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Impact of Male Reproductive Hormones on Sperm Activity via Regulation of Semen Antioxidant Levels

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Abstract

New relationship between Hormones and Antioxidants has been proposed. The levels of several hormones correlate well with different Antioxidants activities in different tissues. The study conducted to find a relationship between serum male reproductive hormones andsemen Antioxidantlevels and sperm activity.

Samples of infertile group consist of Asthenozoospermia ASZ (n=31) and healthy fertile men as a control (n=24). The patients have been selected and examined according to **(**W.H.O 2010**)** semen analysis manual criteria**.** In addition th biochemical analysis of seminal plasma including (Total antioxidant capacity TAC, Glutathione Reductase GRD, glutathione and Malondialdehyde MDA) and serum (LH, FSH and Testosterone) were assayed for each sample.

Results show a decrease in TAC level of ASZ group as compared to control (439.1±207.9 and 834.5±253.7 µM), and a significant decrease in seminal glutathione levels of (127.77±59.08 and 174.12±61.25 µM) respectively, and show an increase in MDA level (131.45±119.61 and 44.13±13.07 nmol/ml) respectively, while no significant difference in GRD level (24.00±17.69 and 16.02± 9.66 U/mL) respectively. Also there is an increase in LH and FSH hormones level (5.26±2.92, 5.81±5.46 mIU/ml), in ASZ as compared to control (2.29±1.15, 1.970±1.547 IU/ml) respectively, and no significance difference in the testosterone level (5.60±1.92 and 4.35±1.65 ng/mL).

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Introduction

Uncontrolled production of ROS that exceeds the antioxidant capacity of the seminal plasma leads to oxidative stress (OS) which is harmful to spermatozoa (SPZ), all cellular components including lipids, proteins, nucleic acids, and sugars are potential targets of OS [(1)]. ROS induces lipid peroxidation (LPO) and impairs sperm membrane integrity, leading to the complete inhibition of motility and energy metabolism of sperm cells [(2)]. New relationships

between Hormones and Antioxidants are proposed, recently a relationship between reproductive hormones and plasmatic total antioxidant capacity have been observed [3], [4], and levels of several hormones (e.g., growth hormone and prolactin) shown to correlate well with different antioxidant enzyme activities in different tissues [5]. Mancini *et al.* (2009) have reported that both testosterone and estradiol are shown to increase the effects of AO enzymes such as GPx [4] , and Angelini *et al.* (2011) have reported that TAC is significantly correlated with total testosterone in male [3].

Methods and Materials

The study groups consists of (31) Asthenozoospermia (ASZ); whose progressive motility less than 35%, all of them have no children for at least one year , and control fertile group (24) who have one or more children during last year. Semen samples has been collected and examined according to **(**W.H.O 2010**)** criteria [6]. Following sample liquefaction each semen samples were examined, sperm total count was examined using a hematocytometer; sperm vitality was determined using Hypo-osmotic swelling test and sperm morphology was examined by staining with india ink**.** After semen analysis sample was centrifuged and seminal plasma separated and preserved at (-45 C°) until analysis. The following biochemical parameters were assayed in the seminal plasma; Total antioxidant capacity (TAC) using (Cell Biolabs assay kit, USA), Glutathione Reductase (GRD) using (Oxford assay kit, UK), glutathione (GSH) and Malondialdehyde (MDA). In addition to analysis of serum reproductive hormones (LH, FSH and Testosterone) using (MonoBind, USA) ELISA kits.

Results and Discussions

a significant decrease in sperm activity in ASZ group compared to control (43.66±16.58 and 57.44±11.11%) and in progressive motility (17.97±8.06 and 43.88±14.96 %) respectively were seen (table 1), While no significant difference in the other sperm functions parameters of as compared to fertile group.

Sperm Parameters	ASZ (n=31)	Control (n=24)	P value
Count (millions/mL)	78.04±46.81	58.31±30.70	0.001
Live %	69.76±13.84	74.50±10.01	0.002
Active %	43.66±16.58	57.44±11.11	0.001
Progressive %	17.97±8.06	43.88±14.96	0.001
Non progressive %	25.68±14.65	13.56±11.92	0.003
Sluggish %	26.10±12.70	17.06±7.84	0.015
Normal morph. %	50.95±14.48	61.04±11.79	0.063

Data in the table 2 show a significant decrease in TAC level for ASZ as compared to fertile (439.1±207.94 and 834.5±253.7 µM), no significant difference in GRD (24.00±17.69 and 16.02±9.66 mU/mL), and significant decrease in Glutathione level for ASZ group as compared to fertile group (127.77±59.08 and 174.12± 61.25 µmol/mL**)**. Also there is a significant increase in the MDA level for ASZ group (131.45±19.61. nmol/mL) as compared to fertile (44.13±13.07 nmol/mL).

Table 2. Mean ±SEM of antioxidant biomarkers in the seminal plasma of ASZ infertile and fertile groups

Semen Biomarkers	ASZ (n=31)	Control (n=24)	P value
TAC µM	439.1±207.9	834.5±253.7	0.001
GRD activity mU/mL	24.00±17.69	16.02±9.66	0.58
Gluthatione µmol/mL	127.77±59.08	174.12±61.25	0.05
LPO (MDA nmol/mL)	131.45±19.61	44.13±13.07	0.01

These results agree with Gholinezhad *et al.* (2011) they reported a decrease in the TAC level in the asthenoteratospermic group [(7)], and disagree with Lakpour *et al.* (2012) [(8)] , also agree with Tarish (2009) and Shiva *et al.* (2011) [(9)] , who reported an increased MDA levels in ASZ [(10)]. Also our results agree with Shete *et al.* (2012) who reported a decrease in glutathione level in the ASZ group [(11)].

shows a significant difference in the levels of LH and FSH hormones for ASZ group (5.26±2.92 and 5.81±3.46 mIU/mL) as compared to fertile group (2.29±1.15 and 1.970±1.547 mIU/mL) (table 3), while no significance difference in the testosterone level for ASZ as compared to control (5.60±1.92 and 4.35±1.65 ng/mL). These results agree with a study by Zedan *et al.* [(12)], they reported a significance increase in LH level of ASZ as compared with the control group.

Table3. Mean ±SEM of serum reproductive hormones of ASZ infertile and fertile groups

Serum Hormones	ASZ (n=31)	Control (n=24)	P value
LH mIU/mL	5.26±2.92	2.29±1.15	0.001
FSH mIU/mL	5.81±3.46	1.970±1.547	0.039
Testosterone ng/mL	5.60±1.92	4.35±1.65	0.05

Healthy mature spermatozoa reflect appropriate testicular tissue function in response to androgens [(8)]. Maturation of SPZ during passage through the epididymis involves changes in motility, metabolism, morphology, biochemical properties and the development of the ability to fertilize ova [(13)] , they remain in the epididymis for about 14 days can be exposed to the

toxic element such as ROS that is generated in low quantities as a by-product of normal cellular metabolism [14], they also exposed to the forward motility protein (FMP), a specific epididymal protein, responsible to linear movement of SPZ [13]. ROS exposure is especially dangerous because during the transit along the epididymis, the plasma membrane of SPZ contains increasing amounts of polyunsaturated fatty acids (PUFAs) leading to increase LPO and decreasing motility. However, beside the function of epididymis in the maturation, storage and survival of SPZ, this organ play an important role in the AO protection of SPZ through the production of AO enzymes [14], therefore epididymis has a role in the defense against OS [15].

Epididymis, a hormonally sensitive tissue, may undergo changes, this epididymal senescence may lead to decreased SPZ motility [16]. The gonadotropin produces changes in the morphology of epididymal epithelial cells *in vitro* similar to those observed in Leydig cells, the release of Estradiol (E_2) hormone by the epididymal epithelial cells is controlled by LH, as it takes place in Leydig cells [14]. FSH also binding to Sertoli cells stimulates testicular fluid production and synthesis of intracellular androgen receptor proteins [17]. Mancini *et al.* (2009) has suggested a hormonal control the TAC in human semen. They previously demonstrated that TAC is negatively correlated with FSH levels in patients with varicocele, [15].

In the epididymis high level of ROS due to low AOs in semen causes epididymis proteins destruction and impaired sperm motility as a result of protein destruction. Epididymis is hormonally controlled [16]**,** decrease TAC level in epididymis enhance hypothalamus to increase the secretion LH and FSH. Low AOs level cause to decrease reduced glutathione level in the epididymis (127.77±59.08 µmol/mL) as compared to fertile group (174.12±61.25 µmol/mL).

Glutathione protects sperm cell by directly reaction with ROS by its free sulphydryl groups, When present in extracellular space, GSH reacts directly with cytotoxic aldehydes produced during LPO and thus protects the sperm plasma membrane [18].Glutathione deficiency can lead to instability of the mid-piece, resulting in defective motility; as a result of LPO of ASZ sperm plasma membrane as seen in our results (131.45±119.61nmol/mL) as compared to fertile group (44.13±13.07 nmol/mL). Because of mammalian SPZ are rich in PUFAs and, thus, are very susceptible to ROS causing damage of the membrane, LPO cause ATP depletion rapidly in the sperm cell resulting in decreased and cause impairment of motility [19].

Low TAC level increases ROS level in human semen. The excessive production of ROS may result in peroxidation of PUFs of the plasma membrane; As a result, the fluidity of the SPZ

membrane is assured by the complex network of PUFs is compromised by the ROS and inhibits proper membrane fusion with the oocytes [20]. LPO process results in loss of membrane fluidity due to disorganization of membrane architecture and reduction in the activity of membrane enzyme. As a result, SPZ are unable to initiate the necessary biochemical reactions associated with acrosome reaction, zona pellucida binding and oocyte penetration [19] . LPO in cell membranes can damage cell membranes and disrupting fluidity and permeability of the cell [21], at the level of SPZ acrosome LPO causes disruption in acrosome membrane and loss of its ability to fertilize the ovum (figure 1).

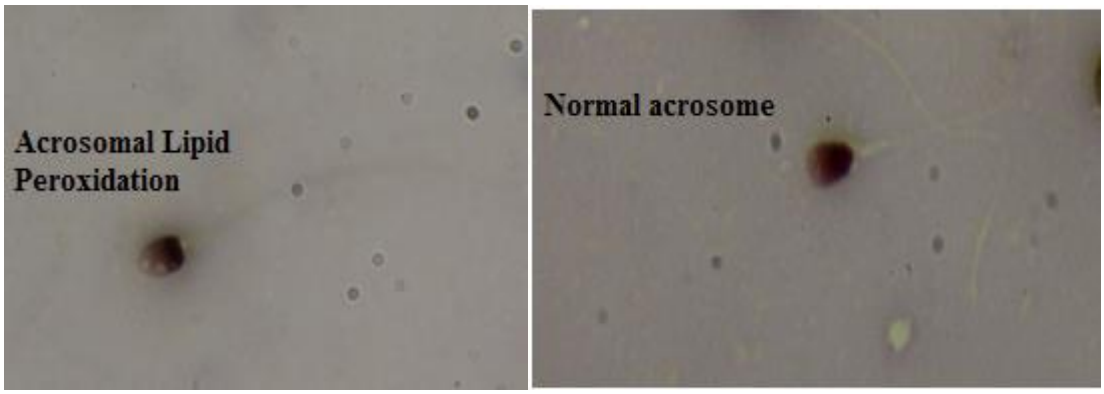

Figure 1: sperm membrane peroxidation (left), Normal membrane (right)

Results show a negative correlation (r=-0.451, p=0.005) between FSH and activity, (figure 2).

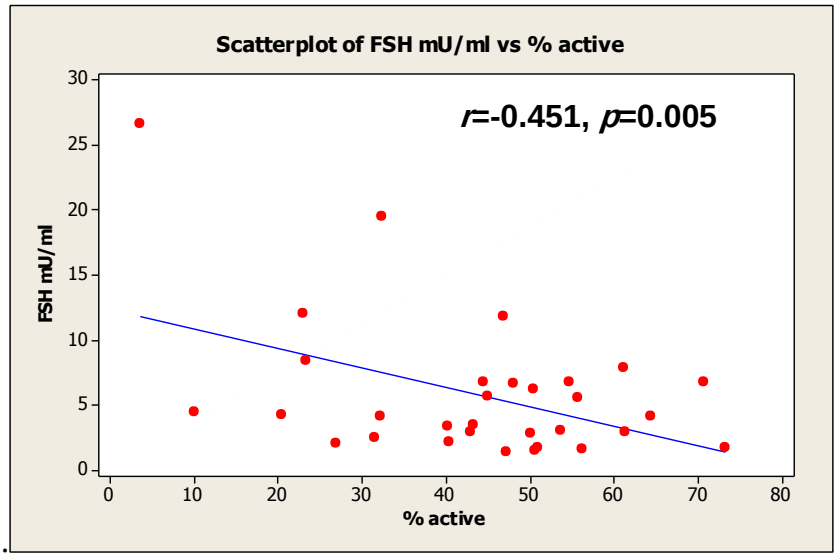

Figure 2: The Correlation between Sperm Activity and S.FSH Level in ASZ

this is confirm the role of FSH on sperm motility through FSH action on epididymis, OS state in the epididymis destroys FMP protein by oxidation causing decrease in progressive motility [13]. Also results show a positive correlation between LH and FSH levels (r= 0.847, p=0.00) as

shown in (figure 3). This can be explained as described earlier by the sharing the same source, GnRH. the secretion of GnRH that stimulates the secretion of FSH and LH from the pituitary gland [(22).]

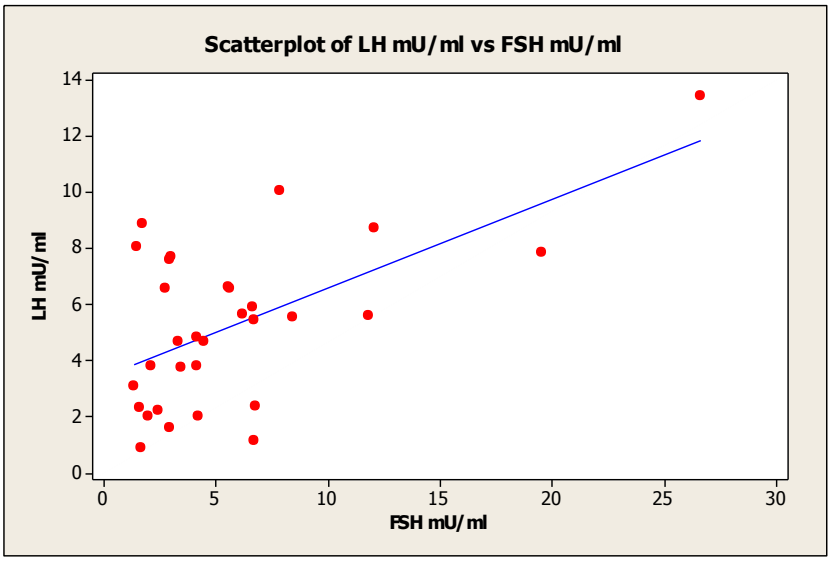

Figure 3: The Correlation between S.LH and S.FSH Level in ASZ

Conclusion

LH and FSH hormones have effect on sperm activity via regulation of the level of antioxidants in human semen through controlling the epididymis.

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