clean tube until time of analysis.

Five biochemical parameters were evaluated, these are: total protein (TP), cholesterol, calcium, creatine kinase (CK), and fructose. These parameters were measured in the seminal plasma of sixty patients. Also, they were measured after addition of OT at the concentration of 2 IU/ml in the supernatant of sperm suspension of control and OT-treated samples. The biochemical parameters were measured as the following:

- 1.Total Protein:** Total protein was determined according to the Biuret method reported by Silverman *et al.*⁽¹³⁾, Linear kit was used in this colorimetric method.
- 2.Cholesterol:** Cholesterol concentration was determined according to the method of Richmond⁽¹⁴⁾, Linear kit was used in this enzymatic colorimetric method.
- 3.Calcium:** Calcium concentration was estimated applying the method reported by Baginski *et al.*⁽¹⁵⁾, kit of Giesse was used in this colorimetric method.
- 4.Creatine Kinase:** Kinetic determination method was followed in estimation of CK activity⁽¹⁶⁾. Enzyme activity was assayed with Syrbio kit.

5.Fructose: Fructose level was estimated according to the method of Mann⁽¹⁷⁾.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 10.5, computer software. All data were expressed as mean \pm SD. Statistical comparisons between groups were made using student's-t-test and a P value of < 0.05 was considered significant. Differences between groups were analyzed using an analysis of variance (ANOVA), the significance level was set at $P < 0.05$ ⁽¹⁸⁾.

Results

Seminal Fluid Analysis:

The physical characteristics of the semen samples (Table 1) revealed that mean of liquefaction time was on the maximal side of the normal value, while mean of semen volume was on the minimal side of the normal value. All semen samples had normal alkaline pH, which was within the normal limit. Sperm concentration was within the normal limit, while the percentage of motile sperm was below the normal limit, also, mean of sperm grade activity was below the normal range of Macleod scale.

The percentage of sperm abnormality was on the maximal side of the normal limit. The percentage of sperm agglutination was above the normal limit. Regarding seminal fluid infection, the results revealed that, the concentrations of leukocytes and phagocytes were above WHO normal limit.

Table (1)
Semen physical characteristics of the patients.

Semen Characters	Value (Mean \pm SD)	Normal Values
Liquefaction time (minute)	35.17 \pm 8.46	< 30
Semen volume (ml)	2.50 \pm 0.93	2-6 ml
pH	7.84 \pm 0.15	7.4-8.2
Sperm concentration (million/ml)	44.20 \pm 20.53	> 20
Sperm motility (%)	34.66 \pm 11.32	> 60
Grade of activity (Macleod scale)	1.62 \pm 0.44	≥ 3
Sperm abnormality (%)	48.82 \pm 8.65	< 50
Sperm agglutination (%)	3.72 \pm 2.14	Negative
Leukocytes concentration (million/ml)	2.52 \pm 1.19	< 1
Phagocytes concentration (million/ml)	2.86 \pm 1.44	< 0.5

Effect of Oxytocin on Sperm Function Tests:

Results shown in (Table 2) indicated that addition of OT at 2, 4, and 6 IU/ml did not cause any significant change ($P > 0.05$) in all sperm function tests except the grade of activity which

rose significantly ($P < 0.05$) at the concentration of 2 IU/ml in comparison with the control samples.

Table (2)
Effect of different concentrations of oxytocin (2,4, and 6 IU/ml) on sperm function tests.

Parameters	Control	OT- treated (2 IU/ml)	Control	OT- treated (4 IU/ml)	Control	OT- treated (6 IU/ml)
Sperm concentration (million/ml)	35.20 ± 13.59	35.50 ± 13.57	30.60 ± 10.31	30.90 ± 10.63	32.90 ± 12.28	33.50 ± 12.40
Sperm motility (%)	58.40 ± 10.73	58.72 ± 9.22	48.55 ± 8.18	49.00 ± 8.10	48.72 ± 9.11	47.84 ± 9.34
Grade of activity	2.62 ± 0.34	2.98* ± 0.38	2.56 ± 0.26	2.54 ± 0.24	2.46 ± 0.22	2.43 ± 0.23
Sperm abnormality (%)	48.45 ± 5.12	49.00 ± 4.58	45.10 ± 4.63	44.50 ± 4.48	48.14 ± 5.35	48.50 ± 4.86
Sperm agglutination (%)	2.12 ± 1.39	2.18 ± 1.42	2.20 ± 1.50	2.00 ± 1.67	2.15 ± 1.60	2.15 ± 1.58
Leukocytes concentration (million/ml)	1.20 ± 0.79	1.20 ± 0.92	1.32 ± 0.68	1.22 ± 0.68	1.20 ± 1.03	1.10 ± 0.94
Phagocytes concentration (million/ml)	2.00 ± 0.92	1.90 ± 1.00	1.92 ± 1.20	1.68 ± 0.98	1.40 ± 1.11	1.20 ± 0.95
Number of samples	10		10		10	

Values are means ± standard deviation of means. *Significant difference ($P < 0.05$).

Effect of Oxytocin on Sperm Function Tests

Addition of OT (2 IU/ml) to the semen revealed that, in presence of SPM (Table 3), there were a high significant decrease ($P < 0.001$) in sperm concentration and a significant increase ($P < 0.05$) in the percentage of sperm motility and sperm grade activity after activation for the control and OT-treated samples as compared to their values before

activation. The percentage of sperm motility and sperm grade activity significantly improved ($P < 0.05$) after the application of OT (2 IU/ml) as compared with the control samples. When SPM was absence (Table 4), a high significant decrease ($P < 0.001$) was observed in sperm concentration in the two groups as compared to their values before activation. An increase

(although it is not significant) was observed in the percentage of sperm motility and sperm grade activity only for the OT-treated samples. When a comparison was made between the same activation technique with or without SPM, no significant difference ($P > 0.05$) was found in regard to sperm concentration

between the two preparation techniques (Fig.1). A significant improvement ($P < 0.05$) was registered in the percentage of sperm motility and sperm grade activity when SPM was used (Fig. 2 and 3). While no significant differences ($P > 0.05$) were observed in the other sperm parameters as shown in (Figs. 4, 5, 6 and 7).

Table (3)

Effect of oxytocin (2 IU/ml) on sperm function tests following sperm activation with sperm preparation medium.

Sperm function tests	Before activation	After activation	
		Control	OT-treated
Sperm concentration (million/ml)	45.60 ± 15.95	32.30 ^{a**} ± 13.20	32.93 ^{a**} ± 12.82
Sperm motility (%)	30.93 ± 12.17	37.70 ^{a*} ± 14.49	40.78 ^{b*} ± 14.27
Grade of activity	1.61 ± 0.41	2.06 ^{a*} ± 0.44	2.48 ^{b*} ± 0.46
Sperm abnormality (%)	49.87 ± 9.70	48.83 ^a ± 11.32	48.66 ^a ± 11.36
Sperm agglutination (%)	3.82 ± 2.84	3.24 ^a ± 1.84	3.36 ^a ± 1.54
Leukocytes concentration (million/ml)	2.02 ± 1.30	1.92 ^a ± 0.84	1.82 ^a ± 0.94
Phagocytes concentration (million/ml)	2.33 ± 1.42	2.16 ^a ± 0.90	2.24 ^a ± 0.88

Values are means ± standard deviation of means. *Significant difference ($P < 0.05$). **Significant difference ($P < 0.001$). Means carrying similar small letters indicate a non-significant difference ($P > 0.05$). Means carrying different small letters indicate a significant difference ($P < 0.001$).

Table (4)

Effect of oxytocin (2 IU/ml) on sperm function tests following sperm activation without sperm preparation medium.

Sperm function tests	Before activation	After activation	
		Control	OT-treated
Sperm concentration (million/ml)	43.53 ± 14.95	31.27 ^{**} ± 12.38	31.53 ^{**} ± 12.73
Sperm motility (%)	30.00 ± 12.36	30.07 ± 10.69	31.66 ± 11.54
Grade of activity	1.60 ± 0.43	1.63 ± 0.56	1.72 ± 0.58
Sperm abnormality (%)	50.73 ± 6.61	49.79 ± 6.97	49.57 ± 6.20
Sperm agglutination (%)	3.42 ± 2.45	3.14 ± 1.95	3.22 ± 1.85
Leukocytes concentration (million/ml)	2.23 ± 1.53	2.12 ± 0.84	2.15 ± 0.72
Phagocytes concentration (million/ml)	2.47 ± 1.38	2.42 ± 0.92	2.48 ± 0.89

Values are means ± standard deviation of means. **Significant difference ($P < 0.001$).

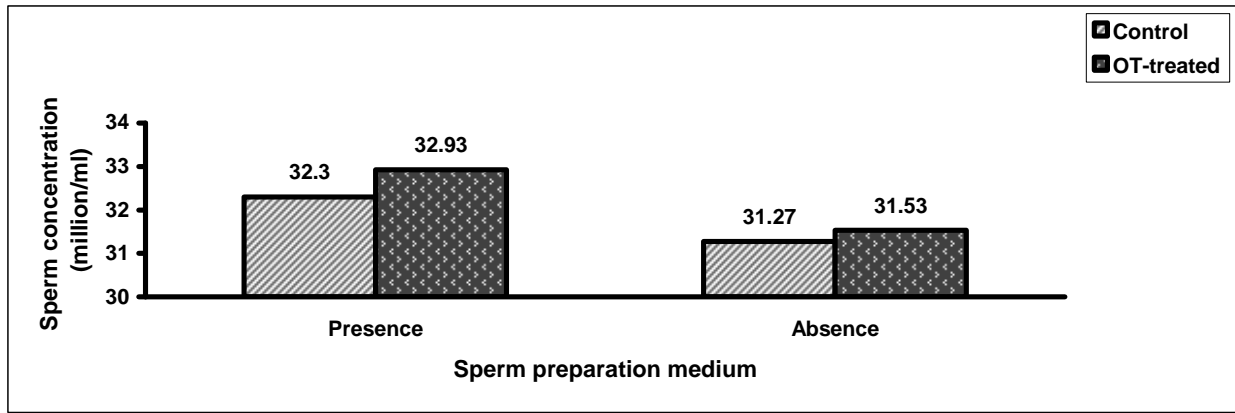


Fig.(1) : Sperm concentration of the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• Non-significant difference ($P > 0.05$) from corresponding group.

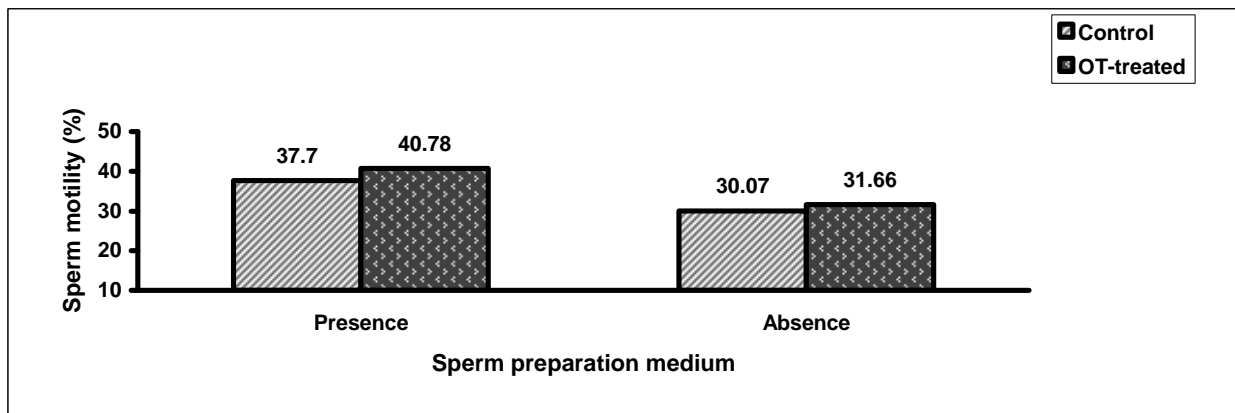


Fig. (2): The percentage of sperm motility of the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• * Significant difference ($P < 0.05$) from corresponding group.

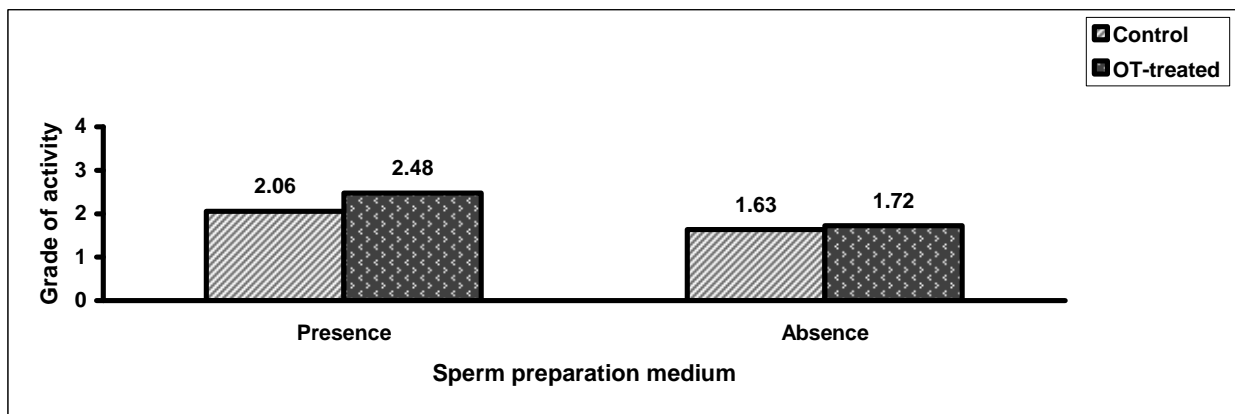


Fig. (3) : Sperm grade activity of the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• *Significant difference ($P < 0.05$) from corresponding group.

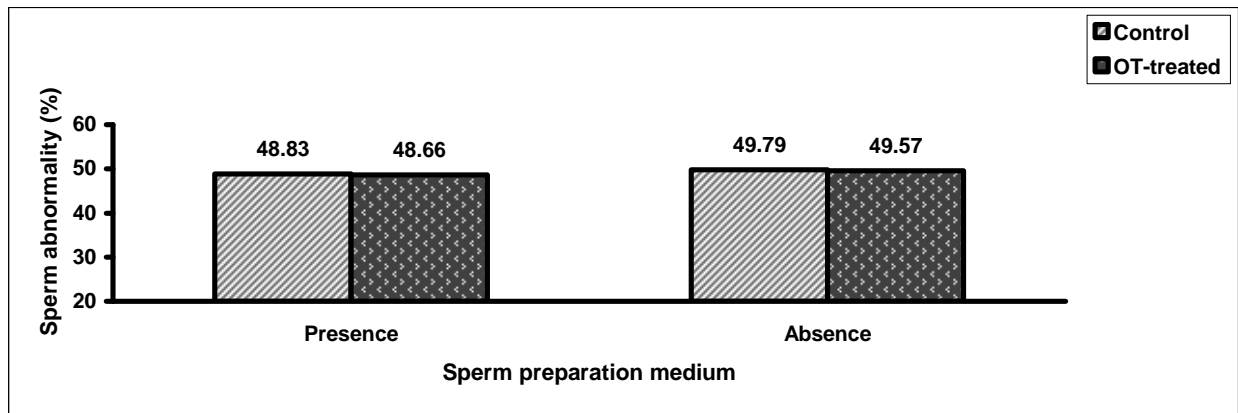


Fig. (4) :The percentage of sperm abnormality of the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• Non-significant difference ($P > 0.05$) from corresponding group.

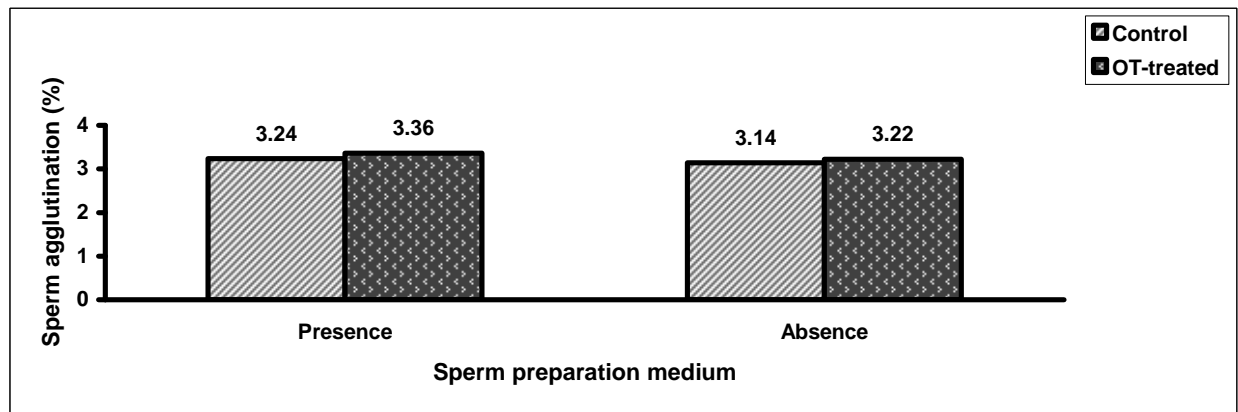


Fig. (5) :The percentage of sperm agglutination of the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• Non-significant difference ($P > 0.05$) from corresponding group.

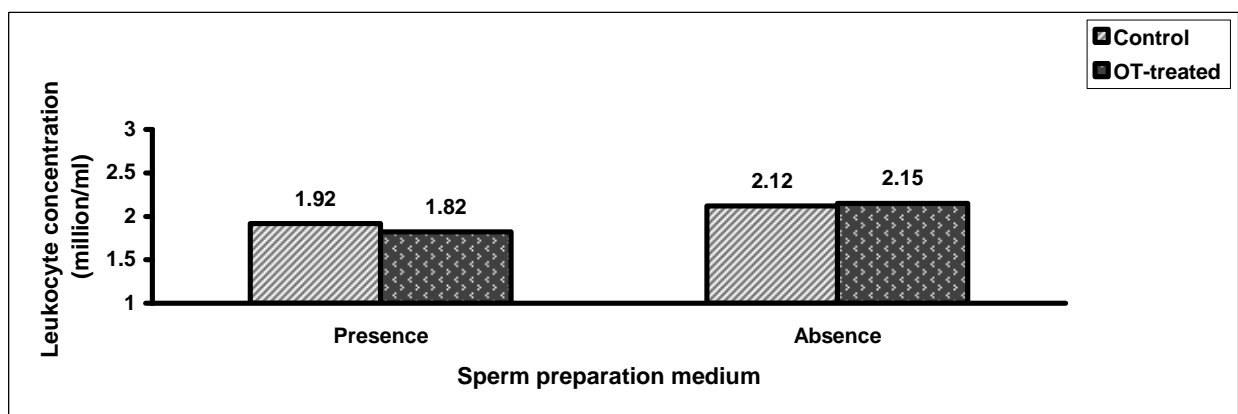


Fig. (6) : Leukocytes concentration of the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• Non-significant difference ($P > 0.05$) from corresponding group.

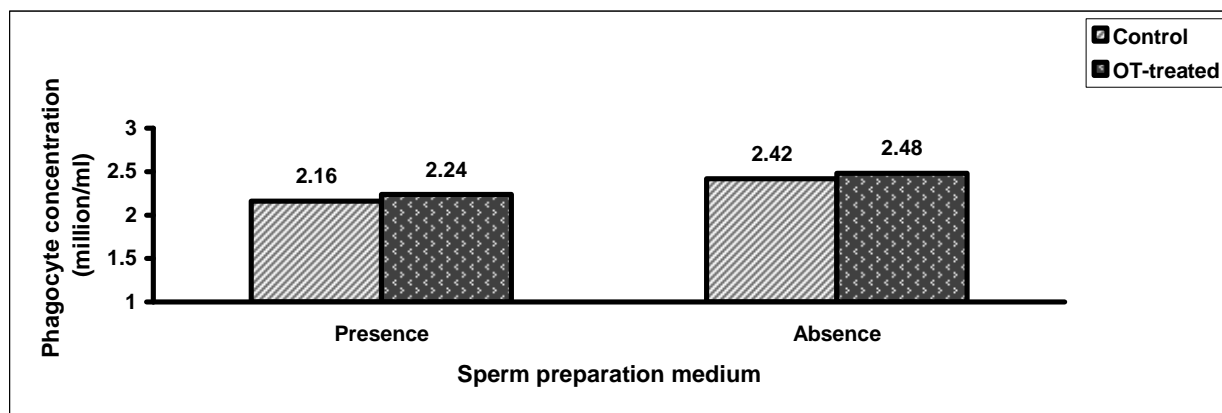


Fig. (7) : Phagocytes concentration of the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

- Non-significant difference ($P > 0.05$) from corresponding group.

Effect of Oxytocin (2 IU/ml) on The Seminal Biochemical Parameters:

In presence of SPM (Table 5), all the biochemical parameters significantly decreased ($P < 0.001$) after sperm activation in control and OT-treated samples as compared to their values before activation. When these biochemical parameters were compared between the OT-treated samples and control samples, the results

revealed that there was no significant difference ($P > 0.05$) in level of TP and CK in the OT-treated samples versus the control samples, while there was a high significant increase ($P < 0.001$) in level of cholesterol, and a high significant decrease ($P < 0.001$) in level of both calcium and fructose in the OT-treated samples versus the control samples.

Table (5)

Effect of Oxytocin (2 IU/ml) on the seminal biochemical parameters following sperm activation with sperm preparation medium.

Biochemical parameters	Before activation	After activation*	
		Control	OT-treated
Total Protein (g/100ml)	4.62 ± 0.65	2.85 ± 0.34	2.92 ± 0.39
Cholesterol (mg/100ml)	31.49 ± 12.50	20.15 ± 8.04	23.65** ± 8.75
Calcium (mg/100ml)	16.75 ± 6.78	12.68 ± 3.76	10.12** ± 3.14
Creatine Kinase (IU/L)	218.23 ± 48.44	164.20 ± 39.56	165.20 ± 39.38
Fructose (mg/100ml)	250.07 ± 63.93	184.58 ± 43.20	180.42** ± 43.16

Values are means ± standard deviation of means. *Significant differences ($P < 0.001$) in all the biochemical parameters between before and after activation. **Significant difference ($P < 0.001$).

In absence of SPM (Table 6), a high significant decrease ($P < 0.001$) was also observed in all the biochemical parameters in control and OT-treated samples as compared to their values before activation. Effect of OT on the biochemical parameters was obvious as a slight

increase and a slight decrease (non-significant) in levels of cholesterol and calcium, respectively, of OT-treated samples as compared with control samples. While level of fructose in the OT-treated samples was significantly ($P < 0.05$) lower than control samples.

Table (6)
Effect of Oxytocin (2 IU/ml) on the seminal biochemical parameters following sperm activation without sperm preparation medium.

Biochemical parameters	Before activation	After activation*	
		Control	OT-treated
Total Protein (g/100ml)	4.80 ± 0.74	2.81 ± 0.46	2.85 ± 0.40
Cholesterol (mg/100ml)	31.86 ± 12.85	20.54 ± 9.13	21.98 ± 8.67
Calcium (mg/100ml)	17.27 ± 5.80	14.17 ± 2.98	13.12 ± 2.68
Creatine Kinase (IU/L)	220.47 ± 44.51	166.22 ± 33.83	165.82 ± 36.98
Fructose (mg/100ml)	253.80 ± 50.83	188.22 ± 45.22	184.71** ± 43.31

Values are means ± standard deviation of means. *Significant differences ($P < 0.001$) in all the biochemical parameters between before and after activation. **Significant difference ($P < 0.05$).

When a comparison was made between the same activation technique with or without SPM, non-significant differences ($P > 0.05$) were found in regard to levels of TP and cholesterol between the two preparation techniques in control and OT-treated samples as shown in (Fig.8 and 9) respectively, while level of calcium significantly decreased ($P < 0.05$) following the use of SPM in the two groups

(Fig.10). The results also revealed that non-significant difference ($P > 0.05$) was observed in regard to level of CK between the two preparation techniques in the two groups (Fig.11), while level of fructose significantly decreased ($P < 0.05$) following the use of SPM in the two groups (Fig.12).

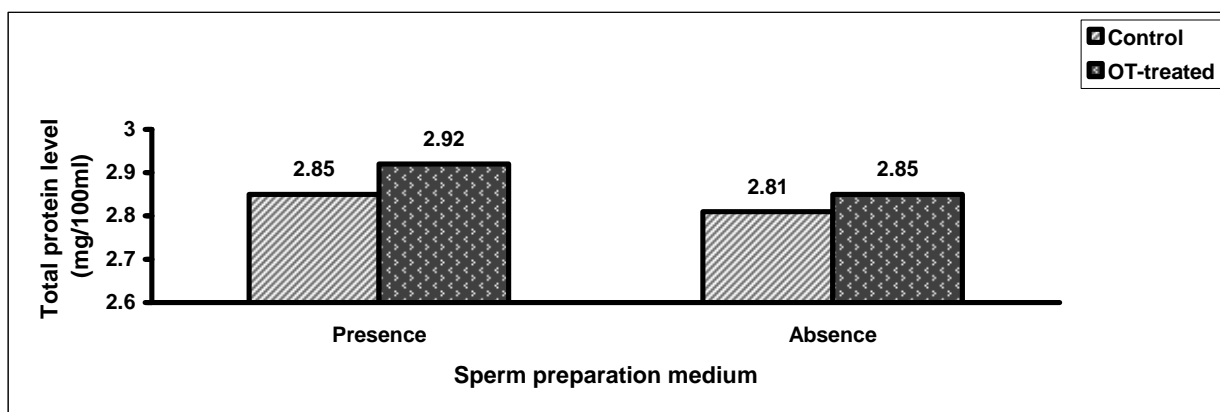


Fig. (8) : Level of total protein in the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

- Non-significant difference ($P > 0.05$) from corresponding group.

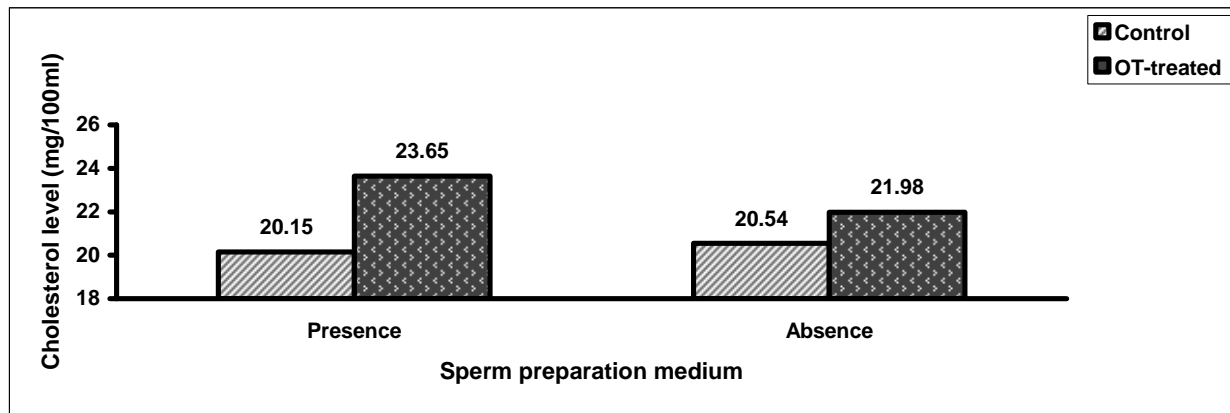


Fig. (9): Level of cholesterol in the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• Non-significant difference ($P > 0.05$) from corresponding group.

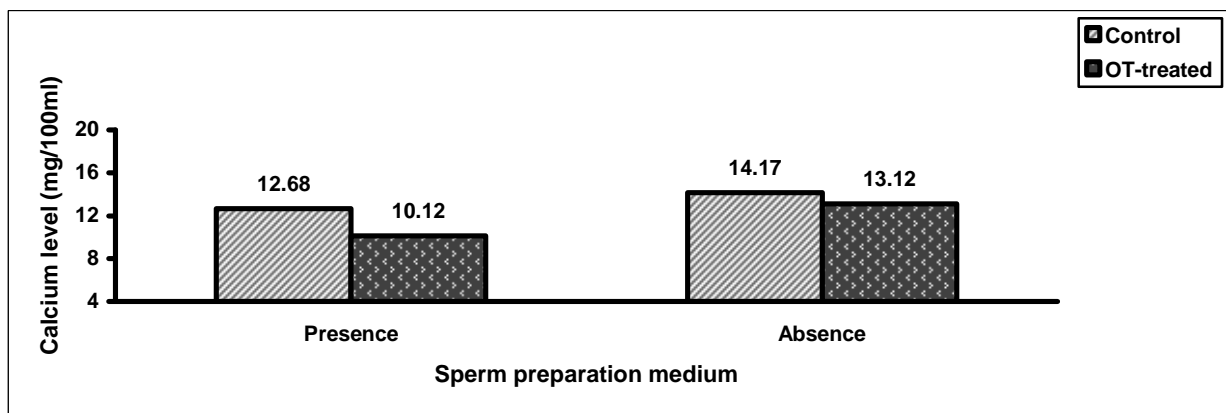


Fig.(10) : Level of calcium in the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• *Significant difference ($P < 0.05$) from corresponding group.

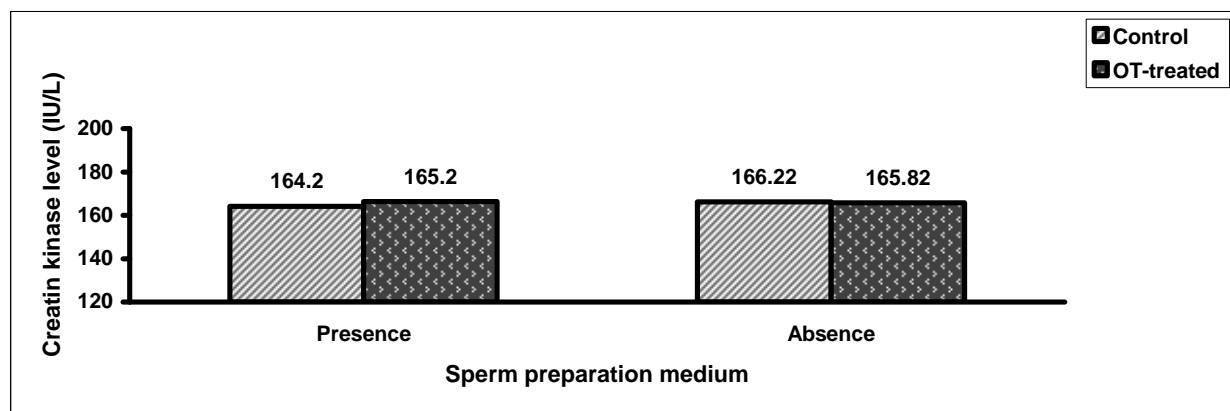


Fig.(11) : Level of creatine kinase in the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

• Non-significant difference ($P > 0.05$) from corresponding group.

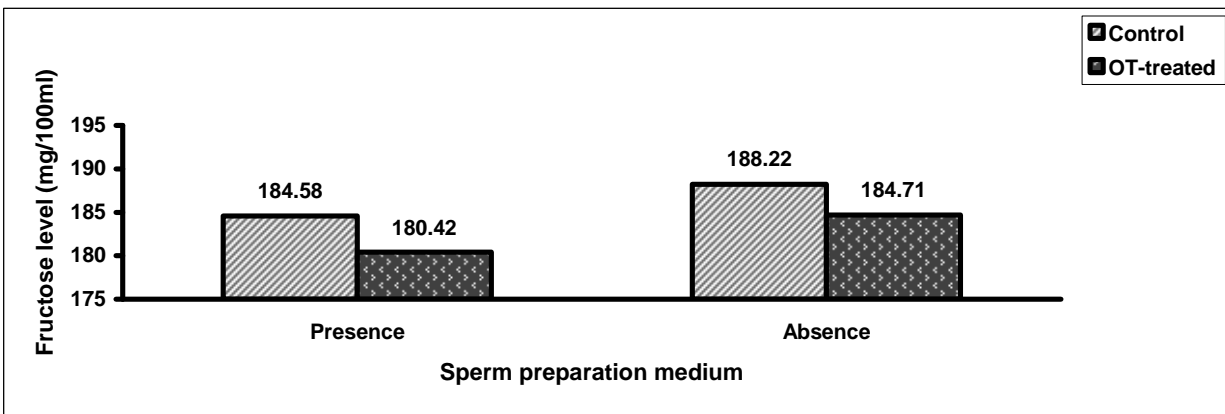


Fig.(12) : Level of fructose in the control and OT-treated samples following sperm activation divided according to presence and absence of sperm preparation medium.

- *Significant difference ($P<0.05$) from corresponding group.

Discussion

Regarding semen analysis of ninety asthenospermic patients, the results indicate a significant increase in liquefaction time associated with a significant decrease in semen volume as compared to the reported values in the available literature⁽¹¹⁾. Inadequate liquefaction of the semen may indicate a deficiency of prostatic enzymes⁽¹⁹⁾. Low semen volume can be indicative of retrograde ejaculation, ejaculation duct obstruction, and androgen deficiency⁽²⁰⁾. The mean of sperm concentration of the studied cases was within normal limit, majority of them had sperm concentration below the normal limit. The decline in sperm concentration may be due to the lifestyle risk factors such as: cigarette smoking, alcohol consumption, chronic stress, and nutritional deficiencies⁽²¹⁾. The low percentage of sperm motility and sperm grade activity of the semen samples in the present study may be due to many factors such as: spermatozoal structural defects, genital tract infection, antisperm antibodies, and partial ductal obstruction⁽²²⁾. The presence of abnormal sperm forms in the studied cases might reflect disturbances during spermiogenesis, spermiation, and sperm passage during epididymis⁽²³⁾. Increase percentage of agglutinated sperm in the semen of the studied cases may attributed to infections of the male genital tract and presence of antisperm antibodies in the seminal plasma or

to infections of the male genital tract. Mazumder and Levine⁽²⁴⁾ found that infections of the male genital tract are associated with antisperm antibodies formation and sperm agglutination. Elevation of leukocytes and phagocytes concentrations of the semen samples above the normal limit stated by WHO⁽¹¹⁾ can be due to a variety of pathologic changes. The previous reports showed that excessive presence of leukocytes in semen of the infertile men can interfere with the quality of the semen and more importantly with the functional status of sperm fertilizing ability^(25; 26).

The significant increase in sperm grade activity, when OT was added at concentration of 2 IU/ml and the tendency for a decrease in this parameter with increasing OT concentration are in agreement with a previous study⁽¹²⁾ who suggested that 2 IU/ml of OT is the best dose which exhibit varying degree of stimulation on sperm grade activity. In presence of SPM, the significant reduction observed in sperm concentration after the incubation period in the OT-treated and control samples may be due to semen dilution during sperm activation. The significant improvement observed in the percentage of sperm motility and sperm grade activity in both groups may be attributed to presence of SPM which consists of a basic salt solution and energy sources which cause an increase in sperm activity⁽²⁷⁾. The results showed that OT caused an increase in sperm motility

and sperm grade activity, this indicates that OT has got a stimulatory effect on sperm movement. In a previous study, Al-Abayachi⁽¹²⁾ concluded that OT increases sperm progressive movement (grade of activity) when proper artificial medium is available after half an hour, while it increases sperm vigorous (hyperactivity) movement in activated sperm after 2-4 hours of incubation. In absence of SPM, the results showed lack of any clear statistically significant change in all the semen parameter following the incubation period in both OT-treated and control groups except the sperm concentration which reduced significantly. On the other hand, the results showed that OT induced responses (although non significant) in both sperm motility and grade of activity as compared to the control. These results can be interpreted on the ground that sperm motility induced by OT require essential nutrients in SPM which can not be provided by normal saline alone. It is well known that hyperactive motility occurs when the sperm were incubated in culture medium that is a balanced salt solution containing energy providing substances such as lactate, pyruvate, glucose, and a protein such as albumin⁽²⁸⁾.

Regarding the effect of OT on the seminal biochemical parameters, the results showed, in presence of SPM, a significant reduction observed in all the biochemical parameters after incubation period in OT-treated and control samples, this may be attributed to semen dilution as a result of addition of SPM, OT, and normal saline during sperm activation. The results revealed no significant differences in the level of TP between the OT-treated and control samples, while cholesterol level in OT-treated samples was significantly higher than the control samples. This finding suggests that OT may be caused loss of cholesterol from the plasma membrane of the sperm, because we found increase level of this component in the external medium. It has been reported that when sperm are incubated in a capacitating medium *in vitro*, the sperm cholesterol content gradually decreases^(29;30). A significant decrease in level of calcium in the external medium of the OT-treated samples as compared with the control

samples suggests that presence of OT combination with SPM may modify cellular calcium transport by incorporating into the sperm cell plasma membrane and transporting calcium across the plasma membrane, resulting in high intracellular calcium concentrations due to an influx of extracellular calcium. This finding suggests that OT stimulates human sperm motility at least in part by a mechanism which increased intracellular calcium concentration. It has been reported that the ability of the mature mammalian sperm to maintain a coordinated and forward motility is dependent upon intracellular free calcium⁽³¹⁾. When the level of CK was compared between the OT-treated group and control group, no significant differences were observed between the two groups. In a previous study, Huszar *et al.*⁽³²⁾ measured the value of sperm CK activity in the initial specimens and in the swim-up fractions of fertile and infertile men, they demonstrated that CK activities in the self-migrated sperm fractions were significantly improved downward compared to the initial semen specimens. Considering the fructose level in the OT-treated and control samples, it is obvious that it was lower in the former group than in the later group. This finding may be explained on the ground that increased utilization of fructose by the activated sperm in the OT-treated samples as compared with the control samples. This finding also indicated that sperm of OT-treated samples were more active than control samples because the more active cells used more fructose⁽³³⁾. In absence of SPM, a slight increase and a slight decrease (non-significant) in levels of cholesterol and calcium, respectively were observed in OT-treated samples as compared with the control samples, while the level of fructose in the OT-treated samples was significantly lower than that of the control samples.

It is of great interest to note that these findings provide another evidence for the role of OT as a stimulating agent to improve the sperm motility of the asthenospermic specimens. In the same time, these data revealed indirectly the importance of SPM in improving the motility

characteristics of sperm since a comparison between the two cases (presence and absence of SPM) indicated that the most significant changes were noted only when SPM was used.

Conclusions

Addition of OT (2 IU/ml) to semen of asthenospermic patients caused a significant increase in the percentage of sperm motility and grade of activity, and presence of SPM was found to have synergistic action on the improvement of sperm motility characteristics. The mechanism of action of OT on human sperm and its stimulatory effect on sperm motility parameters may be due to two mechanisms:

- a. Increasing Ca^{2+} transport across the plasma membrane that increase intracellular calcium concentration.
- b. Modification of the cholesterol content of the plasma membrane that cause loss of cholesterol from the plasma membrane of the sperm.

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الخلاصة

تم فحص عينات المنى لـ 90 مريضاً يعانون من وهن النطف، ثم قسمت إلى ثلاثة مجاميع رئيسية. في المجموعة الأولى، تم استقصاء إضافة الأوكسيتوسين بثلاثة تراكيز

(6,2,4 وحدة دولية/مل) مع وسط تحضير النطف، وقد استخدمت التغيرات في اختبارات وظيفة النطف وكيميائية المنى كمؤشر لتأثير الإضافة. أظهرت النتائج زيادة معنوية في درجة نشاط النطف عند التركيز 2 وحدة دولية/مل مع الميل إلى تناقص هذا المعيار الحركي في التركيز الأعلى للأوكسيتوسين. تم تطبيق التركيز 2 وحدة دولية/مل للأوكسيتوسين مع وجود وسط تحضير النطف وعدم وجوده في المجموعتين الثانية والثالثة، على التوالي. عند وجود وسط تحضير النطف، لوحظت زيادة معنوية في خصائص الحركة (النسبة المئوية للنطف المتحركة ودرجة نشاط النطف). أما عند غياب وسط تحضير النطف، أظهرت النتائج عدم حدوث أي تغيير معنوي واضح في هذين المعيارين. تشير هذه النتائج إلى التأثير المحفز للأوكسيتوسين عند التركيز 2 وحدة دولية/مل على خصائص حركة النطف، وقد أدى استعمال وسط تحضير النطف إلى دعم ذلك التأثير.

تم قياس مستوى البروتين الكلي، الكوليستيرول، الكالسيوم، إنزيم الكرياتين كيناز، والفركتوز في السائل المنوي وفي الجزء الراق من عالق النطف في عينات السيطرة والعينات المعاملة بالأوكسيتوسين. وفقاً للنتائج، ربما يكون الأوكسيتوسين سبباً في فقدان الكوليستيرول من الغشاء البلازمي للنطف وبالتالي زيادة مستواه وبشكل ملحوظ في الوسط الخارجي. كذلك فإن الأوكسيتوسين ربما أدى إلى تحويل نقل الكالسيوم الخلوي عبر الغشاء البلازمي وبالنتيجة زيادة تركيز الكالسيوم داخل الخلية وانخفاض مستواه وبشكل معنوي ملحوظ في الوسط الخارجي. بالإضافة إلى الانخفاض المعنوي الملحوظ في مستوى الفركتوز في الوسط الخارجي.