SEMEN pH;
PATIENTS ATTENDING INFERTILITY CLINIC AT MUHIMBILI NATIONAL HOSPITAL, TANZANIA

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ABSTRACT... Background: Male infertility is a frequent reproductive health problem in the world. It is usually related to abnormal sperm production or function and these abnormalities can occur anywhere in the production of sperm including hormonal regulation, storage and transport of sperm. Various factors are known to be responsible for seminal fluid abnormalities. Usually the first step in evaluating for male infertility is semen analysis. **Setting:** The study was conducted at Muhimbili National Hospital mainly in the department of Pathology laboratory, cytology unit. Study design: The study was a hospital based cross-sectional type. **Objective:** To determine semen pH in patients presenting with infertility complains and relate how pH of seminal fluid and other semen parameters influence each other. **Material and methods:** Before semen collection, patients were instructed to abstain from sexual intercourse for a minimum of 48 hours to 7 days and collect semen by masturbation and transport it to the laboratory through shirt pocket. Semen was to reach the laboratory for examination in not more than one hour from time of collection. Semen was examined macroscopically for volume, colour, viscosity and pH by using a pH meter (Consort C830) followed by microscopic examination which included motility of spermatozoa and sperm count by using Neuber counting chamber. The smear was made on glass slides, fixed in 95% ethyl alcohol for 30 minutes then stained by using Papanicolaou's staining technique and then analyzed microscopically for morphological examination. **Results:** In the analysis of the influence of semen parameters on semen pH, there was decrease in seminal fluid pH with age whereby as age increased the pH of seminal fluid decreased.. The general trend observed was that the pH of seminal fluid tended to decrease with an increase in the days of abstinence. pH tended to decrease with an increase of seminal fluid volume. The pH of seminal fluid also increased with an increase in viscosity (Hyperviscosity >Hypoviscosity). The pH of seminal fluid in patients with less than 50% forward progressive movement of spermatozoa was higher when compared to those with more than 50% forward progressive movement. pH had an influence on the motility of spermatozoa. **Conclusions and recommendation:** pH and other parameters tended to have an influence each other during seminal analysis in our study. There is a variation of pH in different parts of the World according to the studies done. It is recommended that pH should be included during seminal analysis because our study has shown that it affects most of the seminal fluid parameters in and contribute to the problem of infertility.

Key words: Infertility, Seminal fluid, pH.

INTRODUCTION

Human semen is a mixture of components produced by several different glands. These components are incompletely mixed during ejaculation and, hence, the initial ejaculate is not an entirely homogeneous mixture. The first portion of the ejaculate, about 5% of it, is made up of secretions from the Cowper (bulbourethral) and Littre glands. The second portion derives from the prostate and contributes from 15% to 30% to the ejaculate. There follow small contributions of the ampulla and epididymis and, finally, of the seminal vesicles, which contribute the remainder, and majority, of the ejaculate. Thus the semen plasma is derived primarily (50-80%) from the seminal vesicles.

The secretions of the organs contributing to the ejaculate differ in composition, and there has been a longstanding interest in evaluating the composition of semen from a diagnostic point of view[2,3,4]. The prostate is the main source of the acid phosphatase, citric acid, inositol, calcium, zinc, and magnesium found in the ejaculate. The seminal vesicles' contribution is rich in fructose, ascorbic acid, and prostaglandins, while the concentrations of L-carnitine and neutral alpha-glucosidase are indications of epididymal function[4]. A small portion of the fructose present originates from ampulla of the ductus deferens.

The pH of the ejaculate is determined predominantly by the basic seminal vesicles secretions and acidic prostatic secretions, which may have a pH between 6.5 and 7.2[5,6]. With advancing age or infections the fluid may become more basic[6]. pH values may indicate the presence of chronic or acute infections that impair fertilization in vitro

or in vivo[7,8].

The pH of the normal ejaculate may vary between 7.2 to 8.0[9,10], 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[8]. Acidic ejaculate (pH<7.2) may be associated with blockage of seminal vesicles. Infection is usually associated with alkaline ejaculate (pH>8.0). In general a pH value outside the normal range is harmful to sperm.

pH of exocervical mucus is always lower than that of mucus in the endocervical canal[4]. Spermatozoa are susceptible to changes in pH of the cervical mucus. Acidic mucus immobilizes spermatozoa while alkaline mucus may enhance motility(4). Excessive alkalinity of cervical mucus (pH greater than 8.5) may however adversely affect the viability of spermatozoa[4]. The pH between 6.0 and 7.0 may still be compatible with sperm penetration.

Another factor influencing the results of the various studies reviewed was the method of sample collection and preparation[9,10]. The most important variable here was usually the length of time since ejaculation. Time after collection is particularly important for pH measurements, since the pH changes as a result of increased CO_2 concentration and lactic acid production. The rheological properties of the semen sample also change with time, as the material first coagulates and then liquefies. This process is accompanied by biochemical changes in composition. If sperm are present in the sample, over time they may influence the measured composition, as a result of binding of plasma components to the sperm and to sperm metabolic activity. Sperm metabolic activity can alter composition due to fructolysis, glycolysis, and the excretion of metabolic wastes. In addition, after ejaculation, some of the sperm cell contents may leak out into the surrounding plasma[9,10].

Semen maintains its pH near neutral in the acidic vaginal environment, providing the sperm with the opportunity to enter the neutral pH cervical mucus. The pH of human semen is a matter of some debate[11,12]; there is considerable variation in the pH measurements reported by different researchers. Most researchers have used one of two techniques for measuring semen acidity—pH indicator paper/colorimetry or a pH electrode (in almost all cases, whole semen was used). One study comparing the two methods found slightly higher values when pH paper was used[13,14]. The measured pH can depend on the length of time since ejaculation, and it tends to increase shortly after ejaculation as a result of loss of CO_2[13]. Further aging of whole semen can result in a substantial decrease in pH resulting from fructolysis and the production of lactic acid.

The measurement of pH is a standard component of the semen analysis. The World Health Organization (WHO) laboratory manual, last revised in 1992, states that normal pH of semen ranges from 7.2 to 8.0[9]. An article in Norway[13] reported that the studied population had pH values consistently> 8.0. The semen pH in our population has not been studied before.

During semen analysis the parameters are analyzed macroscopically and microscopically. Macroscopically semen is analyzed for volume and colour of the eminal fluid, viscosity and pH of the seminal fluid. Microscopically it is analyzed for motility of the spermatozoa in which motility is graded as rapid forward progressive motility (grade 4), slow or sluggish undirected progressive motility (grade 3), no progressive motility (grade 2) and immotility (grade 1). Other parameters analyzed microscopically are sperm count and sperm morphology.

Semen pH value at our center has not been studied before. In all our measurements concerning pH we used pH value ranges of the World Health Organization (WHO) as a reference. pH value is known to affect the fertility of male, however at our centre semen parameters which include semen volume, semen viscosity, colour, odour, motility of spermatozoa and sperm count are done but their relation with pH and how they affect each other has not yet been elucidated. The study is therefore intended to determine the pH of seminal fluid in patients who presented with complains of infertility and relate how pH and other parameters may influence each other in causing infertility.

PATIENTS AND METHODS

The objective was o determine semen pH in patients presenting with infertility complains and relate how pH of seminal fluid and other semen parameters influence each other and contribute to the problem of infertility.

The study was a hospital based cross-sectional study which was conducted for 12 months (Oct.2009 – Sept. 2010 on 221 patiens who presented at Muhimbili National Hospital, Tanzania in the Cytology Unit , department of Pathology

Inclusion and Exclusion criteria

The study included all male patients who presented with infertility problems at Muhimbili National Hospital laboratory from gynecological and family planning clinics. It included patients with 18 years of age and above and who agreed to participate in the study. Those who did not agree to participate in the study were excluded.

Data Collection

Patients were given clean non-sperm toxic containers for sample collection at the pre laboratory and were instructed on how to collect sample either at clinic or at home. The instructions were to abstain for a minimum of 2 days up to 7 days, to collect semen by masturbation and to transport semen in shirt pocket and to make sure semen reach the laboratory within one hour from time of collection. Information was recorded using structured questionnaires.

Examination of the specimen in the laboratory

As soon as the sample reached the laboratory patient's instructions were filled in the questionnaire and the, sample was labeled with patient's name and given identification number and be registered in a register book. The sample was allowed to liquefy at room temperature for 20 minutes followed by seminal fluid analysis which were divided into macroscopic examination and microscopic examination.

MACROSCOPIC EXAMINATION OF THE SEMEN

Volume and colour

The semen sample was evaluated by inspecting the colour and followed by volume measurement of the seminal fluid by using a graduated measuring cylinder.

Viscosity

The viscosity of the semen was estimated by gentle aspiration of seminal fluid into a 5ml pipette and allowing the semen to drop by gravity, the length of the thread formed was recorded. Viscosity was recorded as hypovisid if semen dropped without forming a thread, normal if it formed a thread of 1-2 cm and hypervisid if it formed a thread of more than 2 cm.

pH meter measurement

pH of the seminal fluid was measured with a pH meter Consort C830 a multi parameter analyzer (Manufactured by Consort Electrophoresis power supplies, Turnhout, Belgium) which measures pH in two decimal places. Before semen pH measurements, the pH electrode previously stored in KCl solution was then rinsed in a beaker containing distilled water and thereafter, the electrode was immersed in a container that contained raw semen and the pH value was recorded.

MICROSCOPIC EXAMINATION

Sperm motility

Motility was analyzed by putting a drop of seminal fluid into a clean glass slide and then covered with a cover slip. The freshly made wet preparation was left to stabilize for one minute then examined with a microscope using x 40 magnification and the percentage and grade of motility of spermatozoa was recorded. Percentage motility of spermatozoa was graded as rapid progression motility, slow or sluggish motility, or non motile motility depending on how the motility was observed under the microscope.

Sperm count

The sperm count was calculated using Neuber counting chamber. First semen was diluted with Sodium bicarbonate .The dilution was made by mixing one drop of semen to 19 drops of sodium bicarbonate. The mixture was introduced in the Neuber chamber and observed under the microscope where two large chambers were chosen and the spermatozoa from these chambers were counted. The number of spermatozoa per milliliter of seminal fluid was calculated by applying the formula:

Number of spermatozoa/ml of semen = n/2 x 10 x20 x1000.

Where n = number of spermatozoa counted
10 = depth of the chamber
20 = dilution factor
2 = number of chambers counted
1000 = conversion of a drop into a milliliter

Staining procedure

Smears were made on the glass slides and the slides were fixed in 95% ethyl alcohol for about 30 minutes. The slides were stained by using the conventional Papanicolaou's staining technique. The smears were placed in xylene for removing alcohol and clearing and then mounted in Destrine Plasticizer Xylene (DPX) and a cover slip was applied. The slides were then examined microscopically for morphology.

RESULTS

There were 221 patients with complains of infertility with the mean age of 34 years. The overall mean seminal fluid pH was 8.12. There was a tendency for seminal fluid pH to decrease with increasing age such that as age increased pH of seminal fluid tended to decrease (Table-I).

Out of 221 male patients investigated for infertility, 17.2% were azoospermic 30.3% oligospermic and 52.5% were normospermic. The general tendency observed was that oligospermia and azoopermia increased with an increase in age and was highest (75%) in the age group 50-59 years. (Table-II)

Patients who had volume of seminal fluid less than 2mls had a slightly higher pH than that found in patients with seminal fluid volume of 2 -4mls. In the remaining group of patients who had seminal fluid production of more than 4mls had the highest pH among the three groups.(Table-III). The volume seminal fluid had an influence in the pH such that as the volume increased, the pH also increased (became more basic).

Table-I. The frequency distribution of pH of seminal fluid in infertile men in relation to age

Mean pH	Age groups in years				Total
	20-29	30-39	40-49	50-59	
8.23	45	-	-	-	45
8.12	-	138	-	-	138
7.80	-	-	34	-	34
7.88	-	-	-	4	4
Total	45	138	34	4	221

Table-II. Sperm concentration/ml (count) of seminal fluid in relation to age

Age group	Sperm concentration / ml of seminal fluid in men with infertility			Total
	Oligospermia <20 million/ml	ormospermia ≥20 million/ml	Azoospermia	
20-29	13 (cases)	24 (cases)	08 (cases)	45
30-39	45 (cases)	74 (cases)	19 (cases)	138
40-49	07 (cases)	17 (cases)	10 (cases)	34
50-59	02 (cases)	01 (case)	01 (case)	04
Total	67	116	38	221

Table-III. pH value of patients with infertility in relation to volume of seminal Fluid production

Mean pH value	Volume of seminal fluid			Total
	<2 mls	2-4 mls	> 4 mls	
8.17	33 (cases)	-	-	33
8.16	-	160 (cases)	-	160
8.43	-	-	28 (cases)	28
Total	33	160	28	221

Table-IV. pH value in relation to viscosity of seminal fluid

Mean pH value	Viscosity of seminal fluid			Total
	Normal	Hypo viscosity	Hyper viscosity	
8.08	51 (cases)	-	-	51
8.13	-	88 (cases)	-	88
8.14	-	-	82 (cases)	82
Total	51	88	82	221

Table-V. pH value of seminal fluid in relation to motility of spermatozoa.

Mean pH	% of spermatozoa with forward progressive motility in men with infertility				Total
	Rapid ≥50%	Sluggish <50%	Non motile	Azoospermia	
8.14	67	-	-	-	67
8.13	-	103	-	-	103
7.91	-	-	13	-	13
7.92	-	-	-	38	38
Total	67	103	13	38	221

Patients with hyperviscosity had a highest pH compared to patients with hypoviscosity who had a lower pH. An increase of viscosity of seminal fluid was thus associated with an increase in its pH and vice vesa. (Table-IV).

The pH value of seminal fluid in of patients who had 50% or more of spermatozoa with rapid forward progressive movement was the highest in comparison to other groups of patients with less than 50% forward progressive motility (sluggish movement) and in those with non motile spermatozoa respectively. It should be noted that the motility of the three categories tended to diminish with a decrease in the pH seminal fluid and further confirm that basic pH favours sperm motility rather than acidic pH (Table-V).

DISCUSSION

The normal pH of semen has been defined as ranging from 7.2 to 8.0.This study population of 221 patients had an average pH of 8.12 with no patient falling below the supposedly normal range, 38 (17.2%) patients falling within the normal range and 183 (82.8%) patients were above the WHO reference range. Our study population show slightly higher mean pH than WHO reference range but is similar to the findings of other studies[13,16]. Haugen and Grotmol[16] noted that sperm pH in their population

was consistently higher than the WHO reference values. Another group reported elevated semen pH among healthy medical students[16].

Several inflammatory processes of the prostate and seminal vesicles are thought to alter semen pH and according to the WHO infection should be suspected if the pH exceed 8.00 or below 7.2[7]. In light of this study results, recommendation could possibly lead to over diagnosis of infections and other inflammatory processes as about 28 (56%) patients would have been included. However this needs more studies to be done in order to agree or rule out the presence of infection in infertile men if pH exceeds 8.00 or below 7.2.

It is interesting that the WHO manual[9,16] states that the optimal pH for sperm migration and survival in the cervical mucous is 7.0 to 8.5 which agrees with ours whereby 92% were within this range. Although the findings of our study does not agree with those of Haugen and Grotmol[16,17], where they found no significance difference of pH between patients with normal and abnormal sperm parameters, our findings further extend this by demonstrating that the mean semen pH among patients with normal sperm parameters was pH 8.00 which was different from those with abnormal sperm parameters pH 8.21.

This study on pH reference range reflects the value that has been observed by WHO.

Mears EM[8] noted that with advancing age the pH of seminal fluid may become more basic. Our study disagree with this finding as we found that with advancing age the pH of seminal fluid decreased as it was found that patients with 40-49 years of age had the lowest mean seminal fluid pH of 7.83 compared to other patients in the groups of 30-39 and 20-29 which had a mean pH of 8.16 and 8.19 respectively. The length of time interval from collection of the ejaculate to analysis of the sample influenced our pH measurement whereby there was an increase in the pH for patients with an interval of more than 1 hour (pH 8.23) in comparison to those with 1 hour or less. This is in agreement to other studies[10] where they found similar results in which the change in the pH was related to rheological properties of semen sample which changes with the length time interval and biochemical changes in its composition which follow thereafter. An increase in seminal fluid volume was associated with an increased on its pH. This might have been influenced by excessive secretions from several different glands which participate in production of semen secondary to an inflammatory process because infection makes the fluid more basic[7]. However, this needs further study but in our cases the presence of infection in the seminal fluid was not evaluated. In addition it was also observed that the pH of seminal fluid tended to decrease with an increase in the days of abstinence. In one study[10] it was observed that both a too short period of time since last ejaculation and a too long reduces semen quality. This might be related to biochemical changes occurring in the seminal vesicles as related to time.

The motility of spermatozoa tended to diminish with an increase in the pH of seminal fluid in our study, further confirming that basic pH enhances sperm motility rather than acidic pH which immobilizes spermatozoa[4]. Excessive alkalinity, pH greater than 8.5 may however adversely affect the viability of spermatozoa[4]. Our study agrees with this finding as we found a decreased forward progressive movement and non motile spermatozoa in 7(14%) patients with seminal fluid pH 8.50 or more. Viscosity of seminal fluid tended to affect the concentration of spermatozoa in seminal fluid. We found that fifteen (30%) patients with hyper viscosity had a mean pH of 8.16 compared to 19(38%) patients with hypo viscosity who had a mean pH of 8.09. Viscosity of seminal fluid may be attributed to the presence of various constituents of seminal fluid which may include; spermatozoa concentration, presence of inflammatory cells, presence of micro organisms, electrolytes and length of time from ejaculation to analysis of the sample. Thirty two (64%) patients who had a high pH (mean 8.20) had sperm count of less than 20 million per mil. of seminal fluid. This might have been influenced by an inflammatory process and thus contributed to high pH in contrast to 18 (36%) patients with a pH of 8.03, which was very close to normal range and had a normal sperm count.

Conclusion and Recommendation

pH tended to have an influence on most of parameters during seminal analysis in our study. It is therefore important that pH should to be included during seminal analysis because pH below 7.2 or above 8.00 may be an indication of inflammation of the male accessory organs or chronic disease of the prostate or seminal vesicles. In these circumstances, semen has to be cultured for aerobic and anaerobic infection as well as Chlamydia and Mycoplasma and treatment instituted accordingly. Also the presence of abnormality in the way the testes, connective vessels or penis are constructed, can be indicative of hormonal issues. Hormones play a large role in the pH of semen and other bodily fluid, therefore; tests can be performed to check these hormonal imbalances in patients with low or high semen pH in determining the cause infertility in a particular patient. The study in subgroup of 16 patients had normal sperm parameters with a mean pH of 8.00 which was within the WHO reference range. There is a variation of pH in different parts of the World according to the studies done. It is recommended that pH should be included during seminal analysis because our study has shown that it affects most of the seminal fluid parameters and contribute to the problem of infertility.

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