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The Effects of Age and Ejaculatory Abstinence on Semen Quality and Reproductive Hormones in Africa and the Middle East

Lionel Wildy Moungala[1], Opheelia Makoyo[2]

[1]Androcryos Andrology Laboratory, Johannesburg, South Africa
[2]Department of Obstetrics and Gynaecology, Mere-Enfant Hospital, Libreville, Gabon
Email: u12232310@tuks.co.za

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Abstract

The aim of this study was to retrospectively evaluate the effects of male age and ejaculatory abstinence on semen parameters and reproductive hormones among men residing in Africa and the Middle East. A total of 70,142 semen analysis results were analysed and grouped according to the age intervals (16 - 20, 21 - 30, 31 - 40, 41 - 50, 51 - 60, >60) and ejaculatory abstinence (<2 days, 2 - 5 days and >5 days). Semen parameters *i.e.* volume, concentration, progressive motility, total progressively motile count, morphology, total normal sperm count, DNA fragmentation, viability, sORP, normed sORP were specifically evaluated. Additionally, for each age interval, reproductive hormones *i.e.* estradiol, luteinizing hormone, follicle stimulating hormone, testosterone and prolactin were evaluated. Semen volume, total progressively motile count, sperm morphology and total normal sperm count constantly decrease significantly after the age of 30 years. Sperm concentration started declining significantly after the age of 50 years. There was a constant age-related increase in number of spermatozoa with damaged DNA. sORP constantly increased up to 60 years. Furthermore, constantage-related decreases in FSH, serum testosterone and prolact in were observed from patients aged between 16 years and 60 years. Semen volume, sperm concentration, progressive motility and normal morphology were significantly higher in patients having > 5 days of abstinence. Patients having > 5 days of abstinence had the lowest normed sORP. Male age significantly affects sperm parameters and reproductive hormones in fertile and infertile men residing in Africa and the Middle East. Prolonged abstinence days provides better semen quality.

Keywords

Africa, Middle East, Reproductive Hormones, Semen Quality

1. Introduction

Semen analysis is the cornerstone for the investigation of male infertility [1] [2]. Several factors such as age [3]-[8] and ejaculatory abstinence period [9] [10] [11] can influence semen quality and/or reproductive hormones.

Aging is a natural unavoidable process that occurs in every individual and characterized by a series of physiological changes in the human body [12]. Understanding the impact of aging on male fertility is an important public health issue as an increasing number of men choose to father children at older ages [13]. Aging changes in the male reproductive system can occur in multiple mechanisms. From the anatomical point of view, testicular size, which is an important marker of spermatogenesis, decreases with age [14] [15]. Furthermore, the number of testicular cells such as Sertoli cells [16], and Leydig cells [17] [18] decreases with male age. Aging was found to decrease semen volume [7] [19] [20] [21], sperm concentration [4] [5] [22], sperm progressive motility [7] [19] [21] [22] [23] [24], morphology [7] [19] [22] [23], DNA fragmentation [12] and vitality [25].

From ahormonal level aging can affect the central regulation of the hypothalamic-pituitary-testicular (HPT) axis which can cause hypogonadism [26]. Hypogonadism is a condition in which the endogenous secretion of testosterone is either insufficient or inadequate to maintain serum testosterone levels within normal range, with a late-onset hypogonadism defined as a decrease in serum testosterone level in older men compared to young men [27]. Hypogonadal testosterone levels increase to 20% in males aged over 60 years, to 30% for those over 70 years old and to 50% in male aged over 80 years [28]. Aging can decrease the ability of Leydig cells to produce testosterone in response to Luteinizing Hormone (LH) stimulation [18] [29] [30]. This is a result of the reductions in cAMP production and protein kinase A (PKA) activities in the Leydig cells [20] [31]. Significant age-related increases in luteinizing hormone (LH), follicle stimulating hormones (FSH) and sex hormone binding globulin were found [32]. Estradiol concentration was found to decrease with advanced male age [33]. However, advanced male age seems not to significantly impact prolactin concentration [34]. Most previous studies have reported the effects of aging in America [5] [8] [35], Asia [21] [22] [36] [37] [38] and Europe [39] [40] [41]. Limited data are available for men residing in African and Middle East Countries.

Sexual abstinence has been reported to be a major factor that influences semen parameters [42] [43]. Prolonged abstinence periods were found to favour the accumulation of spermatozoa in the epididymis, which can consequently increase the exposure time of spermatozoa to the detrimental effects of ROS [44]. The World Health Organization manual recommends 2 - 7 days before having a semen test [45] [46]. However, this recommendation was based on clinical studies on normozoospermic fertile men [45] [46]. Furthermore, the American Urological Association recommends a shorter period of ejaculatory abstinence of

2 - 3 days [47]. The impact of sexual abstinence periods on semen quality needs further investigations [48].

Consequently, this study was performed to determine the impact of age on semen quality, functional sperm tests and hormones in fertile and infertile men residing in Africa and Middle East regions. Furthermore, the study evaluated the effects of abstinence periods on semen characteristics.

2. Methodologies

This retrospective study (2005-2019) involved semen analysis results together with patient age, geographic locations, date of semen collection, ejaculatory abstinence period reports from andrology laboratories located in Africa and Middle East. All samples were collected through masturbation. Semen samples collected before 2010 were examined according to the techniques recommended by the World Health Organization [49] while those obtained after 2010 were analysed following the methods described by the WHO (2010). Normal values were defined based on the WHO standards. Sperm DNA fragmentation was evaluated using the Halosperm® technique [50]. Data received was organized in a Microsoft Excel spreadsheet. To evaluate the age-related changes in semen parameters and reproductive hormones, data was divided into four groups based on age and following previous published study by Pino *et al.* (2020) [51]. The following age groups were used in this study: 16 - 20 years, 21 - 30 years; 31 - 40 years; 41 - 50 years; 51 - 60 years and more than 60 years. To determine the effects of sexual abstinence periods on semen characteristics, semen analysis reports were categorized according to sexual abstinence period into 3 groups: <2 days, 2 - 5 days, and >5 days as previously reported by Comar *et al.* (2017) [52]. Semen parameters *i.e.* volume (mL), concentration (millions/mL), total sperm count (millions), progressive motility (%), total progressively motile count (millions), total normal sperm count (%), sperm morphology (%); functional sperm tests *i.e.* DNA fragmentation (%), sperm viability (%), sORP (mV)and normed sORP ($mV/10^6$ sperm/mL) and hormones *i.e.* Testosterone (nmol/L), estradiol (pmol/L), LH (IU/L), FSH(IU/L) and prolactin (mIU/L) were evaluated according to age categories.

Statistical analysis was performed using the MedCalc® statistical software version 19.5 (MedCalc Software Ltd, Ostend, Belgium; https:www.medcalc.org; 2020). The Chi-Square test was used to determine the distribution of all the data sets. Based on the distribution of data, non-parametric statistical analyses were applied. The Mann-Whitney Test was used to evaluate the statistical differences between groups. For all statistical tests, a P-value of < 0.05 was considered statistically significant.

3. Results

The Chi-Square test for normality shows that all parameters are not normally distributed and are therefore reported and analysed using non-parametric statis-

tical tests based on the median and inter-quartile range (IQR).

3.1. Effects of Male Age on Semen Parameters and Reproductive Hormones

This study included data from 70,142 men with a median (IQR) age of 38 (34 - 43) years. The highest number of semen analysis reports was obtained from patients aged between 35 and 40 years (34%) and most patients (56%) categorised as 30 - 45 years old (Figure 1).

Semen Parameters: Table 1 illustrates the differences in semen parameters according to age groups. Significant decreases in semen volume, total progressively motile count, sperm morphology and total normal sperm count after the age of 30 years were found. Sperm concentration started declining after the age of 50 years. The oldest age group (more than 60 years) had a significantly ($P < 0.01$) lower semen volume (median (IQR) = 1.50 (1 - 2.8) mL) than the other age groups. The same age group (more than 60 years) was also indicated to have the lowest sperm concentration (median (IQR) = 26 (4.9 - 74) $\times 10^6$/mL), total sperm count (median (IQR) = 37.50 (27 - 210) $\times 10^6$), progressive motility (median (IQR) = 15.5 (0 – 30)%), total progressively motile count (median (IQR) = 4.41 (3.1 - 21) $\times 10^6$), total normal sperm count (median (IQR) = 2.45 (0.31 - 9.7)%), and percentage of spermatozoa with normal morphology (median (IQR) = 4 (2 – 7)%).

Functional Sperm Tests: The results obtained for the functional sperm tests are summarized in Table 2. There was a constant age-related increase in number of spermatozoa with damaged DNA and a constant decrease in the number of viable spermatozoa with age from 21 years old. Patients under 30 years old had a significantly ($P < 0.05$) lower number of spermatozoa with DNA fragmented (median (IQR) = 22 (15 - 34)%) than patients older than 41 years old, and significantly higher number of viable spermatozoa (median (IQR) = 50 (38.5 - 64.5)%) compared to patients aged between 41 - 60 years old. Although, sORP

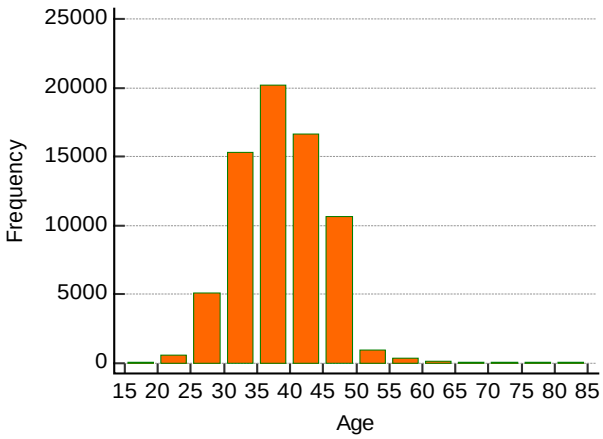

Figure 1. Frequency of semen analysis reports collected per age group.

Table 1. Basic semen parameters by age decade Africa and the Middle East, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

Age groups		Volume (mL)	Concentration ($\times 10^6$/mL)	Total sperm count ($\times 10^6$)	Progressive motility (%)	Total progressively motile count ($\times 10^6$)	Total normal sperm count (%)	Sperm morphology (%)
16 - 20 years (n = 96) A	Mean ± SD Median (IQR) n	2.74 ± 1.70 2.60 (1.5 - 3.3) 65	40.89 ± 45.00 28.00 (8 - 57.5) 65	111.06 ± 122.77 72.50 15.7 - 157.3) 65	25.32 ± 16.96 25.00 (14.2 - 35.7) 31	35.06 ± 56.90 18.00 (3.5 - 38.4) 31	10.82 ± 15.3 4.95 (1.2 - 15.8) 50	7.08 ± 5.47 5.00 (3 - 10) 50
21 - 30 years (n = 8470) B	Mean ± SD Median (IQR) n	3.17 ± 1.60 3.00 (2 - 4) 7,445	57.07 ± 61.40 40 (15 - 78) 7,423	173.16 ± 200.09 112 (37.5 - 240) 7,401	30.76 ± 18.23 32.00 (16 - 46) 2,525	64.17 ± 87.77 35.28 (7.9 - 87.9) 2,525	17.22 ± 26.3 7.50 (1.9 - 21.4) 5,187	7.28 ± 5.61 6.00 (4 - 10) 5,194
31 - 40 years (n = 36,900) C	Mean ± SD Median (IQR) n	3.06 ± 1.60 2.90 (2 - 4) 32,587	58.59 ± 62.48 41.00 (15.4 - 80) 34,526	169.68 ± 197.60 108 (37.5 - 232) 32,402	30.39 ± 18.24 32.00 (15 - 46) 6,066	62.83 ± 84.99 31.58 (5.9 - 85) 6,066	15.54 ± 24.8 6.30 (1.6 - 18.7) 13,490	6.83 ± 5.04 6.83 (3 - 10) 13,497
41 - 50 years (n = 22,154) D	Mean ± SD Median (IQR) n	2.84 ± 1.55 2.50 (1.8 - 3.6) 17,806	59.27 ± 62.55) 41.00 (15 - 82) 22,642	159.55 ± 193.54 97.50 (31.5 - 216) 17,695	28.34 ± 19.22 30.00 (10 - 45) 1780	51.52 ± 81.19 21.74 (2.27 - 66) 1780	12.79 ± 23.2 4.93 (1.1 - 14) 4449	6.20 ± 4.57 5.00 (3 - 8) 4450
51 - 60 years (n = 2359) E	Mean ± SD Median (IQR) n	2.37 ± 1.55 2.00 (1.2 - 3.1) 843	54.69 ± 74.26 30.00 (6.5 - 74.5) 828	115.83 ± 166.47 55.00 (10.8 - 153.3) 827	24.29 ± 20.67 22.00 (1 - 43) 287	48.29 ± 88.12 13.06 (0.00 - 56) 287	10.67 ± 22 3.46 (0.7 - 11.4) 712	5.76 ± 4.78 5.00 (3 - 7) 713
> 60 years (n = 163) F	Mean ± SD Median (IQR) n	2.02 ± 1.44 1.50 (1 - 2.8) 128	51.29 ± 69.23 26.00 (4.9 - 74) 122	98.86 ± 168.56 37.50 (27 - 210) 122	17.97 ± 18.18 15.50 (0 - 30) 48	34.48 ± 74.42 4.41 (3.1 - 21) 48	8.74 ± 16.21 2.45 (0.31 - 9.7) 98	5.26 ± 3.97 4.00 (2 - 7) 98
P-value (A vs. B)		0.011	0.019	0.006	0.079	0.019	0.094	0.480
P-value (A vs. C)		0.055	0.009	0.007	0.098	0.046	0.287	0.864
P-value (A vs. D)		0.459	0.072	0.037	0.341	0.440	0.985	0.556
P-value (A vs. E)		0.052	0.579	0.425	0.721	0.725	0.208	0.182
P-value (A vs. F)		0.009	0.998	0.051	0.057	0.109	0.066	0.104
P-value (B vs. C)		<0.0001	0.296	0.296	0.394	0.047	<0.0001	<0.0001
P-value (B vs. D)		<0.0001	0.0025	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
P-value (B vs. E)		0.0014	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P-value (B vs. F)		<0.0001	0.006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P-value (C vs. D)		<0.0001	0.1786	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P-value (C vs. E)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P-value (C vs. F)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P-value (D vs. E)		<0.0001	<0.0001	<0.0001	0.0009	0.0051	<0.0001	0.0018
P-value (D vs. F)		<0.0001	0.0016	<0.0001	0.0002	0.0025	0.0024	0.0268
P-value (E vs. F)		0.0051	0.4910	0.0482	0.0441	0.0968	0.2477	0.3749

Table 2. Functional semen parameters by age decade Africa and the Middle East, with statistically significant values obtained using the Mann–Whitney test for in dependent values. Bolded P-values indicate statistical significance.

Age groups		DNA Fragmentation (%)	Viability (%)	sORP (mV)	Normed sORP (mV/10^6 sperm/mL)
16 - 20 years	Mean ± SD	13.50 ± 2.12	51.50 ± 19.09	45.20 ± 16.32	2.66 ± 2.90
(n = 96)	Median (IQR)	13.50 (12 - 15)	51.50 (38 - 65)	42.60 (34.7 - 56)	1.39 (0.9 - 4.1)
A	n	2	2	19	18
21 - 30 years	Mean ± SD	27.21 ± 17.41	49.71 ± 18.57	54.41 ± 32.41	4.98 ± 9.16
(n = 8,470)	Median (IQR)	22.00 (15 - 34)	50.00 (38.5 - 64.5)	49.30 (36.4 - 66.9)	1.77 (0.9 - 4.1)
B	n	194	131	709	696
31 - 40 years	Mean ± SD	28.92 ± 19.08	47.41 ± 18.51	57.70 ± 37.35	5.16 ± 11.26
(n = 36,900)	Median (IQR)	23.00 (15 - 38)	50.00 (35 - 60)	52.35 (37.7 - 70)	1.89 (1 - 4.3)
C	n	443	324	1526	1482
41 - 50 years	Mean ± SD	36.29 ± 22.15	43.63 ± 19.90	57.29 ± 39.55	5.69 ± 10.01
(n = 22,154)	Median (IQR)	30.00 (20 - 50)	47.00 (26.5 - 57.5)	50.70 (37.1 - 68.8)	1.92 (1 - 4.9)
D	n	161	151	528	504
51 - 60 years	Mean ± SD	44.46 ± 22.99	38.14 ± 19.80	65.19 ± 58.21	3.88 ± 8.10
(n = 2,359)	Median (IQR)	42.0 (25 - 59)	45.0 (19.5 - 53.5)	53.75 (38.7 - 73)	1.83 (1 - 3.4)
E	n	30	48	112	113
> 60 years	Mean ± SD	50.62 ± 25.70	44.18 ± 18.02	49.40 ± 51.42	3.85 ± 6.36
(n = 163)	Median (IQR)	55.00 (28 - 60)	37.00 (15 - 54.5)	37.00 (21 - 56)	1.31 (1 - 2.5)
F	n	8	11	22	23
P-value (A vs. B)		0.152	0.970	0.167	0.490
P-value (A vs. C)		0.150	0.734	0.074	0.345
P-value (A vs. D)		0.057	0.536	0.113	0.248
P-value (A vs. E)		0.035	0.413	0.039	0.495
P-value (A vs. F)		0.066	0.429	0.314	0.979
P-value (B vs. C)		0.501	0.249	0.030	0.237
P-value (B vs. D)		<0.0001	0.0106	0.295	0.077
P-value (B vs. E)		<0.0001	0.0013	0.048	0.861
P-value (B vs. F)		0.0076	0.3284	0.9824	0.412
P-value (C vs. D)		0.0001	0.0367	0.4492	0.3330
P-value (C vs. E)		0.0001	0.0044	0.2783	0.4285
P-value (C vs. F)		0.0123	0.5253	0.6866	0.2541
P-value (D vs. E)		0.0477	0.1277	0.1747	0.2208
P-value (D vs. F)		0.1063	0.2843	0.8426	0.2048
P-value (E vs. F)		0.4732	0.4078	0.4123	0.4734

constantly increased up to 60 years, no significant difference in normed sORP was observed.

Reproductive Hormones: Table 3 shows an age-group related decrease in median (IQR) serum testosterone and prolact in observed from patients aged between 16 years old and 60 years old. Furthermore, in Table 3, the youngest

Table 3. Reproductive hormones by age decade in MENA region, with statistically significant values obtained using the Mann-Whitney test for independent values. Bolded P-values indicate statistical significance.

Age groups		Estradiol (pmol/L)	Luteinizing hormone (IU/L)	Follicle stimulating hormone (IU/L)	Testosterone (nmol/L)	Prolactin (mIU/L)
16 - 20 years	Mean ± SD	88.66 ± 29.73	4.57 ± 3.18	2.35 ± 1.71	20.87 ± 4.88	262.42 ± 73.37
(n = 96)	Median (IQR)	94.00 (62 - 115)	3.50 (2.62 - 5.7)	2.20 (1.2 - 2.3)	21.50 (19.4 - 24.5)	248.90 (218.5 - 281)
A	n	32	35	35	34	25
21 - 30 years	Mean ± SD	106.87 ± 53.44	4.62 ± 4.40	4.22 ± 8.68	19.27 ± 9.72	250.28 ± 231.64
(n = 8,470)	Median (IQR)	98.00 (71.75 - 126)	4.00 (2.9 - 5.3)	2.70 (1.7 - 4.6)	17.10 (12.8 - 22.8)	204.65 (149.7 - 282)
B	n	642	649	649	637	609
31 - 40 years	Mean ± SD	104.15 ± 50.19	4.72 ± 12.29	5.05 ± 10.14	18.06 ± 8.75	241.13 ± 274.60
(n = 36,900)	Median (IQR)	96.00 (72 - 125)	3.60 (2.6 - 5.1)	3.20 (2.1 - 5.8)	16.32 (12.5 - 21.6)	196.30 (146 - 267)
C	n	812	873	873	864	731
41 - 50 years	Mean ± SD	103.94 ± 46.14	4.41 ± 2.91	5.54 ± 4.69	17.88 ± 9.99	208.65 ± 115.26
(n = 22,154)	Median (IQR)	97.00 (73.7 - 126)	3.70 (2.7 - 5.1)	3.90 (2.6 - 7.2)	16.10 (12 - 20.9)	180.20 (137.7 - 247.7)
D	n	617	709	709	683	565
51–60 years	Mean ± SD	122.43 ± 69.20	4.83 ± 3.07	5.32 ± 4.50	15.86 ± 7.06	214.60 ± 119.35
(n = 2,359)	Median (IQR)	109.5 (79 - 132)	4.00 (2.5 - 6.4)	4.43 (2.7 - 6.7)	14.80 (11.7 - 17.2)	175.30 (132.5 - 292.5)
E	n	308	329	329	312	209
> 60 years	Mean ± SD	84.80 ± 44.74	4.37 ± 3.25	5.36 ± 3.61	20.94 ± 7.74	248.48 ± 116.04
(n = 163)	Median (IQR)	82.00 (49.7 - 102.7)	3.45 (3 - 5.4)	5.50 (2.7 - 6.1)	21.21 (12.6 - 25.4)	208.2 (176 - 269)
F	n	12	65	65	62	41
P-value (A vs. B)		0.4767	0.6906	0.1405	0.1952	0.1678
P-value (A vs. C)		0.5643	1.000	0.0305	0.0906	0.0934
P-value (A vs. D)		0.5693	0.8213	0.0073	0.0766	0.0286
P-value (A vs. E)		0.2697	0.7727	0.0112	0.0193	0.0840
P-value (A vs. F)		0.7150	1.000	0.0737	0.7751	0.3914
P-value (B vs. C)		0.7139	0.0616	0.0002	0.0956	0.3914
P-value (B vs. D)		0.8669	0.4915	<0.0001	0.0740	0.0131
P-value (B vs. E)		0.2309	0.6457	0.0014	0.0164	0.1945
P-value (B vs. F)		0.2583	0.8225	0.1602	0.4225	0.6617
P-value (C vs. D)		0.9232	0.3301	0.0010	0.6252	0.0323
P-value (C vs. E)		0.1604	0.2626	0.0885	0.0728	0.3070
P-value (C vs. F)		0.2770	0.9354	0.4118	0.2773	0.4868
P-value (D vs. E)		0.1939	0.4861	0.9403	0.1309	0.9790
P-value (D vs. F)		0.2761	0.9285	0.8624	0.2198	0.2429
P-value (E vs. F)		0.1254	0.6486	0.7452	0.1329	0.2563

age group (16 - 20 years) had a significantly higher ($P < 0.05$) testosterone concentration (median (IQR) = 21.50 (19.4 - 24.5) nmol/L) in comparison to the oldest age group (>60 years old) which had a median (IQR) testosterone level of

21.21 (12.6 - 25.4) nmol/L). Patients aged between 41 - 50 years had a significantly ($P < 0.05$) lower prolactin concentration (median (IQR) = 180.20 (137.7 - 247.7) mIU/L) than the younger age groups: 31 - 40 years (median (IQR) = 196.30 (146 - 267) mIU/L); 21 - 30 years (median (IQR) = 204.65 (149.7 - 282) mIU/L) and 16 - 20 years (median (IQR) = 248.90 (218.5 - 281) mIU/L).

3.2. Influence of Ejaculatory Abstinence Periods on Semen Parameters

The results on the influence of different periods of abstinence on semen volume, sperm concentration, progressive motility, normal morphology, sperm DNA fragmentation and normed sORP, are summarized in Table 4. Significantly ($P < 0.01$) higher semen volumes (median (IQR) = 3.00 (2 - 4) mL) and sperm concentrations (median (IQR) = 48.33 (15 - 95) × 10^6/mL) are found in patients having > 5 days of abstinence than in those having < 2 days abstinence (median (IQR) = 1.80 (1 - 2.6) mL and 32 (15 - 60) × 10^6/mL, respectively) and the group of patients having 2 to 5 days of abstinence (median (IQR) = 2.8 (2 - 3.8) mL and 40 (15 - 80) × 10^6/mL, respectively). Additionally, patients who have the longest abstinence days (>5) have a significantly ($P < 0.01$) greater progressive motility (median (IQR) = 35 (18 - 46)%), normal morphology (median (IQR) = 6 (64 - 10)%) and lowest normed sORP (median (IQR) = 1.75 (0.9 - 3.9) mV/10^6 sperm/mL).

4. Discussion

Evaluating the effects of male age on semen quality and reproductive hormones

Table 4. Comparison between semen parameters and sexual abstinence periods. Statistically significant values obtained using the Mann-Whitney test for independent values. Bolded P-values indicate statistical significance.

		Volume (mL)	Concentration (×10^6/mL)	Progressive motility (%)	Normal morphology (%)	DNA Fragmentation (%)	sORP (mV)	Normed sORP (mV/10^6 sperm/mL)
<2 days (n = 29,743) A	Mean ± SD	2.02 ± 1.48	43.60 ± 41.99	13.14 ± 13.84	5.55 ± 8.67	23.42 ± 13.71	49.24 ± 26.07	4.82 ± 9.28
	Median (IQR)	1.80 (1 - 2.6)	32.00 (15 - 60)	10.00 (0 - 21)	4.00 (2 - 8)	22.00 (12.7 - 28.7)	44.55 (33.2 - 65.6)	2.00 (0.9 - 4.2)
	n	175	245	85	98	19	74	74
2 - 5 days (n = 19,729) B	Mean ± SD	2.96 ± 1.55	57.08 ± 60.80	29.35 ± 18.81	6.61 ± 4.87	29.54 ± 19.51	55.29 ± 37.57	5.11 ± 10.35
	Median (IQR)	2.80 (2 - 3.8)	40.00 (15 - 80)	30.00 (13 - 45)	5.00 (3 - 9)	25.00 (15 - 37)	50.00 (37 - 67.1)	1.88 (1 - 4.5)
	n	15,571	19,300	6067	13,822	558	1913	1870
>5 days (n = 428) C	Mean ± SD	3.28 ± 1.75	68.98 ± 75.92	31.85 ± 17.62	7.17 ± 5.06	36.30 ± 21.86	65.54 ± 37.65	1.75 ± 10.54
	Median (IQR)	3.00 (2 - 4)	48.33 (15 - 95)	35.00 (18 - 46)	6.00 (4 - 10)	30.00 (20 - 50)	59.10 (42.6 - 78.8)	1.75 (0.9 - 3.9)
	n	8406	8627	2866	6,096	157	542	521
P-value (A vs. B)		<0.0001	0.009	<0.0001	<0.0001	0.227	<0.0001	0.761
P-value (A vs. C)		<0.0001	<0.0001	<0.0001	0.001	0.013	<0.0001	0.802
P-value (B vs. C)		<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	0.951

is becoming a major public health issue, due to the increasing number of men who decide to have their children at older ages [6] [13]. Even though, based on birth rate data in the USA, the peak age for fathers was reported as 25 - 29 years old, followed by the 30 - 34 years old group [13]. The median (IQR) age of the full cohort in our retrospective study was 38 years (34 - 43). This is reflected in the age distribution, with the largest percentage (34%) of patient records categorised as 35 - 40 years old, and most patients (56%) categorised as 30 - 45 years old (**Figure 1**).

A steady decline in various semen parameters with increasing age has been proposed in the literature. In non-smoking males without known fertility concerns between 22 and 80 years of age, semen volume decreased by 0.03 ml per year, as did sperm total motility, progressive motility and total progressively motile sperm (0.7%, 3.1% and 4.7% per year, respectively) [5]. A peak semen volume of 3.51 ± 1.76 ml at age ≥30 to <35 years and a peak motility of 44.39% ± 20.69% at age <25 years were previously reported in 6022 semen samples with normal sperm concentration [53]. A decline in sperm motility has been reported with age in healthy males aged 22 - 80 years of age [54]. In a meta-analysis of 90 studies and 93,839 males, age related declines are found for semen volume, progressive motility, total motility, normal morphology and unfragmented cells, independent of confounding variables [7]. This is reflected in the results of our study where a constant significant decrease in semen volume, total progressively motile count, sperm morphology and total normal sperm count after the age of 30 years were observed.

Increasing evidence suggests constant age-related decrease in sperm concentration [3] [5] [20] [55]. In North America, a study conducted in 5081 men showed that sperm concentration and normal morphology declined after 40 years [35]. In South America, males aged above 50 years old were significantly more likely to have anomalies in semen volume, sperm concentration, and sperm DNA fragmentation [12]. Similar trends were found in China, where sperm concentration declines just after 55 years old in a study involving 71,623 infertile Men [8]. This is reflected in our study where a significant decline in sperm concentration after the age of 50 years in men residing in Africa and the Middle East was observed.

In the current study, some DNA fragmentation was found to increase steadily through each advancing age group (**Table 2**). Considering that the data for sperm DNA fragmentation is from the MENA region only, it is limited in sample size particularly at the extremes of the age groups analysed. However, these results are supported by numerous previous studies that have reported a positive correlation between Sperm DNA fragmentation and male age [56]-[61]. Furthermore, men aged more than 50 years are 4.8 times more likely to present increased sperm DNA fragmentation compared to those aged between 21 and 30 years [12]. Sperm DNA fragmentation is associated with a longer time to conceive, impairment of embryo development and higher miscarriage rates [62] [63]

[64]. Oxidative stress is one of the main factors triggering sperm DNA fragmentation [65].

Numerous studies suggest that oxidative stress associated with advancing age negatively affects sperm parameters and sperm DNA fragmentation, in turn affecting male fertility, pregnancy outcomes and the health of the offspring [66] [67]. Even though normed sORP generally increased with advancing age groups in this study, these changes were not generally significant. However, there was a non-significant reduction of normed sOPR in the over 60 years of age group compared to all groups analysed (**Table 2**). The sample size of normed sORP however is small, with 136 patient reports available for patients aged more than 50 years, compared to the 2,700 reports from patients aged less than or equal to 50 years. Cocuzza *et al.* (2008) [68], Koh *et al.* (2016) [69] and Nago *et al.* (2021) [67] suggested that male age could impact seminal oxidative stress. Cocuzza *et al.* (2008) [68] suggested that older men have increased levels of ROS and/or decreased antioxidant capacities in semen. Mitochondrial disruption due to aging results in increased ROS production, decreased ATP production, and apoptosis [70]. This leads to a decrease in antioxidant capacity in the body, and potential oxidative damage to spermatic DNA [12].

The data for reproductive hormones in this study was obtained from men residing in the MENA region. A constant age-related decline in testosterone and prolactin levels, and an increase in FSH, was found from 16 years to 60 years of age in men residing in the MENA region. However, both testosterone and prolactin were found to be increased again in the greater than 60 years age group compared to younger ages (**Table 3**). These results are similar to previous findings by Feldman *et al.* (2002) [71] in middle-aged men, reporting increases in FSH and decreases in testosterone levels with age. Although the level of testosterone seems to be higher for men aged 60 years and above in this study, the sample size of men aged 60 years and above (n = 62) is remarkably lower than the 51 - 60 years group (n = 312), the 41 - 50 years (n = 683), 31 - 40 years (n = 864) and the 21 - 30 years (n = 637). No significant influence of age on LH was observed in males in the MENA region (**Table 3**). This result is different from the significant increase in LH and FSH with age previously reported [32] [72].

In the current study, no significant impact of age on estradiol was observed in males in the MENA region (**Table 3**). However, increasing age is associated has been associated with a loss of estradiol [73], which is important in spermatogenesis [74]. Furthermore, decreases in prolactin concentration were observed in each age category until 60 years old. The highest level (248.90 mIU/L / 11.66 ng/ml) found in the 16 - 20 years old group is less than the upper normal limit (20 ng/ml) suggested by Thapa and Bhusal (2022) [75]. The lowest level (175.30 mIU/L / 8.23 ng/ml) found in the 61 - 60 years old group is higher than the normal average basal limit (5 ng/ml) reported by Thapa and Bhusal (2022) [75]. Prolactin is a hormone that was found to significantly increase with age [76] [77]. Although in the current study, different trends were observed it is impor-

tant to note that most results were not significant.

Several studies have been undertaken to determine the influence of ejaculatory abstinence on various semen parameters. The World Health Organization (WHO) recommends an ejaculatory abstinence period of 2 - 7 days before semen collection for evaluation [45] [46]. Shorter (1 day or less) ejaculatory abstinence was associated with increasesin sperm motility [48] [77], normal sperm morphology [48], oxidative activityand sperm function [77] in both normozoospermic and olizoospermic men, while other studies have shown a significant increase in semen volume, pH, sperm concentration, total sperm count, with abstinence length [9] [78]. Based on a systematic review, the weight of evidence suggests reduced semen volume and sperm concentration with shorter abstinence, not all studies have the same conclusion, and the relationships are complex [10] [43].

In the current study, an increased duration of abstinence significantly increased semen volume, sperm concentration, progressive motility, normal morphology and sperm DNA fragmentation. Similarly, in 2,458 fertile and infertile men, there was a significantly increased semen volume and sperm concentration with an abstinence period of more than 5 days compared to an abstinence period of 2 to 5 days and less than 2 days [52]. It was previously found that shorten ejaculatory abstinence period decreases sperm DNA fragmentation [9] [79] [80]. This is consistent with our results which showed a significant lower sperm DNA fragmentation in patients with the shortest abstinence period. Although, limited studies have evaluated the effects of ejaculatory abstinence period on oxidative activity, intracellular oxidative activity was significantly lower after 1 day abstinence period compared to 4 days ejaculatory abstinence [77]. Furthermore, a decrease in ROS production was associated with a decrease in abstinence period [78]. The results of this cohort study are contrary to these reports. Although there was a downward trend in normed sORP with increasing abstinence in this study cohort, this was not significant. There is no published data on the impact of duration of abstinence on sORP and normed ORP identified in the literature. Therefore, the current study provides a novel in the investigation on the influence of ejaculatory abstinence on oxidative stress evaluated using the sORP.

5. Conclusion

Age was found to negatively influence semen parameters in Africa and the Middle East, specifically affecting sperm concentration, progressive motility and normal morphology, with increasing sperm DNA fragmentation and normed sORP. In the MENA region, an age-related decline in testosterone and prolactin, and increase in FSH was found, with no significant changes for LH and estradiol with age. An increased sexual abstinence period positively impacted semen volume, sperm concentration and progressive motility, where reduced abstinence improved sperm DNA fragmentation. The current study provides significant data for men residing in Africa and the Middle East region, and useful in-

formation in the clinical management of male infertility.

Ethical Considerations

The current study was conducted in line with the Declaration of Helsinki for medical research. Institutional approval was granted by the Biomedical Research Ethics Committee (BMREC), University of Western Cape (UWC), South Africa (Ethics Reference Number: BM19/9/7). Permissions to use data confidentially were obtained from participating laboratories. No personal identification data such as name, ID or laboratory requisition number was extracted.

Limitations

The large sample size of data subjects this study to random errors, inherent biases and confounding. Furthermore, information about patient's lifestyle behaviors (e.g. smoking, nutritional patterns, and drinking habits), occupational and environmental exposures and comorbidities (e.g. obesity and diabetes mellitus) have not been available as potentially confounding variables. Finally, there is no regression analysis to estimate the relationship between dependent variables such as semen parameters and hormones and independent variables such as age, abstinence periods.

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Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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