SPERM PARAMETERS OF THE INFERTILE PATIENTS IN RELATION TO SOCIO DEMOGRAPHIC FACTORS

Ahmed N. Altimimi[a]*, Mohsin K. Almurshdi[a]

Article History: **Received**: 30.05.2022 **Revised**: 29.06.2022 **Accepted**: 17.07.2022

Abstract: Objective: To investigation the effect of age, obesity, smoking, infections, varicocele, period of abstinence and liquefaction, sperm parameters. **Patients and methods**: The study was carried out between January 2022 and July 2022 which including (60) selected infertile patients who attended the fertility center in Al-Sadr Medical City-Najaf-Iraq. The Parameters for the semen analysis were analyzed and classified according to the WHO criteria (WHO, 1999). Patients should give their semen for analysis in the laboratory by masturbation. **Results**: The results showed that significant (P<0.01) difference in semen volume, sperm concentration, progressive motility percent (A+B) and normal morphology in relation to age, body mass index and smoking, also the results revealed a significant (P<0.01) reduction in semen volume, progressive motility (A+B) and normal morphology in leukocytospermia. The results in current study also showed a significant (p<0.01) difference in Semen volume, sperm concentration and progressive motility (A+B) in relation to duration of abstinence, a significant (P<0.01) decrease was noticed via 2-3 days compared to other groups of duration of abstinence, there was a significant (p<0.01) decrease in sperm concentration, progressive motility (A+B) and normal morphology in varicocele positive, a significant (P<0.01) decline in sperm concentration, progressive motility (A+B) and normal morphology was showed with group ≥30 minutes of Liquefaction time. **Conclusion**: Advance paternal age, obesity and smoking lead to a deterioration in sperm parameters and thus contribute as one of the causes of male infertility, also the infections, varicocele, period of abstinence and liquefaction time have a negative impact on the semen quality, which leads to decrease male fertility.

Keywords: age, obesity, smoking, infections, varicocele, period of abstinence, liquefaction time, semen, seminal fluid analysis, socio demographic factors.

[a]. Department of Laboratory Investigation, Collage of Science, Kufa university, Iraq

***Corresponding Author**

E-mail: ahmednaser896@gmail.com

DOI: 10.31838/ecb/2022.11.04.012

INTRODUCTION

Infertility has been defined by the World Health Organization as a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. In general, infertility is defined as not being able to get pregnant (conceive) after one year (or longer) of unprotected sex [2]. Infertility can be primary or secondary, the primary infertility is when a pregnancy has never been achieved by a person, and the secondary infertility is when at least one prior pregnancy has been achieved. Infertility may be caused by a number of different factors, in either the male or female reproductive systems. However, it is sometimes not possible to explain the causes of infertility [3]. In the male reproductive system, infertility may be caused by the obstruction of the reproductive tract causing dysfunctionalities in the ejection of semen [4]. This blockage can occur in the tubes that carry semen (such as ejaculatory ducts and seminal vesicles). Blockages are commonly due to injuries or infections of the genital tract, hormonal disorders leading to abnormalities in hormones produced by the pituitary gland, hypothalamus and testicles [5]. Hormones such as testosterone regulate sperm production, example of disorders that result in hormonal imbalance include pituitary or testicular cancers, testicular failure to produce sperm, for example due to varicoceles or medical treatments that impair sperm producing cells (such as chemotherapy) [6]. abnormal sperm function and quality. Conditions or situations that cause abnormal shape (morphology) and movement (motility) of the sperm negatively affect fertility.

There are many influences that effected to the sperm or semen, Environmental and lifestyle factors such as smoking, excessive alcohol intake and obesity can affect fertility [7]. In addition, age plays a much more important role in predicting female infertility, couples in which the male partner is 40 years old or older are more likely to report difficulty conceiving, Use of marijuana, Exposure to testosterone, exposure to radiation, exposure of the testes to high temperatures, exposure to certain medications such as flutamide, cyproterone and etc. [8]. Also, varicoceles are widely considered the most common correctable cause of male infertility [9], leukocytospermia was associated with decreased sperm numbers and impaired sperm motility [10], high white blood cell (WBC) concentrations within semen are an indicator of infection; this condition, marked by pus in the semen [11]. resulting in their influence decreased numbers and poor-quality sperm, leading to infertility.

MATERIAL AND METHODS

The study was carried out in the Fertility Center in AL-Sader Medical City and local laboratories, through June 2022 and April 2022 which including (60) infertile men. The parameters for the semen analysis were analyzed and classified according

to the WHO criteria (WHO, 1999). The population in this study were divided into:

According to age, it was divided into:

- Patients with ages Less than 20 years.
- Patients with ages between 20-29 years.
- 30-39 years.
- ≥ 40 years.

According to Body Mass Index, it was divided into:

- Normal weight (18.5-24.9 kg /m²).
- Overweight (25-29.9 kg/m²).
- Obese (≥ 30 kg /m²).

According to Smoking habit, it was divided into:

- Smoking.
- Non-Smoking.

According to Leukocyte concentration, it was divided into:

- Patients with non Leukocytospermia concentration (< 1 x 10^6/ml).
- Patients with Leukocytospermia concentration (>1x10^6/ml).

According abstinence Period, it was divided into:

- Patients with abstinence Period 2-3 days.
- Patients with abstinence Period 4-5 days.
- Patients with abstinence Period 6-7 days.

According varicocele, it was divided into:

- Patients with varicocele (+ve)
- Patients without varicocele (-ve)

According Liquefaction time, it was divided into:

- Patients with liquefaction time less than 30 minute (<30 minute).
- Patients with liquefaction time 30 minute or more than (≥30 minute).

Demographic and medical history information was taken from the patients before starting the evaluation of semen analysis. This information was recorded in a prepared data.

Statistical Analysis

It was done by using of SPSS (Statistical package for Social Science) version (21) in which we use frequency with percentages and mean with standard deviation as description statistics, for analysis we use independent Sample t-test. To compare the parameters of three groups or more analysis of variance (ANOVA) was used, the differences between Values were considered statistically significant at (p<0.01).

RESULT

The present study aimed to investigate the impact effect of some sociodemographic factors such as age, body mass index, smoking, varicocele, period of abstinence, leukocyte and liquefaction time. The results in table (1) showed that significant (P<0.01) difference in semen volume, sperm concentration, progressive motility percent (A+B) and normal morphology in relation to age. A significant (p <0.01) reduction was noticed via ≥40 years compared to other groups of age. The results were recorded a significant (p<0.01) difference in Semen volume, sperm concentration, progressive motility (A +B) and normal morphology in relation to body mass index a significant (P<0.01), that observe in table (2), it's decrease in the obese compared to other groups of body mass index. The results also presented a significant (P< 0.01) difference in semen volume, sperm concentration, progressive motility (A+B) and normal morphology in relation to smoking, observe in table (3). A significant (P<0.01) reduced were appeared in smokers compared to non-smokers groups. The results in table (4) revealed a significant (P<0.01) reduction in semen volume, progressive motility (A+B) and normal morphology in leukocytospermia compared to non-leukocytospermia, while the results was observed non significant (P>0.01) difference in sperm concentration between two leukocyte groups, The results in current study also showed a significant (p<0.01) difference in semen volume, sperm concentration and progressive motility (A+B) in relation to duration of abstinence, a significant (P<0.01) decrease was noticed via 2-3 days compared to other groups of duration of abstinence, but no significant (P>0.01) difference was appeared in normal morphology among groups of duration of abstinence, that observe in table (5). There was a significant (p <0.01) decrease in sperm concentration, progressive motility (A+B) and normal morphology in varicocele positive compared to varicocele negative, while the results were showed non-significant (P>0.01) difference in semen volume between two groups of varicocele, that observe in table (6). Lastly, the table (7) show the result that a significant (P<0.01) decline in sperm concentration, progressive motility (A+B) and normal morphology was showed with group ≥30 minutes compared to <30 minutes of Liquefaction time, non significant (p>0.01) was recorded in semen volume between two groups of liquefaction time.

Table 1: Effect the age on the sperm parameters of infertile patients.

Sperm Parameters	**Age (years)**				**P. Value**
	<20 (n=12) Mean ± SD	**20-29 (n=15) Mean ± SD**	**30-39 (n=20) Mean ± SD**	**≥40 (n=13) Mean ± SD**	
Semen volume (ml)	3.13±0.74	3.08±0.68	2.62±0.37	1.89±0.17	<0.001
Sperm concentration (million/ml)	33.13±11.58	29.57±9.87	23.66±91.00	18.13±5.35	0.003
Progressive motility (%)	47.12±14.33	45±14.77	33.54±11.47	27.19±9.29	<0.001
Normal sperm morphology (%)	31.55±14.33	30.32±10.57	21.22±8.31	17.43±5.21	0.002

Significant difference (p<0.01)

Table 2: Effect of the body mass index on the sperm parameters of infertile patients.

Sperm Parameters	Normal weight (n=24) Mean ± SD	Over weight (n=22) Mean ± SD	Obese (n=14) Mean ± SD	P. Value
Semen volume (ml)	3.15± 0.43	2.36±0.62	1.93±0.84	<0.001
Sperm concentration (million/ml)	33.51±13.31	24.33±11.62	18.72±8.43	<0.005
Progressive motility (%)	44.84±16.53	37.19±13.83	29.72±11.82	0.002

Normal sperm morphology (%)	37±14.37	31±12.39	28.51±10.34	<0.001

Significant difference (p<0.01)

Table 3: Effect of the smoking on the sperm parameters of infertile patients.

Sperm Parameters	**Smokers (n=25) Mean ± SD**	**Non-Smokers (n=35) Mean ± SD**	**P. Value**
Semen volume (ml)	2.1 ± 0.25	2.8±0.42	0.001
Sperm concentration (million/ml)	21.38 ±12.31	33.75 ± 14.33	0.002
Progressive motility (%)	36.43 ±15.22	43.00 ± 17.54	<0.001
Normal sperm morphology (%)	31.00 ±13.45	36.84±15.36	0.004

Significant difference (p<0.01)

Table 4: Effect of the Leukocytes on the sperm parameters of infertile patients

Sperm Parameters	**Leukocytes ($x10^6$/ml)**		**P. Value**
	Leukocytocytospermia (>$1x10^6$/ml) (n=25) Mean ± SD	**Non Leukocytocytospermia (<$1x10^6$/ml) (n= 35) Mean ± SD**	
Leukocytes ($x10^6$/ml)	3.37 ± 0.51	0.86 ± 0.062	0.002
Semen volume (ml)	2.50 ± 0.59	3.09 ± 0.72	0.002
Sperm concentration (million/ml)	17.34 ±7.55	19.98 ±7.58	0.541
Progressive motility (%)	39.86 ±13.31	48.89±17.57	0.003
Normal sperm morphology (%)	21.23 ±6.61	29.70±9.46	<0.001

Significant difference (p<0.01)

Table 5: Effect of the duration of abstinence on the sperm parameters of infertile patients

Sperm Parameters	**Duration of abstinence (days)**			**P. Value**
	2-3 (n=20) Mean ± SD	**4-5 (n= 22) Mean ± SD**	**6-7 (n= 18) Mean ± SD**	
Semen volume (ml)	2.2 ± 0.36	2.4 ± 0.43	2.7±0.49	<0.001
Sperm concentration (million/ml)	21.41 ±7.88	26.55 ±9.32	34.31±11.12	0.002
Progressive motility (%)	36.27 ±10.21	40.13±12.83	48.31±16.22	<0.001
Normal sperm morphology (%)	28.91 ±8.38	29.34±9.31	29.13±10.21	0.425

Significant difference (p<0.01)

Table 6: Effect of the varicocele on the sperm parameters of infertile patients

Sperm Parameters	**Varicocele (positive –infertile) (n=25) Mean ± SD**	**Varicocele (negative –infertile) (n=35) Mean ± SD**	**P. Value**
Semen volume (ml)	3.13 ±0.83	3.09 ± 0.76	0.42
Sperm concentration (million/ml)	12.62 ± 3.47	18.73±4.31	0.005
Progressive motility (%)	37.22 ±8.22	43.51±11.31	0.002
Normal sperm morphology (%)	20.91 ±7.33	29.36±5.36	0.003

Significant difference (p<0.01)

Table 7: Effect of the Liquefaction time on the sperm parameters of infertile patients

Sperm Parameters	**Liquefaction time**		**P. Value**
	<30 minutes (n=25) Mean ± SD	**≥30 minutes (n=35) Mean ± SD**	
Liquefaction time (minutes)	20.76 ± 5.06	47.63 ± 16.83	0.001
Semen volume (ml)	3.2 ±0.56	3.0 ± 0.49	0.13
Sperm concentration (million/ml)	35.48 ± 17.32	26.92 ±12.33	<0.001
Progressive motility (%)	43.72 ±13.28	36.55±11.98	0.002
Normal sperm morphology (%)	45.48 ±15.29	36.64±12.34	0.005

Significant difference (p<0.01)

DISCUSSION

Defective sperm parameters indicate an important role in diagnosis fertility and was closely correlated to fertilization and Pregnancy rate in the natural fertilization process as well as in ART [12]. Our current study may be expanding the knowledge of male infertility and its association with different and potential sociodemographic factor which effect the sperm Parameters.

Sperm parameters of the infertile patients in relation to age

The present study clearly reveals that age has a significant negative effect on Semen volume, sperm concentration, progressive motility and normal morphology are represented in table (1). The semen volume was significantly decrease with the increase of paternal age, and they were particularly lowest in the proportion of patients who were 40 years or above. The decrease in volume may be related to seminal vesicle insufficiency because seminal vesicle fluid composes most of the ejaculate volume [13] or changes in prostate such as reduction in water and protein contain which might affect ejaculation volume [14] many studies suggested that the pronounced changes occur in men aged > 45 years. Semen volume declines from a mean of (2.80 ml) in those aged 45-47 years to n 1.95 ml in men more than 56 years [14-15]. Also similar to present study results by [16] who found decrease in semen volume (3-22%) when compared 30 years old man to 50-years old men. Contrary to our study, another study by Zofnat [17] were found no significant change in semen volume with older age patients our study revealed a declining trend in sperm concentration with advancing age, this is agreed with many studies which indicate fall in all the semen parameters with advancing male age [18] study reported a decrease in sperm concentration. of up to 3.3 % per year of age [19], another study including 1283 men, found sperm concentration lower at both extremes of age as compared to men aged 26-45 years [20]. This decrease in sperm concentrate with age degenerative changes occur in germinal epithelium, leading to fall in number and functions of Leydig cells, thereby affecting spermatogenesis through a decrease in testosterone Level [21]. Contrary to this study comprising of 22,759 infertile men found significant increase in the sperm concentration of 0.7% per year of age [22]. Several study results support the finding that progressive motility decrease with advancing age, present study also observed significant decrease in progressive motility with aging. Reduction in progressive motility by 0.17-0.6% per year with aging resulting in 3-12% decline in motility over 20 years [23]. Also, recent Study found that progressive motility decreases by 0.8% per year of age [14]. In addition to progressive motility, normal morphology also shows to be affected with increasing male age. Our study also observed decrease in normal sperm morphology with age. Similar that indicate a decrease in normal morphology of 0.2 % - 0.9% per year of age [23]. The zhu performed sperm analysis in men ranging between 20 and 60 years, showing that age has negative effect on normal morphology.

Sperm parameters of the infertile patients in relation to body mass index

Obesity and overweight are the most frequent chronic medical problems and characterized by excessive storage of adipose tissue in the organism [24]. This results in table (2) shows a significant decrease in all sperm parameters (semen volume, sperm concentration, progressive sperm motility and normal sperm morphology) with increase BMI. Our study is agreement with Chavarro [25] found that the measured volume of ejaculate decreased with increased BMI. Several studies have observed negative association between obesity, increasing BMI and basic sperm parameters, including semen volume, sperm concentration, progressive sperm motility and normal sperm [26-28], while Mac Donald reported no significant relationship between BMI and Semen volume [29]. In our study sperm concentration in infertile patients appeared a significant decline with BMI, agreed to present results [27] showed 21.6% significant reduction in sperm concentration in patients with BMI >25 compared with those classified as normal. Study by Martini [30] also found a negative association between sperm concentration and BMI. Progressive sperm motility was significantly decrease with BMI [31]. Similar to our results which relevant a significant decline in progressive sperm motility (A+B) with increasing BMI. Our finding also is consistent with recent study reporting an association of BMI with decreased sperm motility and sperm concentration [32]. Failed to found any relationship between BMI and sperm motility was reported by Macdonal [30] which showed did not reach a significant correlation between BMI and sperm motility. In addition to Progressive sperm motility, our results reported a significant decline in normal sperm morphology with BMI in overweight and obese compared to normal weight. Same results were by other study by Macdonal [30] found negative impact on normal sperm morphology to only. This finding was in contrary to study with Chavaro [26] who did not observe statistically significant difference in sperm morphology. Also, the Hofny found that BMI had significant negative association with normal sperm morphology [33].

Sperm parameters of the infertile patients in relation to Smoking

Smoking is an eminent risk factor for reproductive health [34]. Smoking causes reproductive hormone disorders, impairment in spermatogenesis and maturation of sperm, and compromised functioning of spermatozoa [35]. This study has shown a significant decrease in parameters level in smokers as compared to non-smokers. Many tobacco smoke components have effects on semen parameters. Nicotine has been reported to impair male reproductive hormone system by causing Leydig cell apoptosis and inhibition of synthesis of androgen [36]. The study has shown evaluated the effects of cigarette consumption on semen volume, pooled results indicated that volume was lower in smokers ($p<0.001$) than in nonsmokers, these results are similar to a few other studies [37-38]. The semen parameters (sperm concentration, progressive sperm motility and sperm morphology) are decreased in infertile smokers as compared to infertile non-smokers, smoking caused a significant decrease in sperm parameters, the study compared with [39].

Sperm parameters of the infertile patients in relation to Leukocyte count

Leukocytospermia can be a result of infection or inflammation anywhere along the genitourinary tract, particularly the prostate gland, seminal vesicles, testicles, and bulb urethral glands, which produce the fluid in semen. The significance of leukocytes in the semen remains highly controversial, while our study has correlated seminal leukocyte elevation with impaired semen parameters, especially semen volume, sperm progressive motility and sperm morphology, that similar with [39]. The concentrations, however the study show negative effects. The semen volume in our study has a significantly ($p<0.01$), semen

volume is compatible with data reported in similarly controlled studies. The study appear change in sperm motility with leukocytospermia (39.86±13.31), the result close with Henkel [37].

Sperm parameters of the infertile patients in relation to Abstinence period

The findings of this study are in accordance with result in table (5) that noted a high abstinence time improves the percentage of semen volume, sperm concentration and sperm motility. One of the reasons for the relatively fast reduction in this parameter can be related to the increased production of ROS (reactive oxygen species). Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants. Within the WHO recommended abstinence period of 2-7days for performing diagnostic semen analyses, we observed a significant increases in semen volume, sperm concentration and sperm motile, when effect of reducing ejaculated to 1 day and increasing to 7 days However, prolonged stay without sex (5-6) days, makes a man to produce a higher volume of semen, sperm concentration and sperm motility, similar study by Falcone. Low parameter on the first day seemed to be the most prominent parameter to increase the results on third day. In this case, the sperm morphology assessment is negative effect to sperms produced within a shorter or longer period of abstinence, it may be due to the reason that the abnormal shape must undergo treatment, so the period of abstinence does not affect the shape, contrary study by Goldstein [14] show a significant with sperm morphology.

Sperm parameters of the infertile patients in relation to Varicocele

A varicocele is a common cause of male infertility, the results demonstrated that patients with the varicocele had lower sperm concentration, sperm motility and sperm morphology compared to the control group, which is similar with reported results [15-17]. The results of this study showed that the number of sperm with abnormal morphology was significantly higher in the varicocele group.

Sperm parameters of the infertile patients in relation to Liquefaction time

In liquefaction time, it normally takes less than 30 minutes for semen to change from a thick gel into a liquid. An unusually long liquefaction time may indicate an infection, there is discrepancy between the expected chance of normal couple conceiving and the above-mentioned parameters, there was a very highly significant correlation of prolonged liquefaction time with sperm concentration, sperm motility and sperm morphology. The study suggested a possible relationship between the coagulation-liquefaction property of human ejaculates and their semen parameters, all these results in table (4-7) supported our conclusion that there is a significant correlation between prolonged time of liquefaction and defects in other parameters of semen analysis that measured by the conventional methods expect semen. An unusually long liquefaction time may indicate an infection. Semen is analyzed for fructose when there is no sperm in the ejaculate (azoospermia) or if the semen volume is very low (less than 1 ml).

CONCLUSION

advance paternal age, obesity and smoking lead to a deterioration in sperm parameters and thus contribute as one of the causes of male infertility, Infections, varicocele, period of abstinence and liquefaction time have a negative impact on the semen quality, which leads to decrease male fertility.

Ethical approval: This study was approved by the ethics committee of the
Kufa University (#MEC-62).
Funding details: N/A
Conflict of interest: N/A
Informed Consent: Permission was taken from patients and kept the receipts in the Kufa General Hospital

REFERENCES

i. W*orld Health Organization* (2020). https://www.who.int/
ii. Central of Disease Control and Prevention, (2021). https://www.cdc.gov/
iii. Gore AC, Chappell VA, Fenton SE, et al. (2015). EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews*. 36(6): E1-E150, doi:10.1210/er.2015-1010.
iv. Yetter R. (2015). *Value Receptacles. Noûs*. 49(2): 322-332, doi:10.1111/nous.12023
v. Fenton M (2015) Social care and child welfare in Ireland: Integrating residential care, leaving care and aftercare.
vi. Gore AC, Chappell VA, Fenton SE, et al. (2015) EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews*. 36(6): E1-E150, doi:10.1210/er.2015-1010
vii. Thalia R, Segal, Linda C. Giudice (2019) Before the beginning: environmental exposures and reproductive and obstetrical outcomes, *Fertility and Sterility*. 112(4): 613-621.
viii. Dubin L, Amelar RD. (1970) Varicocele size and results of varicocelectomy in selected sub fertile men with varicocele. *Fertil Steril*. 21(8): 606-9, doi:10.1016/s0015-0282(16)37684.
ix. Gerhard K, Lenhard W, Kruse E, Runnebaum B (1992) Clinical data which influence semen parameters in infertile men. *Human Reproduction*. 7(6): 830-837.
x. Dickerman Z, Sagiv M, Segenreich E, Levinsky H, Singer R (2009) The Evaluation of Routine Andrological Parameters in Human Semen/Auswertung der Routine-Parameter der Andrologie am Sperma. *Andrologia*. 492-501, doi.10.1111/j.1439-0272.
xi. Burger HG, Teede HJ, Bone KM (2013) Vitex agnuscastus extracts for female reproductive disorders: a systematic review of clinical trials. *Planta medica*. 79(07): 562-575.
xii. Zhu QX, Meads C, Lu ML, Wu JQ, Zhou WJ, Gao ES (2011) Turning point of age for semen quality: a population-based study in Chinese men. *Fertil Steril*. 96: 572-576
xiii. Sloter E, Schmid TE, Marchetti F, Eskenazi B, Nath J, Wyrobek AJ (2006) Quantitative effects of male age on sperm motion. *Hum Reprod*. 21: 2868-2875

xiv. Hellstrom WJ, Overstreet JW, Sikka SC, Denne J, Ahuja S, Hoover AM, Sides GD, Cordell WH, Harrison LM, Whitaker JS (2006) Semen and sperm reference ranges for men 45 years of age and older. *J Androl*. 27: 421-428

xv. Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, Moore D (2003) The association of age and semen quality in healthy men. *Hum Reprod*. 18: 447-454.

xvi. Kidd SA, Eskenazi B, Wyrobek AJ (2001) Effects of male age on semen quality and fertility: a review of the literature. *Fertil Steril*. 75: 237-248.

xvii. Zofnat WM, Auslandr R, Dimfeld M (2012) Advanced paternal age and reproductive outcome. *Asian J. Androl*. 14: 69-76.

xviii. Rolf C, Behre HM, Nieschlag E (1996) Reproductive parameters of older compared to younger men of infertile couples. *Int J Androl*. 19: 135-142

xix. Auger J, Kunstmann JM, Czyglik F, Jouannet P (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med*. 332: 281-285

xx. Fisch H., Goluboff ET, Olson JH, Feldshuh J, Broder SJ, Barad DH (1996) Semen analyses in 1283 men from the United States over a 25-year period: no decline in quality. *Fertil Steril*. 65, 45-49.

xxi. Harman SM, Metter EJ, Tobin JD, Blackman MR (2001) Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore longitudinal study of aging. J. Clin. Endocrinol. *Metab*. 86, 724-731.

xxii. Andolz P, Bielsa MA, Vila J (1999) Evolution of semen quality in North-eastern Spain: a study in 22,759 infertile men over a 36-year period. *Hum Reprod*. 14: 731-735.

xxiii. Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT, Meikle AW (2008) Male obesity and alteration in sperm parameters. *Fertility and sterility*. 90(6): 2222-5

xxiv. Chavarro JE, Toth TL, Wright DL, Meeker JD, 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. *Fertility and sterility*. 93(7): 2222-31.

xxv. Jensen T, Andersson A, Jorgensen N, Andersen A, Carlsen E, Petersen J, et al. (2004) Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril*. 82:863-70

xxvi. Bieniek JM, Kashanian JA, Deibert CM, Grober ED, Lo KC, Brannigan RE, et al. (2016) Influence of increasing body mass index on semen and reproductive hormonal parameters in a multi-institutional cohort of sub fertile men. *Fertil Steril*. 106: 1070-5.

xxvii. Koloszár S, Fejes I, Závaczki Z, Daru J, Szöllősi J, Pál A (2005) Effect of body weight on sperm concentration in normozoospermia males. *Arch Androl*. 51: 299-304.

xxviii. MacDonald AA, Herbison GP, Showell M, Farquhar CM (2010) The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Hum Reprod Update*. 16: 293-311

xxix. Martini AC, Molina RI, Ruiz RD, Fiol de Cuneo M (2012) Obesity and male fertility. *Review Revista de la Facultad de Ciencias Médicas Cordoba*. 69(2).

xxx. Braga DP, Halpern G, Figueira Rde C, Setti AS, Iaconelli A Jr, Borges E (2012) Food Intake and Social Habits in Male Patients and Its Relationship to Intracytoplasmic Sperm Injection Outcomes. *Fertility and Sterility*. 97(1): 53-59.

xxxi. Hofny ER, Ali M, Abdel-Hafez HZ, Kamal EED, Mohamed EE, Abd El-Azeem HG, et al. (2010) Semen parameters and hormonal profile in obese fertile and infertile males. *Fertility and sterility*. 94(2): 581-4

xxxii. Dai J, Zhan C, Xu W, Wang Z, Nie D, Zhao X, Qiao Z (2015) Nicotine elevates sperm motility and induces Pfn1 promoter hypomethylation in mouse testis. *Andrology*. 3(5), 967–978.

xxxiii. Sharma R, Harlev A, Agarwal A, Esteves SC (2016) Cigarette smoking and semen quality: A new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. *European Urology*. 70(4): 635-645.

xxxiv. Mostafa RM, Nasrallah YS, Hassan MM, Farrag AF, Majzoub A, Agarwal A (2017) The effect of cigarette smoking on human seminal parameters, sperm chromatin structure and condensation. *Andrologia*. e12910.

xxxv. Anifandis G, Bounartzi T, Messini CI, Dafopoulos K, Sotiriou S, Messinis IE (2014) The impact of cigarette smoking and alcohol consumption on sperm parameters and sperm DNA fragmentation (SDF) measured by Halosperm. *Archives of Gynecology and Obstetrics*. 290(4), 777-782.

xxxvi. Lackner JE, Märk I, Sator K, Huber J, Sator M (2008) Effect of leukocytospermia on fertilization and pregnancy rates of artificial reproductive technologies. *Fertil Steril*. 90: 869-871.

xxxvii. Henkel RR. Leukocytes and oxidative stress (2011) Dilemma for sperm function and male fertility. *Asian Journal of Andrology*. 13(1): 43-52.

xxxviii. Rao M, Zhao XL, Yang J, Hu SF, Lei H, Xia W, Zhu CH (2015) Effect of transient scrotal hyperthermia on sperm parameters, seminal plasma biochemical markers, and oxidative stress in men. *Asian Journal of Andrology*. 17(4): 668-675

xxxix. Du CQ, Yang YY, Chen J, Feng L, Lin WQ (2020) Association Between Sleep Quality and Semen Parameters and Reproductive Hormones: A Cross-Sectional Study in Zhejiang, China. *Nat Sci Sleep*. 12: 11-18.