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Semen parameters of some infertile patients attending Aminu Kano Teaching Hospital, Kano, Nigeria

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Abstract
Semen analysis is the first test usually carried in the laboratory towards determination of the influence of genital pathophysiology on the reproductive capacity of men; even though certain parameters might not be of any clinical significance. Semen parameters are essential in the determination of infertility of a male subject where semen analysis is used to evaluate male fertility. The study was carried out on 111 infertile subjects attending Aminu Kano Teaching Hospital between the months of September and November, 2017. The study was done by manual method adhering to WHO guideline 2010. Infertility and in particular poor semen quality is a serious health problem worldwide. Among the semen parameters, the ones that have greatest effect on fertility are concentration, motility and morphology. The finding in this study shows that sperm quality has oligoasthenozoospermia 12 (10.8%). While 13 (11.7%) have Oligozoospermia, 13(11.7%) 13 (11.7%) oligoteratozoospermia, 16 (14.4%) of azoospermia asthenoteratozoospermia 39 (35.1%) oligoasthenoteratozoospermia, 58 (52.3%) asthenozoospermia, and 61 (55.0%) teratozoospermi. Generally, semen parameters of infertile men are low compared to the WHO standard lower limit; abnormal semen quality remains a significant factor to the overall infertility in our community, with abnormal morphology and low progressive motility being the most common abnormal semen parameters.

Keywords: Azoospermia, Asthenoteratozoospermia, Infertility, Oligozoospermia Oligoasthenoteratozoospermia, Semen, Teratozoospermia,

Introduction
Infertility is the inability to achieve pregnancy after one year of unprotected intercourse. Therefore, if couples do not achieve pregnancy within the stipulated period, they are considered infertile (Albert *et al.,* 2014). The World Health Organization (2013) sees infertility as a disease of the reproductive system while the United States National reproductive endocrinologists society view infertility as the inability to conceive within reproductive age in the absence of contraceptives. Infertility is a global problem and more worrisome in developing countries and a common gynecological consultation in most Nigerian clinics (Ujaddughe *et al.,* 2015). Infertility in male also refers to the inability of a man to impregnate a normal healthy woman after 12 months of regular and unprotected sexual intercourse (Patrick *et al*., 2015). In male, infertility could also be define as the inability of the male reproductive cells to produce mature, actively motile and morphologically normal

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functional spermatozoa in sufficient amount that will ensure fertilization of a released ovum in the fallopian tubes (Festus *et al.*, 2013)

Infertility can be categorized into primary and secondary, the primary infertility refers to those who have never been pregnant while secondary infertility refer to those who have at least one previous pregnancy (Friday, 2005). The prevalence of primary infertility has increased since 1990, but secondary infertility has decreased. Rates decreased (although not prevalence) of female infertility in high-income Central/Eastern Europe, and Central Asia regions (Mascarenhas *et al.,* 2012).

Spermatogenesis is the process by which the male gametes called spermatozoa (sperms) are formed from the primitive spermatogenic cells (spermatogonia) in the testis. It takes 74 days for the formation of sperm from a primitive germ cell to develop to maturity (Sembulingam and Sembulingam, 2010). Throughout the process of spermatogenesis, the spermatogenic cells have cytoplasmic attachment with Sertoli cells. Sertoli cells supply all the necessary nourishment for spermatogenesis which takes place in the Testes (Friday, 2005). Prostate secretion gives milky appearance to the semen. Secretions from seminal vesicles and bulbourethral glands provide mucoid consistency to the semen (Pramanik, 2010). During ejaculation, semen is produced from a concentrated suspension of spermatozoa, stored in the paired epididymis, mixed with, and diluted by, fluid secretions from the accessory sex organs. Semen has two major quantifiable attributes, first the total number of spermatozoa; this reflects sperm production by the testes and the patency of the post-testicular duct system then secondly the total fluid volume contributed by the various accessory glands: this reflects the secretory activity of the glands. The nature of the spermatozoa (their vitality, motility and morphology) and the composition of seminal fluid are also important for sperm function (WHO, 2005).

Semen analysis is the examination of a male's ejaculate, carried out to determine if the cause of a couple's infertility is attributed to the male's inability to fertilize the ovum (Arvind *et al*., 2012).
Semen analysis is the first test usually carried in the laboratory towards determination of the influence of genital pathophysiology on the reproductive capacity of male, even then certain parameters might not be of any clinical significance. Manual method is widely used in most laboratories (especially developing countries; Nigeria inclusive) to evaluate semen volume, sperm count, motility and morphology (Chaurasia *et al*., 2016). The aim of this research is to assess the quality and parameters of seminal fluid of infertile male patients attending Aminu Kano teaching hospital which have never being done on AKTH clients.

Materials and Methods

Study area

This study was carried out in Medical Microbiology Laboratory of Aminu Kano Teaching Hospital Kano, Kano State, Nigeria (AKTH). AKTH Kano is a tertiary institution situated within Kano metropolis of Tarauni local government area of Kano state. Kano is situated between latitude 11^0 30` N and longitude, 8°30`E and lies at about 1580feet above sea level. Its total land area is 20760 sq^2 (Ado, 2009). Kano State borders Katsina to the north-west, Jigawa State to the north-east, Bauchi Ttate to the south-east and Kaduna State to the south-west.

Study design
This study was cross-sectional study.

Sample size determination
The sample size of this study was calculated and determined using the formula as follows (Naing *et al.,* 2006).

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where
n= number of samples
z = statistic for level of confidence at 95% = 1.96
p = prevalence = 7.1% = 0.071
d = allowable error of 5%, (0.05) (Ashok *et al*., 2015).

$n = \frac{1.96^2 \times 0.071(1-0.071)}{0.05^2} = 101.3$ with attrition of 10%, sample size =111.1 (≈ 111).

Ethical consideration
Ethical approval to conduct the research was sought and received from the research ethics committee of AKTH. A written informed consent was obtained from all subjects before inclusion using approved protocol.

Sample collection and delivery
The subjects were given clear, written and spoken instructions concerning the collection and transportation of the semen samples. The samples were collected after 3 days abstinence from masturbation or sexual intercourse; this is to ensure freshness, vitality and optimum volume. It was emphasized that the semen sample needs to be complete, i.e. all the ejaculate need to completely collected, including the first sperm-rich portion, and that the subject must report any loss of any fraction of the sample. The container was sterile, clean, dried and well labeled with name of subject and identification number. The subjects recorded the time of semen production and to ensure that sample was delivered to the laboratory within 1 hour of collection and during transport to the laboratory; the sample was kept between 20 °C and 37 °C (WHO, 2010).

Sample processing
Macroscopic examination
The semen sample was liquefied within 30 minutes at 37 °C while those samples that could not liquefy within 60 minutes were recorded as abnormal liquefaction. Samples appeared either homogeneous or grey-opalescent.
The viscosity of the sample was estimated by gently aspirating semen into a plastic disposable pipette, semen was allowed to drop by gravity and length was observed for any thread. A normal sample leaves the pipette in small discrete drops while abnormal viscosity was recorded when the thread exceeded 2cm (WHO, 2010).
The liquefied semen was measured with 10ml disposable pipette and the volume was recorded between 1.5 to 5ml.
The pH of the semen was determinined by placing a drop of semen on a pH paper after which the color change was observed and then compared with standard pH color.

Microscopic examination
Microscopic examination comprises of sperm concentration, total sperm count, sperm motility and sperm morphology.

Sperm count
Concentration of sperm was performed using improved Neubauer haemocytometer. The semen was diluted with Sodium bicarbonate formalin 1 in 20. The Neubauer counting chamber was prepared and charged with the diluted seminal fluid and was allowed to stand for 5 minutes, then 1-5 large squares of the counting chamber were examined microscopically with x40 objective lens and the number complete sperm cells seen counted.
The Sperm concentration was calculated using the expression below:
Sperm concentration = n × 10^6/ml (n = number of sperm)
Total sperm count = n×10^6×volume of semen (WHO, 2010).

Sperm motility
Categories of sperm motility are rated as being progressive, non-progressive or immotile. One drop of well-mixed liquefied semen was placed on a clean grease free slide and covered with a cover slip and 100 fields were examined microscopically with x10 and x40 objectives lens. Percentage and type of motility was recorded based on their characteristic movement.

Sperm morphology
Thin smear were made from well-mixed liquefied semen on a clean grease free slide and were allowed to air dried. The smears were fixed with 95% v/v ethanol for 5 minutes. The smears were washed with sodium bicarbonate formalin solution to remove any mucus which may be present and rinsed with water. Smears were covered with diluted carbol fuchsin and allowed to stain for 3 minutes and was washed with water and was counterstained with diluted Loeffler's methylene blue for 2 minutes and was also washed off with water, drained and air dried. The stained slides were then examined with x40 and x100 objectives to observe for normal and abnormal spermatozoa.

Statistical analysis
Data was evaluated using graph pad prism version 7.02, the results were tabulated and comparisons were done using chi-square test. The p-value < 0.05 was considered to be significant.

Results
Table 1: Shows a semen quality of 111 samples out of which 13 (11.7%) have Oligozoospermia, 16(14.4%) are Azoospermia, 61(55.0%) are teratozoospermia, 58(52.3%) are asthenozoospermia, 13(11.7%) are oligoteratozoospermia, 12(10.8%) are oligoasthenozoospermia, 39(35.1%) are asthenoteratozoospermia and 13(11.7%) are oligoasthenotereatozoospermia.

Table 2: Shows the macroscopic and pH value of the semen by distribution of normal and abnormal numbers. , Out of 111 total semen samples analyze, 64(57.7%) have normal liquefaction and viscosity and 47(42.3%) sample have abnormal semen liquefaction and viscosity, 100(90.1%) sample have normal pH and 11(9.9%) sample have abnormal semen pH. Also 97(87.4%) sample have normal semen volume and 14(12.6%) sample have abnormal semen volume.

Table 3: Shows comparison in sample collection between coitus interruption and masturbation in relation to semen parameters where 84 (91.3%) have normal pH and 8(8.7%)

have abnormal pH using coitus interruption but 11 (84.7%) have normal pH using masturbation with 2(15.4%) abnormal pH, 79 (85.9%) have normal volume and 13(14.1%)have abnormal volume using coitus interruption but 12(92.3%) have normal volume using masturbation with 1(2.9%) abnormal volume, 51 (55.4%) have normal liquefaction and viscosity and 41(44.6%)have abnormal liquefaction and viscosity using coitus interruption but 8 (61.5%) have normal liquefaction and viscosity using masturbation with 5(38.5%) abnormal liquefaction and viscosity, 72 (78.3%) have normal sperm count and 20(21.7%)have abnormal sperm count using coitus interruption but 9(69.2%) have normal sperm count using masturbation with 4(30.7%) abnormal sperm count,39 (42.4%) have normal progressive motility and 53(857.6%)have abnormal progressive motility using coitus interruption but 6(46.2%) have normal progressive motility using masturbation with 7(53.8%) abnormal progressive motility, 37 (40.2%) have normal sperm morphology and 55(59.8%) have abnormal sperm morphology using coitus interruption but all 13 sample collected by masturbation have abnormal sperm morphology.

Table 4: Shows comparison between primary infertility and secondary infertility in relation to semen parameters where 65 (84.4%) have normal volume and 12(15.6%) have abnormal volume in primary infertility but 31(91.2%) have normal volume in secondary infertility with 3(8.8%) abnormal volume, 70 (90.9%) have normal pH and 7(9.1%) have abnormal pH in primary infertility but 31(91.2%) have normal pH in secondary infertility with 3(8.8%) abnormal pH, 41(84.4%) have normal liquefaction and viscosity and 36(46.8%)have abnormal liquefaction and viscosity in primary infertility but 24(70.6%) have normal liquefaction and viscosity in secondary infertility with 10 (29.4%) abnormal liquefaction, 60 (77.9%) have normal sperm count and 17 (22.1%)have abnormal sperm count in primary infertility but 30(88.2%) have normal sperm count in secondary infertility with 4(11.8%) abnormal sperm count,37 (48.1%) have normal progressive motility and 40(51.9%)have abnormal progressive motility in primary infertility but 13 (38.2%) shave normal progressive motility in secondary infertility with 21(61.8%) abnormal progressive motility, 31(40.3%) have normal sperm morphology and 46(59.7%)have abnormal sperm morphology in primary infertility but 12(35.3%) have normal sperm morphology in secondary infertility with 22(64.7%) abnormal sperm morphology.

Table 1: Quality of semen parameters

Parameters	Frequency	Percentage (%)
Oligozoospermia	13	11.7
Teratozoospermia	61	55.0
Asthenozoospermia	58	52.3
Oligoteretozoospermia	13	11.7
Oligoasthenozoospermia	12	10.8
Asthnoteratozoospermia	39	35.1
Oligoasthnoteratozoospermia	13	11.7
Azoospermia	16	14.4

Table 2: Distribution of normal and abnormal values of macroscopy and pH semen parameters

Parameters	**Normal (%)**	**Abnormal (%)**
Volume	97 (87.4)	14 (12.6)
Liquefaction	64(57.7)	47 (42.3)
Viscosity	64(57.7)	47 (42.3)
pH	100(90.1)	11 (9.9)

Table 3**:** Comparison in sample collection between coitus interruption and masturbation in relation to semen parameters

parameters	Coitus interrupts		Masturbation		
	Normal (%)	Abnormal	Normal (%)	Abnormal (%)	p- value
Volume	79(85.9)	13(14.1)	12(92.3)	1(2.9)	0.8388
Liquefaction	51(55.4)	41(44.6)	8(61.5)	5(38.5)	0.9072
Viscosity	51(55.4)	41(44.6)	8(61.5)	5(38.5)	0.9072
pH	84(91.3)	8(8.7)	11(84.6)	2(15.4)	0.7917
Sperm count	72(78.3)	20(21)	9(69.2)	4(30.7)	0.7092
Progressive motility	39(42.4)	53(57.6)	6(46.2)	7(53.8)	0.7975
Normal morphology	37(40.2)	55(59.8)	0(0)	13(100)	0.0114

Table 4: Comparison of primary infertility and secondary infertility in relation to semen parameter values

Parameters	Primary infertility		Secondary infertility		P-value
	Normal (%)	Abnormal (%)	Normal (%)	Abnormal (%)	
Volume	65(84.4)	7(9.1)	31(91.2)	3(8.8)	0.5097
Liquefaction	41(53.2)	36(46.8)	24(70.6)	10(29.4)	0.1335
Viscosity	41(53.2)	36(46.8)	24(70.6)	10(29.4)	0.1335
Ph	70(90.9)	7(9.1)	1(91.1)	3(8.8)	0.9638
Sperm count	60(77.9)	17(22.1)	30(88.2)	4(11.8)	0.3097
Progressive motility	37 (48.1)	40(51.9)	13(38.2)	21(61.8)	0.0206
Normal morphology	31(40.3)	46(59.7)	12(34.3)	22(64.7)	0.7766

Discussion

Proper collections of human ejaculates are necessary for semen analysis and infertility treatment purposes. Infertility and in particular poor semen quality is a serious health problem worldwide, Among semen parameters, those that have the greatest effect on fertility are concentration, motility and morphology, the length of abstinence must be taken in to account when analyzing the sperm. According to the findings by several authors, that length of abstinence is responsible for many variations in the semen parameters.

The finding in this study shows that sperm quality has 14.4% with Azoospermiais which is below what was reported by Fetus *et al.* (2013) who found 8.8%. Another higher percentage of 24.4% was also reported by Aulia *et al*. (2017) which may be due to Environmental factors such as heat, chemical and lifestyle including diet, frequency of intercourse, smoking and alcohol which is more common among people in the study area more especially polygamous individuals. Other possible causes of semen abnormalities are stress (emotional and physical), insomnia, tight brief, and hot tubs which are known to have adverse effects on sperm parameters (Ugwuja *et al*., 2008). Similarly 11.7% have Oligozoospermia which is lower than the percentage found by Peter *et al.* (2016) who reported 34.8%. Also 55.0% have teratozoospermia, 52.3% have asthenozoospermia and 11.7% have oligoteratozoospermia, which is in contrast with a 4.2% as reported by Peter *et al.* (2016). This could possibly be due to environmental factor.

The finding of 10.8% oligoasthenozoospermia in this study is far lower compared to the finding of Fetus *et al.* (2013) who reported 20.2%. Also 35.1% have asthenoteratozoospermia which is higher than the one reported by Peter *et al.* (2016) with 5.9%. Oligoasthenoteratozoospermia was found to be 11.7% which is also higher than the one reported by Emmanuel *et al.* (2015). This could be due to environmental factor.

In this study the comparison of primary infertility and secondary infertility in relation to semen parameters value indicated no statistical significant difference between them; in primary infertility 84.4% have normal volume and 15.6% abnormal volume while 91.2% have normal volume in secondary infertility with 8.8% abnormal volume. 90.9% have normal pH and 9.1% abnormal pH in primary infertility while 91.2% have normal pH in secondary infertility with 8.8% abnormal pH. 84.4% have normal liquefaction and viscosity and 46.8% abnormal liquefaction and viscosity in primary infertility whereas 70.6% have normal liquefaction and viscosity in secondary infertility with 29.4% abnormal liquefaction. 77.9% have normal sperm count and 22.1% abnormal sperm count in primary infertility while (88.2%) have normal sperm count in secondary infertility with 11.8% abnormal sperm count. 48.1% have normal progressive motility and 51.9% abnormal progressive motility in primary infertility but 38.2% share normal progressive motility in secondary infertility with 61.8% abnormal progressive motility. 40.3% have normal sperm morphology and 59.7% abnormal sperm morphology in primary infertility but 35.3% have normal sperm morphology in secondary infertility with 64.7s% abnormal sperm morphology. Both the comparison did not show any significant difference at P-value > 0.05 except in progressive motility where secondary infertility showed high abnormal when compare with abnormal progressive motility in primary infertility with p-value (P = 0.0206). This finding is in keeping with the finding of Albert *et al.* (2014) who reported that sperm motility among primary and secondary infertile couples are significant.

Comparison between coitus interruption and masturbation as methods of sample collection indicate no significance statistical difference in most of the semen parameters. Only sperm morphology, where masturbation has 100% abnormal sperm cells and coitus interruption

has 59.7% with significance p-value (P = 0.0114). This study is similar with report of Valli *et al.* (2004) on sperm morphology but differs from the report of Zarmakoupis *et al.* (1999) who reported no significant difference.

Conclusion

In conclusion, masturbation remains the methods choice for the collection of semen sample for analysis, variability in semen parameter is dynamic from region to region therefore, the need to adhere strictly to the WHO standard range. Semen analysis when properly carried out, the semen parameters remains an essential tool that can aid in the diagnosis and treatment of infertility.

Recommendations

The following recommendations were made from the findings of the study;

i. The hospital should provide room dedicated for semen sample collection, more especially for those client residing far from the hospital.
ii. People should be educated on method of masturbation during sample collection
iii. Triangulation of these parameters will also produce a significant guide on the diagnosis and treatment of infertility.
iv. Further studies should be undertaken to find the exact cause(s) of abnormal sperm morphology more especially in secondary infertility patients

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