

## Original Articles

# Relationship of Seminal Biochemical Parameters and Serum Reproductive Hormones with Sperm Function Tests in Asthenospermic Patients

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### Abstract

**Aim:** The aim of this study is to determine the correlation between levels of certain seminal biochemical parameters and serum reproductive hormones, on the one hand, and sperm function tests, on the other hand, in asthenospermic patients.

**Patients and Methods:** Sixty asthenospermic patients and twenty fertile men as a control group were included in this study. Semen samples were collected to perform seminal fluid analysis. Total protein, cholesterol, calcium, creatine kinase, and fructose were measured in the seminal plasma. Blood samples were collected for hormonal assay of serum reproductive hormones: testosterone, prolactin, luteinizing hormone, and follicle-stimulating hormone.

**Results:** The results revealed a significant positive correlation between the seminal cholesterol level and sperm concentration. A similar correlation was observed between the seminal calcium level and motility characteristics (the percentage of sperm motility and sperm grade activity). A significant negative correlation was found between the seminal fructose level and sperm concentration; in addition, a negative correlation was found between the fructose level and sperm motility characteristics. A significant negative correlation was found between the level of serum prolactin and sperm concentration. Also, a similar negative correlation was found between the level of serum follicle-stimulating hormone and sperm concentration.

**Conclusions:** The evaluation of seminal biochemical parameters and serum reproductive hormones provide useful information for clinical studies in the status of male infertility, especially when these parameters are correlated with sperm function tests.

**Keywords:** Asthenospermia, seminal biochemistry, reproductive hormones, sperm function tests.

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### Introduction

The laboratory evaluation of male infertility can be separated into three main components: the semen analysis, endocrine parameters, and

immunologic parameters.<sup>1</sup> Semen analysis provides information concerning the production of spermatozoa and their abnormalities, and disturbances in the function of the genital accessory glands that affect sperm function.<sup>2</sup> The

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objectives for obtaining male reproductive hormone data in clinical situations are usually to allow diagnosis of endocrine deficiencies, predict deficiencies in semen quality, or explore the potential to predict the likelihood of obtaining usable sperm samples for assisted reproductive techniques.<sup>3</sup>

Seminal biochemistry provides useful diagnostic information at the onset of an infertility evaluation and during specific treatment when information about seminal markers for the secondary sex glands is of value.<sup>4</sup> Seminal plasma contains chemical substances or factors that influence fertilizing capacity,<sup>5</sup> these chemical substances can be assigned to specific organs or compartments of the male genital system. These substances can thus serve as diagnostic indicators.<sup>6</sup>

## **Materials and Methods**

### **Patients**

Sixty asthenospermic patients were included in this study during their attendance at the Institute of Embryo Research and Infertility Treatment, University of Al-Nahrain, Iraq. Twenty fertile men were matched with the age of the patients that served as the control group. Informed consent was obtained from all subjects. The study was approved by the local Ethical Committee.

### **Seminal Fluid Analysis (SFA)**

Semen samples were collected by masturbation after an intercourse abstinence period ranging between 2-7 days, and incubated at 37°C for liquefaction. After liquefaction, semen samples were examined macroscopically and microscopically to evaluate semen quality according to published recommendations.<sup>7</sup> Sperm grade activity (quality of forward movement) was assessed on the Macleod Scale (0-4).<sup>8</sup>

### **Measurement of Seminal Biochemical**

### **Parameters**

Five biochemical parameters were measured in the seminal plasma of the patients, as follows:

- **Total Protein (TP):** Total protein was determined according to the Biuret method reported by Silverman et al.<sup>9</sup> A linear kit was used in this colorimetric method.
- **Cholesterol:** Cholesterol concentration was determined according to the method of Richmond.<sup>10</sup> A linear kit was used in this enzymatic colorimetric method.
- **Calcium:** Calcium concentration was estimated applying the method reported by Baginski et al.<sup>11</sup> A kit of Giesse was used in this colorimetric method.
- **Creatine Kinase (CK):** The kinetic determination method was followed in the estimation of CK activity.<sup>12</sup> Enzyme activity was assayed with a Syrbio kit.
- **Fructose:** The fructose level was estimated according to the method of Mann.<sup>13</sup>

### **Hormonal Examination**

Serum concentrations of four reproductive hormones [testosterone, prolactin (PRL), luteinizing hormone (LH), and follicle-stimulating hormone (FSH)] were measured by using mini-VIDAS (VIDAS 12 model, 1992) through an enzyme linked fluorescent assay (ELFA) technique.

### **Statistical Analysis**

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS), version 10.5, computer software. The results were reported as a mean  $\pm$  SD. Statistical comparisons between groups were made using the 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), where  $p < 0.05$  was regarded as statistically significant. Pearson correlation coefficients were calculated to check the relationship between variables.<sup>14</sup>

## Results

### Seminal Fluid Analysis (SFA)

The results of SFA (Table 1) revealed that the patients had higher liquefaction times than the control group ( $P < 0.05$ ), while semen volume was lower in the patients when compared to the control group ( $P < 0.05$ ). All semen samples had normal alkaline pH value, which were within the normal limit. No significant difference between the studied cases and the control was seen in regard to sperm concentration. The percentage of motile sperm and mean of sperm grade activity (the quality of forward movement) were lower in the patients when compared to the control group ( $P < 0.001$ ). The patients had more abnormal sperm morphology ( $P < 0.05$ ) with a higher percentage of sperm agglutination ( $P < 0.05$ ) when compared with the control group. Regarding seminal fluid infection, the results revealed that the concentrations of leukocytes and phagocytes were higher in the patients when compared to the control group ( $P < 0.05$ ).

### Levels of the Seminal Biochemical Parameters and their Correlation with Sperm Function Tests

The means of the seminal biochemical parameters of the patients are shown in Table (2). The correlation between values of these parameters and sperm function tests indicated that there was no significant correlation ( $P > 0.05$ ) between the level of seminal TP and all sperm function tests. However, a significant positive correlation ( $r = +0.29$ ,  $P < 0.05$ ) was found between the level of seminal cholesterol and sperm concentration (Figure 1). A significant positive correlation was found between the level of seminal calcium and the percentage of sperm motility ( $r = +0.27$ ,  $P < 0.05$ ) and sperm grade activity ( $r = +0.37$ ,  $P < 0.05$ ) (Figures 2 and 3). A non-significant correlation ( $P > 0.05$ ) was found between the level of seminal CK and sperm function tests. The level of seminal fructose revealed a significant negative correlation with sperm concentration ( $r = -0.41$ ,  $P < 0.001$ ), the percentage of sperm motility ( $r = -0.28$ ,  $P < 0.05$ ), and sperm grade activity ( $r = -0.32$ ,  $P < 0.05$ ) as shown in Figures 4, 5 and 6, respectively.

**Table (1): Semen physical characteristics of the patients and the control group.**

Semen Characters	Value in patients (Mean $\pm$ SD)	Value in control (Mean $\pm$ SD)	Normal Value*
Liquefaction time (minute)	35.20* $\pm$ 8.52	24.56 $\pm$ 3.48	< 30
Semen volume (ml)	2.54* $\pm$ 0.95	3.83 $\pm$ 0.72	2-6 ml
pH	7.85 $\pm$ 0.14	7.62 $\pm$ 0.12	7.4-8.2
Sperm concentration (million/ml)	47.57 $\pm$ 15.45	50.76 $\pm$ 8.75	> 20
Sperm motility (%)	30.47** $\pm$ 12.25	70.42 $\pm$ 5.92	> 60
Grade of activity (Macleod scale)	1.61** $\pm$ 0.42	3.15 $\pm$ 0.55	$\geq$ 3
Sperm abnormality (%)	50.30* $\pm$ 7.53	32.28 $\pm$ 5.43	< 50
Sperm agglutination (%)	3.62* $\pm$ 2.46	1.54 $\pm$ 0.68	Negative
Leukocytes concentration (million/ml)	2.13* $\pm$ 1.42	0.58 $\pm$ 0.23	< 1
Phagocytes concentration (million/ml)	2.40* $\pm$ 1.40	0.24 $\pm$ 0.15	< 0.5

\* Significant difference ( $P < 0.05$ ), \*\* Significant difference ( $P < 0.001$ ).

● World Health Organization Reference values of semen variables. In: WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. 3<sup>rd</sup> ed. Cambridge University Press. Cambridge, 1992. <sup>7</sup>

**Table (2): Level of the biochemical parameters in the seminal plasma of the patients.**

Biochemical parameters	Mean $\pm$ SD	Range
Total Protein (g/100ml)	4.71 $\pm$ 0.70	2.80 – 6.40
Cholesterol (mg/100ml)	31.58 $\pm$ 12.57	8.50 – 56.80
Calcium (mg/100ml)	16.58 $\pm$ 4.77	8.00 – 30.00
Creatine Kinase (IU/L)	218.80 $\pm$ 46.64	122.00 – 320.00
Fructose (mg/100ml)	251.93 $\pm$ 57.29	128.00 – 355.00

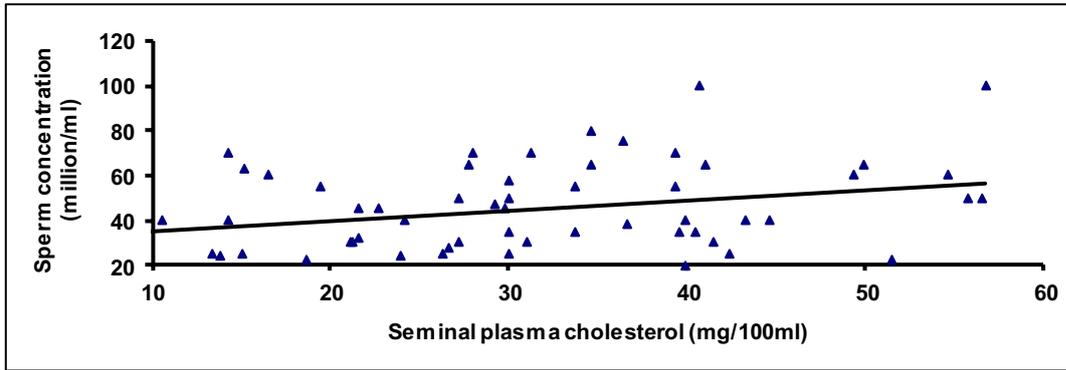


Figure (1): Correlation of seminal cholesterol level and sperm concentration in the patients ( $r = +0.29$ ,  $P < 0.05$ ).

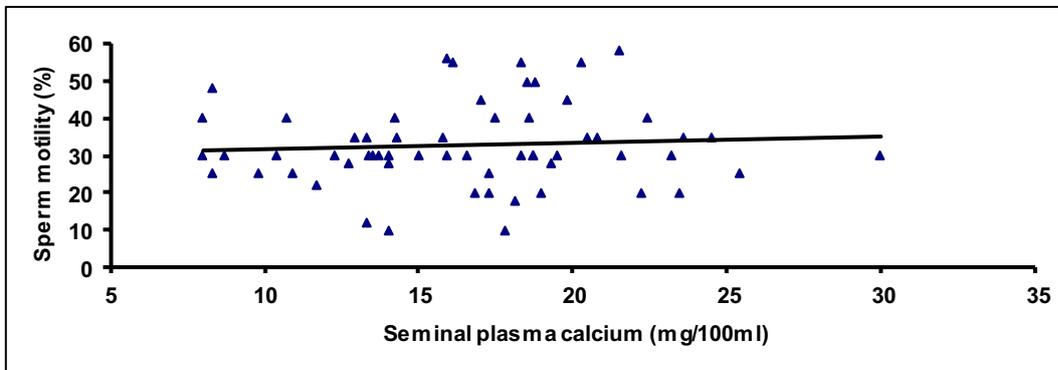


Figure (2): Correlation of seminal calcium level and the percentage of sperm motility in the patients ( $r = +0.27$ ,  $P < 0.05$ ).

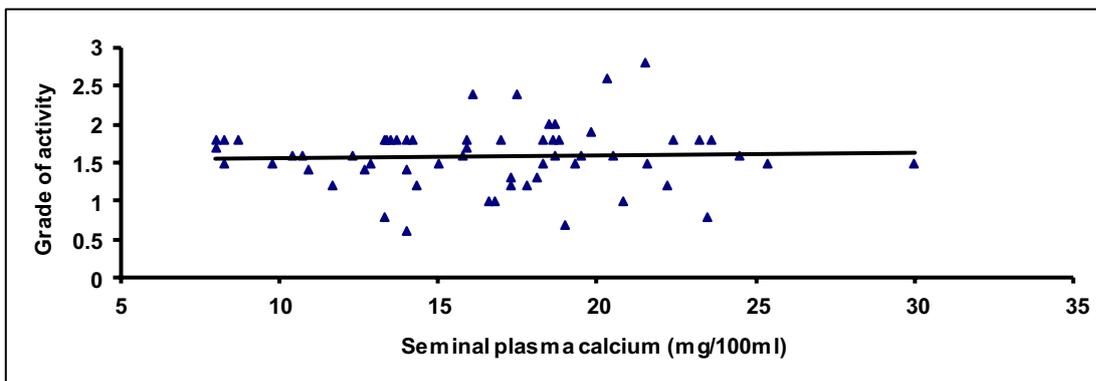


Figure (3): Correlation of seminal calcium level and sperm grade activity in the patients ( $r = +0.37$ ,  $P < 0.05$ ).

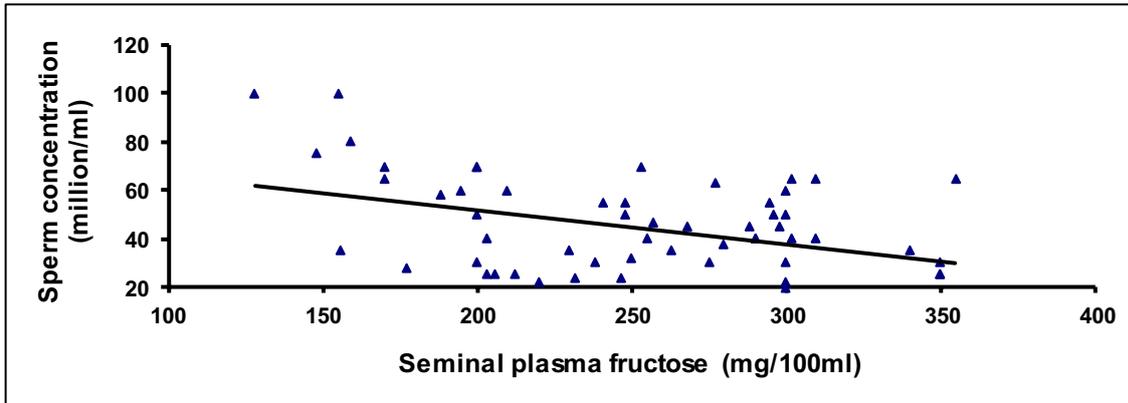


Figure (4): Correlation of seminal fructose level and sperm concentration in the patients ( $r = -0.41, P < 0.001$ ).

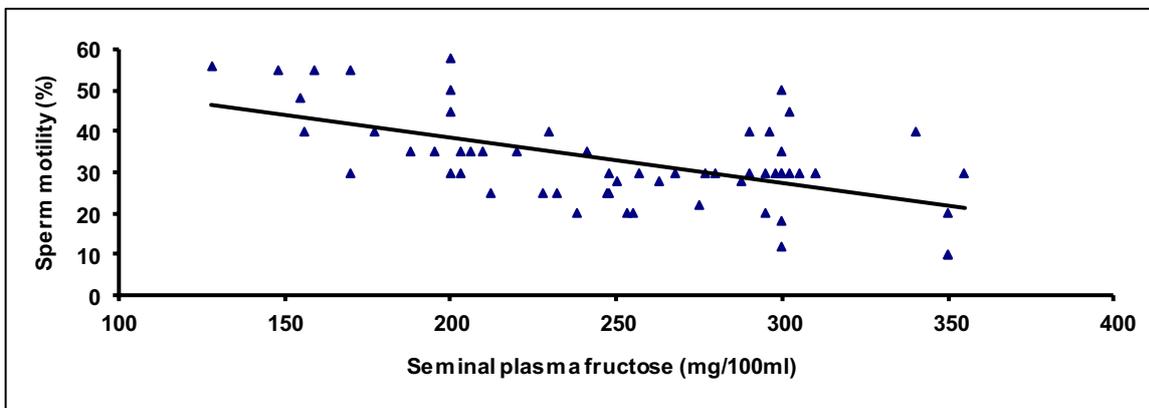


Figure (5): Correlation of seminal fructose level and the percentage of sperm motility in the patients ( $r = -0.28, P < 0.05$ ).

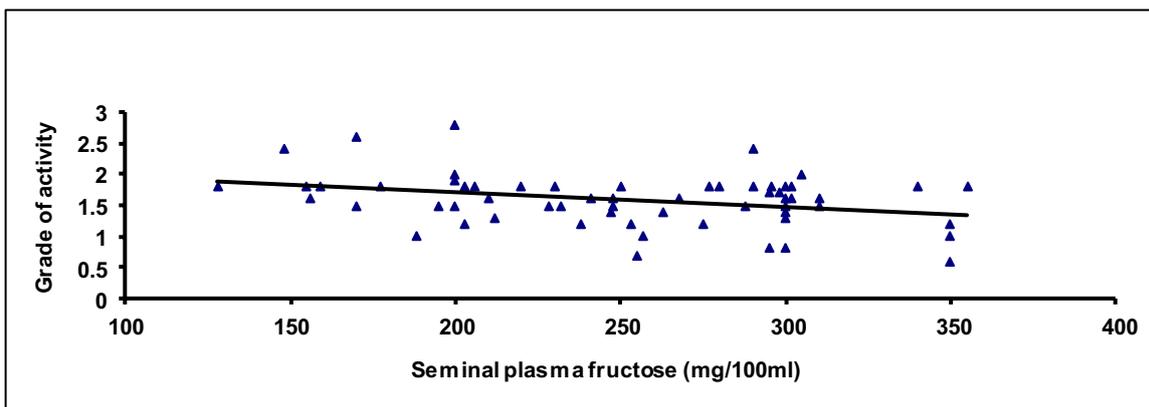


Figure (6): Correlation of seminal plasma fructose level and sperm grade activity in the patients ( $r = -0.32, P < 0.05$ ).

### Level of the Serum Reproductive Hormones and their Correlation with Sperm Function Tests

The levels of the reproductive hormones in the serum of the patients and the control group are shown in Table (3). The levels of these hormones were within the normal values except PRL which was significantly higher ( $P < 0.001$ ) in the patients than the control group, and LH which was significantly lower ( $P < 0.05$ ) in the patients than the control group.

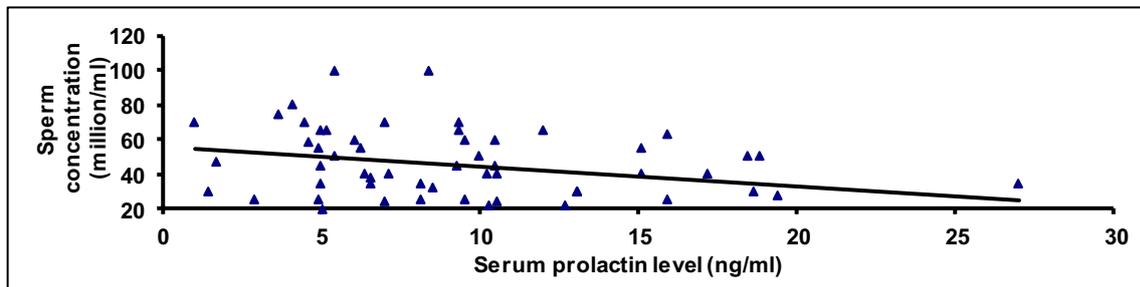
Studying the correlation between the levels of these hormones and sperm function tests revealed that non-significant correlation ( $P > 0.05$ ) was observed between the levels of both serum testosterone and serum LH with various sperm function tests, while a significant negative correlation was found between the levels of both serum PRL ( $r = -0.29$ ,  $P < 0.05$ ) and serum FSH ( $r = -0.28$ ,  $P < 0.05$ ) with sperm concentration as shown in Figures (7 and 8), respectively.

**Table (3): Level of the reproductive hormones in serum of the patients and the control group.**

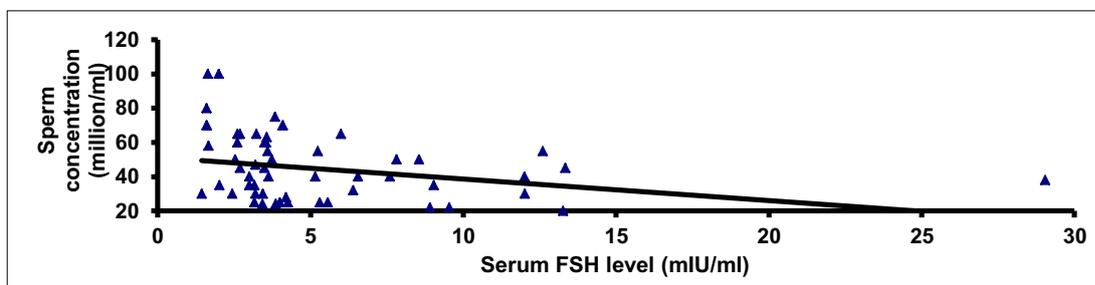
Hormone	Value in patients (Mean $\pm$ SD)	Value in control (Mean $\pm$ SD)	Normal Value*
Testosterone (ng/ml)	6.43 $\pm$ 2.66	7.23 $\pm$ 1.45	3.00 – 9.00
Prolactin (ng/ml)	9.88** $\pm$ 5.23	4.78 $\pm$ 2.15	1.50 – 7.50
Luteinizing hormone (mIU/ml)	3.25* $\pm$ 1.82	5.83 $\pm$ 1.54	4.60 – 10.0
Follicle-stimulating hormone (mIU/ml)	5.31 $\pm$ 4.66	6.21 $\pm$ 3.12	1.70 – 12.00

\* Significant difference ( $P < 0.05$ ), \*\* Significant difference ( $P < 0.001$ ).

● Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. Laboratory values of clinical importance (Appendices) In: Harrison's Principles of Internal Medicine. 15th ed (Vol. 2). McGraw-Hill Companies. USA. 2001. <sup>15</sup>



**Figure (7): Correlation of serum prolactin level and sperm concentration in the patients ( $r = -0.29$ ,  $P < 0.05$ ).**



**Figure (8): Correlation of serum FSH level and sperm concentration in the patients ( $r = -0.28$ ,  $P < 0.05$ ).**

## **Discussion**

Focusing on the evaluation of the patients' semen analyses, the results indicate a significant increase in the liquefaction time associated with a significant decrease in semen volume when compared to the control group. Inadequate liquefaction of ejaculated semen may indicate a deficiency of prostatic enzymes.<sup>16</sup> Low semen volumes could be attributed to abnormalities in accessory sex gland fluid synthesis or secretion.<sup>17</sup> The low percentage of sperm motility and sperm forward progression in the studied cases may be due to many factors such as spermatozoal structural defects, genital tract infection, antisperm antibodies, and partial ductal obstruction.<sup>18</sup> The presence of abnormal sperm forms in the studied cases can be due to a variety of insults including infection, testicular stress (e.g. varicocele, poor sperm production, or environmental toxins), or a hormonal imbalance.<sup>16</sup> An increased percentage of agglutinated sperm in the semen of the patients may be attributed to the presence of antisperm antibodies in the seminal plasma or to infections of the male genital tract. Shibahara et al.<sup>19</sup> reported that antisperm antibodies bound to sperm surfaces causing a relatively high incidence of asthenospermia. Most of the semen samples showed elevated leukocytes and phagocytes concentrations above the normal limit stated by WHO.<sup>7</sup> Leukocytes in the semen can originate from various sources, namely, the urethra, prostate, seminal vesicles, and epididymidis; therefore, their presence represents a variety of pathologic changes.<sup>16</sup>

In agreement with other investigators,<sup>20, 21</sup> we have found a wide range of protein levels in the seminal plasma of the infertile patients. Non-significant correlation was found between the level of seminal TP and sperm function tests. Regarding sperm concentration, this may indicate that spermatozoa do not contribute protein significantly to seminal plasma. These results agree with the finding of a previous study.<sup>21</sup> The level of seminal cholesterol in the patients was lower than those of other workers.<sup>20, 22</sup> This could be attributed to the different methods used

to perform the biochemical analysis in this study when compared with the previous works. A significant positive correlation was found between the seminal cholesterol level and sperm concentration. This finding is in agreement with the finding of a previous study.<sup>20</sup> These results may be due to the possibility of the loss of cholesterol molecules from the spermatozoa cell membrane. The exchange of cholesterol between sperm cells and seminal plasma was shown by the striking correlation between the lipid composition of seminal plasma with that of sperm.<sup>23</sup> The level of seminal calcium in the patients was lower than those reported in previous studies.<sup>24, 25</sup> On the other hand, it was higher than the value which was reported in another study.<sup>26</sup> These differences may be due to the degree of asthenospermia in the original semen samples. A significant positive correlation was found between the level of seminal calcium and the motility characteristics of the sperm (sperm motility and sperm grade activity). These observations are in agreement with the findings of Prien et al.,<sup>26</sup> whereas they disagree with results of Hong et al.<sup>27</sup> In spite of all these conflicting results, findings of our study suggest that, sperm motility is indeed related to the seminal  $Ca^{2+}$  content, and this relationship between  $Ca^{2+}$  and sperm motility characteristics seems logical according to the suggestion that  $Ca^{2+}$  may activate ATP and drive flagella movement of sperm.<sup>28</sup>

In the current study, the level of seminal CK was lower than that recorded by other investigators.<sup>29</sup> This difference may be due to the choice of patients, since patients' selection in the present study included asthenospermic infertile patients only while in that study infertile patients with various types of infertility were included (i.e. oligozoospermia, asthenospermia, and teratospermia). A non-significant correlation was found between the level of seminal CK and sperm function tests. These results are in broad agreement with those of a previous research.<sup>29</sup> In agreement with previous studies<sup>30, 31</sup>, we observed that the level of seminal fructose was within the normal levels reported in available literature. We also observed a wide range of

fructose levels in the seminal plasma. This finding may be due to the fact that although the fructose level in human semen is under androgenic regulation, many factors such as storage, frequency of ejaculation, blood glucose levels, and nutritional status can also affect the seminal fructose level.<sup>32</sup> Fructose levels in the seminal plasma of the patients showed a significant negative correlation with sperm concentration. A similar result was reported by other researchers.<sup>22,21</sup> This result is logical, since the spermatozoa utilize fructose as metabolic energy.<sup>33</sup>

Also, the present result could be explained on the grounds that the feedback mechanism of low spermatogenesis induces an increase in pituitary gonadotrophins thus stimulating testosterone production which in turn induces fructose synthesis.<sup>34</sup> Our results are also in agreement with Lewis-Jones et al.<sup>35</sup> with regard to the significant negative correlation between the level of seminal fructose and sperm motility parameters (sperm motility and sperm grade activity). These results suggest that the more active sperm used more fructose as a source of energy, since fructose has been reported to be the source of energy needed for the motility of the sperm.<sup>36</sup>

In the present study, a non-significant difference was found between the patients and the control group in regarding to the level of serum testosterone. This finding may be explained on the grounds that normal testosterone levels could be seen in cases of germinal compartment failure, some cases of partial androgen resistance, and sometimes in cases with possible hypogonadotropic hypogonadism.<sup>37, 38</sup> A non-significant correlation was found between the level of serum testosterone of the asthenospermic patients and their sperm function tests. Uhler et al.<sup>39</sup> reported that a non-significant correlation was found between serum testosterone and any semen quality variable in normal couples. The significant elevated levels of PRL when compared with the control group were in agreement with those found in a previous study.<sup>40</sup>

A significant negative correlation was found between the level of serum PRL and the concentration of sperm. This negative correlation may reflect impaired spermatogenesis in the patients who complained of hyperprolactinemia. It has been reported that men with hyperprolactinemia usually are sexually impotent, and often have a low sperm concentration.<sup>41</sup>

The significant reduction in the level of serum LH in the asthenospermic patients when compared with the control group could be due to some sort of pituitary or hypothalamic defect (hypogonadotropic hypogonadism) especially in the presence of hyperprolactinemia in the studied cases.<sup>42</sup> A non-significant correlation was observed between the level of serum LH and various sperm function tests. The same finding was reported in normal couples.<sup>39</sup> A non-significant difference was found between the patients and the control group in regards to the level of serum FSH. The current results showed that the serum FSH was significantly negatively correlated with sperm concentration. Similar results were also reported by previous studies in infertile men<sup>43</sup> and in fertile men.<sup>39</sup>

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## علاقة المعايير الكيمياءحياتية المنوية والهرمونات التكاثرية للمصل مع اختبارات وظيفة النطف في مرضى وهن النطف

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### الملخص

**الهدف:** دراسة علاقة الترابط بين مستوى بعض معايير الكيمياء الحيوية الخاصة بالسائل المنوي والهرمونات التكاثرية في المصل من جهة، واختبارات وظيفة النطف من جهة أخرى في مرضى وهن النطف.

**المرضى وطرق البحث:** تضمن هذا البحث 60 مريضاً يعانون من وهن النطف و20 رجلاً من الأصحاء الخصيين كمجموعة سيطرة. جمعت عينات المنى لإجراء تحليل السائل المنوي. تم قياس كل من البروتين الكلي، الكوليستيرول، الكالسيوم، إنزيم الكرياتين كينيز، والفركتوز في السائل المنوي. جمعت عينات الدم لإجراء التحليل الهرموني لأربعة هرمونات تكاثرية في المصل وهي: التيستوستيرون، والبرولاكتين، والهرمون اللوتيني، والهرمون المحفز للجريب.

**النتائج:** لوحظت علاقة موجبة معنوية بين مستوى الكوليستيرول المنوي وتركيز النطف، وقد لوحظت علاقة مشابهة بين مستوى الكالسيوم المنوي وخصائص الحركة (النسبة المئوية للنطف المتحركة ودرجة نشاط النطف). بينما كان هناك ترابط سالب وبشكل معنوي ملحوظ بين مستوى الفركتوز المنوي وتركيز النطف من جهة وبشكل معنوي بين مستوى الفركتوز المنوي وخصائص الحركة (النسبة المئوية للنطف المتحركة ودرجة نشاط النطف) من جهة أخرى. وجدت علاقة ترابط سالبة وبشكل معنوي بين مستوى بروتين المصل وتركيز النطف، وقد وجدت علاقة مشابهة أيضاً بين مستوى الهرمون المحفز للجريب في المصل وتركيز النطف.

**الختامة:** تقييم معايير الكيمياء الحيوية الخاصة بالسائل المنوي والهرمونات التكاثرية في المصل يوفر معلومات مفيدة للدراسات السريرية المتخصصة في العقم في الذكور، خصوصاً عند دراسة علاقة الترابط بين هذه المعايير واختبارات وظيفة النطف.

**الكلمات الدالة:** وهن النطف، الكيمياء الحيوية للمني، الهرمونات التكاثرية، اختبارات وظيفة النطف.