DOI: 10.24996/ ijs.2017.58.3B.7

ISSN: 0067-2904

Effect of Body Mass Index on Sperm Parameters and Sex Hormone Level in Sample of Infertile Iraqi Men

Jabaar H. Yenzeel
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

The objective of the present study was to investigate the effect of body mass index (BMI) on semen parameters, level of sex hormone and sperm DNA integrity. Semen samples were collected from (85) infertile men and (40) healthy fertile men with range of age (38.191 ± 0.84) years during their attendance at High Institute of Infertility Diagnosis and ART, Al-Nahrain University from March to June 2016. Semen samples were obtained by masturbation after 72 hours of abstinence. Seminal fluid analyses included semen volume, sperm concentration, percent sperm motility, percent sperm morphology, and sperm chromatin integrity DNA fragmentation index (DFI]). Serum samples were collected from each subject for determination the level of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin (PRL), and Testosterone by ELISA method. The results revealed a highly significant ($P \leq 0.01$) increase in BMI and immotile sperm (%), and significant($P \leq 0.05$) increase in semen liquefaction time, non-progressive motility (%), round cells counts and sperm DNA fragmentation in infertile men as compared to control group, while there was a highly significant ($P \leq 0.01$) decrease in progressive motility (%), and a significant ($P < 0.05$) decrease in the sperm concentration, sperm motility (%) and normal sperm morphology (%). The results showed statistically significant ($P < 0.05$) positive correlations between body mass index and sperm motility, progressive motility, non-progressive motility, immotile sperm, normal sperm morphology and sperm DNA fragmentation. No significant correlations were observed between body mass index and semen liquefaction time, semen PH, sperm concentration, round cells counts and age. In respect with level of serum hormones a significant ($P < 0.05$) decrease in level of FSH ,LH and testosterone was found ,while the level of prolactin showed a significant ($P < 0.05$) increase in infertile men when compared with control group. Significant ($P < 0.05$) negative correlation was observed between body mass index and serum level of prolactin and testosterone, while non-significant correlations were observed between body mass index and serum level of FSH and LH. In conclusion, this study has shown that body mass index has major effect on semen characteristics and sex hormones.

Keywords: Body mass index, Semen quality, Male sex hormones, DNA fragmentation

تاثير مؤشر كتلة الجسم على بعض معايير النطف ومستوى الهورمونات الجنسية في عينه من الرجال العراقيين غير الخصيبين

جبار حميد ينزيل

قسم علوم الحياة ، كلية العلوم ، جامعة بغداد ، بغداد، العراق.

*Email: Dr.jabbaryenzeel@yahoo.com

الخلاصة

هدف هذه الدراسة هو التحري عن تاثير مؤشر كتلة الجسم على معايير النطف ومستوى الهورمونات الجنسية وسلامة المادة الوراثية للنطف. جمعت عينات السائل المنوي من 85 رجل غير خصيب و 40 رجل سليم وبمعدل عمر (38.191 ± 0.84) سنة عند حضورهم الى المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب في جامعة النهرين للفتره من اذار الى حزيران للعام 2016. تم الحصول على عينات السائل المنوي عن طريق الاستمناء بعد 72 ساعة من التوقف عن الاتصال الجنسي . تضمنت تحاليل السائل المنوي حجم السائل و تركيز النطف والنسبة المؤية لحركة النطف ومظهر النطف ومعامل تشظي الدنا. جمعت عينات المصل من كل شخص لغرض تحديد مستوى الهورمون المحفز للجريبات والهورمون اللوتيني وهورمون الحليب وهورمون الشحمون الخصوي بطريقة امتصاصية الانزيم المرتبط (الاليزا). توصلت نتائج الدرسة الى وجود ارتفاع معنوي عالي ($P\leq 0.01$) في مؤشر كتلة الجسم وعدد النطف غير المتحركة في الرجال غير الخصيبين مقارنة مع الاصحاء، فضلا عن حدوث ارتفاع معنوي ($P< 0.05$) في وقت تميع النطف والحركة غير التقدمية وعدد الخلايا المستديره وتشظي الدنا في الاشخاص غير الخصيبين مقارنة مع الاصحاء، في حين كان هناك انخفاض معنوي عالي ($P\leq 0.01$) في الحركة التقدمية وانخفاض معنوي ($P< 0.05$) في تركيز النطف وحركة النطف والشكل الطبيعي في الاشخاص غير الخصيبين.اظهرت النتائج وجود ارتباط معنوي ($P< 0.05$) موجب بين مؤشر كتلة الجسم وبين كل من حركة النطف والحركة التقدميه ووغير التقدمية وغير المتحركه والشطل الطبيعي للنطف وتشظي الدنا للنطف. ولم يظهر ارتباط معنوي بين مؤشر كتلة الجسم وكل من وقت التميع ودالة الحموضه وتركيز النطف والخلايا المستديره والعمر. فيما يتعلق بمستوى الهورمونات فقد لوحظ وجود انخفاض معنوي ($P< 0.05$) في مستوى هورمون المحفز للجريبات والهورمون اللوتيني وهورمون الشحمون الخصوي في الرجال غير الخصيبين مقارنه مع الاصحاء ، في حين لوحظ ارتفاع معنوي في مستوى هورمون الحليب في الرجال غير الخصيبين ولوحظ وجود ارتباط معنوي سالب بين مؤشر كتلة الجسم وهورمون الحليب في حين لم يكن هناك ارتباطا معنويا مع بقيه الهورمونات. يستنتج من الدراسة ان مؤشر كتلة الجسم له تاثير كبير على معايير النطف والهورمونات الجنسية.

Introduction

Obesity is a medical condition in which excess body fat, or white adipose tissue, accumulates in the body to the extent that the excess fat adversely affects health, the fundamental cause of obesity and overweight is an energy imbalance, where the energy consumed exceeds the energy expended. Global increases in overweight and obesity are attributable to a number of factors, including a shift in diet toward increased intake of energy-dense foods that are high in fat and sugars, and a trend toward decreased physical activity [1]. Body mass index (BMI) defined as the weight in kilograms divided by the square of the height in meters (kg/m2) and calculated from a person's weight and height .It is a simple index of weight-for- height, that is commonly used to classify people as underweight, normal weight, overweight and obese, an individual is normally defined as being overweight if their BMI is between 25 and 30 kg/m 2 and obese if it exceeds 30 kg/m 2[2].

Infertility is defined as "the inability of a couple to achieve conception or bring a pregnancy to term after 12 months or more of regular, unprotected sexual intercourse" [3]. Infertility affects up to 15% of couples. A male factor is solely responsible in about 20% of infertile couples and contributory in 30–40% of cases [4]. While obesity has been associated with a host of cardiovascular diseases, metabolic syndrome, and a wide variety of endocrine abnormalities, recent data suggested a potential link between obesity and male infertility [5, 6].However, with the increasing prevalence of sedentary life styles and dietary changes, obesity is emerging as an important cause of adverse health outcomes, including male infertility [7]. Obesity has recently been proposed for addition to the list of known aetiologies of male infertility.

Obese males usually express a characteristic hormonal profile described as "hyperestrogenic hypogonadotropic hypogonadism". Total body fat, intra-abdominal fat, and subcutaneous fat have all been associated with low levels of total and free testosterone [8]. Body mass index has been associated with alterations in sperm parameters in several reports [9-11]. Many studies have

highlighted impairments of sperm quality for patients with a high or very high body mass index (BMI), notably a decrease in seminal sperm concentration [12- 14].

Sperm DNA integrity is another factor that may be affected in obese subjects, possibly resulting from increased damage due to oxidative stress. A reduced number of studies have assessed the impact of BMI on sperm DNA integrity, with controversial results due to low numbers of cases studied and disparity in the techniques used. [14-17]. There are now several but little population-based studies showing that overweight and obese men have up to 50 % higher rate of sub-fertility when compared to men with normal weight [18, 19].

Many studies have shown that overweight and obese men present hormonal changes such as lower plasma levels of sex hormone-binding globulin, total and free testosterone, luteinizing hormone (LH) and follicle- stimulating hormone [11], Nevertheless, lower sperm count, decreased normal- motile sperms and increased DNA fragmentation index [11] .Decreased ejaculate volumes and lower fertilization rate were also reported in rats and mouse [20- 22]. Increase in the DNA fragmentation index (DFI) accompanied an increase in BMI, demonstrating that obesity might compromise the integrity of sperm chromatin, their only genetic material. An increase in the BMI above 25 kg/m 2 causes an increase in sperm DFI and a decrease in the number of normal chromatin-intact sperm cells per ejaculate, relative to the degree of obesity [10].

Materials and Methods

Subjects:

This study includes (85) infertile men and(40) healthy men during their attendance at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al- Nahrain University from March to June 2016. The required information was recorded for all subjects assessed in this study, and semen samples obtained by masturbation after 72 hours of abstinence.Seminal fluid analysis was done according to criteria WHO [23], included semen volume, sperm concentration, percent sperm motility, percent sperm morphology (normal forms), and sperm chromatin integrity DNA fragmentation index (DFI). Serum samples were collected from each subject were analysed for determination the level of Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL), and testosterone by ELISA method.

Assessment of DNA Fragmentation index (DFI)

Sperm chromatin integrity was done according to [24] by used Acridine Orange fluorescence. Sperm chromatin status was evaluated; using the method described by [25]. Spermatozoa with normal, intact double-stranded DNA stained green and those with denatured ones showed red or orange fluorescence were assessed. DNA fragmentation is quantified using the DFI, which expresses the amount of fragmented DNA as a percentage total DNA

Statistical analysis

Statistical package for social science (SPSS/PC) software (version 18) were used to analyze the obtained data. Sperm parameters were analyzed using complete randomized design (CRD) of one way analysis of variance (ANOVA).

Differences among means were computed using the Duncan multiple ranges test [26].

Results

The results of semen analyses parameters are shown in (Table-1). In this table the results revealed a highly significant ($P\leq 0.01$) increase in BMI(28.344 ± 0.45) and immotile sperm (32.989 ± 2.74) as compared with control (21.70 ± 1.06) and (16.650 ± 1.40) respectively, Also there was a significant increase in semen liquefaction time(42.468 ± 1.45), non-progressive motility (23.755 ± 0.99) and round cells counts (7.894 ± 0.28) in compared with control (36.140 ± 1.08),(18.300 ± 0.93) and (4.439 ± 0.49) respectively . The results revealed a highly significant ($P\leq 0.01$) decrease in progressive motility (31.266 ± 1.54) and normal sperm morphology (34.755 ± 1.18) as compared with control (58.410 ± 2.61) and (64.494 ± 1.55) respectively, while there was a significant ($P\leq 0.05$) decrease in sperm concentration (41.340 ± 3.38), sperm motility (55.021 ± 0.93) as compared with control (55.360 ± 2.08) and (76.71 ± 2.13).The semen PH showed non- significant($P\geq 0.05$) decrease (7.467 ± 0.06) when compared with control (7.763 ± 0.03). The results of sex hormones in infertile men revealed a significant ($P\leq 0.05$) decrease in serum concentration of follicles stimulating hormone(FSH) (4.46 ± 1.01 Ng/ml), luteinizing hormone(LH) (4.23 ± 0.36ng/ml) and testosterone (18.021 ± 1.93 Ng/ml) as compared with control groups (5.75 ± 0.7 Ng/ml),(5.76 ± 1.03 Ng/ml) and (28.71 ± 2.13 Ng/ml) respectively, While, a significant ($P\leq 0.05$) increase in the serum

concentration of prolactin (PRL) (198.3 ± 7.38ng/ml) as compared with control group (176.36 ± 5.08 Ng/ml) (Table- 2).

Figure -1 showed the influence of body mass index on sperm DNA fragmentation for all semen samples. With respect to sperm DNA fragmentation, a significant ($P \leq 0.05$) increase was observed in infertile men (32.65± 1.87) as compared with control (19.34 ± 1.03).

Table 1- Sperm pparameters of infertile and healthy men (mean ± SE).

Sperm Parameters		**Control**	**Infertile**	**T-test and Sig**
Semen liquefaction time (minute)		36.140 ± 1.08	42.468 ± 1.45	4.893 *
Semen PH		7.763 ± 0.03	7.467 ± 0.06	0.472 NS
Sperm concentration (million/ml)		55.360 ± 2.08	41.340 ± 3.38	4.314 *
Sperm motility (%)		76.71± 2.13	55.021± 0.93	7.657 *
Sperm grade activity (%)	Progressive motility (%)	58.410 ± 2.61	31.266 ± 1.54	5.207 **
	Non-Progressive motility (%)	18.300 ± 0.93	23.755 ± 0.99	2.612 *
	Immotile sperm (%)	16.650 ± 1.40	32.989 ± 2.74	5.758 **
Normal sperm morphology (%)		64.494 ± 1.55	34.755 ± 1.18	5.184 **
Round cells counts (HPF)		4.439 ± 0.49	7.894 ± 0.28	1.769 *
Sperm DNA fragmentation		19.34 ± 1.03	32.65± 1.87	3.55 *
BMI (kg/m2)		21.70 ± 1.06	28.344 ± 0.45	3.977 **

No. of Infertile = 85: No. of Control = 40. * (P<0.05), ** (P<0.01), NS: Non-significant.

Table 2-Level of sex hormones in Infertile and healthy men (mean ± SE)

Hormones	**Control**	**Infertile**	**T-test and Sig.**
Follicles Stimulating Hormone(FSH) (ng/ml)	5.75 ± 0.7	4.46 ± 1.01	0.893 *
Luteinizing Hormone(LH) (ng/ml)	5.76 ± 1.03	4.23 ± 0.36	0.62 *
Prolactin (PRL) (ng/ml)	176.36 ±5.0	198.3 ± .38	9.43 *
Testosterone (ng/ml)	28.71± 2.13	18.021±1.93	3.57 *

No. of Infertile = 85, No. of Control = 40. Significant.* (P<0.05)

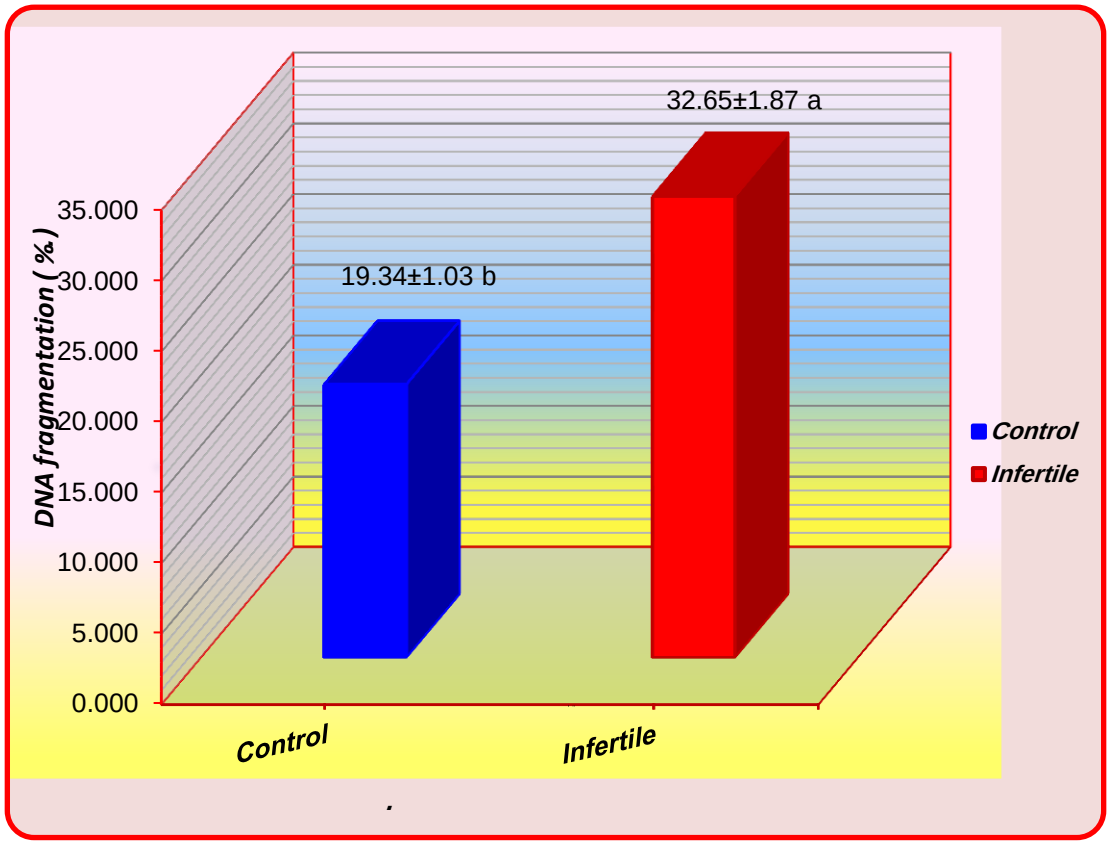

Figure 1-DNA fragmentation of sperm for infertile and healthy men.
Means with different superscripts within each column are significantly different (P<0.05).

The results of correlation showed statistically significant (P≤ 0.05) positive correlations between body mass index and sperm motility (r= 0.26), progressive motility (r= 0.16), non-progressive motility (r= 0.22), immotile sperm (r= 0.18), normal sperm morphology(r= 0.21) and sperm DNA fragmentation(r= 0.21).Non- significant (P≥ 0.05) correlations were observed between body mass index and semen liquefaction time (r=0.07), semen PH(r=0.006), sperm concentration(r=0.09), round cells counts(r= 0.002) and age(r= 0.10) (Table- 3). Non-significant (P≥ 0.05) negative correlations were observed between body mass index and serum level of prolactin(r= - 0.058), FSH(r= - 0.065) and LH(r= - 0.074), while non-significant (P≥ 0.05) positive correlations were observed between body mass index and serum level of testosterone(r= 0.001) (Table- 4).

Table 3- Correlation coefficient between body mass index and Sperm Parameters

BMI with parameters	Correlation coefficient-r	Level of sig.
Semen liquefaction time (minute)	0.07	NS
Semen PH	0.006	NS
Sperm concentration (million/ml)	0.09	NS
Sperm motility (%)	0.26	*
Progressive motility (%)	0.16	*
Non-Progressive motility (%)	0.22	*
Immotile sperm (%)	0.18	*
Normal sperm morphology (%)	0.21	*
Round cells counts (HPF)	0.002	NS
Sperm DNA fragmentation	0.21	*
Age (year)	0.10	NS
* (P<0.05), NS: Non-significant.		

Table 4- Correlation coefficient between body mass index and sex hormones

BMI with hormones	Correlation coefficient-r	Level of sig.
Follicles Stimulating Hormone(FSH)	- 0.065	NS
Luteinizing Hormone(LH)	- 0.074	NS
Prolactin (PRL)	- 0.058	NS
Testosterone	0.001	NS
* (P<0.05), NS: Non-significant.		

Discussion

The results of current study showed significant increase in BMI in an infertile men as compared with healthy, also semen analyses parameters revealed significant increase immotile sperm semen, liquefaction time, non-progressive motility and round cells counts in an infertile man as compared with control, while there was significant decrease in progressive motility, normal sperm morphology, sperm concentration and sperm motility. This results in agreement with previous studies [9- 14].A possible association between metabolic syndrome (MS) and male infertility has been hypothesized. Male obesity and (MS) are associated with hypogonadism[27]. Insulin and leptin, which are important regulators of male reproduction via modulation of the hypothalamus-pituitary-testes (HPT) axis, were detected in the seminal fluid of obese infertile men [28]. One such adipose-derived hormone is leptin, which is best known as a regulator of food intake and energy expenditure via hypothalamic-mediated effects. Excess leptin may be an important contributor to the development of reduced androgens in male obesity.

Leptin receptors are not only present in testicular tissue but also on the plasma membrane of sperm suggesting that leptin may directly affect sperm via the endocrine system, independent of changes in the HPG axis [29] . As mentioned previously, adipocytes secrete various adipokines (e.g., tumor necrosis factor a (TNF- a), interleukin 6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), and tissue factor). A number of these adipokines have been connected to infertility and testicular cancer. Also, increased release of adipokines from excess white adipose tissue, resulting in inflammation, can have a toxic effect on spermatozoa through the release of excess reactive oxygen species (ROS) and reactive nitrogen species (RNS) [30].

Many authors have noted that obesity and several of its causative agents, namely insulin resistance and dyslipidemia, are associated with increased oxidative stress .This association is most likely the result of the elevated metabolic rates that are required to maintain normal biological processes and increased levels of stress in the local testicular environment, both of which naturally produce ROS. The local influences of biologically active substances (cytokines) released by activated leukocytes in the course of the inflammatory response to obesity may damage sperm and inhibit spermatogenesis. Agarwal *et al.* [31] found that abnormal patterns of increased ROS were associated with male factor infertility and are responsible for abnormal sperm concentration, motility, and morphology found in obese males.

In respect with level of serum hormones the results of current study showed a significant decrease in level of FSH, LH and testosterone, while the level of prolactin showed a significant increase in infertile men when compared with control group.The reproductive hormonal profiles of most obese men deviate from what is considered the normal. Obese men tend to present with elevated estrogen and low testosterone and FSH levels [32].

Androgen deficiency or hypogonadism found in males who are obese or have metabolic syndrome can account for problems with erectile dysfunction and spermatogenesis. However, many other hormones associated with obesity may alter the male reproductive potential. In morbidly obese individuals, reduced spermatogenesis associated with severe hypotestosteronemia may contribute to infertility [33]. This estrogen excess is explained by over activity of the aromatase cytochrome P450 enzyme, which is expressed at high levels in white adipose tissue and is responsible for a key step in the biosynthesis of estrogens. High levels of estrogens in obese males result from the increased conversion of androgens into estrogens, owing to the high bioavailability of these aromatase enzymes [34]. Visceral obesity can serve as a major endocrine disrupter and can also influence the endocrine interactions by reducing the levels of luteinizing hormone (LH) and testosterone, resulting in hypogonadotropic hypogonadism, a condition which contributes to male infertility [35].

Excess body weight can impair the feedback regulation of the HPG axis, and all of the factors above might contribute to or be a result of this dysregulation, contributing to apparent semen quality abnormalities. Sex steroids and glucocorticoids control the interaction between the hypothalamic–pituitary–adrenal (HPA) and the HPG axes, and any amount of disturbance might, in turn, affect spermatogenesis and male reproductive function. Men of normal weight with low levels of testosterone regularly present with elevated levels of LH and FSH, in contrast with obese men, who usually present with low LH and FSH levels [36].

The results of this study showed significant increase percentage of sperm DNA fragmentation in infertile men as compared to control group. This results in agreement with other studies that explained, the integrity of DNA in the sperm nucleus is an important determinant of semen quality since it defines not only the success of fertilization but also the normality of embryonic development and the health trajectory of the offspring. As a consequence, DNA damage in these cells is associated with the impairment of fertility, an increase in the incidence of miscarriage, and a variety of defects in the progeny ranging from neurological conditions such as autism to cancer [37].

A major cause of DNA damage in spermatozoa is oxidative stress mediated by a variety of reactive oxygen species (ROS) including free radicals such as superoxide anion ($O_2^{-\bullet}$), nitric oxide ($NO^{\bullet}$), or the hydroxyl radical ($OH^{\bullet}$) as well as powerful oxidants such as hydrogen peroxide (H_2O_2) or peroxynitrite ($ONOO^-$). Spermatozoa are particularly prone to oxidative stress because their antioxidant defensive capacity is limited, due to the removal of a majority of their cytoplasm during spermatogenesis and a consequential reduction in cytoplasmic antioxidants such as catalase or superoxide dismutase. Furthermore, these cells are professional generators of ROS, with a vast majority of these reactive oxygen metabolites being generated as a consequence of electron leakage from the sperm mitochondria [38].

References

1. Stothard, KJ., Tennant, PW., Bell, R. and Rankin, J. **2009**. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *JAMA*., **301**(6): 636-50.
2. World Health Organization **2006.** *BMI Classification*. WHO Global Database on Body Mass. WHO Press; p. 34–74.
3. World Health Organization **2010**. *WHO laboratory manual for the examination and processing of human semen*. 5th ed. Geneva: WHO Press; p. 7–114.
4. Jarow, JP., Sharlip ID, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, Schlegel PN, Howards SS, Nehra A, Damewood MD, Overstreet JW, Sadovsky R**. 2010.** Male infertility best practice policy committee of the American Urological Association Inc: best practice policies for male infertility. *J Urol*. **167**: 2138–44.
5. Pasquali, R. **2006.** Obesity and androgens: facts and perspectives. *Fertil. Steril.* **85**: 1319-40.
6. Ferris, WF., Crowther NJ. **2011**. Once fat was fat and that was that: our changing perspectives on adipose tissue. *Cardiovasc J Afr*; **22**: 147-54.
7. Hammoud, AO., Gibson, M., Petersonm CM. and Meikle, AW. **2008**. Impact of male obesity on infertility: a critical review of the current literature. *Fertil Steril*; **90**: 897-904.
8. Tsai, E.C., Matsumoto, A.M., Fujimoto, W.Y. and Boyko, E.J. **2004**. Association of bioavailable, free, and total testosterone with insulin resistance, influence of sex hormone-binding globulin and body fat. *Diabetes Care*. **27**: 861 –868.
9. Koloszar, S., Fejes, I., Zavacki, Z., Daru, J. and Szollosi, J. **2005**. Effect of body weight on sperm concentration in normozoospermic males. *Arch Androl*. **51**: 299-304.
10. Kort, H.I., Massey, J.B., Elsner, C.W., Mitchell-Leef, D., Shapiro, D.B., Witt, M.A., Roudebush, W.E. **2005**. Impact of body mass index values on sperm quantity and quality. *J Androl*. **27**: 450 – 452.
11. Magnusdottir, E.V., Thorsteinsson, T., Thorsteinsdottir, S., Heimisdottir, M. and Olafsdottir, K. **2005**. Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Hum Reprod*, **20**: 208 –215.
12. Chavarro, JE., Toth TL., Wright, DL., Meeker, JD. and Hauser, R. **2010.** Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertil Steril*, **93**: 2222–31.
13. Hammiche, F., Laven, JS., Boxmeer, JC., Dohle, GR., Steegers, EA. et al. **2011**.Sperm quality decline among men below 60 years of age undergoing IVF or ICSI treatment. *J Andro*l, **32**: 70–6.
14. **14**- Tunc O, Bakos HW, Tremellen K. **2011**. Impact of body mass index on seminal oxidative stress. *Andrologia*, **43**: 121–8.
15. **15**- Sermondade N, Faure C, Fezeu L, Levy R, Czernichow S. Obesity and increased risk for oligozoospermia and azoospermia. Arch Intern Med 2012, **172**: 440–2.
16. **16**- Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP. **2013**. BMI in relation to sperm count: an updated systematic review and collaborative meta- analysis. *Hum Reprod* Update; **19**: 221–31.
17. Rybar, R., Kopecka, V. and Prinosilova, P. **2011.** Male obesity and age in relationship to semen parameters and sperm chromatin integrity. *Andrologia*; **43**: 286–91.
18. WHO, World Health Organization. **2005**. The SuRF report 2: *The Surveillance of chronic disease Risk Factors*. Geneva: WHO, Press, p. 23–78.
19. .Sunderam, S., Chang, J., Flowers, L., Kulkarni, A., Sentelle, G. and Jeng, G. **2009**. Centers for Disease Control and Prevention (CDC). Assisted reproductive technology surveillance *Surveill Summ*, **58**: 1-25.
20. Bakos, HW., Mitchell, M. and Setchell, BP. **2011**. The effect of paternal diet induced obesity on sperm function and fertilization in a mouse model. *Int J Androl*, **34**: 402-10.
21. Fernandez, CDB., Bellentani, FF., Fernandes, GSA., Perobelli, JE., Favareto, APA., Nascimento, F . **2011**. Dietinduced obesity in rats leads to a decrease in sperm motility. *Reprod Biol Endocrino* **l(**9): 32.
22. Ghanayem, BI., Bai, R., Kissling, GE., Travlos, G. and Hoffler, U. **2010**. Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamideinduced reproductive toxicity. *Biol Reprod*, **82**: 94-104.

23. World Health Organization (WHO). **2010**. *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction*. 5th edition. Cambridge: Cambridge University Press; pp: 162-224.
24. Tejada, R.I., Mitchell, J.C., Norman, A., Marik, J.J. and Friedman, S. **1984.** A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. *Fertil. Steril*. **42**(1): 87-91.
25. Acosta, A. and Kruger, TF. **1996.** *Human Spermatozoa in Assisted Reproduction. Baltimore:* Williams and Wilkins. 319-324.
26. Duncan, D B. **1955.** Multiple range and multiple F tests. *Biometrics*, **11**(1): 1-42.
27. Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. **2010**. The effect of obesity on sperm disorders and male infertility. *Nat Rev Urol*. **7**:153–61.
28. Leisegang K, Bouic PJ, Menkveld R, Henkel RR.**2014**. Obesity is associated with increased seminal insulin and leptin alongside reduced fertility parameters in a controlled male cohort. *Reprod Biol Endocrinol*. **7**(12): 34.
29. Jope, T., Lammert, A., Kratzsch, J. and Paasch, U. **2003**. Leptin and leptin receptor in human seminal plasma and in human spermatozoa. *Int J Androl*. **26**(6): 335–41.
30. Fraczek M, Kurpisz M.2007. In fl ammatory mediators exert toxic effects of oxidative stress on human spermatozoa. *J Androl*. **28** (2): 325–33.
31. Agarwal, A., Sharma, RK., Nallella, KP. and Thomas, Jr. AJ. **2006**. Reactive oxygen species as an independent marker of male factor infertility.*Fertil Steril*. **86** (4): 878–85.
32. Strain, GW., Zumoff, B. and Kream, J. **1982**. Mild hypogonadotropic hypogonadism in obese men. *Metabolism*. **31**(9): 871–5.
33. Tsai, EC., Matsumoto, AM., Fujimoto, WY., Boyko, EJ. **2004** .Association of bioavailable, free, and total testosterone with insulin resistance: in fl uence of sex hormone-binding globulin and body fat. *Diabetes Care*. **27**(4): 861–8.
34. Roth MY, Amory JK, Page ST.**2008**.Treatment of male infertility secondary to morbid obesity. *Nat Clin Pract Endocrinol Metab*. **4**(7): 415–9.
35. Athyros, VG., Tziomalos, K., Karagiannis, A., Anagnostis, P., Mikhailidis, DP. **2010**. Should adipokines be considered in the choice of the treatment of obesity-related health problems? *Curr Drug Targets*. **11**(1): 122–35.
36. Jarow, JP., Kirkland, J., Koritnik, DR. and Cefalu, WT. **1993.**Effect of obesity and fertility status on sex steroid levels in men. *Urology*. **42**(2): 171–4.
37. Aitken, RJ., Bronson, R. and Smith, TB. **2013.** The source and significance of DNA damage in human spermatozoa; diagnostic strategies and straw man fallacies. *Mol Hum Reprod* **19**(8): 475–485. doi: 10.1093/molehr/gat025
38. Koppers, AJ., De Iuliis, GN. and Finnie, JM . **2008.** Signifi cance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. *J Clin Endocrinol Metab*, **93**(8): 3199–3207. doi: 10.1210/jc.2007-2616.