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Semen Characteristics of Male Infertile Couples in the Kumasi Metropolis: A Study of Primary and Secondary Infertile Couples

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Authors' contributions

Author AO designed the study, did the literature searches and performed the semen analysis under the guide and supervision of Authors KBA and FAK. Author DB and DYQwrote the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To assess the semen characteristics of primary and secondary male infertile couples in the Kumasi metropolis.
Study Design: A cross-sectional study.
Place and Duration of Study: Department of Obstetrics and Gynaecology, Komfo Anokye Teaching Hospital, Kumasi; between February 2012 and May 2013.
Methodology: The study involved 150 men whose female partners reported to the Obstetrics and Gynaecology Department of Komfo Teaching Hospital (KATH) in Ashanti Region of Ghana for infertility treatment. Semen of the respondents were examined for various characteristics (viscosity, pH, volume, presence of RBC and bacteria, motility, etc).

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Results: Eighty-eight partners involved in the study representing 59% of total respondents were partners of primary infertile couples whereas 41% were partners of secondary infertile couples. Semen abnormalities were reported in terms of sperm motility, sperm concentration, pH and presence of bacteria and these were significantly different among primary and secondary infertile couples.
Conclusion: Sperm abnormalities influence infertility among males of infertile couples. There is the urgent need to include male partners in the screening, detection and treatment of infertility among couples.

Keywords: Infertility; Azoospermia; sperm motility; semen; Kumasi.

1. INTRODUCTION

One of the realities that some married couples have to face is their inability to have children [1]. This phenomenon is called infertility. Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse [2]. It is a major reproductive health problem affecting 10% to 15% of couples, with approximately equal contributions [3]. Conception is normally achieved within 12 months in 80 to 85% of couples who are not using contraceptive measures; which means an estimated 15% of couples attempting their first pregnancy, may experience difficulty in conceiving. However if couples do not achieve pregnancy within the stipulated period, they are considered infertile [4]. The most common causes of infertility are: male factors [5,6,7,8,9] such as sperm abnormalities [7,5], female factors [6,7,9,10] such as ovulation dysfunction [6,11] and tubal pathology [6,7],combined male and female factors [7,9,12] and unexplained infertility; where no obvious cause could be detected [6,7]. According to University of Utah Health Sciences Center [13], Male infertility refers to the inability of a male to achieve a pregnancy in a fertile female. In humans it accounts for 40-50% of infertility [14,15]. A large proportion of infertile men fail to impregnate their female counterpart because of lack of sperm (azoospermia) or too little sperm (oligozoospermia).

Infertility may also be due to abnormal sperm morphology (teratozoospermia) and insufficient sperm motility (asthenozoospermia) [16]. Abnormal semen quality remains a significant contributor to overall infertility with asthenozoospermia being the most common seminal quality abnormality [17]. Oligozoospermia and asthenozoospermia also remain the most common aetiological factors responsible for male infertility [7]. Again, male infertility is commonly due to deficiencies in semen; semen quality is used as a surrogate measure of male fecundity [18]. There are evidences to show that sperm counts have been declining over the last 50 years, with a consequent increase in male infertility. Carlsen and others [19] analyzed a total of sixty-one studies from 1938-1991 and found that there was a significant decline in mean sperm density from 113 million/ml in 1940 to 66 million/mL in 1990. The seminal volume was also observed to have decreased from an average of 3.4 to 2.75 ml ($P = 0.027$). Their results showed a 20% drop in volume and 58% decline in sperm production in the last 50 years. According to Rubestein and others [20], the initial evaluation of the male patient should be rapid, noninvasive, and cost-effective, as nearly 70% of conditions that cause infertility in men can be diagnosed with history, physical examination, and hormonal and semen analysis alone.

The prevalence of infertility was 11.8% among women and 15.8% among men as put forward by [21] in their study in Ghana among rural population. This shows that infertility in males is real problem among countries in both developed and developing countries including Ghana.

In most of the studies about male infertility, the main cause has been seminal fluid abnormality. However in Ghana and for that matter Kumasi metropolis there is not much study on male infertility in relation to seminal abnormality. The study therefore sought to investigate semen quality of male partners in infertile couples in the metropolis.

2. MATERIALS AND METHODS

2.1 Study Population and Sample

The study was conducted in the Kumasi metropolis in the Ashanti region of Ghana. The number of participants who were selected for the study was one hundredandfifty (150) men whose female partners reported to the Obstetric and Gynaecology Department of Komfo Teaching Hospital (KATH) in Ashanti Region of Ghana for infertility treatment. Such women were asked to bring their male partners for fertility assessment. They were then contacted and details of the study were explained to them. Those who agreed to be part of the study were made to sign a consent form. This study was approved by the Research Ethics Committee of the Hospital. Each participant was registered with a unique identification code that corresponded with the code on the containers for both semen and blood samples.

2.2 Semen Examination

Normal semen sample liquefies within 60 minutes at room temperature; usually this occurs within fifteen minutes [22]. The liquefaction time was determined by placing the sample on a moving tray in an orbital mixer (37^{o}C). All samples produced outside the laboratory were warmed up to a temperature of 37^{o}C in an incubator for 10 minutes before examination. After liquefaction, the samples appearance was observed and grouped into two main categories: Normal and Abnormal. Seminal volume of each sample was also observed and recorded. The thickness (viscosity) of the samples was analyzed and classified either as low normal or high. The semen pH impregnated paper test was used to observe the pH of the samples. Wet mount slides were then prepared for microscopy to determine sperm motility, while the dye exclusion method was employed to determine sperm vitality. The concentration of the sperm was determined using the haemocytometer method on two separate preparations of the semen samples, one on each side of the counting chamber.

2.2.1 Sperm Morphology Determination

This was determined with two smears for duplicate assessment of each sample. The slides were thoroughly cleaned, washed in 70% ethanol and air-dried. 5µl of semen was applied to each slide. Another slide faced down, was placed on top so that the semen spreads between them. The two slides were gently pulled apart to make two smears simultaneously. These slides were fixed while still wet with 95% v/v ethanol for 10 minutes, and air-dried. The smear was washed with sodium bicarbonate-formalin solution to remove any mucus which may be present and afterwards rinsed several times with changes of water. The smears were then flooded with crystal violet solution and allowed to stain for 2 minutes and then the stain was washed off with water. Lugol's solution (mordant) was added to the smear for 1 minute and washed with distilled water. The smear was then counterstained with safranin (0.1%) solution for 2 minutes and washed with distilled water, drained and air-dried.

The ×40 objective was used to observe the slide and oil-immersion bright-field objective was used to corroborate the morphology of the spermatozoa and the other cellular elements in

the smear. The slides were then examined systematically from one microscopic field to another and 100 spermatozoa were assessed and the percentages of normal and abnormal spermatozoa were recorded. The following abnormalities were all grouped under abnormal sperms: Head(greatly increased or decreased in size, abnormal shape and tapering head-pyriform, acrosomal cap absent or abnormally large, nucleus contains vacuoles or chromatin unevenly distributed, two heads, additional residual body); Middlepiece(absent or markedly increased in size, appears divided-bifurcated, angled where it meets tail); Tail(absent or markedly reduced in length, double tail, bent or coiled tail).

2.3 Data Handling and Analysis

All questionnaires and interview results from the field were checked for completeness and internal errors during data collection. Questionnaires were sorted, numbered and kept in files confidentially. Data were coded and entered using SPSS software. Data were analyzed using STATA 11. Descriptive statistics were done using frequencies and percentages and results presented using graphs and tables. Associations between the various factors and semen quality were tested using chi-square analysis at significant levels of $p<0.05$.

3. RESULTS AND DISCUSSION

Majority of the respondents were 30 years and above with 24.7% being above 39 years. All the respondents were married. Fifty-nine male partners constituting 39.3% of the respondents were traders or businessmen, 29.3% were public servants whereas 12% were civil servants. Only 9.4% were farmers. About 47% resided in urban areas whereas 19.3% resided in rural communities. With respect to their educational background, about 11% had no formal education; and the majority, 46% had basic education (primary and Junior High School). Nineteen respondents (12.6%) had tertiary education.

The results indicate that 88 partners involved in the study representing 59% of total respondents were partners of primary infertile couples whereas 62 (41%) were partners of secondary infertile couples. Tables 1 and 2 present description of abnormalities of semen of male partners of infertile couples. The mean percent of sperms with normal morphology among partners with primary infertile couples was 70.95 and this was significantly lower than that of partners of secondary infertile couples (77.47). The percentage mean of motile sperms were also significantly lower among primary infertility partners as compared to secondary infertility partners (48.52 versus 54.51; $p=0.031$). The analysis also indicated a significant variation between the mean percentage immotile or dead cells between primary and secondary infertile couples (36.47 against 34.67; $p=0.011$). The mean pH of primary infertile and secondary infertile couples was 8.22 and 7.98 respectively and the variation was statistically significant. Mean liquefaction times for primary and secondary infertile couples were 36.47 minutes and 39.09 minutes respectively. The sperms in the semen of partners of primary infertility couples were lower than that of those of secondary infertility couples (mean = 36.44×10^6 and 43.31×10^6 respectively; $p=0.045$).

Table 1. Characteristics of sperms by type of infertility

Variables	Primary (n=88)		Secondary (n=62)		p-value
	Mean	SD	Mean	SD	
Morphology (normal)/%	70.95	3.49	77.47	3.33	0.021
Morphology (abnormal)/%	15.41	1.86	15.44	1.97	0.142
Motility /%	48.52	3.10	54.51	3.99	0.031
Rapid progressive motility/%	35.90	2.84	40.80	3.91	0.125
Slow progressive motility%	11.00	0.65	10.80	0.75	0.540
Immotile or dead cells/%	36.47	2.67	34.67	3.67	0.011
pH	8.22	0.038	7.98	0.052	0.001
Liquefaction time/min	36.47	2.02	39.09	2.89	0.001
Abstinence	6.97	0.36	6.48	0.56	0.043
Volume /mL	4.43	0.60	3.67	0.18	0.062
Sperm count ($x10^6$)/mL	36.44	4.91	43.31	6.00	0.045
Short and broken tail/%	11.43	1.43	12.40	1.92	0.308
Large/small oval head/%	2.09	0.50	0.89	0.23	0.158

Statistical test: ANOVA

Table 2. Characteristics of sperms by type of infertility

Variables	Primary N (%)	Secondary N (%)	p-value
RBC			
– Present	2 (2)	4 (6)	0.001
– Absent	86 (98)	58 (94)	
Epithelial cell			0.069
– Present	2 (9)	6 (10)	
– Absent	84 (91)	56 (90)	
Appearance			
– Normal	68 (77)	60 (97)	0.001
– Abnormal	20 (23)	2 (3)	
Viscosity			
– Low	28 (32)	16 (26)	0.571
– Normal	58 (66)	46 (74)	
– High	2 (2)	0 (0)	
Pus cell (hcp)			
– None	2 (2)	6 (10)	0.001
– 0-5	66 (75)	56 (90)	
– 6-10	6 (7)	0 (0)	
– 11 and above	14 (16)	0 (0)	
Gram stain			
– Bacteria	6 (7)	15 (24)	0.004
– None	82 (93)	47 (76)	

Statistical test: Fischer's exact test

As shown in Table 2, the percentage of semen with presence of RBC was generally low. The percentage of semen with RBC was significantly different among partners with secondary infertility couples and those with primary infertility couples (2% and 6%; p=0.001). Majority of the semen analyzed was normal in appearance and there was statistically significant difference between the semen of primary and secondary infertility couples with respect to the

appearance. Majority of semen analyzed had no bacteria whereas majority of the semen analyzed had pus cells of between 0-5. None of the semen of male partners of secondary infertile couples had pus cells more than 5. The difference in pus cell concentration between partners of primary and secondary infertile couples was statistically significant (p=0.001). Comparatively, 7% of primary infertile couples had bacteria present as against 24% of those of secondary infertile couples and this difference was statistically significant.

3.1 Discussion

This study was conducted to determine the total number of spermatozoa which reflects sperm production by the testes and the patency of the post-testicular duct system, the total fluid volume contributed by the various accessory glands which reflects the secretary activity of the glands, the nature of the spermatozoa (their vitality, motility and morphology) and the composition of seminal fluid which are all important for sperm function. Sperm analysis was conducted on 150 samples from male partners of infertile couples, of which 88 (59%) were partners of primary infertile couples whereas 41% were partners of secondary infertile couples. Many studies have linked male infertility with deficiencies in semen characteristics [18]; [17]. In their study in Nigeria, [23] also proposed high rate of semen fluid abnormalities among the male partners of infertile women. Chiamchanya and others [24] reported abnormal semen analysis as the one of the main cause of male infertility.

3.1.1 Motility

Asthenozoospermia (or "asthenospermia") is the medical term for reduced sperm motility. Complete asthenozoospermia (100% immotile spermatozoa in the ejaculate) is reported at a frequency of 1 of 5000 men [25]. The World Health Organization (WHO) standards of normal sperm indicates that motility (movement of the sperm) value should be greater than or equal to 50% with forward progression within 60 minutes of ejaculation [26]. The results however indicate that less than 50% of the sperm cells of both primary and secondary infertile couples demonstrated proper motility. This was however significantly lower among primary infertility partners as compared to secondary infertility partners. These data indicate the extent of influence of motility on the infertility in males. This observation is similarly to a study in Nigeria by Adeniji and others [17], which cited asthenozoospermia as the most common seminal quality abnormality that contributes to overall infertility. Again, Feng [16] also associated infertility with insufficient sperm motility.

3.1.2 Volume

The average volume of semen produced at ejaculation is 2 to 5mL and volumes consistently less than 1.5ml (hypospermia) or more than 5.5mL (hyperspermia) are probably abnormal [27]. Results from this study indicate that the mean sperm volume of primary and secondary infertile couples were 4.4mL and 3.7mL respectively. Comparatively, the mean volumes of semen of both primary and secondary infertile couples were all within the normal average volume produced at ejaculation (2 – 5mL). According to Calsen and others [19] sperm volume has been decreasing over the past 50 years (1938-1991) with an increase in male infertility. However, semen volume might not be a contributing factor in infertility among Ghanaian male infertility couples.

3.1.3 Sperm counts

The WHO shows that the total number of spermatozoa in the ejaculate should be at least 40 million [26]. In this study however, the sperms concentration of partners of primary infertility couples was 36.44 million as compared to 43.31 among partners of secondary infertility couples. This indicates that the mean sperm count of primary infertility couples could be a significant factor for their infertility. Generally, half of the respondents had very low sperm counts (oligospermia) whereas 36.7% had normal sperm concentration in the semen. Consistently, the study by [19] also found that there was a significant decline in mean sperm density from 113 million/mL in 1940 to 66 million/mL in 1990 with decline in male fertility over the years. Feng [16] also established an association between infertility and lack of sperm (azoospermia) or too little sperm (oligozoospermia). Again, a similar study conducted at the Nnamdi Azikiwe University Teaching Hospital in South-east Nigeria showed that oligozoospermia (35.9%) and asthenozoospermia(32.3%) were the most common aetiological factors responsible for male infertility [7].

3.1.4 pH

The WHO puts the pH of normal semen at 7.2-8.0. Acidic ejaculate (pH<7.2) may be associated with blockage of seminal vesicles whereas infection is usually associated with alkaline ejaculate (pH >8.0) [18]. In this study, the mean pH of the primary infertility male partners was alkaline (8.2) and this could account for their infertility. The pH of the secondary infertility couples was however within the normal range (7.92). The pH of the semen was significantly associated with the kind of infertility among the male partners of infertility couples (p=0.001).

3.1.5 Bacteria

This study further analyzed the presence of bacteria in the semen of respondents. Infections of the male genitourinary tract account for up to 15% of cases of male infertility [28]. Majority of semen analyzed had no bacteria. Comparatively, 7% and 24% of primary and secondary infertile couples had bacteria present respectively. This indicates that the presence of bacteria could account for infertility in both primary and secondary infertile couples. Recent studies have shown that the simple presence of bacteria in semen samples may compromise the sperm quality [29];[30]. Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process causing qualitative and quantitative sperm alterations [31]; [32]; [29].

4. CONCLUSION

The study reported various abnormalities in the semen of male partners which also differed significantly among primary and secondary infertile couples. This included sperm motility, sperm concentration, pH (the mean pH of the primary infertility male partners was alkaline, 8.2) and presence of bacteria (7% and 24% of primary and infertile couples had bacteria present respectively). The present data indicate that the sperm abnormalities influence male infertility. There is therefore the urgent need to include male partners in the screening, detection and treatment of infertility among couples.

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CONSENT

All authors declare that prior to recruiting respondents; a written informed consent was obtained from them after the purpose of the study had been fully explained to them. The respondents were also assured of the privacy of information giving and their voluntary participation in the study. Again, 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report.

ETHICAL APPROVAL

Ethical clearance for the study was obtained from the Committee on Human Research, Publications and Ethics (CHPRE) of the Kwame Nkrumah University of Science and Technology (KNUST) and KATH. The researcher did not in any way expose participants of the study to physical or psychological harm. Participation in the study was strictly voluntary with the informed consent of participants that guaranteed their right to privacy. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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