

The Impact of Age on Semen Parameters of Northern Sudanese Couples

Mohammed Ahmed Ibrahim Ahmed¹, Mubarak Elsaed Mustafa Elkarsany², Nahla Ahmed Mohammed Abderahman³, Elameen Elawad Ali⁴, Mosab Nouraldein Mohammed Hamad^{5*}

¹Assistant Professor of Microbiology, Faculty of Medicine, Department of Microbiology, Nile Valley University, Atbara, Sudan

²Associate Professor of Microbiology, Faculty of Medicine, Department of Microbiology, Karary University, Omdurman, Sudan

³Assistant Professor of Biochemistry, Faculty of Medicine, Department of Biochemistry, Nile Valley University, Atbara, Sudan

⁴Consultant in Obstetrics and Gynecology, River Nile State, Atbara, Sudan

⁵Phylum of Medical Parasitology, Department of Medical Laboratory Sciences, Faculty of Health Science, Elsheikh Abdallah Elbadri University, Berber, Sudan

Abstract: Background: There are several physiological changes brought on by the inevitable process of aging. For exploring the potential role of aging in male infertility; we examined the semen quality in Northern Sudanese sub fertile men. **Objectives:** This study aimed to look into possible association between male age and different sperm parameters derived from semen analysis taking into account the World Health Organization (WHO) reference values of semen indicators. **Methods:** A retrospective descriptive study included 150 Northern Sudanese Couples men presenting with primary or secondary infertility who underwent semen analysis at Modern Specialized Laboratory (MSL), Atbara, River Nile State, Sudan, between August 2021 and April 2023. Seminal fluid were collected in a sterile, wide mouth container, by dry masturbation, after at least 48 hours of abstinence and brought immediately to the laboratory. Color, viscosity, PH and volume were examined macroscopically, and motility under the microscope within 20 minutes to one hour of collection time and was stained by 10% Giemsa stain after fixation by spirit for 10-15min. Morphology and abnormal sperms were checked manually to assess the abnormalities in the head, midpiece and the tail of the sperm. This manual method was allowed the discrimination of immature sperm from white blood cells (WBCs) and then counting the WBCs. Data were collected by predesigned closed ended questionnaires, through direct personal interview with patients and questionnaires were filled after an informed consent was taken from each patient. The statistical analysis was done with the help of statistical software for social sciences SPSS 21. The characteristics of the semen, personal and demographic data were analyzed according to the standard protocols. **Results:** There was significant positive association between age and the different measured parameters except for the reaction and presence of microorganism and, a significant difference was found with the presence of microorganism by $p=0.004$. **Conclusion:** Age differences between participants were not observed to have an impact on semen parameters.

Keywords: Semen parameters, Age, Atbara, Sudan.

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Research Paper

*Corresponding Author:

Mosab Nouraldein
Mohammed Hamad

Phylum of Medical Parasitology,
Department of Medical Laboratory
Sciences, Faculty of Health
Science, Elsheikh Abdallah Elbadri
University, Berber, Sudan

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INTRODUCTION

The revised glossary exemplifies infertility as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (Dalal Salih Abdel-aziz *et al.*, 2021). The measure of semen quality is composed of multiple indicators. Accurate assessment of semen quality from original semen analysis data reports has always presented a complex challenge for andrologists (Wang N *et al.*, 2022). According to a

number of researches, aging is linked to a drop in semen parameters (Sharon A, 2001). In reality, from the age of 35 forward, age-related semen quality deterioration can be seen, and from the ages of 40 to 50, these changes become more prominent (Rosiak-Gill A, *et al.*, 2019). With men having children at earlier ages than ever before, it is critical to know that sperm quality also declines with age, according to new research (Collodel G, *et al.*, 2021). Nevertheless, a number of studies showed a connection between aging and structural and functional changes in the endocrine system, sexual

organs, and other sexually active tissues. These findings raise the possibility that these changes may have an impact on sperm quality and fertility (Pino V, *et al.*, 2020). The reported fall in sperm quality was verified; however non-Western nations did not show a similar decline. Since 1980, the population of fertile males has been subject to declining normative values for these indicators under World Health Organization (WHO) recommendations. There is yet no definitive research on how paternal age affects semen parameters (Li WN, *et al.*, 2019). However, it has been shown in recent research that a number of semen characteristics, including volume, total sperm count, motility, and shape, deteriorate with age. Additionally, men's fertility rates drastically decline beyond the age of 40 (Nago M, *et al.*, 2021). The issue of increasing male fertility has grown essential. According to studies, male fertility rates have been declining globally and go lower as people get older. The World Health Organization's (WHO) Human Semen Examination and Processing Laboratory Manual is the primary source for the current male fertility evaluations (Chen GX, *et al.*, 2022). In some studies, body mass index (BMI), drinking, smoking, dietary habits, physical activity, stress, place of residence, abstinence period, and age have been shown to be related to semen quality in some populations. However, other studies have found no such relationship (Wang N, *et al.*, 2022).

There are no sufficient researches and very limited data in Sudan regarding the effect of age on semen parameters with small number of patients in a narrow time and limited resources. This study aim to study the effect of leucocytospermia among Northern Sudanese Couples Sudanese males attending the Modern Specialized Laboratory in Atbara city.

PATIENTS AND METHODS

Study design, area and population: A retrospective, cross-sectional, hospital based study was carried out at Modern Specialized laboratory, Atbara, River Nile State, Sudan. One hundred fifty Sudanese patients attending to the Modern Specialized Laboratory, during period from August 2021 to April 2023.

Inclusion criteria: Sudanese males at reproductive ages married for >1 year, having primary or secondary infertility, without sexual disorders, and resident in the study area.

Exclusion criteria: Patients outside the study area, married <1 year, patients diagnosed with other sexual disorders (e.g erectile dysfunction) or other causes of male infertility e.g trauma, varicocele, smoking and alcohol users, patients meet inclusion criteria and refuse to be enrolled in this study.

Sample size and selection technique: The following formula was used: $N = (P \times Q \times Z^2) / d^2 + 10\%$ (non-responder rate) for selection of sample size.

Randomized cluster design was used to sample participants. The suspected cases were numbered in regular manner. Seminal fluid samples were aseptically collected from each participant after dry masturbation after at least 48 hours of abstinence, brought immediately to the laboratory and were examined macroscopy and microscopy following WHO 2010 gridlines. Seminal fluid analysis was performed color, viscosity, PH and volume were macroscopically, and motility, morphology, count, leukocytes, RBCs, epithelial cells bacterospermia was examined under the microscope within 20 minutes to one hour of collection time and was stained by 10% Giemsa stain after fixation by spirit 10-15min. Morphology and abnormal sperms were checked manually to assess the abnormalities in the head, midpiece and the tail of the sperm. The concentration of WBC was counted as described in the WHO laboratory manual: $WBC \text{ (white blood cells)} = N \times S / 100$. N is the number of given cell type (white blood cells) counted in the same fields as 100 spermatozoa, while S is the sperm concentration in Mio/mL. Blood and macConkey agar were used for cultivating of microorganisms.

Data collection and management: Data were collected by pre-designed closed ended questionnaires, through direct personal interview with patients after taking informed consent from each respondent. Collected data were revised for completeness and accuracy. Data were entered using the Statistical Package for Social Sciences (SPSS) version 21 after data auditing and clearance. Descriptive analysis was carried out and the relationships between different study variables were tested for statistical significance using Chi-square test. The significant is calculated at p-value ≤ 0.05 .

Ethical Consideration

Considering patients generously provided semen samples for this study, which included non-invasive approaches. Ethical approval was obtained from ethical committee of Faculty of Medicine, Nile Valley University as well as River Nile State Ministry of Health. Data were collect using coding system where each record is assigned with code; the data was never being used for any purpose rather than the objectives of the study. Safe keeping of the collected data sheet where all sampled records were filled, the information was use in data analysis and raw sheet were discarded after the completion of analysis by the study period. Confidentiality and strict anonymity were maintained throughout.

RESULTS

Demographic characteristics of study participants

150 male couples participated in a cross-sectional laboratory based study to evaluate the impacts of aging on seminal fluid parameters in Atbara, River Nile State who were attending the Modern Special Laboratory and suffering from primary or secondary infertility. The general characteristics of the participants

included age, which ranged from 20 to 84 years in different occupations, including farmers, government employees, workers, and others. Their minimum duration of liquefaction was 15 minutes and reached 130 in maximum with a mean of 34.4. The semen volume of respondents ranged from 0.4 to 7.5 ml, with a mean of 7.7 ml. The reaction of the study group ranged from 6.6 0 as acidic to 8.8 as alkaline, with a mean of 7.7. The counting of sperm among study couples ranged from azoospermia (0 sperm) to normospermia (90 millions), with a mean of 25.2 million, Table 1.

Comparison between age groups with regard to various aspects of semen analysis:

In Table 2 we compare the study variables depending on the age. The ANOVA results represented that there was significant positive association between age and the different parameters except for the reaction and presence of microorganism, and non-significant difference was found except for presence of microorganism by $p=0.004$.

Table 1: General characteristics of the study group

Variable	Minimum	Maximum	Mean	Std. Error	Std. Deviation
Age					
- 20-40 Year = 118(78.7%)	20	84	40.33	0.92	11.31
- 41-60 Years = 24(16.0%)					
- > 60 Years = 8(5.3%)					
Liquated\minutes	15	130	34.40	2.40	29.38
Absent\day	2	7	5.33	0.12	1.42
Infertility Duration\years	1	2	1.24	0.04	0.43
Volume	0.40	7.50	2.92	0.12	1.47
Reaction	6.60	8.80	7.71	0.03	0.36
Count	0	90	25.23	1.83	22.37

Table 2: Distribution of study participants' variables responding to age

Variable	Group	Count N=150	Age group\years			p-value	ANOVA Association
			20-40	41-60	> 60		
Liquifaction/ minutes	=<60	132	78.8%	16.7%	4.5%	0.452	0.002
	> 60	18	77.8%	11.1%	11.1%		
Absent	5-7=Normal	126	77.8%	16.7%	5.6%	0.831	0.004
	<5=Decreased	24	83.3%	12.5%	4.1%		
Occupation	Farmer	23	82.6%	17.4%	0.0%	0.863	0.008
	Government employee	54	75.9%	16.7%	7.4%		
	Worker	49	79.6%	16.3%	4.1%		
	Other	24	79.2%	12.5%	8.3%		
Infertility Duration/Years	<5	114	78.9%	77.8%	6.1%	0.626	<0.0001
	>5	36	14.9%	19.4%	2.8%		
Infertility Type Group	Primary	25	72.0%	20.0%	16.7%	0.647	0.006
	Secondary	125	80.0%	15.2%	4.8%		
Volume	=>1.5=Normal	126	77.8%	16.7%	5.6%	0.831	0.002
	< 1.5= Decreased	24	83.3%	12.5%	4.2%		
Reaction	<7.2 Acidic	5	80.0%	20.0%	0.0%	0.927	0.891
	>7.2 Alkaline	143	78.3%	16.1%	5.6%		
	=7.2 Neutral	2	100.0%	0.0%	0.0%		
Motility	=>40% Normal	91	80.2%	13.2%	6.6%	0.499	0.004
	<40% Decreased	31	71.0%	25.8%	3.2%		
	No sperms	28	82.1%	14.3%	3.6%		
Morphology	=>4% Normal	94	78.7%	75.9%	81.5%	0.899	0.001
	<4% Abnormal	29	14.9%	20.7%	14.8%		
	No Sperm	27	6.4%	3.4%	3.7%		
Count/Million	=<15=Normospermia	94	78.7%	58.3%	6.4%	0.870	0.002
	1-14.9=Oligospermia	28	75.0%	21.4%	3.6%		
	Zero=Azoospermia	28	82.1%	14.3%	3.6%		
Pus Cells	Normal <=5	67	85.1%	11.9%	3.0%	0.209	0.020
	Increasesd >5	83	73.5%	19.3%	7.2%		
Red Blood Cell	=< 2=Normal	65	76.9%	16.9%	6.2%	0.882	
	>2=Hemospermia	85	80.0%	15.3%	4.7%		

Variable	Group	Count N=150	Age group\years			p-value	ANOVA Association
			20-40	41-60	> 60		
Epithelial Cells	<2 cells	108	81.5%	13.9%	4.6%	0.402	0.002
	>2 cells	42	71.4%	21.4%	7.1%		
Type of sperm abnormalities	Normozoospcrrnia	93	78.5%	15.1%	6.5%	0.855	0.011
	Oligo-asthenoteratozoospermia	28	75.0%	21.4%	3.6%		
	Azoospermia	29	82.8%	13.8%	3.4%		
Presence of microorganism	Staphylococcus	2	0.0%	100.0%	0.0%	0.004	0.069
	Streptococcus	2	0.0%	100.0%	0.0%		
	Escherichia	23	73.9%	17.4%	8.7%		
	Corynebacterium	1	0.0%	100.0%	0.0%		
	Neisseria	1	100.0%	0.0%	0.0%		
	Enterococcus	14	85.7%	7.1%	7.1%		
	No microorganism isolated	107	82.2%	13.1%	4.7%		

DISCUSSION

Clinics of infertility consider age as attention factor for infertility of male although of healthy appearance and nonsmoker (Sharon A, *et al.*, 2001).

In this current study, there was no association between age and leukocytospermia with non-statistically significant difference ($p = 0.209$). 118(78.7%) of participants were in the age range between 20-40 years, 24(16.00%) between 41-60 years and only 8% (5.3%) of participants were above 60 years. Leukosytospermia and age, were revealed significant positive correlation by ($p=0.02$). These finding were in agreement with that of study of Indian males with advancing age; their result confirmed that there was considerable decline in healthy sperm quality and mobility in respect to increase age (Asif, *et al.*, 2023). Decreased in sperm quality appears in sperm quantity, rate of progressive motility and normal morphology according to a Turkish study (Ulubay M, *et al.*, 2022). In the current investigation, age and type of isolated microorganism were statistically significantly correlated by ($p=0.004$). This was matching the study of Ahmed MAI, *et al.*, (2023) which confirmed that type of microorganism (Escherichia coli, Enterococcus, and Staphylococcus) was recognized as potentially effect of sperm quality parameters and causes leukocytospermia (Ahmed MAI, *et al.*, (2023). These findings were in contrast to Fady Sharara *et al.*, 2023 study which presented that motility and volume did not decreased with age (Sharara FI, *et al.*, 2023). Furthermore, our data did not match up with study of Gustavo Luis Veron, *et al.*, 2018 which concluded that: aged male and unfavorable environments are key factors in the reduction of sperm quality and approved a negative association between age and common sperm parameters (Veron, *et al.*, 2018). Spermatozoa viability and progressive motility were decline with advancing age. This finding raises the possibility that the patient's age plays a role in male infertility and highlights the need for a deeper comprehension of the complex mechanisms and risks involved (Silea C, *et al.*, 2019). In different study

male partners of infertile couples aged 21-50 years did not significantly experience deterioration in semen quality with age in a cross-sectional observational research include 390 participants (Zabihullah M, *et al.*, 2023). The intricate link between age and semen quality is highlighted by these findings, which also underline the need for more studies to find the effect of age in semen parameters and male fertility.

Limitations

The research under consideration has inherent constraints. This study may have biases due to the fact that it is a single center-based cross-sectional study. Moreover, a control group of healthy, fertile males is lacking. The study's small sample size is its last challenge, rendering it necessary to confirm the conclusions with further patients.

CONCLUSION

Age differences between participants were not observed to have an impact on semen parameters.

RECOMMENDATIONS

The precise detailed clinical characteristics of the patients, their demographics, and their hormone profiles were outside the purview of this study and are thus suggested for future research.

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DECLARATION OF CONFLICTING INTERESTS

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DATA AVAILABILITY

The data set used or analyzed during the current study is available from the corresponding author upon reasonable request.

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