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Patterns of Semen Analysis of Male Patients Undergoing *in Vitro* Fertilization Treatment in a Private Fertility Center in Ghana

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Abstract

Male infertility is known to contribute to about half of all infertility cases. In Ghana, the prevalence of male infertility is higher (15.8%) than in females (11.8%). We evaluated descriptive parameters of semen analysis obtained from males in the workup of infertility in some men undergoing sub-fertility/*in vitro* fertilization (IVF) treatment. We conducted a prospective cohort study in a specialized IVF center in Ghana involving 558 men who reported with their female partners for sub-fertility treatment (clinically diagnosed). Patient selection criteria were based on sub-fertility partners participating in an IVF/ Intracytoplasmic Sperm Injection (ICSI) program. The clients were considered eligible for inclusion in the study if the male partner had motile ejaculated spermatozoa during preliminary semen analysis and before controlled ovarian stimulation (COS). The age group of the men was 27 to 65 years. Exclusion criteria were: previous disease(s) or surgery associated with reproductive function (including varicocele, cryptorchidism, epididymitis, mumps, azoospermia); vasectomy and vasectomy reversal. Clients were questioned regarding their careers, smoking habits, alcohol consumption, medical and family histories. All cases of severe alteration in spermatogenesis, including frozen and surgically retrieved sperm, prostatitis, and or patients who had prior cancer therapy were excluded from this study. Semen samples were then collected by masturbation into sterile containers and analyzed following WHO guidance for semen analysis within 60 minutes after ejaculation and collection. The total number of spermatozoa, and percentages of progressively

motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits (Normozoospermia) were observed in men less than 40 years (13.38%) and compared to men between 40 - 50 years (13.71%) and those above 50years. The highest percentage of progressively motile (PR) spermatozoa below the lower reference limit (Asthenozoospermia) was observed in men older than 50 years (35.00%) as compared to men less than 40 years (5.43%) and those between 40 and 50 years (4.68%). The highest total number of spermatozoa below the lower reference limit (Oligozoospermia) was observed in men older than 50 years (30.00%) as compared to men less than 40 years (27.20%) and those between 40 and 50 years of age (22.41%). Semen analysis from a small population of some Ghanaian men undergoing IVF treatment showed that morphologically normal spermatozoa below the lower reference limit (Teratozoospermia) were highest (35.15%) in men less than 40 years as compared to those between 40 and 50 years (25.27%) and more than 50 years of age (10.00 %). Knowledge of the factors that influence sperm quality in this geographical region can contribute to informed decisions on the effective management of infertility in Ghanaian men.

Keywords

Sperm, Semen. Semen Analysis, IVF/ICSI, Ghana, Fertility Center, WHO

1. Introduction

The primary functions of the male reproductive system are the production of sex steroid hormones and the production of gametes, which ensure the survival of the species [1]-[3]. Men are therefore specifically equipped and geared towards this process of gametogenesis [4]-[6]. Sperm production continues virtually throughout a normal male's life, and at peak, up to 20 - 200 million sperm can be produced daily [7]. To generate this large number of sperm, the spermatogonia renew themselves continuously through cell division [4] [8] [9]. The first observation of human spermatozoa dates from as early as 1677 [10]. Using an improved microscope, Leeuwenhoek and Hamm were the first to demonstrate human spermatozoa, but they did not understand the role of the spermatozoon in fertilization [11]. They thought the human sperm contained a miniature human being (the preformation theory) and that the miniature human being would grow once the spermatozoon had penetrated an oocyte [12]. Earlier, Malpighi, in observing what he considered to be unfertilized hen's eggs, suggested the idea that the egg contained a miniature chick [12]. This controversial preformation theory was overturned in 1775 when Spallanzani proved that both the oocyte and the spermatozoon were necessary to form a new individual [11]. In 1830, Prevost and Dumas were able to prove that spermatozoa were essential for fertilization [13], and in 1841, Von Kolliker discovered that spermatozoa were the end product of cell divisions in the seminiferous tubules of the testis [14] [15]. In 1878, Flemings' chromosome research led him to assume that chromosomes have a role in fertilization [16]-[18].

In 1883, Van Beneden discovered that mature germ cells (gametes) contained a reduced number of chromosomes [19] [20] and described meiosis as the process by which the number of chromosomes is reduced [4]. The number of chromosomes was finally determined as 46 in 1956 [21]. The primary reproductive organ in men is the testis, while the secondary reproductive organs are: the scrotum, epididymis, ductus deferens, seminal vesicles, urethra, prostate gland, bulbourethral glands, and penis [21]-[24]. The genital tract in males consists of vasa efferentia (efferent ductules), epididymis, vas deferens, ejaculatory duct, and urethra [25]-[27] and carries the sperm produced in the testis to the urethra, from where they are deposited in the vagina during sexual intercourse [21] [28]. It is helpful to examine two or three samples of patients to obtain baseline data [29]-[31]. While measurements made on the whole population of ejaculated spermatozoa cannot define the fertilizing capacity of the few that reach the site of fertilization, semen analysis nevertheless provides a comprehensive view of essential information on the clinical status and reproductive functioning of the male individual [32] [33]. It includes assessments of sperm count (which examines sperm production, transport through the male genital tract, and ejaculatory function), sperm motility (a basic functional marker of likely sperm competence), sperm vitality (to distinguish between dead spermatozoa and live immotile spermatozoa), sperm form (aspects of sperm production and maturation), sperm morphology (full description of the detailed structure for the different sperm regions for normality, thus; the sperm head, midpiece or neck, and the tail) and the physical appearance of the ejaculate [29] [32] [33].

2. Materials and Methods

2.1. Ethical Consideration

Ethical approval for this study (number: CHRPE/AP/276/17) was provided by the Committee on Human Research, Publication, and Ethics (CHRPE) of the School of Medicine and Dentistry, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST). We obtained written consent from all participants before the data collection.

2.2. Study Design and Site

This prospective cohort study was carried out at the Airport Women's Hospital (AWH) and Fertility Centre in Ghana between January 2017 and December 2019. The AWH is one of the renowned and leading assisted reproductive technology (ART) private fertility hospitals in Ghana and conducts 50 to 60 IVF treatments annually. It was established in 2012, and attendance at the hospital is generally high by local standards, with patients mostly from across Ghana and the West African sub-region.

2.3. Study Population

The study included 558 men who reported with their female partners for sub-

fertility treatment (clinically diagnosed). Patient selection criteria were based on sub-fertility partners participating in an IVF/ Intracytoplasmic Sperm Injection (ICSI) program. The clients were considered eligible for inclusion in the study if the male partner had motile ejaculated spermatozoa during preliminary semen analysis and before controlled ovarian stimulation (COS). The primary aim of studying asthenozoospermia is to measure the degree of reduced sperm motility. For this to be done, some level of motility must be present. If all sperm are immotile, motility cannot be meaningfully graded or analyzed. Including only men with motile sperm helps differentiate asthenozoospermia (reduced but not absent motility) from more severe conditions, such as Necrozoospermia, when all sperm are non-viable and immotile, and Azoospermia, complete absence of sperm in the ejaculate. These conditions are clinically different and may require exclusion depending on the study's scope. Measuring motility by motile spermatozoa ensured that standardized motility parameters (like percentage of progressive motility) were collected and compared according to WHO guidelines. The age group of the men was 27 to 65 years. Exclusion criteria were: previous disease(s) or surgery associated with reproductive function (including **varicocele**, cryptorchidism, epididymitis, mumps, and azoospermia); vasectomy and vasectomy reversal. Clients were questioned regarding their careers, smoking habits, alcohol consumption, and medical and family histories. All cases of severe alteration in spermatogenesis, including frozen and surgically retrieved sperm, prostatitis, and or patients who had prior cancer therapy were excluded from this study.

2.4. Sample Collection

Written information for the clients about the purpose of the investigation (semen analysis) and the circumstances that could interfere with the interpretation of the results was supplied directly to the client when the appointment for semen analysis was made with the Andrology laboratory. Special attention was given to ejaculation (the relevance of correct abstinence time and the importance of collecting the entire ejaculate). Further oral information was given when the client came to the Andrology laboratory to pick up the sample container. On the day of sample collection, relevant information about sample collection and abstinence time was recorded.

2.5. Semen Analysis

Semen analysis was performed following the World Health Organization (WHO, 2010, 5th Edition) manual. Analysis of the ejaculate provided a comprehensive view of the reproductive functioning of the male partner. It included assessments of sperm count (which examines production, transport through the male genital tract and ejaculatory function), sperm motility (a basic functional marker of likely sperm competence), sperm vitality (to distinguish between dead spermatozoa and live, immotile spermatozoa), sperm morphology (aspect of sperm structure, defects and maturation) and the physical appearance of the ejaculate (semen pro-

duction).

2.6. Physical Examination

Parameters used to describe the appearance of the semen sample were grouped under this term. Although strictly speaking, a biochemical characteristic, pH, is also included in this group. These parameters were simple to perform and were mainly executed by the use of the eye. A normal liquefied semen sample has a homogeneous, grey-opalescent appearance. It appears less opaque if the sperm concentration is very low. If the semen is red-brown due to the presence of red blood cells, it is called haemospermia. It is yellow in jaundiced/patients, taking certain vitamins or medication. Although semen has a strong, distinctive odor derived from prostatic secretions, the parameter is seldom used. A change in odor from the normal can, however, be an indication of an inflammation. Semen samples take on a viscous, gelatinous, and sometimes flocculent-lumpy to sago-like consistency immediately after ejaculation. Coagulation is the transformation of the semen from a liquefied state into a semisolid state (coagulum) under enzymatic influence (protein kinase), immediately after ejaculation. The coagulating enzymes originate from the seminal vesicles and, as such, are an important indicator of the presence of the vas deferens and the seminal vesicles [34]. Semen was allowed to liquefy within 30 - 60 minutes. The proteolytic enzymes, fibrinolysin, fibrinogenase, and aminopeptidase are known to be involved in the process. Liquefaction, therefore, serves as an indicator of normal prostate function, being a source of the proteolytic enzyme fibrinolysin [29]. Immediately after the semen samples were received from clients, specimen containers were placed on the bench (a laminar flow cabinet - class II) at room temperature (20 - 25˚C) for liquefaction to avoid large temperature changes that may affect the spermatozoa after they are ejaculated. Liquefaction and appearance of the semen were assessed within 30 - 60 minutes, and the volume was measured. Semen is typically a semisolid coagulated mass, and within a few minutes at room temperature, the sample liquefied (became thinner), at which time a heterogeneous mixture of lumps was seen in the fluid. As liquefaction continued, the semen became more homogeneous and quite watery, and in the final stages, only small areas of coagulation remained. Some samples achieved complete liquefaction within 30 minutes at room temperature, while others took up to 60 minutes or more. Samples that did not achieve complete liquefaction within 1 hour were recorded. Semen samples that were collected at home were liquefied by the time they were received in the laboratory. Normally, liquefied semen samples contain jelly-like granules (gelatinous bodies) that do not liquefy; these do not appear to have any clinical significance, but the presence of mucous strands, however, may interfere with semen analysis [28]. Liquefaction can be recognized both macroscopically, as described above, and microscopically. Immobilized spermatozoa gained the ability to move as the semen liquefied, while more time (30 minutes) was allowed for complete liquefaction for immobilized spermatozoa observed on light microscopic examination. During

liquefaction, continuous gentle mixing or rotation of the sample container on a two-dimensional shaker, at room temperature, assisted in producing a homogeneous sample. After liquefaction, the viscosity of the sample was estimated by gently aspirating it into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity, and observing the length of any thread (the drop method). A normal viscous sample leaves the pipette in small, discrete drops. Samples with abnormal viscosity were observed to have semen drops from a sterile pipette, forming a thread more than 2 cm long. An increased viscosity could be a result of inflammation, abnormal prostate function, unsuitable plastic containers, frequent ejaculation, and the psychological state of the patient [35]. Increased viscosity was treated by adding a preparation medium (sperm preparation media, Origio, Copenhagen, Denmark) to the semen sample, followed by careful pipetting. Extremely viscous semen was treated by the addition of proteolytic enzymes like alpha chymotrypsin [36]. Samples were mixed with an equal volume of protein-free semen preparation medium containing 5 mg alpha chymotrypsin [Type IV-S from the bovine pancreas. Sigma CHY-5S or CHY-100S] [36]. Alternatively, the viscosity was evaluated by introducing a glass rod into the sample, and the length of the thread that formed upon withdrawal of the rod was observed. Viscosity was recorded as abnormal when the thread exceeded 2 cm. Normal viscous semen samples examined exhibited homogeneous stickiness and consistency and did not change with time. High viscosity was recognized by the elastic properties of the samples, which adhered strongly to itself when attempts were made to pipette it. High viscosity interferes with the determination of sperm motility, sperm concentration, the detection of antibody-coated spermatozoa, and the measurement of biochemical markers. The volume of the ejaculate is contributed mainly by the seminal vesicles, the prostate gland, with a small amount from the bulbourethral glands and the epididymides. Accurate measurement of semen volume was essential in the evaluation of semen because it allowed for the total number of spermatozoa and non-sperm cells in the ejaculate to be calculated. A permanent marker pen was given to clients to write on the vessel itself or a label. The labels used for recording the weight were attached before the empty container was weighed. The volume was measured by weighing the sample in the vessel in which it was collected. The weight was recorded on the container before it was given to the client. The semen sample was collected in a pre-weighed, clean, disposable container, and the vessel with semen in it was weighed. The weight of the container only was subtracted from the final volume to obtain the volume of the sample, with the density of semen assumed to be 1.000 g/mL [37] [38]. Semen density usually ranges from 1.043 to 1.102 g/mL [38]. The normal volume of ejaculation after 2 - 7 days of sexual abstinence is 2 - 6 mL. A semen volume less than 1 mL was an indication of an "incomplete" sample. This was confirmed by asking the client about the method of collection and the period of abstinence. Low semen volume could be a result of collection problems (loss of a fraction of the ejaculate), partial retrograde ejaculation, or androgen deficiency,

and a possible characteristic of obstruction of the ejaculatory duct caused by previous infection, frequent ejaculation, retrograde ejaculation, or congenital bilateral absence of the vas deferens (CBAVD) [1]. High volume could be a reflection of active exudation in cases of active inflammation of the accessory organs and a long abstinence period. The possibility of retrograde ejaculation was investigated by asking some clients to urinate after masturbation or coitus interruptus and then examining the urine for the presence of spermatozoa. Measuring volume by aspirating the sample from the specimen container into a pipette or syringe, or decanting it into a measuring cylinder, was not done because not all the sample would be retrieved, and the volume would be underestimated [1]. Normal semen is cloudy milky-whitish with a grey to light grey. A high degree of cloudiness showed high concentrations of spermatozoa present in the seminal fluid. A transparent and watery consistency often indicates low concentrations of spermatozoa, which may be caused by an inflammation or extreme cases of cancer. In cases of inflammation, a more yellowish color may exist. pH of semen reflects the balance between the pH values of the different accessory gland secretions, mainly the alkaline seminal vesicular secretion and the acidic prostatic secretion. The pH was measured after liquefaction at a uniform time, preferably within 30 to 60 minutes of ejaculation, since it is influenced by the loss of carbon dioxide (CO_2) that occurs after production. The pH was measured using a special indicator paper (range 6.4 - 8.0 Merck #9557). pH paper in the range of 6.0 to 10.0 was used. The semen sample was mixed well, and a drop was spread evenly onto the pH paper. After waiting (<30 seconds) for the color of the impregnated zone to become uniform, it was compared against the color scale of the calibration strip to read the pH. The pH of a normal ejaculate varies from 7.2 and 8.0. Above or below this range may be an indication of inflammation of the male accessory sex organs or chronic disease of the prostate gland and seminal vesicles. The accuracy of the pH paper was checked against known standards. For viscous samples, the pH of a small aliquot of the semen was measured using a pH meter designed for the measurement of viscous solutions [7]. Semen samples with a pH less than 7.0, with low volume and low sperm numbers, may be an indication of ejaculatory duct obstruction or congenital bilateral absence of the vas deferens [39]. Semen pH increases with time, as natural buffering decreases, so high pH values provide little clinically useful information.

2.7. Quantitative Analysis (Macroscopic Examination)

Quantitative analysis begins soon after liquefaction, preferably within one hour but no longer than 60 minutes after ejaculation to prevent dehydration or changes in temperature from affecting semen quality. A phase-contrast microscope (Nikon E4, Japan) was used for all examinations of unstained preparations of fresh semen. An initial microscopic examination of the sample involved scanning the preparation at a total magnification of ×100. This provided an overview of the sample to reveal: mucous strand formation; sperm agglutination, the presence of

cells other than spermatozoa (epithelial cells, leukocytes, and immature germ cells), and isolated sperm heads or tails. The preparation was then observed at ×200 or ×400 total magnification. This permitted the assessment of sperm motility and the determination of the dilution required for accurate assessment of sperm number.

2.8. Categories of Sperm Motility

The motility of each spermatozoon is graded as follows: Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed. Non-progressive motility (NP): all other patterns of motility with an absence of progression (swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed). Immobility (IM): no movement. The previous edition of the WHO manual recommended that progressively motile spermatozoa should be categorized as rapid or slow, with a speed of >25 ms^{-1} at 37˚C defining "grade a" spermatozoa, "grade b" as slow or sluggish progressive motility, and "grade c" as nonprogressive motility and categorized as immobility. However, it is difficult for andrologists to define the forward progression so accurately without bias [38]. When discussing sperm motility, it is important to specify total motility (PR + NP) or progressive motility (PR). Spermatozoa were scored in two stages (That is, PR first, followed by NP and IM from the same area) until a count of 200 spermatozoa was achieved. Before all motility categories from that area were scored, counting continued beyond 200 spermatozoa until all categories were counted to avoid bias towards the motility category scored first. That is because it is common to overestimate sperm motility; the order of analysis (NP and IM first) was reversed using an eyepiece reticule.

2.9. Sperm Concentration

The concentration of spermatozoa was calculated from the number of spermatozoa present in the ejaculate during semen evaluation. Samples were well mixed and dilutions prepared. The haemocytometer chamber was loaded, and spermatozoa were allowed to settle in a humid chamber. The samples were assessed within 10 - 15 minutes (after which evaporation has noticeable effects on sperm position within the chamber), and at least 200 spermatozoa were counted per replicate. Replicate counts were compared to see if they were acceptably close. If so, calculations were done; if not, new dilutions were prepared. The concentration of spermatozoa per ml was then calculated.

2.10. Sperm Morphology

Pre-stained, ready-to-use slides (Testimplets) were used for the sperm count. Testsimplet slides have been used to classify sperm morphology since 1978 [40]. Characteristically, it is very easy to prepare specimens quickly, and sperm morphology is seen clearly for the classification of sperm. Ten (10 mL) of semen was placed on the pre-stained slide and then covered with a coverslip. The sample was

read at ×1000 magnification with oil immersion within 1 hour of slide preparation. Two hundred spermatozoa in two replicates for each staining method were examined [40]. The evaluation of the percentage of morphologically normal and abnormal spermatozoa was performed according to the WHO guidelines [41]. In addition, among the spermatozoa with abnormal morphology, the rate of head, midpiece, and flagellum defects was scored.

2.11. Semen Vitality

Sperm vitality was estimated by assessing the membrane integrity of the cells, which was determined, importantly, for samples with less than about 50% progressively motile spermatozoa. This provided a check on the motility evaluation since the percentage of dead cells should not exceed (within sampling error) the percentage of immotile spermatozoa. The percentage of viable cells normally exceeds that of motile cells. The percentage of live spermatozoa was assessed by identifying those with an intact cell membrane from dye exclusion or by hypotonic swelling. The dye exclusion method is based on the principle that damaged plasma membranes, such as those found in non-vital (dead) cells, allow entry of membrane-impermeant stains. Sperm vitality was assessed as soon as possible after liquefaction of the semen sample to prevent observation of deleterious effects of dehydration or changes in temperature on vitality. It is clinically important to know whether immotile spermatozoa are alive or dead. Vitality results were assessed in conjunction with motility results from the same semen sample. The presence of a large proportion of vital but immotile cells may be indicative of structural defects in the flagellum, while a high percentage of immotile and non-viable cells (necrozoospermia) may indicate epididymal pathology. The semen sample was mixed well. A 50 µL aliquot of semen was taken and mixed with an equal volume of eosin-nigrosine suspension in a porcelain spot plate well or test tube. After 30 seconds, the semen sample was remixed before removing a replicate aliquot. A smear was made on a glass slide and allowed to dry in the air for each.

3. Statistical Analysis

Pearson's chi-square (χ^2) test of association was used to compare categorical variables (proportion of men with asthenozoospermia, athenoteratozoospermia, Leukocytospermia, normozoospermia, oligozoospermia, oligoasthenozoospermia, oligoasthenoteratozoospermia, oligoteratozoospermia, and teratozoospermia) between the age groups. The t-test of difference in proportions was used to compare proportions of sperm concentration, progressive motility, and morphology. Results were expressed as proportions, 95% confidence intervals (CI) for the difference in population proportions, and p-values. Significance was assigned to events with a p-value < 0.05. Data analysis was carried out on Minitab Statistical Software (version 22.2.0) and SPSS (version 31). All p-values < 0.05 were considered statistically significant Data was plotted using pie charts and bar charts.

4. Results

A total of 558 men were evaluated. The age of men ranged from 27 to 65 years with an average age of 46 ± 2 years. Out of this, semen analysis showed that, 34 men [5.7%] showed asthenozoospermia, 76 men [13.6%] showed asthenoteratozoospermia, 11 men [2.0%] showed Leukocytospermic, 16 men [2.9%] showed Oligoasthenozoospermia, 24 men [4.3%] showed Oligoasthenoteratozoospermia, 18 men [3.2%] showed Oligoteratozoospermia, 152 men [27.2%] showed Oligozoospermia, 155 men [27.8%] showed Teratozoospermia and 74 men [13.3%] showed Normozoospermia. The analysis made on semen samples was compared with the WHO manual (5th Edition, 2010) for human semen examination variables. To make decisions about patient management and thresholds for clinical investigations. Only complete semen samples - one per man (the first where several were given), obtained following 2 - 7 days of abstinence, were included in this analysis. In this study, patterns of semen samples of male partners undergoing IVF/ICSI treatment were examined as part of the assessment of infertility evaluation in males. Complete medical and sexual history, as well as a thorough physical examination alongside semen analysis required for complete diagnostic assessment, were done. The quality of sperm was assessed by measuring viscosity, volume of ejaculate, color, pH, motility, concentration, morphology, and vitality. The distribution of semen analysis for the study participants from 2017 to 2019 is recorded in Table 1. From Table 1, the results showed that 32 men out of 558 participants [5.7%] were asthenozoospermia, 76 men [13.6%] were asthenoteratozoospermia (ATS), 11 men [2.0%] were Leukocytospermic, 16 men [2.9%] were Oligoasthenozoospermia (OA), 24 men [4.3%] were Oligoasthenoteratozoospermia (OATS), 18 men [3.2%] were Oligoteratozoospermia, 152 men [27.2%] were Oligozoospermia, 155 men [27.8%] were Teratozoospermia and 4 men [13.3%] were Normozoospermia. The percentage distribution of semen analysis categorized by age from 2017 to 2019 was recorded in Table 2. From Table 2, the results showed that 239 men out of the 558 participants [42.83%] were less than

Table 1. Distribution of semen analysis for study participants from 2017 to 2019.

Nomenclature related to semen analysis	2017 (n = 160)	2018 (n = 198)	2019 (n = 200)
Asthenozoospermia	10 (6.25)	12 (6.10)	10 (5.00)
Asthenoteratozoospermia	20 (12.50)	16 (8.10)	40 (20.00)
Leukocytospermic	5 (3.12)	3 (1.50)	3 (1.50)
Normozoospermia	25 (15.63)	29 (14.65)	20 (10.00)
Oligoasthenozoospermia	5 (3.12)	5 (2.53)	6 (3.00)
Oligoasthenoteratozoospermia	3 (1.88)	11 (5.56)	10 (5.00)
Oligoteratozoospermia	2 (1.25)	10 (5.00)	6 (3.00)
Oligozoospermia	40 (25.00)	62 (31.31)	50 (25.00)
Teratozoospermia	50 (31.25)	50 (25.25)	55 (27.50)

Table 2. Percentage distribution of semen analysis categorized by age from 2017 to 2019.

Nomenclature related to semen analysis	*Age group (years)*			
Male diagnosis	**<40 (n = 239)**	**40 - 50 (n = 299)**	**>50 (n = 20)**	**Total (n = 558)**
Asthenozoospermia	13 (5.43)	14 (4.68)	7 (35.00)	34 (6.09)
Asthenoteratozoospermia	22 (9.20)	51 (17.06)	2 (10.00)	75 (13.44)
Leukocytospermic	6 (2.51)	7 (2.34)	0 (0.00)	13 (2.33)
Normozoospermia	32 (13.38)	41 (13.71)	1 (5.00)	74 (13.26)
Oligoasthenozoospermia	9 (3.77)	6 (2.01)	2 (10.00)	17 (3.05)
Oligoasthenoteratozoospermia	6 (2.51)	20 (6.69)	0.00	26 (4.66)
Oligoteratozoospermia	2 (0.84)	16 (5.35)	0.00	18 (3.23)
Oligozoospermia	65 (27.20)	67 (22.41)	6 (30.00)	138 (24.73)
Teratozoospermia	84 (35.15)	77 (25.75)	2 (10.00)	163 (29.21)

40 years of age. Out of the 239 men, 13 [5.43%] were asthenozoospermia, 22 [9.20%] were asthenoteratozoospermia, 6 men [2.51%] were Leukocytospermic, 9 [3.77%] were Oligoasthenozoospermia, 6 men [2.51%] were Oligoasthenoteratozoospermia, 2 [0.84%] were Oligoteratozoospermia, 65 [27.20%] were Oligozoospermia, 84 [35.15%] were Teratozoospermia and 32 [13.38%] were Normozoospermia. Data obtained in Table 2 also showed that 299 men out of the 558 participants [53.58%] were between 40 - 50 years of age. Out of the 299 men, 14 [4.68%] were asthenozoospermia, 51 [17.06%] were asthenoteratozoospermia, 7 [2.34%] were Leukocytospermic, 6 [2.01%] were Oligoasthenozoospermia, 20 [6.69%] were Oligoasthenoteratozoospermia, 16 [5.35%] were Oligoteratozoospermia, 65 [27.20%] were Oligozoospermia, 84 [35.15%] were Teratozoospermia and 32 [13.38%] were Normozoospermia. From Table 2, the results showed that 20 men out of the 558 participants [3.58%] were above 50 years. Seven men [35.00%] were asthenozoospermia, 2 [10.00%] were asthenoteratozoospermia, 0 [0.00%] were Leukocytospermic, 2 [10.00%] were Oligoasthenozoospermia, 0 [0.00%] were Oligoasthenoteratozoospermia, 0 [0.00%] were Oligoteratozoospermia, 6 [30.00%] were Oligozoospermia, 2 [10.00%] were Teratozoospermia and 1 [5.00%] were Normozoospermia. Table 3 shows a summary table of Strict WHO standardized reference values to define semen abnormalities based on large population studies of fertile men (WHO, 2021). Table 4 shows a summary of research and observations related to infection rates, environmental toxins, and lifestyle factors in Ghanaian men and how these may have contributed to the unexpectedly high rates of teratozoospermia (abnormal sperm morphology). Sperm concentration ($p = -0.084$). and progressive motility ($p = -0.317$) were significantly lower in the patient group greater than 50 years compared to those in categories less than 40 years and between 40 to 50 years. Age group > 50 is negatively correlated with all semen parameters measured, as shown in Table 5.

Table 3. Summary table of Strict WHO standardized reference values to define semen abnormalities based on large population studies of fertile men (WHO, 2021).

Parameter	WHO Lower Reference Limit (2021)	Abnormal Condition
Semen volume	1.4 mL	Hypospermia
Sperm concentration	16 million/mL	Oligozoospermia
Total sperm number	39 million	—
Progressive motility	30%	Asthenozoospermia
Total motility	42%	Asthenozoospermia
Morphology (strict)	4% normal forms	Teratozoospermia
Vitality	54% live sperm	Necrozoospermia
Semen pH	≥7.2	Acidic semen
WBCs	<1 million/mL	Leukocytospermia

Table 4. Summary of research and observations related to infection rates, environmental toxins, and lifestyle factors in Ghanaian men and how these may contribute to unexpectedly high rates of teratozoospermia (abnormal sperm morphology).

Category	Specific factor in Ghana	Impact on teratozoospermia (abnormal morphology)
Infections	Bacteriospermia (~22% prevalence)	Direct sperm damage and inflammation → abnormal morphology
Lifestyle: Smoking	~15% smoking rates	Lower normal morphology, motility, and concentration
Lifestyle: Alcohol	Alcoholic bitters consumption	Significant decrease in sperm morphology and motility
Sitting /Sedentary / Heat	> 4hrs/day, laptop heat/impact via heat stress	Increased immobility; probable morphology.
Psychological Stress	Elevated among clinic attendees	Linked to poorer morphology
Environmental toxins /Industrial/pesticide pollutants	Urban areas in Ghana	Reduce sperm morphology across studies globally

Table 5. Correlation between age with semen parameters.

Age group (years)	Progressive motility (%)	Concentration ($\times 10^6$/ml)	Morphology (%)
<40	0.087	−0.149[b]	−0.166[b]
40 - 50	0.399[a]	0.223[a]	0.207[a]
>50	−0.317[a]	−0.084	−0.450[a]

Correlation is significant at the $p < 0.01$ level (2-tailed). [b]Correlation is significant at the 0.05 level (2-tailed). Correlation. Coefficient. r, correlation coefficient; PR, progressive motility; NP, non-progressive motility; Conc., sperm concentration.

This finding suggests a trend rather than a definitive conclusion, warranting further investigation with larger sample sizes. Figure 1 shows a bar chart of the percentage distribution of the total value of semen analysis across the study groups. It was observed that morphologically normal spermatozoa below the lower reference limit (Teratozoospermia) were highest (35.15%) in men less than 40 years as compared to those between 40 and 50 years (25.27%) and more than 50 years of age (10%). The highest total number of spermatozoa below the lower reference limit (Oligozoospermia) was observed in men older than 50 years (30.00%) ($p = -0.149$) as compared to men less than 40 years (27.20%) ($p = 0.012$) and those between 40 - 50 years of age (0.223%) as indicated in Table 5. The total number of spermatozoa, and percentages of progressively motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits (Normozoospermia), were observed in men less than 40 years (13.38%) and compared to men between 40 - 50 years (13.71%) and those above 50 years. A pie chart in Figure 2 shows the percentage distribution of semen analysis categorized

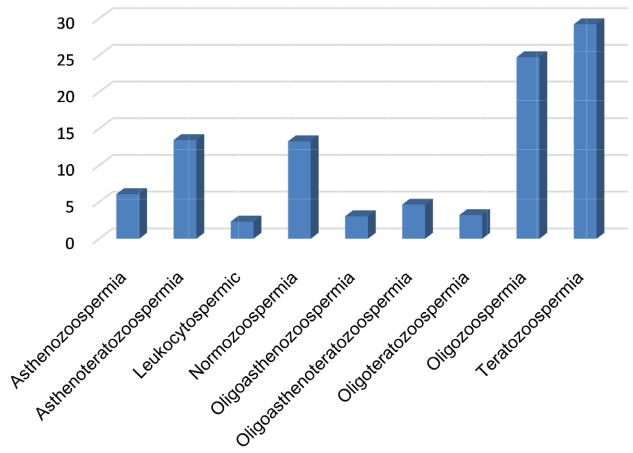

Figure 1. The percentage distribution of the total value of semen analysis across the study groups.

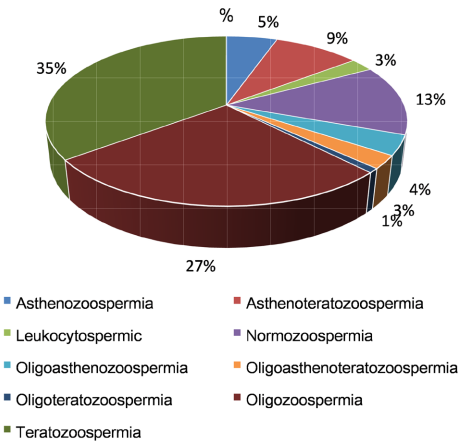

Figure 2. Percentage distribution of semen analysis categorized by age group <40 years.

by age group less than 40 years. A pie chart in Figure 3 shows the percentage distribution of semen analysis categorized by age group between 40 - 50 years. The highest percentage of progressively motile (PR) spermatozoa below the lower reference limit (Asthenozoospermia) was observed in men older than 50 years (35.00%) as compared to men less than 40 years (5.43%) and those between 40 and 50 years (4.68%), as shown in Figure 4. A pie chart in Figure 4 shows the percentage distribution of semen analysis categorized by age group greater than 50 years.

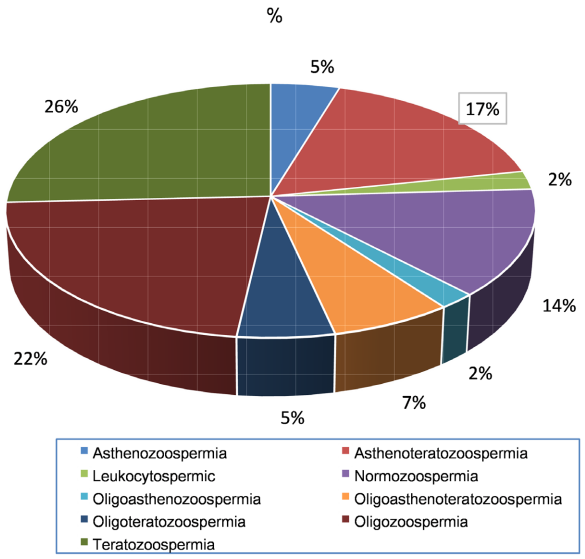

Figure 3. Percentage distribution of semen analysis categorized by age group, 40 - 50 years.

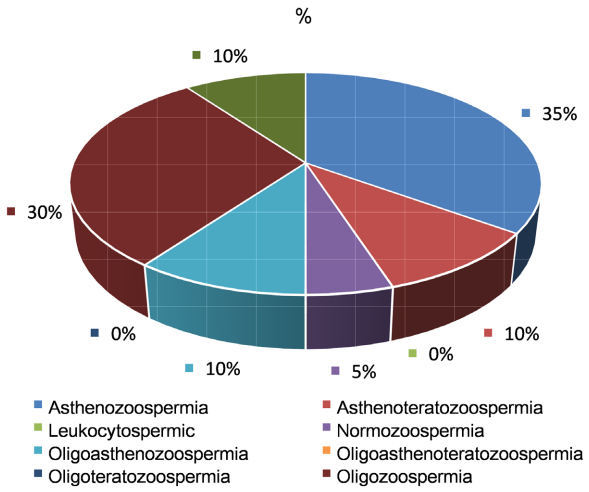

Figure 4. Percentage distribution of semen analysis categorized by age group >50 years.

5. Discussion

In this study, semen samples of 558 study participants were examined. It was ob-

served that 155 men (27.8%) examined showed Teratozoospermia. Studies have revealed that abnormalities in sperm morphology (teratozoospermia) are suggestive of a spermatogenesis problem [33]. Sperm morphology has been shown in some studies to be a predictor of fertilization potential [32]. For the valuation of sperm morphology, the whole spermatozoon was considered [33]. A spermatozoon without any morphological "defect" has been considered morphologically "normal", although it is now recommended that the term typical should be used instead [29] [33]. The definition of morphologically typical spermatozoon is based on the modal form seen after spermatozoa have migrated through good, peri-ovulatory cervical mucus either *in vivo* or *in vitro* [33]. This principle was first adopted in the 1992 WHO manual for human semen examination and confirmed as the standard method in the 1999 and 2010 WHO manual for the examination of human spermatozoa [32] [35]. However, a study in the same year, in a publication stated that: "whether the morphologic assessment of sperm has a significant impact on pregnancy rates after IVF/ICSI or not, it remained controversial" [41].

Research has shown that men with low sperm count <15 million/mL (Oligozoospermia) with clinical findings suggestive of endocrine pathology, could be attributed to the influence of lifestyle habits such as diet, smoking, alcohol consumption, and environmental factors such as exposure to X-rays, chemical pollution or abnormalities in their hormone levels such as total testosterone and follicle-stimulating hormone (FSH) [42]-[45]. Men with low sperm count (Oligozoospermia), low testosterone, high FSH, and high LH could be an indication of primary hypogonadotropic hypogonadism (which affects both the spermatogenesis and Leydig cell function) [7] [44]. Normal testosterone and normal LH, high FSH in oligozoospermic men is an indication of seminiferous tubule damage without Leydig cell dysfunction and abnormal spermatogenesis while low sperm count, low testosterone, normal or low FSH and normal or low LH or hypogonadotropic hypogonadism could be an indication of pituitary hormone deficiencies including thyroid function (free T4) and cortisol [44] [46]. High prolactin levels are suggestive of a prolactin-secreting tumor [44]. Low sperm count, high testosterone, and high LH, and normal FSH an indications of partial androgen resistance syndrome in men, causing androgen receptors not to function optimally in such men [47]-[49]. They are phenotypically male with gynaecomastia and with a variable presentation from hypospadias to normal male genitalia [21]. Low sperm count, normal testosterone, normal LH and normal FSH, and normal testis size an indications of genital tract obstruction [50]. Low sperm count and low LH in muscular men are an indication of androgen abuse [51]. We also observed that 32 out of 558 participants (5.7%) were Asthenozoospermic. This condition possibly arose from ejaculatory duct obstruction secondary to infections like chlamydia, gonorrhea, tuberculosis, or vasectomy [52] [53]. Low sperm motility by itself does not have a significant impact on the likelihood of natural conception unless a very high proportion of sperm is immotile [54]. In such cases, artificial reproductive techniques (ART) such as intracytoplasmic sperm injection (ICSI) were used to

help treat male infertility. A high number of immotile and non-viable sperm may be due to structural defects in the flagellum and epididymal pathology [55]-[57]. Semen samples of 11 men [2.0%] were Leukocytospermic. The presence of round cells in the ejaculate assessed with peroxidase activity and leukocyte markers in men with >1 million leukocytes /mL (pyospermia) was an indication of genital tract inflammation or infection [58] [59]. Male fertility patterns vary greatly among countries and even within regions in the world [2] [43] [58]-[60]. A combination of social habits such as alcohol intake, cigarette smoking, environmental conditions, and genetics appears to contribute to these variations [42] [43] [61]. According to the World Health Organization and other studies, approximately one-third of the world's male adult population (above 15 years of age) smokes [54] [61]-[63]. More than 90% of male factor infertility is characterized by a low number of sperm (Oligozoospermia) in semen or the production of spermatozoa of poor quality [64]. Olsen observed that there has been a genuine decline in semen quality over the past 50 years [65]. As fertility in men is to some extent correlated with sperm count, motility, vitality, and morphology, a reduction in each of these parameters may reflect an overall reduction in male fertility [66]. A study in Kumasi (Ghana) found bacterial infections (bacteriospermia) in 22.3% of semen samples from male partners of infertile couples [67]. The identified bacteria included E. coli, Staphylococcus aureus, Ureaplasma urealyticum, and Chlamydia trachomatis, among others [67]-[69]. These infections were shown to negatively impact key semen parameters, including sperm morphology [70]-[74]. These high infection rates may directly damage sperm structure, potentially contributing to elevated teratozoospermia rates among the study participants (young men in Ghana) [67] [70] [71]. Broadly speaking, global evidence links exposure to organochlorine pollutants like DDT, PCBs, and hexachlorobenzene to decreased semen quality and abnormal morphology [71]-[74]. In Ghana, industrialization, urban growth, and exposure to pesticides and pollutants are cited as risk factors negatively affecting sperm quality [67] [68]. Though direct measurements of these toxins in Ghanaian semen are lacking, these general trends suggest that environmental pollutants (particularly in urban areas) may damage developing sperm and contribute to morphological abnormalities [67]. A Ghana-specific study among 212 clinic-attending men (Teshie, Greater Accra) in **Table 4** summarizes several modifiable lifestyle factors, such as smoking: ~15% were current smokers. Smoking was significantly associated with reduced sperm morphology, motility, and concentration ($p < 0.05$) [68]. Alcohol and Alcoholic bitters (around 60% consumed alcohol; alcoholic bitters were linked to decreased sperm morphology and progressive motility (strong negative correlations; $r \approx -0.58$ to -0.68, $p = 0.000$) [68]. High BMI/Obesity: The mean BMI was around 31.6 kg/m^2 - within the obese range. Elevated BMI was strongly negatively associated with sperm morphology ($r \approx -0.403$, $p = 0.004$). Another study in Ghana noted that prolonged sitting (>4 hours per day) was linked with higher sperm immotility, but morphology was not directly mentioned in the specific study [67] [68]. Laptop Use (Heat Exposure):

Laptop use on laps correlated negatively with testosterone levels and, by extension, may impact morphology [68] [73] [74]. Elevated stress was significantly associated with poor morphology, motility, and concentration [68] [73] [74]. Caffeine generally had a positive relationship with motility but not morphology, and thus is not likely to be a major contributor [68] [70]. While many of the Ghanaian studies include a broad age range, younger men may be particularly affected due to Increased exposure to lifestyle risks. Younger individuals may engage more frequently in alcohol (especially bitters), smoking, prolonged sedentary behavior (computer work), and stress due to urban and occupational pressures [68] [73] [74]. Rising obesity trends among younger populations, potentially exacerbated by dietary shifts toward western-style, processed foods (linked globally to poor sperm morphology) [68] [74]. Finally, Ghanaian young men residing in or working in urban areas with higher levels of industrial or agricultural toxins (environmental pollutants exposure) may affect semen quality even at subclinical levels.

We will recommend broader public health efforts to reduce smoking and harmful alcohol consumption, promoting active lifestyles, healthy diets to combat obesity, awareness about minimizing scrotal heat (avoid laptops on laps, reduce prolonged sitting), enhancing STI screening and treatment, especially for men seeking fertility evaluation, and environmental monitoring and regulation to curb exposure to reproductive toxins in Ghana.

6. Limitations of the Study

Requiring motile ejaculated spermatozoa in the inclusion criteria ensured valid motility assessment but created a selection bias that likely led to underestimation of the prevalence and severity of asthenozoospermia. A non-representative study of semen samples may be required to reduce the generalizability of findings to the wider infertile male population in Ghana.

7. Conclusion

The study revealed that the patients under 40 years old have the highest number of morphologically normal spermatozoa below the lower reference limit (Teratozoospermia) (35.15%), followed by those over 50 years old (30.00%). Patients under 40 years old have the highest total number of spermatozoa. Patients over 50 years old have the highest percentage of progressively motile spermatozoa below the lower reference limit (Asthenozoospermia). Lifestyle measures, such as avoiding alcohol, healthy eating, exercise, and smoking cessation, can improve male factor fertility.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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