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Semenogram Characteristics in Correlation with Hormonal Studies among Different Age Groups of Men - Damietta Governorate

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ABSTRACT

Background: The average age of first reproduction has increased dramatically throughout the world in recent decades. New evidence suggests that older parents have lower fertility and less healthy children. Most of these studies focused on men over the age of 60 who were infertile. Few studies have looked at how age affects normally fertile men under the age of 60.

Aim of the work: Assess the semenogram using Computer assisted semen analysis [CASA] and Hormonal studies that will include total and free testosterone, FSH, LH, Estrogen, prolactin and TSH in Egyptian men between age groups of 20 and 50 years in Damietta governorate, to assess if there is a decline in the overall parameters of sperm.

Patients and Methods: Two hundred adult males were recruited from the Dermatology outpatient clinic at the Damietta Campus of the Al-Azhar University School of Medicine for this cross-sectional study. All participants gave written consent and underwent a thorough history and physical examination, and their sperm was analyzed for a variety of parameters and their sexual hormone levels was measured after receiving approval from the local research committee.

Results: The semen volume, the total sperm count, sperm motility, progressive sperm motility and abnormal sperm morphology were statistically significantly lower in group C [40-49 years] compared to group A [20-29 years] and group B [30-39 years]. The FSH concentration and LH concentration was statistically significantly higher in group C [40-49 years] compared to group A [20-29 years] and group B [30-39 years].

Conclusion: In healthy, fertile males, we found that as age increased, semen impairment also increased. The hormonal profile revealed changes that correlate with a reduction in the semen parameters, such as an increase in FSH, LH, Estradiol, and TSH levels.

Keywords: Semen; Elderly; Semenogram; Infertility; Sex hormones.

INTRODUCTION

Between the ages of 22 and 80 years, sperm volume and motility dropped steadily in a sample of healthy males drawn from a nonclinical setting. There was no proof of a threshold age [1].

Pino and colleagues discovered a probable connection between some sperm characteristics and male age in their investigation. It was found that males over 50 are far more likely to experience issues with sperm volume, sperm concentration, and sperm DNA fragmentation [2].

It is important to know if older paternal age is associated with worse semen quality and an increased risk of infertility because more and more couples are seeking assistance from infertility clinics to establish babies later in life. Age was found to have a substantial impact on sperm volume, motility, and fast advancing motility [3].

Retrospective investigation of the association between age and semen parameters was performed on men with normal sperm concentration; the results showed a statistically significant and inverse link between patient age and sperm volume and quality. Despite the fact that the guys had been engaged in sexual activity for a considerable amount of time, this bond remained strong. The investigation was conducted in a research fertility and IVF unit using computer-generated data [4].

Retrospective reviews of sperm analysis records from infertile males who were referred to the Andrology Laboratory were conducted. The groups included people between the ages of 20 and 55. Researchers noticed a significant linear fall in sperm viability, shape, and motility as men aged who were unable to father children [5].

The purpose of the study is to analyze the relationship between various sex hormones in serum and male sperm quality since circulating sex hormones can act as non-invasive indicators to enhance the assessment of sperm quality. The early diagnosis and treatment of male infertility may be improved with the help of this non-invasive tool. The results may clarify the independent relationship between the aforementioned hormones and offer clues as to the precise impact that each hormone has on the sperm's quality.

PATIENTS AND METHODS

The Dermatology, Venereology and Andrology outpatient clinic at Damietta Faculty of Medicine, Al-Azhar University, undertook this cross-sectional descriptive and analytical statistics study from September 2022 to April 2023.

Two-hundred adult males from the Damietta Governorate, aged 20 to 29, 30 to 39, and 40 to 49, participated in this study. This classification technique was selected because it has been used in research with comparable goals in the past and because it appears to be the most effective in terms of reaching the target audience and yielding important statistical findings.

Males who met the following criteria were excluded from the study: those who were younger than 20 or older than 50; those with known confirmed infertility; smokers; those who had undergone a vasectomy, chemotherapy, radiation; those who had a history of prostate cancer or an undescended testicle; those with chronic illnesses; those with congenital anomalies, etc.

The study was carried out in compliance with the 2013 revision of the Helsinki Standards [6]. After receiving approval from the local ethics committee, the faculty of medicine at Al-Azhar University [Damietta], and after getting the subjects' written or verbal informed consent, the study was carried out.

The subjects underwent a clinical examination to rule out any systemic diseases as well as a history taking that covered personal information like marital status, sexual activity, infertility, medication or disease history, surgical history, and special habits of medical significance.

Hormonal analysis: Venous blood samples [3 ml] were drawn, centrifuged at a speed of 2000-3000 rounds per minute for 20 minutes, and the sera were collected and kept at 20 °C until the hormone serum level analysis. According to the manufacturer's instructions, serum samples were tested using an automated chemiluminescence assay system [Immulite TM 2000, Diagnostic Products Corporation, Siemens, Los Angeles, USA] for FSH, LH, estrogen [estradiol [E2]], prolactin, total testosterone, and free testosterone. TSH levels using chemiluminescence immunoassays with high specificity solid-phase techniques.

Semen analysis

After a minimum of two days and a maximum of seven days without engaging in sexual activity, semen samples were taken. Masturbation was used to extract sperm into a sterile plastic container.

Within an hour of the samples' completion of the 37 °C liquefaction process, they were all tested in accordance with the WHO laboratory manual for the examination and processing of human semen: World Health Organization; 2021 [7].

The variables used for the semen analysis were volume [in milliliters], concentration of sperm [in million per milliliter], motility [% of motile spermatozoa], and morphology [% of spermatozoa with normal morphology].

A spermatozoon is regarded as normal if it has an oval head with a smooth surface and an acrosome that fills between 40% and 70% of the sperm head. The amount of cytoplasm in a sperm head cannot exceed 50%.

The Makler chamber, which counts sperm, was used to determine the number of spermatozoa. According to the WHO handbook, sperm motility was assessed using a wet preparation that was 20 m deep and was categorized as linear progressive sperm motility [PR], non-progressive sperm motility [NP], or immotile [IM]. In order to evaluate morphology, semen smears were used.

Statistical analysis of data: The data was coded, processed, and analyzed using SPSS version 27 for Windows® [Statistical Package for Social Sciences] [IBM, SPSS Inc., Chicago, IL, USA]. Quantitative data was reported as a percentage and a number [frequency]. The two groups were compared using the Monte-Carlo test or the Chi-Square test. The Kolmogorov-Smirnov test determined whether quantitative data was normal. Parametric data were shown as median SD as opposed to non-parametric data, which were presented as median [Range]. The Kruskal-Wallis test was performed if the data were skewedly distributed, and the One-way analysis of the variance [One-way ANOVA] test was used to compare three groups with regularly distributed quantitative variables. For all tests, P values of 0.05 or less are considered significant.

RESULTS

According to age, the participants were split into three groups, as shown in table [1]. Group A, which consisted of adults aged 20 to 29 [76 participants, or 38%], Group B, which consisted of people aged 30 to 39 [72 participants, or 36%], and Group C, which consisted of people aged 40 to 49 [52 participants, or 26%]. The three groups' means were significantly different statistically as a result [p 0.001]. There was higher prevalence of rural residence in the three study groups [55.3%, 55.6% and 61.5% in group A, B and C respectively]. There was no statistically significant difference between the three study groups regarding the residence [p= 0.744]. There was a statistically significant difference between the three study groups regarding the marital status [p= 0.015]. There was more prevalence of single males in group A, while Widowed/divorced males increased in group C.

Table [2] shows that there was a statistically significant difference between the three study groups regarding the semen volume [p< 0.001]. The semen volume was statistically significantly lower in group C [40-49 years] compared to group A [20-29 years] and group B [30-39 years]. There was a statistically significant difference between the three study groups regarding the total sperm count [p= 0.002]. The total sperm count was statistically significantly lower in group C [40-49 years] compared to group A [20-29 years] and group B [30-39 years]. The total sperm concentration did not differ statistically significantly [p=0.333] between the three study groups. Between the three study groups, there was a statistically significant difference in sperm motility [p 0.001]. Compared to groups A [20-29 years] and B [30-39 years], the sperm motility was statistically substantially decreased in group C [40-49 years]. Regarding the progressive sperm motility, there was a statistically significant difference between the three study groups [p 0.001]. In comparison to groups A [20-29 years] and B [30-39 years], the progressive sperm motility was statistically substantially lower in group C [40-49 years]. The percentage of abnormal sperm morphology varied amongst the three study groups in a statistically significant way [p=0.044]. In comparison to groups A [20-29 years] and B [30-39 years], group C [40-49 years] had statistically substantially more abnormal sperm morphology.

Table [3] It shows that the concentration of FSH varied between each of the three groups in a statistically significant way [p 0.001]. Group C [40-49 years old] had a statistically significantly higher FSH concentration than group A [20-29 years] and B [30-39 years] did. Furthermore, compared to group A [20-29 years], group B [30-39 years] showed a statistically significant higher FSH concentration. Between the three study groups, there was a statistically significant distinction in the levels of LH [p 0.001]. The LH concentration was statistically higher in group C [40-49 years] compared with group A [20-29 years] and B [30-39 years]. The concentration of prolactin varied between the three study groups in a statistically significant way [p 0.001]. In comparison to groups A [20-29 years] and C [40-49 years], group B [30-39 years] had a

statistically significant increased prolactin concentration. Additionally, group A [20-29 years] had a statistically significant greater prolactin concentration than group C [40-49 years]. The total testosterone level [p=0.096] and the free testosterone level [p=0.430] did not statistically differ between the three study groups. The concentration of estradiol varied between the three study groups in a statistically significant way [p 0.001]. In comparison to groups B [30-39 years] and C [40-49 years], group A [20-29 years] had an estradiol concentration that was statistically substantially greater. Additionally, group C [40-49 years old] had a statistically significant greater estradiol concentration than group B [30-39 years old]. The concentration of TSH varied between the three study groups in a statistically significant way [p 0.001]. In comparison to group A [20-29 years], the TSH concentration was statistically substantially higher in group B [30-39 years].

Table [1]: Demographic data in the subjects of the study groups

Demographic data		Group A [Age 20-29 years] N= 76	Group B [Age 30-39 years] N= 72	Group C [Age 40-49 years] N= 52	Test of significance
Age [years]		23.99 ±2.66	34.47 ±2.59	45 ± 3.09	F= 911.025 **P < 0.001*** **P1 < 0.001*** **P2 < 0.001*** **P3 < 0.001***
Residence	Urban	34 [44.7%]	32 [44.4%]	20 [38.5%]	χ^2= 0.592 P = 0.744
	Rural	42 [55.3%]	40 [55.6%]	32 [61.5%]	
Marital status					
Single		38 [50%]	17 [40.5%]	8 [15.4%]	MC = 6.482 **P = 0.015***
Married		34 [44.7%]	42 [58.3%]	30 [57.7%]	
Widowed/divorced		4 [5.3%]	13 [18.1%]	14 [26.9%]	

χ^2: Chi-Square test; MC: Montecarlo test; F: One-way ANOVA test; P1: Comparing between Group A [Age 20-29 years] and Group B [Age 30-39 years]; P2: Comparing between Group A [Age 20-29 years] and Group C [Age 40-49 years] P3: Comparing between Group B [Age 30-39 years] and Group C [Age 40-49 years]; *: Statistically significant [P≤ 0.05].

Table [2]: Semen parameters in the subjects of the study groups

Semen parameters	Group A [Age 20-29 years] N= 76	Group B [Age 30-39 years] N= 72	Group C [Age 40-49 years] N= 52	Test of significance
Semen Volume [ml]	3.56 ± 0.89	3.28 ± 0.99	2.80 ± 0.84	F= 19.157 **P < 0.001*** P1 = 0.148 **P2 < 0.001*** **P3 < 0.001***
Total Sperm count [10^6]	232.36 ± 102.47	224.74 ± 115.72	165.66 ± 72.08	KW= 12.674 **P = 0.002*** P1 = 0.890 **P2 = 0.001*** **P3 = 0.004***
Sperm Concentration [10^6 /ml]	65.84 ± 24.30	67.54 ± 26.56	60.62 ± 22.56	KW= 2.199 P = 0.333 P1= 0.908 P2=0.496 P3 = 0.275
Sperm motility [%]	52.12 ± 9.96	49.56 ± 8.44	40.83 ± 13.24	F= 24.431 **P < 0.001*** P1 = 0.269 **P2 < 0.001*** **P3 < 0.001***
Progressive Sperm motility [%]	32.12 ± 9.96	29.56 ± 8.44	22.54 ± 11.24	KW= 24.406 **P < 0.001*** P1 = 0.252 **P2 < 0.001*** **P3 < 0.001***
Abnormal morphology [%]	3.41 ± 1.14	3.44 ± 1.27	4.08 ± 1.61	KW= 6.239 **P = 0.044*** P1 = 0.988 **P2 = 0.015*** **P3 = 0.023***

F: One-way ANOVA test; KW: Kruskal-wallis test; P1: Comparing between Group A [Age 20-29 years] and Group B [Age 30-39 years]; P2: Comparing between Group A [Age 20-29 years] and Group C [Age 40-49 years]; P3: Comparing between Group B [Age 30-39 years] and Group C [Age 40-49 years]. *: Statistically significant [P≤ 0.05].

Table [3]: Serum hormonal levels in the subjects of the study groups

Serum hormonal levels	Group A [Age 20-29 years] N= 76	Group B [Age 30-39 years] N= 72	Group 6 [Age 40-49 years] N= 52	Test of significance
FSH [mIU/ml]	4.49 ± 2.32	12.20 ± 6.81	16.43 ± 13.78	**KW= 53.874 P < 0.001* P1 < 0.001* P2 < 0.001* P3 = 0.014***
LH [mIU/ml]	5.67 ± 1.88	5.25 ± 2.19	17.15 ± 11.36	**KW= 47.127 P < 0.001* P1 = 0.907 P2 < 0.001* P3 < 0.001***
Prolactin [ng/ml]	22.06 ± 14.57	29.61 ± 22	13.27 ± 6.34	**KW= 18.552 P < 0.001* P1 = 0.015* P2 = 0.009* P3 < 0.001***
Testosterone-T [ng/ml]	4.41 ± 2.19	3.89 ± 1.16	3.73 ± 2.30	**KW= 4.692 P = 0.096 P1 = 0.222 P2 = 0.119 P3 = 0.892**
Testosterone –F [pg/ml]	13.89 ± 7.20	14.67 ± 4.02	13.31 ± 6.33	**KW= 1.687 P = 0.430 P1 = 0.712 P2 = 0.845 P3 = 0.432**
Estradiol [E2] [pg/ml]	28.96 ± 12.59	18.13 ± 3.95	23.73 ± 10.91	**KW= 26.395 P < 0.001* P1 < 0.001* P2 = 0.010* P3 = 0.006***
TSH [uIU/ml]	1.91 ± 1.08	2.82 ± 1.45	2.30 ± 1.39	**KW= 14.522 P < 0.001* P1 < 0.001* P2 = 0.221 P3 = 0.076**

F: One-way ANOVA test; KW: Kruskal-wallis test; P1: Comparing between Group A [Age 20-29 years] and Group B [Age 30-39 years]; P2: Comparing between Group A [Age 20-29 years] and Group C [Age 40-49 years]; P3: Comparing between Group B [Age 30-39 years] and Group C [Age 40-49 years]. *: Statistically significant [P≤ 0.05].

DISCUSSION

The present study on semen profile in 200 men was undertaken in the context of conflicting reports on decline in sperm counts in men over the last few decades. Male factor infertility is commonly defined in terms of the conventional semen profile, which provides descriptive information on the numbers of spermatozoa present in the ejaculate, the proportion that are motile [or progressively motile] and the percentage that are morphologically normal [% normal] [8]. On the basis of such criteria, the **WHO** [7] has suggested a range of arbitrary threshold values for normal human semen. Upon closer inspection of the various sperm parameters in the current study, we have shown significant differences in almost all the semen parameters tested between each of the age groups.

The average age at which men become fathers is constantly increasing, therefore this data is becoming more and more important. There is also growing evidence that sperm fitness reduces with age [9-11]. Numerous theories have been put up in the literature, despite the fact that the precise reasons for this drop-in sperm quality are still unknown. The alteration in testicular function [12], the damage caused by urological illnesses [13], and oxidative stress [14] are all

factors that contribute to the loss in overall sperm quality with paternal age.

The age evaluation of men has brought new focus to environmental influences, lifestyle choices, anatomical anomalies, and inflammatory disorders [15]. Current research on the impact of paternal age on seminal parameters is contradictory [12, 16, 17], especially in light of the fact that study populations were frequently ill-defined and fertile men were infrequently utilized as controls [14].

The current study examined the semenogram using computer assisted semen analysis [CASA] and hormonal studies that included total and free testosterone, FSH, LH, estrogen, prolactin, and TSH in Egyptian men between the ages of 20 and 50 in the Damietta governorate to ascertain whether there has been a decline in the general characteristics of sperm.

The three study groups had greater rates of rural living [55.3 percent, 55.6%, and 61.5% in groups A, B, and C, respectively]. Between the three study groups, there was no statistically significant difference in terms of residence [p= 0.744]. This shows that the randomization method is effective.

The marital status of the three study groups varied in a statistically significant way [p= 0.015]. While single men dominated group A, divorced or widower men climbed in group C. According to the author, Egypt's high rate of divorce and late marriage start are mostly due to economic concerns.

Group C [40-49 years] in the current study displayed statistically substantially lower levels of semen volume, total sperm count, sperm motility, progressive sperm motility, and abnormal sperm morphology compared to groups A [20-29 years] and B [30-39 years]. These measurements weren't significantly different among age groups A and B.

This was in agreement with **Petroianu *et al.*** [18] who found that the group of people aged 41 to 50 years had the highest frequency of the normal oval spermatozoids when compared to the other three groups [p = 0.03]. Between the ages of 41 and 50, abnormal spermatozoids were less common than in the other age groups [p = 0.02].

According to **Kidd *et al.*** [16] increasing paternal age is associated with a decrease in ejaculate volume, sperm morphology, and motility but not sperm concentration. This finding is consistent with the findings of the current study. A significant difference in semen volume [3%–22%], sperm motility [3%–37%], and morphology [4%–18%] was found between two age groups [30 y vs. 50 y].

Sperm volume and sperm count both decline with fathers' aging, following a study by **Hossain *et al.*** [1]. In a sizable prospective research of 3,729 male partners assessed for semen quality and age-specific alterations, a comparable drop in sperm volume and motility was documented [19].

In a study by **Stone *et al.*** [20] that examined sperm samples from 5081 men ranging in age from 16 to 72, the effects of male age on semen parameters were also explored. The likelihood of getting pregnant following sex with a man older than 34 declines after age 35, when considering solely the ages of the men.

In a different study, researchers examined the effect of paternal age on the quality of the sperm from 50 fertile men between the ages of 25 and 65 and 140 infertile men between the ages of 24-76. According to the findings, men's sperm became more concentrated and more likely to be of the diploid kind as they aged. On the other hand, it was discovered that neither morphology nor motility significantly changed as men aged [21].

In a related study, the relationship between men's age and the quality of their semen and the levels of epididymal and accessory gland markers in their seminal fluid were investigated. Semen parameters were shown to have decreased significantly in men over the age of 46 and particularly in those over 35. An increase in the proportion of dead spermatozoa was linked to this [22].

Semen samples from men between the ages of 30 and 40 show an inverse relationship between men's ages and semen traits. Older males had lower semen volume, lower progressive motility, and a smaller percentage of normal morphology, according to other retrospective studies [4, 5, 23] that looked at the link between sperm characteristics and age.

The current study revealed that while sperm concentration remained stable, sperm volume and total sperm count declined with age. The term "sperm concentration" refers to the quantity of spermatozoa per milliliter, which is based on both the overall sperm count and the amount of semen present. Due to the accompanying decrease

in sperm volume and quantity, the concentration is nevertheless normal [24].

Age appears to have a greater impact on sperm motility than other parameters because people in all age groups showed a markedly higher probability of abnormal findings than did males aged 21 to 30. The prostate and epididymis are where spermatozoa develop motility. As a result, the steady decline in endocrine function that people undergo as they age may help to explain the dysfunction associated with aging [2].

Harris *et al.* [25] came to the conclusion that aging has an impact on motility, with annual declines of 0.17–0.8% leading to declines in motility of 3–16% over a period of 20 years.

There was a statistically significant variation between the three study groups for the percentage of aberrant sperm morphology in the current study [p=0.044]. Group C [40-49 years old] had statistically significantly more abnormal sperm morphology than group A [20-29 years] and B [30-39 years].

It's interesting to note that **Harris *et al.*** [25] indicated that oligospermia, asthenospermia, and teratospermia are the most significant alterations in semen quality, demonstrating a progressive loss in normal sperm morphology comparable to 0.2-0.9% each year of age.

In this study, there was a statistically significant difference in the concentration of FSH between the three study groups [p 0.001]. Compared to groups A [20-29 years] and B [30-39 years], the FSH concentration was statistically substantially greater in group C [40-49 years]. Additionally, group B [30-39 years] had a statistically significant greater FSH concentration than group A [20-29 years]. In addition, a significant statistical variation in LH concentration was seen across the three study groups [p 0.001]. The LH concentration was statistically higher in group C [40-49 years] compared with group A [20-29 years] and B [30-39 years].

Testicular morphology and physiology are significantly affected by changes caused by age in the male reproductive system, particularly in the hypothalamus pituitary testicular [HPT] axis. The HPT axis controls the release of sex hormones and controls the initiation and progression of spermatogenesis. In actuality, spermatogenesis and the chemical profile of sex hormones both undergo gradual modifications as we age [26].

With aging, there appears to be a reduction in the amount of synaptic inputs to gonadotrophin-releasing hormone [GnRH] neurons as well as GnRH transcripts and peptides. In longitudinal trials involving aging men, altered pituitary FSH secretion and rising circulatory FSH concentrations have been seen [27].

Age was a negative predictor of LH secretory burst amplitude and a positive predictor of LH secretory burst frequency and basal LH secretory rates. Others reported that the pattern of LH release was fully chaotic in older men [28].

The total testosterone level [p=0.096] and the free testosterone level [p=0.430] did not statistically differ between the three study groups. This is due to the fact that none of the included males were older than 60.

Ageing [greater than 60 years] is linked to reduced pituitary response to GnRH, which is one explanation. These abnormalities in the hypothalamic-pituitary complex will then undoubtedly have an impact on testosterone synthesis in the testicles [29].

Changes in the HPT axis circuits, a decrease in the number of Leydig cells, and a fall in testicular function all contribute to age-related decrease in testosterone levels. Leydig cells in the testis are in charge of making testosterone [30].

Compared to groups A [20-29 y] and C [40-49 y] in the current investigation, group B [30-39 y] showed a statistically significantly higher prolactin concentration. Additionally, compared to group C [40-49 years], group A [20-29 years] exhibited a statistically significantly higher prolactin level. Additionally, compared to groups B [30–39 years] and C [40–49 years], group A [20–29 years] showed a statistically significant higher estradiol concentration. Additionally, group C [40-49 years old] had an estradiol concentration that was statistically significantly higher than group B [30-39 years old]. Additionally, compared to group A [20-29 years], group B [30-39 years] showed a statistically significantly higher TSH concentration.

Conclusion

The results of this study clearly illustrate an effect of aging on semen parameters characteristics in men. Hormonal testing revealed shifts in FSH, LH, Estradiol, and TSH that are consistent with a decline in semen parameters. The decline in

sperm quality noted in the present study could cumulatively have a negative effect on fecundity. This study suggested that the aging effect must be taken into consideration when proposing normal standard values for semen parameters characteristics in routine semen analysis as outlined by the WHO standards. The cut-off values for normal semen parameters characteristics might be too strict when evaluating older men and, thereby, classifying them as subfertile when in fact their sperm characteristics might be within normal limits for their age group. This decline may be attributed to exposure to environmental gonado-toxins like oestrogens either in utero or during childhood and change in life habits. These reports raised considerable alarm regarding the deteriorating reproductive health of men.

In cases of male factor infertility, this aging effect on semen quantity could be significant enough to impact on sperm function. The decline in sperm quality could has a negative effect on these couples‹s overall fecundity. This will be of significant clinical importance when advising the patient regarding his potential for natural conception prior to pursuing assisted reproductive technologies.

Our data has the advantage of reflecting the effect of age alone, as we were able to exclude other factors that may affect the semen parameters or hormonal level by studying only normally fertile males.

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