



COMPARATIVE STUDY OF SEMEN PARAMETERS BETWEEN MANUAL AND SPERM QUALITY ANALYZER TECHNIQUES AMONG INFERTILE PATIENTS IN KANO, NIGERIA

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ABSTRACT

Semen analysis is the primary step towards finding the influence of genital pathophysiology on the reproductive capacity of male, even then certain parameters might not be of any clinical significance. It is used to evaluate male fertility, comparing the results of semen analysis using manual method and automated sperm analyzer technique to have a better alternative to the traditional method. The study was a comparative study of 111 cases of semen analysis carried out at the Aminu Kano Teaching hospital Kano between the months of September to Number 2017. It was done by manual and automated method (using SQAI-P analyzer) according to WHO guideline 2010. The finding in this study observed that sperm count, sperm morphology result of manual method are significantly associated with sperm count, sperm morphology result of SQA technique with p-value ($P=0.00216$ and 0.0040 respectively). Sperm progressive motility results of SQA and manual method are not significantly associated with each other. Automated method of semen analysis is a quicker method for the assessment of male infertility, even though automated sperm analyzer only provides percent normal morphology results without quantifying specific abnormalities and lack of analyzing sperm with very low count. As such it is limited when compared with manual methodology where morphological defects can be identified and quantified even if the sperm count is low. Thus, automated sperm analyzer can be used interchangeably with manual semen analysis for examining sperm concentration, motility and morphology.

Keywords: *Semen, infertility, manual, SQA, parameters, quality, techniques*

INTRODUCTION

The inability of couple to achieve pregnancy after one year of unprotected sexual intercourse is referred to as infertility (Albert *et al.*, 2014). World Health Organization (2013) define 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 worldwide problem particularly in developing countries and a common reason for couples to visit gynecological consultation in majority of Nigerian clinics (Ujaddughe *et al.*, 2015). Male infertility 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). And also the inability of the male reproductive cells to produce mature, actively motile and functional spermatozoa in sufficient amount that will ensure fertilization of a released mature ovum in the fallopian tubes (Festus *et al.*, 2013).

Primary infertility refers to those couples who have never achieved pregnancy in their marital status while secondary infertility refers to those couples who have had at least one pregnancy (Friday, 2005), the prevalence of primary infertility has increased since 1990, but secondary infertility has decreased, the rates also 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 known as spermatozoa (sperms) are formed from the primitive spermatogenic cells, in the testis it takes 74 days for the formation of fully matured sperm cells from the primitive germ cell. Throughout the process of spermatogenesis, the spermatogenic cells have cytoplasmic attachment with Sertoli cells. Sertoli cells supply all the necessary materials for spermatogenesis through the cytoplasmic attachment, testes contribute sperms. Prostate secretion gives milky appearance to the semen. Secretions from seminal vesicles and bulbourethral glands provide mucoid consistency to semen (Pramanik, 2010).

During ejaculation, semen is produced from a concentrated suspension of spermatozoa, stored in the paired epididymis, mixed and diluted by, fluid secretions from the accessory sex organs (Kishore *et al.*, 2011). 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 (Samantha *et al.*, 2016; CDC, 2017). The nature of the spermatozoa (their vitality, motility and morphology) and the composition of seminal fluid are also important for sperm function (WHO 2005).

A semen analysis is the examination of a male's ejaculate, performed 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 primary step towards finding 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 to evaluate semen volume, sperm count, motility and morphology (Chaurasia *et al.*, 2016)

The Sperm Quality Analyzer (SQA) IIC-P is an automated analyzer that is based on the principle of electro-optical signal processing in combination with built-in computer algorithms (SQA IIC-P Guide, 2008). The aim of this research is to compare between manual and SQA techniques in determining semen parameters among infertile male patients attending Aminu Kano teaching hospital.

MATERIALS AND METHODS

This study was carried out in Medical Microbiology Laboratory of Aminu Kano Teaching Hospital Kano, (AKTH), located within Kano metropolis of Tarauni local government of Kano state. Kano is between latitude 11° 30' N and longitude, 8° 30' E and is about 1580 feet above sea level. It has a total land mass of about 20760 sqkm kilometer (Census, 2006). Kano state borders Katsina to the north-west, Jigawa state to the north-east, Bauchi state to the south-east and Kaduna state to the south-west.

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).

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

Ethical clearance was obtained from the Research Ethics committees of AKTH. A written and or verbal informed consent was obtained from all subjects before inclusion in the study using approved protocol. The subjects were given clear written and spoken instructions concerning the collection and transport of the semen sample. The sample was collected after 3 days abstinence through masturbation or sexual intercourse.

Semen samples were collected by masturbation and coitus interruptus, with emphasis on complete semen sample collection without missing any fraction of the sample into the receptacle. The containers were sterile, clean, dried and labeled with patient's name and identification number. Time of semen production was recorded by the patient and samples were delivered to the laboratory within 1 hour of collection and were also transported to the laboratory at temperature of between 20 °C and 37 °C (Moazzam *et al.*, 2015).

The following macroscopic examinations were considered; PH was determined using pH paper and by comparing with standard color Sasikala *et al.* (2014), semen liquefaction was observed within 30 minutes at 37 °C. Liquefaction taking up 60 minutes was recorded as abnormal (Nashmail *et al.*, 2017). 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 (Stefan *et al.*, 2013). A normal sample leaves the pipette in small discrete drops while abnormal viscosity was when the thread exceeded 2cm (WHO 2010). The liquefied semen sample was measured with 10ml disposable pipette and recorded appropriately.

Semen sample was mixed and dilutions were prepared with fixative. Counting chamber was loaded and allowed the spermatozoa to settle in a humid atmosphere. Samples were assessed within 10–15 minutes to avoid evaporation. At least 200 spermatozoa per chamber apartment were counted. The two counts were compared to see if they are acceptably close. If so, proceed with calculations; if not, dilutions were repeated. In calculating the concentration of the spermatozoa per ml, the total number of spermatozoa per ejaculate was calculated (WHO, 2010).

Calculation

Sperm Conc = $n \times 10^6 / \text{ml}$ (n = number of sperm)

Total sperm count = $n \times 10^6 \times \text{volume of semen}$ (WHO, 2010).

Sperm motility

Sperms motility was categorized into progressive motility (PM), non-progressive motility (NPM) and immotile sperm cells (IM). A drop of well-mixed liquefied semen was placed on a clean grease free

slide and covered with cover glass and 100 fields were examined microscopically with x10 and x40 objectives lens and mean percentage of motility of all field was counted (Leyla and Gabor, 2015).

Sperm morphology

A thin smear of semen on a clean grease free slide was made. It was air-dried, fixed and stained. The stained smear was examined with bright field light at $\times 1000$ magnification with oil immersion. Approximately 200 spermatozoa were assessed per slide for the percentage of normal and abnormal forms. Then the two count values were compared to ascertain their acceptably if they are close; if so, proceed with calculations, if not, repeat count (Samuel *et al.*, 2016; Hazir *et al.*, 2015).

Procedure

Thin smear was made from well-mixed liquefied semen on a clean grease free slide and allowed to air dry and was fixed with 95% v/v ethanol for 5 minutes. The smear was washed with sodium bicarbonate formalin solution to remove any mucus which may be present and rinsed with water.

Smear was covered with diluted (1 in 20) carbol fuchsin and allowed to stain for 3 minutes. Stain was washed with water. It was counterstained with diluted (1 in 20) Loeffler's methylene blue for 2 minutes. This was washed off with water and drained. It was then allowed to air-dry and examined with x40 and x100 objectives to confirmed normal and abnormal spermatozoa (WHO, 2010).

Semen analysis by SQA analyzer

The semen was analyzed using SQA 11C-P analyzer by drawing the semen into single-use plastic capillaries and placed in the device and the result was read after within 45 seconds.

Principle of SQA

The capillary is inserted into the SQA IIC-P measurement compartment. Tens of thousands of sperm cells are analyzed as they move through a light beam: The movement of motile sperm cells causes light disturbances. These light disturbances are detected and converted into analog electronic signals with "peaks and valleys" which are then analyzed by proprietary algorithms. (SQA IIC-P user guide, 2008)

Graph pad prism version 7.02 was used to analyze the data's generated, the result was tabulated and comparisons were done using chi-square test. The p-value < 0.05 was considered to be significant.

RESULTS AND DISCUSSION

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 of semen parameters by distribution of normal and abnormal numbers, Out of 111 total semen sample 64(57.7%) sample 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 64. 97(87.4%) sample have normal semen volume and 14(12.6%) sample have abnormal semen volume.

Table 3: Comparison between manual and sperm quality analyzer technique where Out of 111 sample, 70(73.7%) have normal sperm count and 25(26.3%) have abnormal sperm count using manual method, while 34(35.8%) sample have normal progressive motility and the remaining 61(64.2%) sample have abnormal progressive motility using manual method. With respect to sperm morphology, 31(32.6%) sample have normal sperm morphology and 64(67.4%) sample have abnormal sperm morphology using manual method. While as for SQA, out of 111 sample the sperm quality analyzer detect only 73 sample and out of 73 sample 66 (90.4. %) have normal sperm count and 7(9.6%) sample have abnormal sperm count using SQA technique, 32(43.8%) have normal progressive motility using SQA technique and 41(56.2%) sample have abnormal progressive motility using SQA technique, 9(12.3%) sample have normal sperm morphology and 64(87.7%) sample have abnormal sperm morphology using SQA technique.

Table 1: Quality of semen parameters

DEFEC	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	16
Azoospermia	11.7	14.4

Table 2: Distribution of normal and abnormal values of macroscopic 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 between manual and sperm quality analyzer (SQA) techniques

		MANUAL (%)	SQA (%)	P-VALUE
SPERM COUNT	NORMAL	70 (42)	66 (39)	0.00216
	ABNORMAL	25 (15)	7 (4)	
PROGRESSIVE MOTILITY	NORMAL	34 (20)	32 (19)	0.3686
	ABNORMAL	61 (36)	41 (24)	
NORMAL MORPHOLOGY	NORMAL	31 (18)	9(5)	0.0040
	ABNORMAL	64(38)	64(38)	

Proper collection of human ejaculates is necessary for semen analysis and infertility treatment purposes (Franken, 2012. Infertility and in particular poor semen quality is a serious health problem worldwide. Among the 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 (Roshan *et al.*, 2015). Several authors have reported that the period of abstinence is responsible for many variations, in the same subject.

The finding in this study shows that Sperm quality has 14.4% have Azoospermia which is below what was reported by Fetus *et al.*, (2013) with (8.8%). in A higher percentage of Azoospermia of about 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 Other 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 (Roshan *et al.*, 2015; Ugwuja *et al.*, 2008)

Similarly 11.7% have Oligozoospermia which was below the percentage reported by Peter *et al.*, (2016) 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 be due to cultural different or environmental factor.

The findings in this study of 10.8% with oligoasthenozoospermia did not agree with the finding of Fetus *et al.*, (2013) who reported 20.2%. Also 35.1% of asthenoteratozoospermia is higher than the percentage reported by Peter *et al.* (2016) with 5.9% in 2011. Oligoasthnoteratozoospermia was found to be 11.7% which is also higher than that reported by Emmanuel *et al.*, (2015). This could be due to cultural different or environmental factor.

The finding in this study observed that sperm count,

sperm morphology result of manual method are significantly associated with sperm count, sperm morphology result of SQA technique with p-value ($P = 0.00216$ and 0.0040 respectively). Sperm progressive motility results of SQA and manual method are not significantly associated with each other which are similar to the report by Chaurasia *et al.* (2016) and contrary to the report of Tamer *et al.*, (2014).

The comparison in sample collection between coitus interruption and masturbation in relation to semen parameters, both show no statistical significant difference except in abnormal sperm morphology where masturbation has 100% and coitus interruption has 59.7% with p-value ($P=0.0114$). This study is similar with report of Valli *et al.*, (2004) on volume, morphology, sperm count, but different on progressive

motility and different with report of Zarmakoupis *et al.*, (1999).

CONCLUSION

Automated method of semen analysis is a quicker method for the assessment of male infertility, even though automated sperm analyzer only provides percent normal morphology results without quantifying specific abnormalities and lack of analyzing sperm with very low count. As such it is limited when compared with manual method where morphological defects can be identified and quantified even if the sperm count is low. Thus, automated sperm analyzer can be used interchangeably with manual semen analysis for examining sperm concentration, motility and morphology.

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