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Influence of ejaculatory abstinence period on semen quality of 5165 normozoospermic and oligozoospermic Nigerian men: A retrospective study

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Abstract

Background and Aims: Several studies have shown that the length of ejaculatory abstinence alters sperm quality. However, the available data are conflicting and none seems to exist in a Nigerian population. The present study aims to compare the semen quality in normozoospermic and oligozoospermic semen samples of a homogenous Nigerian population, following varying ejaculatory abstinence days (EAD); less than 2, 2–3, and 3–7 days.

Methods: The present retrospective study included 5165 semen samples collected over 5 years, from April 2015 to April 2020.

Results: In normozoospermic samples, sperm count and total sperm count were significantly higher in prolonged EAD. In oligozoospermic patients, semen volume significantly increased with prolonged EAD, while sperm count, total sperm count, and progressive motility were significantly reduced with prolonged EAD. In addition, EAD and sperm volume positively correlated in oligozoospermic patients.

Conclusion: Our findings indicate that EAD affects sperm quality in both normozoospermic and oligozoospermic men with varying impacts. Prolonged EAD increased sperm count and total sperm count in normozoospermic patients, while EAD increased semen volume but reduced sperm count, total sperm count, and progressive motility in oligozoospermic patients.

KEYWORDS

ejaculatory abstinence, male fertility, Nigeria, sperm count, sperm motility, sperm quality

1 | INTRODUCTION

Infertility is the inability of a couple to achieve conception despite regular unprotected sexual intercourse for at least 12 months.[1] It is a global public health challenge with social and financial implications.[2] About 72.4 million couples, accounting for 15% of the global couples, experience infertility.[3] Findings have established that 50% of the world's infertility cases are due to male factors, solely and in combination with female factor(s).[4]

Although advanced studies in male reproduction provide useful information on sperm DNA integrity, conventional semen analysis remains the cornerstone for diagnosing male infertility.[5] Studies have shown that several factors, pathological and physiological such as sexual abstinence, influence semen parameters. Sexual abstinence has been reported to be a major factor that influences semen parameters.[6,7] The World Health Organization (WHO) recommends an ejaculatory abstinence period of 2–7 days before semen collection for evaluation,[4] while the European Society for Human Reproduction and Embryology (ESHRE) and the Nordic Andrology Association (NAA) recommend 3–4 days of abstinence.[8] Interestingly, the American Society for Reproductive Medicine recommends 2–5 days,[9] while the American Urological Association recommends an ejaculatory abstinence period of 2–3 days.[10] Although standardization of ejaculatory abstinence period is aimed to achieve inter-laboratory homogenization of results,[11] whether a prolonged or shorter abstinence period improves semen quality and achieves better outcomes with the use of artificial technologies is still poorly understood.

Although several studies have documented the effects of the ejaculatory abstinence period on sperm quality,[12–20] available data are inconsistent and little to none is existent from an African population. Levitas et al. reported that semen parameters in normozoospermic patients improve with increasing ejaculatory abstinence while motility and morphology inversely relate to ejaculatory abstinence in oligozoospermic patients.[21] It has been shown that although longer ejaculatory abstinence promotes sperm DNA damage,[12,15] it is associated with increased sperm volume, motility, and count.[12,15,17] On the other hand, some studies documented that shorter ejaculatory abstinence, about 4–24 h, is associated with better sperm quality using conventional semen analysis than a longer abstinence period.[14,17,18,20] Interestingly, some recent studies revealed that abstinence length is only arbitrary and does not influence semen quality.[13,19] Hence, standardizing the recommended ejaculatory abstinence period remains pertinent for male infertility evaluation, and a successful spontaneous and assisted conception.

The present study thus sought to determine the impact of the ejaculatory abstinence period on conventional sperm parameters in a Nigerian population. This would help to establish a required ejaculatory abstinence length for optimal sperm quality for diagnostic and therapeutic purposes.

2 | MATERIALS AND METHODS

2.1 | Sample collection

This retrospective study was based on 5165 semen samples obtained from male partners of infertile couples who were presented for semen analysis at Oasis of Grace Hospital, Osogbo, Nigeria, and Union Diagnostic and Clinical Services, Osogbo, Nigeria on account of infertility between April 2015 and April 2020. The study was approved by the Ethics Committee of Oasis of Grace Hospital, Osogbo, Nigeria (OGH/2020/231). Semen samples were analyzed within an hour of sample collection. The analyses were performed by at least two experienced professionals. Results of patients who smoke cigarettes or abused any drug were excluded. Also, patients who had chronic medical conditions or any comorbidity, azoospermia, globozoospermia, or any gross testicular deformity were excluded. Results of men in their reproductive age (37.17 years ±0.23) with either normozoospermia or oligozoospermia were included.

2.2 | Semen analysis

Semen analysis was carried out according to the recommendations of the World Health Organization (WHO)[4] and as reported in our previous studies.[22–25] Briefly, each semen sample was obtained by masturbation into a preweighed clean and sterile wide-mouthed plastic universal sample bottle in a private room within the laboratory facility. The sample bottle was appropriately labeled and placed in an incubator at 37°C and allowed to liquefy. To obtain the volume of the semen, the sample bottle containing the semen was weighed and the preweight was deducted from the final weight. The pH of each sample was determined 30 min after liquefaction. The semen samples were mixed well, and a drop of the mixture was spread onto a pH paper. The color of the impregnated zone was allowed to become uniform, and then the color was carefully compared with the calibration strip to read the pH.

To assess sperm motility, an aliquot of the well-mixed semen sample was placed on a clean prewarmed slide and then covered with a coverslip. This was viewed using a phase-contrast light microscope (Omax) at ×10 and ×40 phase settings (×100 and ×400 magnifications). Approximately, 200 spermatozoa per replicate were assessed for the percentage of different motile categories: progressive motility (fast or slow), nonprogressive motility, and immotility. Only intact spermatozoa were assessed for motility.[4,26]

For sperm count evaluation, an aliquot of the well-mixed undiluted liquefied semen was loaded into an improved Neubauer hemocytometer using a pipette and allowed to settle in the humid chamber. Approximately, 200 spermatozoa were counted per replicate using a light microscope with ×400 magnification. The sperm count per ml of ejaculate was then calculated.[4,25]

For the determination of sperm morphology, a smear of semen is prepared on a glass slide, air-dried, and stained with eosin/nigrosin. The slide is mounted with a coverslip and examined using a light microscope. Approximately, 200 spermatozoa per replicate are examined for normal and abnormal forms.[4]

Using the WHO criteria (2010), semen samples were classified as either normozoospermic (15×10^6/ml) or oligozoospermic ($<15 \times 10^6$/ml). Each of these was further classified based on abstinence period into <2, 2–3, and >3–7 days using the recommended 2–3 days ejaculatory abstinence period of the American Urological Association (AUA)[10,11] and 2–7 of WHO[4] as the basis.

2.3 | Statistical analysis

Statistical analyses were performed using Statistical Programs for Social Sciences (SPSS Inc., version 16.0) software programs. Mann–Whitney–Wilcoxon and Kruskal–Wallis tests were used when appropriate. Pearson's bivariate correlational study was carried out to assess the association between the ejaculatory abstinence period and sperm parameters. The level of significance was assumed as $p < 0.05$.

3 | RESULTS

About 61.28% (3165) of the patients were normozoospermic while 38.72% (2000) were oligozoospermic (Table 1). No sample fell into the category of "normozoospermia <2 days." The classification was based on the recommendation of WHO (2010) guidelines (5th edition). The variation in semen volume, semen pH, sperm count, total sperm count, motility, and morphology at different abstinence periods is shown in Table 2. Overall, semen volume, sperm count, total sperm count, motility, and morphology were significantly different ($p < 0.05$) across the groups.

Although semen volume increased with increasing ejaculatory abstinence period in both normozoospermic and oligozoospermic patients, it was only significantly increased in oligozoospermic patients. Interestingly, sperm count and total sperm count significantly increased with ejaculatory abstinence length in the normozoospermic patients; however, both significantly reduced with increasing ejaculatory abstinence length in oligozoospermic patients. Our findings also revealed that increasing the ejaculatory abstinence period significantly impaired sperm motility evidenced by reduced fast progressive sperm and increased nonprogressive and immotile sperm in both normozoospermia and oligozoospermia.

The relationships of the observed semen parameters to the ejaculatory abstinence period are shown in Table 3. Semen volume was positively correlated with increasing ejaculatory period in oligozoospermia (Table 3).

TABLE 1 Categorization of the semen sample

Categories	Frequency ($n = 5165$)	%
Category of semen		
Normozoospermia	3165	61.28
Oligozoospermia	2000	38.72
Category of sexual abstinence		
<2 days	30	0.58
2–3 days	2190	42.40
3–7 days	2945	57.02

4 | DISCUSSION

To the best of our knowledge, this is the first-ever study comparing the semen quality in normozoospermic and oligozoospermic semen samples with regard to the length of ejaculatory abstinence among Nigerians and possibly, among African descents. The present study evaluated the influence of the duration of ejaculatory abstinence on various semen parameters of normozoospermic and oligozoospermic Nigerian men. Although the major limitation of the study is its restriction to conventional semen analysis, the large sample size is a major strength. Furthermore, the present study did not include data

TABLE 2 Impact of ejaculatory abstinence period on semen parameters in normozoospermic and oligozoospermic patients

	Normozoospermia			Oligozoospermia				
Semen parameters	2–3 days	3–7 days	p value[a]	<2 days	2–3 days	3–7 days	p value[b]	p value[c]
Volume (ml)	2.56 ± 0.08	2.72 ± 0.08	0.396	1.43 ± 0.27	2.82 ± 0.11	3.15 ± 0.10	0.004*	0.000*
pH	8.09 ± 0.03	8.03 ± 0.02	0.377	8.00 ± 0.00	8.14 ± 0.45	8.12 ± 0.04	0.856	0.162
Sperm count (10^6/ml)	49.08 ± 2.01	58.49 ± 2.39	0.014*	28.40 ± 15.93	7.11 ± 0.63	6.11 ± 0.32	0.000*	0.000*
Total sperm count (10^6/ml)	125.64 ± 0.65	159.09 ± 0.70	0.001*	40.61 ± 0.72	20.05 ± 0.71	19.25 ± 0.70	0.000*	0.000*
Sperm motility (%)								
Fast progressive	50.37 ± 0.750	49.01 ± 0.52	0.077	50.00 ± 1.21	36.44 ± 1.03	35.31 ± 0.67	0.035*	0.000*
Non progressive	12.64 ± 0.31	12.54 ± 0.18	0.192	13.33 ± 1.05	13.65 ± 0.49	14.82 ± 0.54	0.275	0.000*
Immotile	36.90 ± 0.84	37.49 ± 0.65	0.340	36.67 ± 2.11	49.17 ± 1.37	49.57 ± 1.17	0.213	0.000*
Sperm morphology (%)								
Normal	64.89 ± 0.62	63.57 ± 0.63	0.333	60.00 ± 0.00	56.37 ± 1.04	56.94 ± 0.93	0.775	0.000*

Note: Data are presented as mean ± SEM.

[a]Among normozoospermic.

[b]Among oligozoospermic.

[c]Between normozoospermic and oligozoospermic groups.

*$p < 0.05$.

TABLE 3 Correlational studies between abstinence and sperm quality in normozoospermic and oligozoospermic subjects

	Normozoospermia		Oligozoospermia	
Semen parameters	Ejaculatory abstinence	*p* Value	Ejaculatory abstinence	*p* value
Volume (ml)	0.018	0.647	0.172	0.001*
pH	0.041	0.299	0.034	0.501
Sperm count (10^6/ml)	0.061	0.122	−0.018	0.724
Total sperm count (10^6/ml)	0.048	0.233	0.228	0.181
Sperm motility (%)				
Fast progressive	−0.030	0.452	−0.085	0.088
Non progressive	0.019	0.640	0.022	0.661
Immotile	0.075	0.058	0.049	0.331
Sperm morphology (%)				
Normal	0.002	0.967	0.029	0.564

*$p < 0.05$.

on sperm DNA fragmentation (SDF) because it is not routinely done and data on SDF was not available. In addition, this study included both normozoospermic and oligozoospermic sperm populations, hence the findings of the study might be extrapolated to a wider population. Observations from the study add to the existing literature by providing extensive information based on conventional semen analysis on the changes in sperm quality as a function of the duration of ejaculation abstinence.

We observed among normozoospermic and oligozoospermic semen a consistent increase in semen volume directly correlated to abstinence length, although this was marginal in normozoospermic but significant in oligozoospermic semen. This finding is in concert with previous studies that reported increased semen volume with increasing ejaculatory duration.[15,16,21] Levitas et al.[21] demonstrated that the mean semen volume positively correlates with ejaculatory abstinence length with a significant increase observed from nil abstinence up to abstinence for 4 days and remaining high at approximately about the same level despite increasing abstinence length.

Furthermore, normozoospermic samples showed increased sperm count and total sperm count with increasing duration of ejaculatory abstinence. This observation was contrary to that in oligozoospermic samples that showed significant reductions in sperm count and total sperm count with increasing ejaculatory period. The sperm count and total sperm count deteriorated from less than 2 days of abstinence to 2–3 days of abstinence, and further deterioration during an abstinence period longer than 3 days. Although this does not align with the findings of Levitas et al.[21] that reported increasing sperm count until Day 4 of abstinence in oligozoospermic samples, the observed increased sperm count and total sperm count with increasing ejaculatory abstinence in normozoospermic samples were consistent with previous reports.[15,16,21] This seems to be the first-ever study to observe an inverse relationship between ejaculatory abstinence and sperm count in oligozoospermic samples. Previous studies that showed a positive correlation between abstinence duration and sperm count in oligozoospermic patients attributed their observation to the sperm transport time through the epididymis which has been reported to be about three times longer in oligozoospermic than normozoospermic men.[16] Furthermore, oligozoospermic patients with supposed idiopathic testicular failure might have partial obstruction. This will expectedly prolong sperm transit within the genital tract with a resultant increase in sperm count as ejaculatory abstinence increases. Since sperm count is a measure of spermatogenesis,[27] our observation that a longer ejaculatory period adversely influences sperm count could infer that spermatogenesis may be impaired in oligozoospermic individuals with reduced frequency of ejaculation.

Spermatozoa are rich in polyunsaturated fatty acids which make them susceptible to reactive oxygen species (ROS)-induced damage.[22,23,27] Studies have shown that higher concentrations of ROS are found in the semen of infertile men when compared with semen from fertile men.[28] During transit and storage, spermatozoa are exposed to a higher level of ROS and reactive nitrogen species (RNS) than elsewhere in the genital tract.[29,30] ROS and RNS attack the mitochondrial and nuclear DNA causing increased sperm DNA fragmentation and apoptosis.[31–33] The observed reduction in sperm count with increasing ejaculatory abstinence duration in oligozoospermic semen may be, at least partly, due to increased ROS- and RNS-induced sperm damage.

Sperm function requires progressive motility,[34] hence sperm motility seems to be the single most essential parameter for sperm function. Remarkably, sperm motility was not significantly altered in normozoospermic subjects, although there was a marginal decline with increasing duration of ejaculatory abstinence. In oligozoospermic samples, sperm motility was significantly reduced with prolonged ejaculatory abstinence. Sperm progressive motility was also observed

to be inversely related to the duration of ejaculation abstinence. This observation could be attributed to ROS-driven lipid peroxidation of the sperm membrane thus promoting alteration in the membrane fluidity with a resultant decline in sperm motility.[35] Similarly, this might explain the reduced percentage of normal sperm forms in both normozoospermic and oligozoospermic semen with prolonged ejaculatory abstinence. This aligns with the study of Keihani et al.[36] which reported a significant decrease in progressive sperm motility as well as the percentage of normal morphology with an increased abstinence period in normozoospermic and oligozoospermic semen.

In conclusion, our findings revealed that the impact of ejaculatory abstinence duration on sperm quality differs between normozoospermic and oligozoospermic semen samples. Our findings indicate that EAD affects sperm quality in both normozoospermic and oligozoospermic men with varying impacts. Prolonged EAD increased sperm count and total sperm count in normozoospermic patients, while EAD increased semen volume but reduced sperm count, total sperm count, and progressive motility in oligozoospermic patients. The import of this is that an increased ejaculatory abstinence period might be beneficial to normozoospermic patients, but detrimental to oligozoospermic patients. Hence, it is credible to infer that recommended ejaculatory abstinence period should be individualized putting in mind whether or not the patient is normozoospermic or oligozoospermic, especially when a repeat semen analysis is required.

AUTHOR CONTRIBUTIONS

Roland E. Akhigbe: conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; validation; writing – original draft; writing – review and editing. **Moses A. Hamed**: data curation; formal analysis; funding acquisition; investigation; methodology; project administration; validation; writing – review and editing. **Sulagna Dutta**: data curation; formal analysis; investigation; methodology; project administration; validation; writing – review and editing. **Pallav Sengupta**: data curation; formal analysis; investigation; methodology; project administration; validation; writing – review and editing.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article. All necessary data and materials have been included in the manuscript.

TRANSPARENCY STATEMENT

Akhigbe RE affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ETHICS STATEMENT

The study was approved by the Ethics Committee of Oasis of Grace Hospital, Osogbo, Nigeria (OGH/2020/231). All authors consented to the publication of the study.

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