

Septin12 Gene Appearance of Human Spermatozoa and its effect on Infertility

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ABSTRACT

Septins belong to a family of polymerizing GTP-binding proteins that are required for many cellular functions, such as membrane compartmentalization, vesicular trafficking, mitosis, and cytoskeletal remodeling. One family member, septin12, is expressed specifically in the testis. The aim of the present study was to determine the association between septin12 gene expression by RT-PCR and fertilization ability of human spermatozoa. The present study included 80 semen samples that were obtained from men attending the andrology Outpatient Clinic, Mansoura University Hospital, Mansoura, Egypt and grouped into four group: Normozoospermia (n=20), Asthenozoospermia (n=20), Astheno-Teratozoospermia (n=20), Oligo-Astheno-Teratozoospermia (n=20). The current study showed a statistically significant highly positive correlation in Septin12 gene expression with sperm concentration, grade A motility, grade (A+B) motility, velocity, linear velocity, linearity index, normal morphology. However, it shows significant negative correlation with WBCs. Septin12 had highly significant negative correlation with FSH, however it had moderately significant positive correlation with testosterone. It also had highly significant positive correlation with DHEA-S. In conclusion, SEPTIN12 gene expression provides clinical useful information for the diagnosis of male infertility. It might be a marker for discrimination between fertile and infertile patients.

Introduction

Asthenozoospermia is one of the major causes of male infertility and in some cases is associated with major morphological abnormalities of the flagella. Misassembly of the axoneme (Afzelius and Eliasson, 1979; Escalier, 1984; Chemes et al., 1998; Mitchell et al., 2006; Moretti et al., 2008) or Misassembly of the accessory flagellar structure, particularly of the fibrous sheath (Escalier, 2003), may cause abnormal sperm cell motility and in some cases severe asthenozoospermia (Escalier and Albert, 2006). Septins are small GTPase proteins forming homo/heteropolymers and associating with cellular membranes, actin and the microtubule cytoskeleton (Kinoshita, 2006; Spiliotis and Nelson, 2006; Weirich et al., 2008). They have been recognized as being essential structural components of human and mouse annulus (Sept1, Sept4, Sept6, Sept7 and

Septin12) (Ihara et al., 2005; Kissel et al., 2005; Steels et al., 2007). There are at least 13 septin genes in mammalian species, and each septin has multiple splice isoforms. Some septins are expressed ubiquitously while others are only expressed in well differentiated cells (eg, neuron of male germ cells). In dividing cells, SEPT2, SEPT6, SEPT7, and SEPT9 have been implicated in the completion of cytokinesis. In activation of SEPT4 in the mouse results in male infertility associated with a complete absence of the annulus structure, a hairpin-like bending of the flagellum at the MP-PP junction and a total lack of sperm motility. Disruption of septin functions has been implicated in the pathology of much disease. Including cancers, neurodegeneration, and male infertility (Ihara et al., 2005 Kissel et al., 2005). The aim of the current study was to investigate the possible association between spermatozoa Septin12 gene expression by semi quantitative RT-PCR and male fertility. Moreover, to correlate the gene expression of Septin12 with the different seminogram parameters and the hormonal profile of the studied subjects.

Patients & Methods

This prospective study was done during the period from August 2013 to January 2015; semen samples were obtained from men attending the andrology Outpatient Clinic, Mansoura University Hospital, Mansoura, Egypt. Semen samples were divided into four groups: Group I: comprised 20 semen samples with Normozoospermia. Group II: included 20 semen samples with Asthenozoospermia. Group III: comprised 20 semen samples with Astheno- Teratozoospermia. Group IV: included 20 semen samples with Oligo- Astheno-Teratozoospermia. All subjects were subjected to full history taking and physical examination. A general physical examination was performed to detect possible abnormality relevant to fertility in the following system: metabolic, endocrine, cardiovascular, respiratory, gastro-intestinal and neurological. Local examination of the testis, epididymis, vas deferens and inguinal region was done for all patient to detect possible abnormalities in order to be excluded. Preparation of samples: Fresh semen samples were collected from cases by masturbation, after 2-7 days sexual abstinence and allowed to liquefy for 15-30 minutes, Each semen sample was directly divided into 2 tubes, the first tube was subjected centrifugation at 7000 rpm for 10 minute washed three times to obtain pure cell free sperm pellet, Then it is shock frozen by liquid nitrogen and used immediately for RNA extraction (Ma et al., 2002 and Liu et al., 2011), The second tube was standard at 37°C for 15-30 minute until complete liquifaction, After complete liquifaction, computer assisted semen analysis test was performed according to the recommendation of the World Health Organization (WHO, 2010).

Sperm morphology was evaluated by phase contrast microscope and Sperm Mac stain (Fertipro, Belgium), WBCs were determined by peroxidase stain (Endtz, 1972). The seminal plasma was obtained by centrifugation of the semen sample at 1000 rpm for 15 minutes. The supernatant was stored at -20°C until analyzed for seminal alpha-glucosidase activity (Guerin et al., 1986). Three blood samples were withdrawn at 15 minutes intervals from each patient and control by standard venipuncture techniques in the morning after 12 hours overnight fasting. Sera were prepared from each sample by

centrifugation at 7000 rpm for 15 minutes, pooled together at equal volume and analyzed as one sample for each subject included in the current study, Collected sera were stored at -20°C and analyzed for hormonal profile by enzyme linked immuno sorbant assay (ELISA) technique which include: follicle stimulating hormone (FSH) by Follicle Stimulating Hormone Human ELISA Kit (Abcam, ab108641), lutenizing hormone (LH) by Luteinizing Hormone Human ELISA Kit (Abcam, ab108651), testosterone by Testosterone ELISA Kit (Abcam, ab108666), dehydroepiandrosterone (DHEA) by DHEA ELISA Kit (Genwaybiotech,GWB-719A7E),dehydroepiandrosterone Sulfate (DHEA-S) by DHEA-S ELISA Kit (Abcam, ab178642), prolactin by Prolactin (human) ELISA Kit (Biovision, catalog # K4687-100) and estradiol (E2) by Estradiol E2 Human ELISA Kit (Abcam, catalog ab108640). Semiquantitative RT-PCR of SEPTIN12 gene: Total RNA was isolated according to the method of Das et al (2010) using Trifast Reagent kit purchased from peqlab Biotechnologie GmbH, Germany.

Total RNA extraction was carried out from each semen sample using Trifast Reagent (peqlab. Biotechnologie GmbH, Germany, Cat.No.7930b) and Rneasy mini kit (Qiagen, cat no. 74104). Each sample was allowed to liquefy for 15-30 minutes, Then centrifuged at 10.000 rpm for 15 minutes. The supernatant was divided into tubes and stored at -20°C until analysis, Sperm pellets were collected for assessment of SEPTIN12 gene expression by RT-PCR (Ma et al., 2002 and Liu et al., 2011) according to the manufacturer's instructions. The concentration of isolated RNA was determined spectrophotometrically by measuring the optical density (OD) at 260 nm using (NanoPhotometer ® P-Class, Implen, Germany). 1µl of solubilized RNA was applied directly to the detection surface of apparatus which was then automatically diluted and assessed spectrophotometrically (Sambrook et al., 1989). The purity of RNA was determined by gel electrophoresis using formaldehyde agarose gel electrophoresis and ethidium bromide staining to show two sharp bands representing 28S and 18S ribosomal RNA.

RT-PCR for extracted RNA:

Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) was performed using Maxima first strand cDNA Synthesis Kit for RT-qPCR provided by Thermo Scientific, cat No. #K1641, U.S.A. The Maxima® First Strand cDNA Synthesis Kit is a convenient system optimized for cDNA synthesis in two step real time quantitative RT-PCR (RT-PCR) applications. The Maxima First Strand cDNA Synthesis Kit is capable of reproducible cDNA synthesis from a wide range of starting total RNA amounts (1 pg - 5 µg) at elevated temperatures (50-65°C). The synthesis reaction can be completed in 15-30 minutes. Components of the Maxima First Strand cDNA Synthesis Kit are premixed to save time and to reduce the possibility of pipeting errors. The reaction passed as follow:

A-Synthesis of cDNA: The components of the kit (100 µL Maxima Enzyme Mix (contains Maxima Reverse Transcriptase and Thermo Scientific RiboLockRNase Inhibitor), 200 µL 5X Reaction Mix (contains the remaining reaction components:

reaction buffer, dNTPs, oligo (dT)18 and random hexamer primers), 1.25 mL Water, nuclease-free) after thawing, were mixed, briefly centrifuged and stored on ice, The following reagents were added into a sterile, nuclease-free tube on ice in the indicated order (4 µl 5X Reaction Mix, 2 µl Maxima Enzyme Mix, 5 µg Template RNA, 9 µl Water, nuclease-free), Each tube was mixed gently and centrifuged briefly, The tubes were incubated for 10 min at 25°C followed by 15 min at 50°C, The reaction was terminated heating at 85°C for 5 min. The reverse transcription reaction cDNA product was used in PCR reaction. Gene specific primers used were: Gene specific primers were purchased from Oligo, Macrogen. Primer sets for the PCR amplification genes were selected based on published sequences. Primer for SEPTIN12 gene (Liu et al., 2011) generate a 322bp fragment as follow:

Sense primer: 5'-CCACAGCTATGAACCTGAGTCTGG-3; Antisense primer: 5'-TGCAGTCTTCATCTCGAACTG-3'. Primer for Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (Yang et al., 2007) generate a 382 bp fragment as follow: Sense primer: 5'- AATCCCATCACCATCTTCC -3'; Antisense primer: 5'-CATCAGCCACAGTTTCC -3'. AMPLIFICATION OF cDNA by PCR: Using Emerald Amp GT-PCR Master Mix (2X) provided by Takara, Japan, code No. RR310A, PCR is used to amplify the template cDNA fragments up to ~10 kilo base pairs (kb). First, cDNA template was added to DreamTaq™ Green PCR Master Mix (2X), and gene specific primers. Then during thermal cycling, the double stranded RNA: Cdnaheteroduplex made by the reverse transcriptase was denatured at 94°C to provide the cDNA strand for amplification. The temperature was then reduced to the primers annealing temperature followed by primer extension by Dream Taq™ DNA polymerase at 72°C. Repeated cycles of denaturation, primer annealing and primer extension was used for cDNA amplification. A final extension at 72°C was done to ensure all molecules were completely synthesized (Saiki et al., 1988). Final PCR products were resolved in agarose gel electrophoresis and a single band of expected size indicated the specificity of the reaction. Thermal cycling reaction was performed using thermal cycler (Minicycler PTC- 150).

B-Detection of amplified RT-PCR product: The products were applied to agarose gel electrophoresis using 1.5% agarose stained with ethidium bromide and visualized via light UV Transilluminator, checked for the product bands, The products bands were photographed and analyzed by Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software (Bio Rad, USA, Cat. No #170-8195) GAPDH produced a single 382bp RT-PCR product. SEPTIN12 produced a single 322bp RT-PCR product. The bands were detected and converted to peaks. Areas under each peak were calculated in square pixels and used in quantification. Gene expression levels were determined by calculating the ratio between the square pixel values of the target gene septin12 in relation to the control gene (GAPDH).

Statistical Analysis: was done by using SPSS (Statistical Package for the Social Sciences) program version 16 on windows 7. The quantitative data were expressed as mean,

standard deviation Mann-Whitney test was used as a test of significance for comparison of two groups. The qualitative data were presented in the form of number and percentage.

Spearman rank correlation coefficient was done to study the relation *Septin12 Gene*

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between variables. The result is considered significant if p-values less than or equal 0.05, if P less than or equal 0.01 is considered moderately significant and if P less than or equal 0.001 is considered highly significant. Receiver Operating Characteristic (ROC) curve was constructed to determine the cut-off value for optimal sensitivity and specificity for SEPTIN12 gene expression.

Assay of alpha- glucosidase activity: Measurement of alpha-glucosidase activity in seminal plasma was done according to the colorimetric method described by Guerin et al. (1986), and Cooper et al. (1988) to evaluate the function of the epididymis as the bulk of the activity of alpha-glucosidase in semen has been shown to depend on secretion by the epididymis (Cooper et al., 1990). The test is based on demonstration of the yellow colored P-nitrophenol released from a chromogenic substance (P-nitrophenyl- α -D-glucopyranoside) by the activity of α glucosidase. Alpha glucosidase was measured using (Episcreen Kit; FertiPro, 32,8730 Lotenhulle, Belgium). Extinction of clear aspirated supernatant was assessed by spectrophotometer at wavelength 405nm against (blank). The concentration of α glucosidase was calculated from the standard curve.

Results:

In the present study, table 1 shows the semen parameters which include concentration (million / ml), grade A motility (%) , grade A+B motility (%) , velocity (μ m / sec) , linear velocity (μ m / sec) , linearity index (%) , normal morphology (%), WBCs (million / ml) , and α - glucosidase (m U/ml) in different groups. (Table 2) show blood hormone levels [LH (mIU /ml), F.S.H (mIU /ml), Prolactin (ng /ml), Testosterone (ng /dl), DHEA-S (ng /dl) and E2 (pg/ml)] in different groups. F.S.H levels were highly significantly increased in oligo-astheno- teratozoospermia group compared with normal group. However, there was highly significant decrease in testosterone levels in oligo-astheno-teratozoospermia compared with the normal group. Also, there was highly significant decrease in DHEA-S levels in asthenozoospermia, astheno-teratozoospermia, oligo-astheno- teratozoospermia compared with the normal group. However, E2 levels were significantly increased in oligo-astheno- teratozoospermia compared with the normal group. Table 3 and figure 3 show Septin/GAPDH mRNA ratio in all studied groups. mRNA gene expression of Septin was highly significantly decreased ($P < 0.0001$) in asthenozoospermia, astheno-teratozoospermia and oligo-astheno-teratozoospermia compared with the normal group. Table 4 shows the correlation of Septin/GAPDH mRNA with sperm parameters of all studied groups. In this table, Septin/GAPDH mRNA expression shows highly significant ($P < 0.0001$) positive correlation with sperm concentration ($r = 0.82$), Grade A motility ($\rho = 0.69$), Grade (A+B) motility ($\rho = 0.72$), Velocity ($\rho = 0.68$), Linear velocity ($\rho = 0.65$), Linearity index ($\rho = 0.63$), Normal morphology ($\rho = 0.77$) and α - glucosidase ($\rho = 0.85$).

However, it shows significant ($P < 0.05$) negative correlation with WBCs ($\rho = -0.3$). Table 5 represents the correlation of Septin/GAPDH mRNA with hormonal profile of all studied groups. Moreover, this table also, shows that Septin/GAPDH mRNA has highly significant ($P < 0.0001$) negative correlation with FSH ($\rho = -0.89$), however it had moderately significant positive ($P = 0.001$) correlation with testosterone ($\rho = 0.36$). It also, shows highly significant ($P < 0.0001$) positive correlation between Septin/GAPDH mRNA and DHEA-S ($\rho = 0.79$), also DHEA ($\rho = 0.82$). However it has no significant correlation with LH, prolactin and E2.

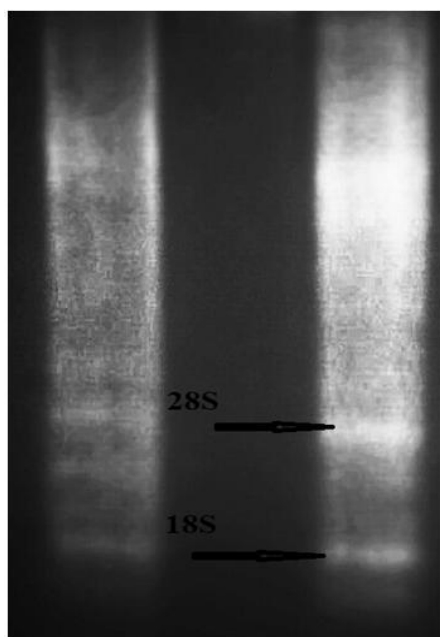


Figure (1): Total sperm RNA agarose gel.

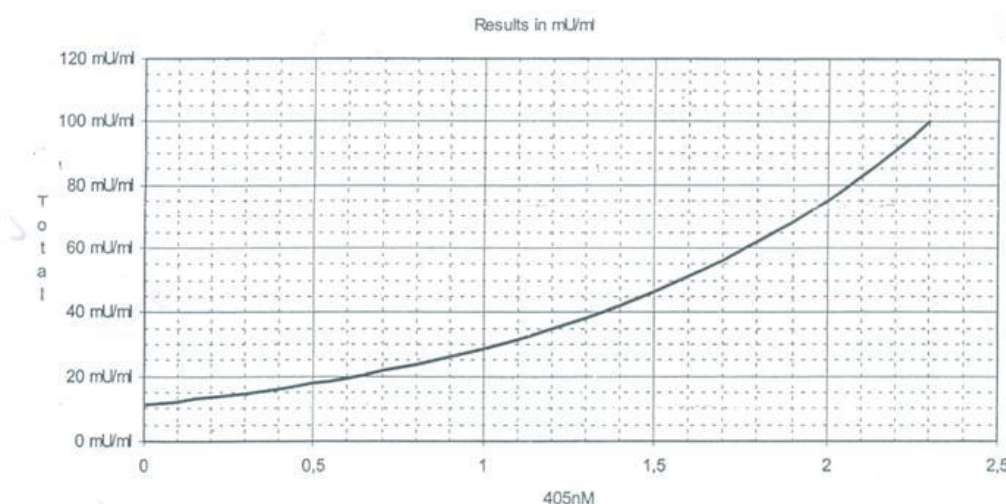


Figure (2): Standard curve of alpha glucosidase

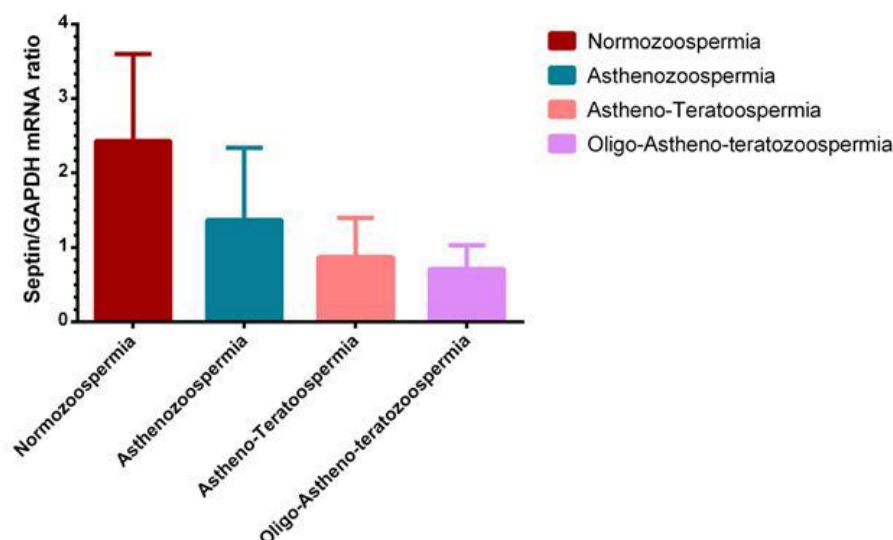


Figure (3): mRNA gene expression of septin12 in all studied groups

Table (1): Semen parameters of all studied groups

	Normozoospermia	Asthenozoospermia	Astheno-Teratoospermia	Oligo-Astheno-teratozoospermia	P
no. of case/group	20	20	20	20	
Concentration (million/ml)	115.73±41.3	63 ±22.4 ^a	29.87± 11.0 ^{ab}	9.3±4.5 ^{abc}	<0.0001
Grade A motility (%)	61±31.2	33±19.2 ^a	13±81 ^{ab}	3±1.1 ^{abc}	<0.0001
Grade (A+B) motility (%)	79±22.1	31± 11.1 ^a	25±9.1 ^{ab}	13±7.3 ^{abc}	<0.0001
Velocity (µm/sec)	67.5±29.8	48.6±28.4 ^a	33.25±19.5 ^{ab}	13.5±11.1 ^{abc}	<0.0001
Linear velocity (µm/sec)	63.4± 29.5	28.9±13.1 ^a	19.67±9.8 ^{ab}	13.7±4.5 ^{abc}	<0.0001
Linearity index %	79±	61± 23.9 ^a	46.9±9.1 ^{ab}	32.1±8.2 ^{abc}	<0.0001
Normal morphology %	54± 33.4	45.5± 28.2 ^a	17±4.1 ^{ab}	7.7±1.1 ^{abc}	<0.0001
WBCs (million/ml)	0.3±0.1	0.5±0.2	0.6±0.21	0.5±0.2	0.2
α- glucosidase (mU/ml)	79.3 ±23.4	49.3±22.1 ^a	35.1±19.1 ^{ab}	26.4±12.1 ^{abc}	<0.0001

Normo. = Normozoospermia, **A** = Asthenozoospermia, **AT** = Asthenoteratozoospermia, **OAT** = Oligo-Astheno- teratozoospermia, **n**= number of cases.

a: significance between Norm group & A, AT, OAT

b: significance between A group & AT, OAT

c: significance between AT & OAT

P: Probability.

Test used: ANOVA followed by post-hoc tukey

Table (2): Blood Hormones of all studied group

	Normo. (n=20)	A (n=20)	AT (n=20)	OAT (n=20)	P
FSH(μ IU /ml)	5.9 $\pm$ 2.5	6.7 $\pm$ 2.8 ^a	7.4 $\pm$ 3.9 ^{ab}	8.3 $\pm$ 3.7 ^{abc}	<0.0001
LH(μ IU /ml)	6.7 $\pm$ 2.8	5.9 $\pm$ 2.1 ^a	5.15 $\pm$ 2.4	5.59 $\pm$ 3.1	0.06
Prolactin(ng /ml)	6.1 $\pm$ 3.1	5.9 $\pm$ 3.2	5.5 $\pm$ 2.7	4.8 $\pm$ 2.8	0.32
Testosterone (ng /dl)	757 $\pm$ 318	739 $\pm$ 229	693.5 $\pm$ 353	653 $\pm$ 415	0.06
DHEA(pg /dl)	9.034 $\pm$ 4.01	6.657 $\pm$ 3.741 ^a	4.589 $\pm$ 2.145 ^{ab}	3.239 $\pm$ 1.245 ^{abc}	<0.0001
DHEA-S(ng /dl)	318 $\pm$ 119	235.7 $\pm$ 245 ^a	154.6 $\pm$ 91.4 ^{ab}	129.4 $\pm$ 43.5 ^{abc}	<0.0001
E2(pg/ml)	27 $\pm$ 11	35 $\pm$ 12 ^a	29 $\pm$ 13 ^{ab}	47 $\pm$ 21 ^{abc}	<0.0001

FSH = Follicle stimulating hormone, LH = Luteinizing hormone,

DHEA = Dehydroepiandrosterone hormone,

DHEA-S = Dehydroepiandrosterone-sulphate hormone,

E2 = Estradiol hormone.

Normo. = Normozoospermia,

A = Asthenozoospermia,

AT = Asthenoteratozoospermia, OAT = Oligo-Asthen- teratozoospermia,

n= number of cases.

a: significance between Norm group & A, AT, OAT

b: significance between A group & AT, OAT

c: significance between AT & OAT

P: Probability.

Test used: ANOVA followed by post-hoc tukey

Table (3): Septin/GAPDH mRNA ratio in all studied groups.

	Normozoospermia	Asthenozoospermia	Asthen- Teratoospermia	Oligo-Asthen- teratozoospermia	P
n	20	20	20	20	
Septin/GAPDH mRNA ratio	2.43 $\pm$ 1.17	1.37 $\pm$ 0.97 ^a	0.87 $\pm$ 0.53 ^{ab}	0.71 $\pm$ 0.32 ^{abc}	< 0.0001

Normo. = Normozoospermia, A = Asthenozoospermia, AT = Asthenoteratozoospermia,

OAT = Oligo-Asthen- teratozoospermia, n= number of cases.

a: significance between Norm group & A, AT, OAT

b: significance between A group & AT, OAT

c: significance between AT & OAT

P: Probability (P $\leq$ 0.05).

Test used: ANOVA followed by post-hoc tukey

Table (4): The correlation of Septin/GAPDH mRNA with sperm parameters of all studied groups.

Variable	Septin/GAPDH mRNA	95% CI for r	P value
Concentration(million/ml)	$\rho = 0.82$	0.74-0.88	$P < 0.0001$
Grade A motility (%)	$\rho = 0.69$	0.56-0.79	$P < 0.0001$
Grade (A+B) motility (%)	$\rho = 0.72$	0.6-0.81	$P < 0.0001$
Velocity ($\mu\text{m}/\text{sec}$)	$\rho = 0.68$	0.54-0.78	$P < 0.0001$
Linear velocity ($\mu\text{m}/\text{sec}$)	$\rho = 0.65$	0.38-0.69	$P < 0.0001$
Linearity index %	$\rho = 0.63$	0.48-0.75	$P < 0.0001$
Normal morphology %	$\rho = 0.77$	0.67-0.85	$P < 0.0001$
WBCs (million/ml)	$\rho = -0.31$	-0.59- -0.22	$P = 0.005$
α - glucosidase (mU/ml)	$\rho = 0.85$	0.77-0.9	$P < 0.0001$

Table (5): Correlation of Septin/GAPDH mRNA with hormonal profile of all studied groups.

Variable	Septin/GAPDH mRNA	95% CI for r	P value
FSH (mIU/ml)	$\rho = -0.89$	-0.92, -0.83	$P < 0.0001$
LH (mIU/ml)	$\rho = -0.22$	-0.49, -0.001	$P = 0.049$
Prolactin (ng/ml)	$\rho = 0.07$	-0.15, 0.29	$P = 0.54$
Testosterone(ng/dl)	$\rho = 0.36$	0.15-0.54	$P = 0.001$
DHEA (pg/ml)	$\rho = 0.82$	0.73-0.88	$P < 0.0001$
DHEA-S (ng/ml)	$\rho = 0.79$	0.69-0.86	$P < 0.0001$
E2 (pg/ml)	$\rho = -0.26$	-0.45, -0.04	$P = 0.01$

ρ = correlation coefficient

*Significant($P \leq 0.05$).

FSH = Follicle stimulating hormone, LH = Luteinizing hormone,

DHEA = dehydroepiandrosterone hormone,

DHEA-S = dehydroepiandrosteronesulphate hormone,

E2 = Estradiol hormone.

Discussion

Mature spermatozoa consist of four major compartments acrosome, nucleus, midpiece, and tail. Lin et al., (2009) found that septin12 protein is widely expressed and forms ring-like structure in different locations of post meiotic germ cells during the terminal differentiation processes, including the peri-acrosome area, peri-nuclear area, midpiece, and tail. Although chimeric mice shared some phenotype reminiscent of those for the Septin 4 Knockout mice, obvious differences exist between the mouse models of these two genes. For the septin4, the reproductive phenotypes including bent-tail, as well as acrosome and mitochondrial dysfunction in mature spermatozoa, were observed only in the Septin4 Knockout mice (Ihara M et al., 2005). However, the reproductive effect is far more profound for septin4 deficiency. Lin et al., (2009) reported that haploin sufficiency of Septin12 is sufficient to cause severe defects of both mature and immature

germ cells. Septins usually mediate their cellular functions through the formation of macromolecular and hetero-oligomeric filaments with other family members (Field CM and Kellogg D, 1999). It is highly documented that septin12 cooperates in interactions with fixed stoichiometry with other septins as septin4 (Dobbelaere J and Barral Y., 2004). In this study, septin12 gene expression was investigated in human spermatozoa of normal and subjects with abnormal seminogram (Asthenozoospermia, Asthenoteratozoospermia and Oligo-Asthenoteratozoospermia), a decrease of septin12 gene expression in infertile groups is found when compared with the group of normal seminogram (fertile normal control group). Also, reduced septin12 expression in spermatozoa with head, neck, or tail defect comes in agreement with Lin YH et al., (2006).

This finding could be explained by the fact that septin12 gene is important in sperm function and fertility because SEPTIN12 is only expressed in postmeiotic germ cell, decreased transcript levels may be secondary to the loss of postmeiotic germ cell in the testicular specimens. Oxidative stress (OS) as a result of an inappropriate balance between oxidants and antioxidants in the semen can lead to sperm damage, impairs the structure and function of spermatozoa and eventually male infertility (Agarwal A. et al., 2009), this OS resulted from Reactive Oxygen Species (ROS) or free radicals produced in semen of such individuals. In physiological conditions, ROS are required for crucial aspects of sperm function, in pathological conditions, excessive levels of ROS can negatively affect sperm quality (Kefer JC et al., 2009). Sperm plasma membrane is particularly susceptible to lipid peroxidation by ROS due to the existence of high concentration of polyunsaturated fatty acids. Lipid peroxidation can lead to loss of membrane fluidity and integrity, as a result of this, reduces sperm-oocyte fusion. Furthermore, they can attack DNA by inducing base modifications, DNA strand breaks, DNA cross-links, and chromosomal rearrangements (Duru NK et al., 2000). Over production of ROS is associated with defective sperm function (Mazzilli F et al., 1994). There are some potential origins of ROS such as, seminal leukocytes with positive peroxidase, immature, morphologically abnormal sperm, can be a cause of male infertility (Plante M et al., 1994). Exposing the spermatozoa to ROS, could lead to DNA damage and lipid peroxidation (Potts RJ et al., 2000).

High lipid peroxidation may lead to reduce acrosomal reaction, fertilization (Aitken RJ et al., 1983) and sperm oocyte fusion. Several studies have shown that lipid peroxidation impacts the sperm concentration, motility, morphology and associated with poor sperm quality (Hsieh YY et al., 2006). The relation between seminal oxidative stress and high sperm morphological deformity may be causal and related to the greater capacity of the morphologically abnormal spermatozoa to produce ROS (Saleh et al., 2002). It has been hypothesised that oxidative damage of mature sperm by ROS-producing immature sperm during their comigration from seminiferous tubules to the epididymis may be an important cause of male infertility (Agarwal et al., 2003). Other authors have found that sperm morphological irregularities are significantly correlated with creatine kinase (CK) activity in human spermatozoa, which is also found to have a positive correlation with the rate of lipid peroxidation. Lipid peroxidation and decreasing

total antioxidant capacity lead to low motility; morphology and sperm count (Aitken, 1999 and Colagar et al., 2013). In the current study, FSH levels were increased and testosterone levels were decreased in oligo-astheno-teratospermia. This finding is in agreement with Wei et al., (2013). They reported decreased testosterone and elevated FSH levels in patients with OAT syndromes. The main sites of testosterone action in spermatogenesis are during meiosis and spermiogenesis. Therefore, an adequate testosterone supply is crucial for Sertoli cell survival and differentiation in early postnatal life. Testosterone, acting alone or with FSH, acts as a surviving factor for spermatocytes and spermatids through regulating intrinsic and extrinsic apoptotic pathways (Ruwanpura et al., 2010).

OAT syndrome might be a consequence of a more vulnerable sperm production to the physiological stress because apoptosis of unhealthy sperm is crucial in spermatogenesis. Hormones, mainly testosterone and FSH, regulate male germ cell development by controlling their survival from apoptosis, rather than by stimulating their proliferation. Since a higher testicular testosterone is necessary for OAT patients to maintain their spermatogenesis, this phenomenon is supported by the context that FSH or LH shows inverse correlation with all the semen parameters. For human spermatogenesis, FSH appears more effective than LH in supporting spermatocytes (Matthiesson et al., 2006). Testosterone and FSH are considered the major hormonal regulators, working either independently or synergistically, for germ cell development at different stages of spermatogenesis (Ruwanpura et al., 2008). Testosterone exerts its biological effects on spermatogenesis via androgen receptors in the Sertoli cells (Silva et al., 2002). In addition, testosterone elicits cellular responses through secondary messengers and signaling pathways in Sertoli cells to facilitate the non-genomic action of FSH, which is different from the classical androgen receptor (AR)-mediated transcription (Walker, 2003). Both of the two pathways of testosterone, synergistically or independently with FSH, are influenced by the LH effect from the hypothalamus-pituitary-gonad axis. Therefore, it was hypothesized that a higher Testosterone/FSH or Testosterone/LH ratio may imply a more sensitive hormonal axis feedback, which results in better sperm production in a patient with OAT syndrome (Wei et al., 2013). Healthy mature spermatozoa reflect appropriate testicular tissue function in response to androgens (Aoki et al. 2006).

Conclusion

SEPTIN12 gene expression provides clinical useful information for the diagnosis of male infertility. It might be a marker for discrimination between normal and abnormal spermatozoa in both fertile and infertile patients. Further studies should focus on SEPTIN12 gene expression and on possible therapeutic and diagnostic strategies directed towards different etiological causes of male infertility that affect SEPTIN12 gene expression.

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