SEMEN, SEMINAL OXIDATIVE STRESS, ZINC AND, SPERM DNA INTEGRITY IN OBESE INFERTILE MEN

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Received :27/ 8 /2018
Accepted: 25/ 9 /2018

ABSTRACT:

Background: *Infertility is a health concern where about 15% of families around the world struggle with; it is noticed that paternal obesity could be a lurking cause and that it may affect semen parameters.*

Aim of the Work: *This work aims to assess obesity's effect on parameters of semen, seminal oxidative stress, and zinc and sperm DNA integrity in the cases of obese men struggling with infertility while comparing the results with men with no infertility issues.*

ıe work consists of two groups: a group of obese infertile men and an age-ɔr all participants, clinical evaluation, conventional semen analysis, and ɔtion and Reactive Oxygen Species (ROS) chemiluminescent assays were ssment of sperm viability by Hypoosmotic Swelling Test (HOS) and DNA fragmentation via staining propidiumio

dide by flow cytometry.

Results: *It was found that infertile overweight males maintained noticeable low sperm motility, viability, and Zn values in semen while maintaininghigh semen ROS and fragmentation of sperm DNA when crosschecked against the control group. Significant negative correlations were found in the participants when comparing BMI with the percentages of sperm mobility, normal morphology, viability, amount of Zn in semen, and concentration of sperm. On the other hand, BMI and both fragmentation of sperm DNA and semen ROS were directly proportional.*

Conclusions: *Obesity negatively affects parameters of semen, oxidative stress, and zinc values in addition to sperm DNA integrity in infertile men; this effect correlated with BMI.*

Keywords: Infertility, Obesity, Sperm DNA, Semen

INTRODUCTION:

Rates of obesity are surging worldwide. Male obesity's repercussions on re-productive health are becoming concerning[1]. Obesity seems to be one of the significant and misdiagnosed infertility causatives. Body mass index (BMI) is an easy way of measuring body fat and assessing obesity. A BMI value exceeding 25 kg/m2establishes the individual as overweight and the said individual is considered obese should their BMI value exceeds 30 kg/m2[2]. Evidence of the relationship between obesity and sub-fecundity is accumulating[3]. Obese people are at greater risk of vascular, metabolic, and endocrine dysfunction that may impair their erectile capacity[4,5]. Despite some conflicts, published data suggested the impairment of spermatogenesis in obese individuals[6,7]. Altered semen parameters were observed in obese men such as lower sperm count, abnormal morphology, and lower sperm motility[8-10]. Spermatogenesis impairment in obese individuals was attributed to physical (scrotal hyperthermia), metabolic, hormonal, and environmental toxin accumulation[11,3,6]. Sperm HOS test, ROS assay, and sperm integrity DNA assessment were shown to play major roles as predictive tests to male fertility potential[12,13]. More sperm with fragmented DNA was found in obese males which can cause fertility problems on its own while suggesting troublesome overall spermatogenesis[14]**.** While some beneficial effects of zinc on semen were acknow-ledged, controversy persists over levels of zinc when comparing various subfertile groups and also the correlation concerning parameters of semen and zinc. Several researchers recorded immensely varying levels of seminal zinc among subfertile and fertile populations, suggesting minimal levels of seminal zinc among subfertile groups[15], while others illustrated that the two groups are not different[16, 17].

AIM OF THE WORK:

To evaluate the impact of obesity on semen parameters, seminal oxidative stress, zinc, and sperm DNA integrity, in infertile obese men. by comparing them to normal-weight men.

PATIENTS AND METHODS:

173 men were enlisted from the Andrology Unit, Assiut University Hospital, and analyzed following their informed

consent and the institutional review board's approval. The men were classified into two groups according to Body Mass Index (BMI). Group 1 consisted of 92obese infertile men (BMI>30 kg/M2), and group 2 comprised of 81normallyweighing men (BMI< 25 kg/M2).

Smokers are occupationally exposed to sperm DNA toxins or may have genital disorders that reduce reproductive potential as varicocele, genital tract infections (leukocyte spermia), mal descended testis, and atrophic testis were excluded from the study. Other patients suffering from diseases of systemic natures prone to reduce reproductive function such as endocrine, hepatic and autoimmune diseases besides those on antioxidants or with female factor infertility were excluded.

The following measures were performed on the participants:

1. History taking, general, and genital examination

2. Conventional semen analysis as well as using Papanicolaou method for sperm morphology evaluation according to WHO 2010 guidelines

3. Sperm Hypoosmotic Swelling (HOS) test[18]

One mL of hypoosmotic pristine-made medium (1.351 g fructose and 0.735 g sodium citrate dehydrate in 100 mL distilled water) was sprinkled with 0.1 mL of liquefied semen and then incubated for 30 minutes at a temperature of 37°C. Using a phase-contrast microscope, spermatozoa were studied, where sperm tail lumping was recognized in 100 spermatozoa and were counted as duplicates.

4. Sperm DNA Fragmentation Percentage Assessment[19-21]:

Fragmentation of sperm DNA evaluation was accomplished on fresh semen via flow cytometry (DAKO-Cytomation, Glostrup, Denmark) procured from Coulter (Beckman Coulter, Fullerton, CA) according to the fluorescence emission of propidium iodide (PI)–stained sperm and argon laser–excitation with a 488-nanometer (nm).

The dimensions varied with PI's capability to cohere histochemically to DNA under suitable conditions concerning staining. Samples of semen were diluted with phosphate-buffered saline (PBS) (pH 7.4) to 2 × 106 sperm/m. then incubated for 15 seconds of 50 µL with 100 µL of lysing reagent. Afterward, 2 mL of PI were added and mingled. Right after staining took place, flow cytometry was used for tube accession, where the frequency of the emission correlated with the content of DNA. Flow-cytometric scanning exhibits a stationary and distinguishable bimodal non artifactual DNA pattern supporting the presence of two particular groups. The principal group is constituted by a peak followed by a shoulder, while the marginal group consists of a group of sperm with a change DNA affliction (a change in the nuclear condensation), capitulating volatile chromatin, which takes more stain. Using the flow cytometer, Damaged DNA sperm cells percentage (sperm DNA fragmentation% was automatically counted following the accession of 5000 spermatozoa.

5. Measuring Semen's Reactive Oxygen Species (ROS)[22]

Levels of ROS were calculated by observation of the chemiluminescence activity via the luminol (5-amino-2, 3 dihydro-1, 4) phtalazindione reagent (C8H7N3O2) supplied by MP Biomedicals. The liquified semen samples were centrifuged at 300×g for seven minutes. After that, the seminal plasma was discarded. The pellet was then rinsed two times (PBS, pH 7.4) by centrifugation at 300xg for five min and resuspended in the PBS at a concentration of 20 x 106 sperm/mL. Ten milliliters of luminol was added to the aliquot, which was utilized as a probe. Levels of ROS were measured by

detecting chemiluminescence activity with an Automat Luminometer (Berthold Technologies, Bad-wildbad, Germany) operating in the integrated mode for 15 minutes. The outcomes were represented in Relative Light Unit (RLU) per 20 million spermatozoa.

6. Measuring of Zinc Level in the Seminal Plasma [23]

For seven minutes, the liquefied semen was centrifuged at 300g and the seminal plasma was taken and chilled at –20°C. The glassware or plastics used were washed with 10% nitric acid overnight and washed meticulously with distilled deionized water. One mL of seminal plasma was left overnight in a glasstube with 2mL of concentrated nitric and 2 mL of perchloric acids. Levels of seminal Zn were measured by a flame atomic absorption spectro-photometer (Buck model 210 VGP; Buck Scientific, Inc., Norwalk, CT) with air-acetylene flame, hollow cathode lamp, lamp current (8 mA), and wavelength (213.9 nm). For each sample, two measurements were gauged. The measurement accuracy was performed with optimal reference materials.

Statistical Analysis:

Data were studied and demonstrated in terms of mean values ± Standard Deviations (SD). SPSS version 16 program (SPSS. Inc, Chicago, USA) was employed for data filtering. When comparing the groups, Unpaired t-tests were utilized for numerical parametric data and while utilizing the Mann-Whitney test for numerical nonparametric data. Pearson's correlation test was used to find out connections between various quantitative variables. Significance was established when P values were below 0.05.

RESULTS:

The current work included 173 men divided into two groups. Group 1 included 92 infertile obese men and group 2 included 81men with normal weight. The two groups were comparable in age (mean of 37.15 ± 5.77 years for group 1 and 34.41± 6.17 years for group 2) and socio-demographic data. The mean BMI was 31.92 ± 4.28 Kg/M2 for group 1 and 21.69 ± 1.76 Kg/M2 for group 2.

The comparison between conventional semen parameters, sperm HOS, semen ROS, and the percentage of fragmentation of zinc and sperm DNA in both populations are illustrated in table 1. The tables show that obese infertile males possess noticeably more inhibited sperm normal morphology, progressive motility, viability percentages, and semen Zn levels. They also had surging percentages of fragmentation of sperm DNA and semen ROS.

Imposing negative associations were observed in the participants that correlated BMI with sperm concentration (r= - 0.26, P<0.01), progressive sperm motility percentage (r= -0.62, P<0.001), normal morphology percentage (r= - 0.51, P<0.001), viability percentage (r= - 0.53, P<0.001), and semen Zn (r= - 0.73, P<0.001). Furthermore, a significantly directly proportional correlation linking BMI and of the percentage of fragmentation of sperm DNA (r= 0.74, P<0.001) and semen ROS (r= 0.67, P<0.001) was found (figures 1 and 2).

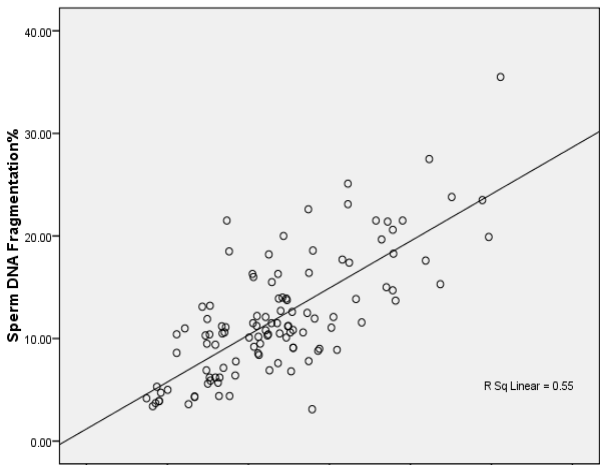

Fig (1) Correlation between BMI and sperm DNA fragmentation%

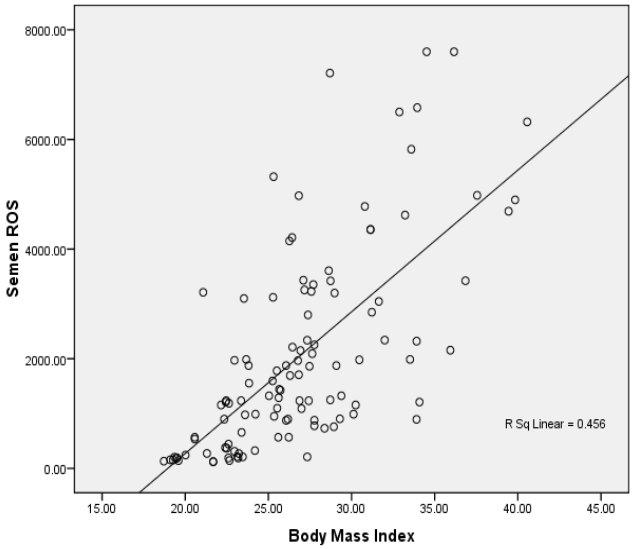

Fig (2) Correlation between BMI and semen ROS

Table (1) Comparison between semen variables in infertile overweight and fertile normally weighing men

Semen Variable	Infertile Overweight (group 1, n=92)	Normal Weight (Control) group2, n=81)	P Value
Semen volume (ml)			
Range	1 - 4	1.5 - 4	
Mean ± SD	2.098 ± 0.77	2.33 ± 0.84	N.S
Sperm concentration(mil/ml)*			
Range	2 - 250	20 - 200	
Mean ± SD	44.74 ± 49.9	72.37 ± 46.06	N.S
Normal sperm morphology%			
Range	0 - 60	30 - 75	
Mean ± SD	28.76 ± 14.62	57.3 ± 11.3	<0.001
Progressive sperm motility %			
Range	0 - 40	32 - 75	
Mean ± SD	16.52 ± 11.59	55.22 ±10.3	<0.001
Hypoosmotic swelling (HOS)%			
Range	15 – 80	40 – 90	
Mean ± SD	49.54 ± 18.23	77.04 ± 11.29	<0.001
Sperm DNA fragmentation %			
Range	6.8 – 35.5	3.4 – 19.9	
Mean ± SD	16.48 ± 5.57	5.92 ± 2.17	<0.001
Semen reactive oxygen species (ROS) level (RLU)/10^6 sperm*			
Range	875 – 7600	120 – 3212	
Mean ± SD	3500 ± 1874	531.67 ± 814.9	<0.001
Semen zinc (Zn) level (µg/mL)			
Range	50-140	105-180	
Mean ± SD	74.96 ± 20.03	140 ± 16.65	<0.001

* Mann-Whitney test

DISCUSSION:

An individual is considered obese when their body mass index (BMI) exceeds 30 Kg/m2 with an increase of the visceral adipose tissue[24]. As a serious public health concern, infertility negatively affects 15% of couples all over the world[25] where half of the cases are resulting from the male factor. Paternal obesity has been suggested as a cause for the decrease in the count of sperms[26] and an increase in the damage of sperm DNA[27]. Our results showed reduced sperm normal morphology, progressive motility, viability percentages and zinc levels, and a high percentage of fragmentation of sperm DNA and semen ROS infertile obese men. It also showed noticeably lower sperm progressive motility and viability percentages and surging fragmentation of DNA of spermin infertile obese men when considered against men with normal weights. This comes in agreement with the study of Kort et al. 2006[28] who found a negative correlation between the BMI and the normal motile sperms number and also found a decreased number of normal chromatin-intact motile sperm cells per ejaculate in males with BMI greater than 25 kg/m2.

Interestingly, our results came in agreement with a research done by Taha et al. 2016[29] on fertile men, and they concluded that obesity and being overweight affected semen variables negatively either in fertile or infertile males, especially in association with other risks as higher age at time of marriage, smoking, and varicocele that may affect the potential fertility for

overweight males in the future. Various mechanisms lead to ED and aberrant parameters of semen in obese overweight men which are physical, hormonal, and genetic factors and adipokine and cytokine factors. The main mechanism is the incorrect regulation of the HPG axis. A hormonal profile with aberrant proportions with high levels of adipokine and adipose-derived hormones can more accurately justify the correlation linking BMI with infertility and seminal abnormalities; it is overly difficult than simply atypical reproductive hormone levels can be considered[30]. Our results were contradictory to the results of Refus et al. 2018[31] who stated that higher BMI did not significantly affect fertility, reassuring infertile males with high BMI that BMI is not a factor in their semen quality. Also, a study done by Bandel et al. 2015[32] exhibited that a significantly higher DFI has been noticed in normal-weight men more than those with overweight with 1.13% as a mean difference (95% CI: 1.05–1.22%); P ¼ 0.001), and they declared no correlation between the integrity of sperm DNA and BMI. The aforementioned outcomes concur with the study of Chavarro et al. 2009[33] who submitted that despite the considerable differences in the level of reproductive hormones with increasing body weight, only morbid obesity could be affecting male reproductive capacity.

In our study, we found that obese infertile patients had lower semen zinc levels, agreeing with Ebisch et al. 2006[15]. The low semen zinc level may influence the semen quality in underlying ways, such as reducing the capacity of antioxidants[34] and restraining the sequel of other heavy metals[35].

Conclusion and Recommendations:

Obesity negatively hinders semen parameters, in addition to sperm DNA integrity and seminal zinc in infertile men.

Limitations of the Study:

There is a need for future research with a larger sample size to further investigate the etiopathogenesis of infertility in obese individuals.

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تاثير السمنه على السائل المنوي ,الاجهاد التاكسدي , الزنك وسلامة الحمض النووي لدي الذكور العقم

ان العقم مشكله عامه تصيب نسبه كبيره من الازواج ويعتقد ان السمنه لدي الذكور قد يكون لها تاثير كبير عليها . لذلك الهدف من هذا البحث هو دراسه تاثير السمنه علي السائل المنوي والاجهاد التاكسدي و الزنك و سلامه الحامض النووي .

قد تم تقسيم المرضى الى مجموعتين المجموعه الاولى بها ذكور لديهم عقم ويعانوا من السمنهاما المجموعه الثانيه هي مجموعه للمقارنه بها ذكور لديهم اعمار متقاربه من المجموعه الاولى.

تم عمل فحص كلينيكي لجميع الحالات و كذلك تحليل سائل منوي وقياس نسبهالاجهاد التاكسدي و الزنك و سلامه الحمض النووي لدى الحيوانات المنويه.

وكانت نتائج المجموعه الاولى بها ضعف في حركه الحيوانات المنويه و قله في عددهم و في نسبه الزنك ذو دلاله احصائيه مقارنة بالمجموعه الثانيه.

كذك وجدت زيادة ذو دلاله احصائيه في نسبة الاجهاد التاكسديو نسبة تكسير الحمض النووي داخل الحيوانات المنويه.

بالاضافه الى وجود علاقه سلبيه بين مؤشر كتلة الجسم و كل من عدد الحيوانات المنويه ,نسبة الحركه,ونسبة الزنك بالسائل المنوي و كذلك علاقه ايجابيه مع كل من نسبة الاجهاد التاكسديونسبة تفتيت الحمض النووي.

النتيجه:السمنه لها تاثير سلبي على قياسات السائل المنوي , الاجهاد التاكسدي , نسبة الزنك و سلامة الحمض النووي و كل ذلك مرتبط بمؤشر كتلة الجسم.