DOI: 10.7860/JCDR/2013/6194.3585

Original Article

Pathology Section

Seminal Plasma pH, Inorganic Phosphate, Total and Ionized Calcium Concentrations In The Assessment of Human Spermatozoa Function

S .OLATUNBOSUN BANJOKO[1], FASIU O. ADESEOLU[2]

ABSTRACT

Introduction: Fertilization in humans is dependent on viability of the male spermatozoa among other factors and there have been conflicting reports on the role of pH, calcium and phosphate concentrations in sperm function. This study therefore aimed to investigate seminal plasma pH, inorganic phosphate, total and ionized calcium concentrations relative to spermatozoa function.

Material and Methods: Seminal plasma concentrations of pH, total calcium, ionized calcium (Ca^{++}); inorganic phosphate, motility and spermatozoa count were determined in 80 males by standard methods.

Results: Forty-nine of the subjects had normal spermatozoa motility (>60%) and 31 had hypomotility (<60%). The hypomotility group exhibited lower calcium ion (Ca^{2}+) concentrations; 0.19+0.01mmol/L compared with normal motility group; 0.24+0.01mmol/L (p<0.001) the latter also had significantly higher inorganic phosphate; 7.83+1.27 while the former had 5.64+1.62mmol/L (p= 0.004). The mean spermatozoa counts for hypomotility and normal motility group were 42.0 ± 13 x 106 , 72.35 + 20 x 106 respectively (p< 0.001). No significant differences were observed in pH, volume of ejaculate and total calcium concentration between the hypomotility and normal motility groups The mean concentrations of pH were 7.51 ± 0.02 and 7.54 ± 0.03 respectively (p= 0.21) and total calcium; 3.10 ± 0.12 and 3.36 ± 0.14mmol/L respectively (p= 0.16). There was a significant difference in percentage of abnormal forms in both groups with hypomotile group having 36% compared to mormal motility group with 5% (p< 0.05).

Conclusion: Correlations were observed between seminal concentrations of calcium ions, inorganic phosphate, spermatozoa count and motility but not with total calcium concentrations and pH and therefore should be considered in understanding male infertility and preparation of media for sperm preservation for in vitro fertilization.

Keywords: Seminal fluid, Biochemistry, Sperm function, Male fertility

INTRODUCTION

Mammalian fertilization is one of the most intricately regulated cell to cell interaction with ions and proteins playing important roles in the binding of spermatozoa to ovum [1]. It had been observed that the presence of spermatozoa in the seminal fluid make little contribution to the total ionic content of the semen and they make up only a small portion of the whole semen contribution of about 1% - 5% of the total volume [2].

Seminal plasma is a mixture of contents from the testes, epididymides and accessory sex glands. The sperm concentration is highest in the first few jets or fractions of the ejaculate and the composition of seminal plasma varies between these fractions because accessory gland secretions are released in a specific order. Semen has a very high buffering capacity, much higher than that of most other fluids in the body. The pH of semen is maintained near neutral in the acidic vaginal environment providing the sperm with the opportunity of entering the neutral pH of the cervical mucus. Citrate is one of the most important anions present in human semen. Although citrate has a high affinity for calcium, magnesium and zinc, the citrate concentration is more than double in the divalent metal concentration, consequently, much of the seminal citrate is strongly anionically charged [3]. Semen owe its high calcium ion buffering capacity to citrate and the latter is probably the major regulator of ionized calcium concentration in seminal plasma and the source of high buffering capacity of semen had been linked to citrate [4].

There had been conflicting data on the pH of human semen which has become a matter of debate. In a study comparing seminal pH values using pH indicator paper, colorimetry and pH electrodes, it was observed that slightly higher values were obtained when pH paper indicator was used [5]. The measured pH may depend on the length of time since ejaculation and it tends to increase shortly after ejaculation as a result of loss of carbon dioxide. Another study observed that in buffering of semen, HCO_3/CO_2 contribution is 24.9%, while protein contributes 28.5% and the other half was contributed by low molecular weight components such as citrate, inorganic phosphate and pyruvate [6]. Therefore, the pH of the seminal fluid may play a significant role in sperm function. The normal pH of seminal plasma is between 7.2 and 8.0. An acidic ejaculate of pH< 7.2 may be an indication of blockage of seminal vesicles while that with an alkaline pH of about 8.0 is usually associated with infections [7].

Estimation of calcium concentration in semen can be of considerable interest as a result of its relationship with sperm motility, metabolism, acrosome reaction and fertilization itself [8]. However, only a small portion; 2% - 4% of the calcium in semen is present in ionized form [9].

Calcium is thought to be the key regulator of human sperm and the beating pattern of sperm tail. To function in these roles, Ca^{2}+ ions concentration must be finely regulated both intracellularly and extracellularly [10]. Changes in intracellular calcium ion concentration are associated with different aspects of sperm function such as sperm motility. The kinetics of changes in free calcium ion concentration in human spermatozoa is complex and crucial to sperm function.

Inorganic phosphate on the other hand had been observed to reflect satisfactorily the functional capacity of the accessory glands of the genital system and was positively correlated with

seminal fructose concentration. Successful treatment of accessory gland infection was observed to result in rise in concentrations of inorganic phosphate, calcium and magnesium [11]. Furthermore, inorganic phosphate was higher in asthenozoospermic but lower in azoospermic than in normospermic individuals. This study therefore aimed to assess the relationship between seminal plasma pH, total calcium concentration calcium ion concentration, inorganic motility, spermatozoa concentration and sperm motility by extension of function.

MATERIAL AND METHODS

The proposal on the study was sent to the University College Hospital University of Ibadan, Nigeria ethics committee on human research for approval, which was granted. A total of 80 semen samples were collected consecutively from male patients attending the infertility clinic of the Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Nigeria.

Estimation of spermatozoa motility and count

Routine semen analysis was performed manually using a binocular microscope and improved neubauer counting chamber for motility and spermatozoa count.

Estimation of Seminal plasma pH

Seminal plasma pH values were determined using Phillips digital pH meter (model PW9409)

The probe was inserted into the liquefied seminal plasma. The pH values were digitally displayed.

Estimation of seminal plasma ionized calcium concentrations

The calcium specific electrode (Orion Space Stat 20) was used to determine calcium ion concentrations, the equipment was calibrated with calcium standards with known concentrations. The electrode was placed in the supernatant sample and determined calcium ion concentrations were displayed digitally.

Estimation of seminal plasma total calcium concentration

Total calcium concentration was determined spectrophotometrically using reagent kit obtained from Randox Laboratories U.K. 20µl of supernatant was added to 1.0mls of O-cresolphthalein complex one in an alkaline medium. The mixture was allowed to stand at room temperature for 10mins and read using a spectrophotometer at 570nm. The concentrations of samples were determined by comparing absorbance with that of a standard with a known concentration.

Estimation of seminal plasma inorganic phosphate concentrations

Inorganic phosphate was determined spectrophotometrically using a reagent kit obtained from Randox laboratories U.K. 1.0ul of supernatant was added to 1.0mls of ammonium heptamolybdate in a strong acidic medium (H_2SO_4). The mixture was allowed to stand at room temperate for 1 minute. The reaction mixture was read at a near UV wavelength of 340nm using a spectrophotometer. Inorganic phosphate concentrations were determined by comparing with the absorbance of a standard phosphate of known concentration.

STATISTICAL ANALYSIS

Results were input into the computer and statistical analysis performed using the Statistical Package for Social Sciences (SPSS) software. The student t-test and chi-square test were utilized in comparing the degree of significance of different parameters estimated.

RESULTS

Results were divided into 2 groups based on motility. (i) The hypomotility group (test group) with motility ≤60% had a mean value of 45.5 + 2.2 % and were 31 in number representing (38.75 %) of study population while (ii) the normal motility group (Control group) with values > 60% with a mean value of 76.3 ± 1.62% were 49 representing (61.25%) of total experimental subjects.

The hypomotility group (Test) exhibited lower calcium ion (Ca++) concentrations with mean values of 0.19+ 0.01mmol/L compared to normal motility group (Control) with mean concentration of 0.24 + 0.01mmol/L ($p < 0.001$). The latter also had significantly higher inorganic phosphate of 7.83 + 1.27mmol/L compared to the former with a mean value of 5.64 + 1.62 mmol/L ($p = 0.004$). The mean total spermatozoa count for normal motility group was 72.35 + 20 x 106 while the hypomotility group had a count of 42.0 ± 13 x 10^6 ($p = 0.001$).

No significant differences were observed in the seminal plasma pH and total calcium concentration between the hypomotility and normal motility groups The mean values of these parameters were pH; 7.51 ± 0.02, 7.54 ± 0.03 respectively ($p = 0.21$) and total calcium; 3.10 ± 0.12, 3.36 ± 0.14mmol/L respectively ($p = 0.16$) [Table/Fig-1].

	Motility		
Parameters	**< 60% (n = 31) (38.75%) Hypomotility (Test) group**	**> 60% (n = 49) (61.25%) Normal motility (Control) group**	**Probability**
Age (Years)	36.0 ± 3.0	37.0 ± 4.0	p = 0.22
Motility (%)	45.5 + 2.2	76.3 + 1.62	p = 0.001*
Sperm Count (x 106)	42 + 13	72 + 20	p = 0.001*
pH	7.51 + 0.02	7.54 + 0.02	p = 0.21
Total Calcium (mmol/L)	3.10 + 0.12	3.36 + 0.14	p = 0.16
Ca++ ion (mmol/L)	0.19 + 0.01	0.24 + 0.01	p = 0.001 *
Inorganic phosphate (mmol/L)	5.64±1.62	7.83 + 1.27	p = 0.04*
Volume (mls)	2.32 ± 0.16	2.42 ± 0.09	p = 0.22
Abnormal forms (%)	36.0	5.0	p < 0.001*

[Table/Fig-1]: Seminal plasma pH, inorganic phosphate, total and ionized calcium concentrations in individuals with normal spermatozoa motility (control) and hypomotility (test)
p is significant at values < 0.05
* denotes significant p values.

DISCUSSION

The male factor infertility is most commonly defined as abnormalities in the number of sperm present and proportion of the motile and morphologically normal sperm. The normal spermatozoa ejaculate is expected to have a count greater than 20 millions/ml, pH value of 7.2-7.8, motility greater than 50% and spermatozoa with normal morphology greater than 40%. Determinant of fertility is a couple-related phenomenon that requires the initiation of a pregnancy therefore semen analysis is not a test of fertility per see but an inclusive assay. It had been shown that 30% of all patents with normal semen analysis have abnormal sperm function. The suggestion that additional factors not included in routine seminal analysis contribute to male infertility is therefore a corollary and an underpinning of this study.

In the study, it was observed that low seminal plasma calcium ions and inorganic phosphate correlates with spermatozoa motility and count [Table/Fig-1]. In another Nigerian study, a significant positive correlation was observed between calcium and percentage motility on one hand, and count and motility on the other in another [12]. The same study which divided its cohort into fertile an infertile male similarly observed seminal plasma calcium ions to be significantly

higher in the former. A contrasting phenomenon was observed in the relationship between calcium and sperm motility by Abou Shakira et al., [13] In another study, elevated ionized calcium was observed to inhibit spermatozoa motility [10]. It was also observed in our study ionized calcium concentration positively correlates with sperm motility, but such relationship was not observed with total calcium concentration. This observation had been corroborated by the study of Prien et al., [14].

Calcium ions apparently have paradoxical effect on sperm motility. In the epididymis, calcium ion stimulate immature sperm whereas in ejaculated semen, it inhibits sperm motility. Maturation processes is therefore thought to change the response to sperm calcium ions. Calcium binding substances and calcium transport inhibitors secreted by male accessory sexual organs are mixed with sperm during ejaculation. In the female general tract, sperm acquire full capacity to fertilize the ovum whereby calcium binding substances and calcium transport inhibitors are removed during the process known as capacitation. Finally, calcium ions trigger the acrosome reaction and facilitate sperm penetration into the ovum [10]. Therefore given the biochemical requirements for Ca^{2+} ions by adenosine triphosphate to drive the flagella, the relationship between calcium and sperm motility seems logical.

Furthermore, calcium and inorganic phosphate were observed by other workers to positively affect motility and fertilization potential of spermatozoa [15,16]. These finding were in line with those observed in this study. In addition, the fact that inorganic phosphate was observed to be higher in asthenozoosperma but lower in azoospermic patients than in normospermic men,in other studies underscores the clinical importance of estimation of seminal plasma phosphate. It had also been suggested that estimation of Ca^{2+}, Mg^{2+} and inorganic phosphate may be of value in the assessment of accessory gland function of the male genitalia [11,17]. Phosphate ion is crucial to the activities of prostatic acid phosphatase (PAP) which has been associated with liquefaction process of semen and adenyl cyclase; the primary regulator of sperm motility [18].

The main aim of the sperm is to locate the egg and this is dependent on efficient motility. The speed at which the calcium concentration in the cell changes, control the swimming behavior of spermatozoa [19].

Sperm only reacts to changes in calcium concentration by calculating the dynamics and become capable of maneuvering even in the presence of high calcium concentration, The effect of calcium ions in the cytoplasm of mature spermatozoa is negatively associated with viability but calcium inflow triggers the capacitation of spermatozoa [10]. Conversely, a positive effect of calcium ions on spermatogenesis had been proven. The relative low concentration [19] of calcium ions is however maintained by calcium ATPase on the sperm's membrane.

A sperm's path to the egg typically includes straight ahead runs alternating within curves and even loops. The sperm's flagellum guides the journey by sensing the egg's released chemo attractants therefore causing calcium spikes in the flagellum and adjusting it's beating to steer in the direction of the egg. Earlier studies suggested that the concentration of calcium ions determine the flagellum beating pattern with high calcium levels spurring the sperm to turn. But recent information on the subject called these findings to question. For example Alvarez et al., [11] demonstrated that the sperm continued on a straight course even when calcium was abundant and responds to a change in calcium concentration in a mathematical time derived terms. It was further shown that the rate of calcium ion increase dictates how sharply the sperm turns whereas the path of the subsequent runs depend on the steepness of the calcium decline.

In addition, experimental evidence using Ethylamine Diamine Tetra Acetic Acid (EDTA),a calcium chelator causing a decrease in calcium concentration to the tune of 65% caused a significant loss of sperm motility. This is an indication of regulatory effect of sperm motility by calcium ions and spermicidal activity of EDTA [19].

Therefore, estimation and interpretation of seminal plasma calcium concentration can be complicated. In addition, binding with other compounds like citrate, phosphate and proteins may reduce the ionization and activity of calcium. Furthermore, semen has a very high calcium buffering capacity. Calcium also binds to the sperm surface which can lead to differences between measurements on whole semen versus seminal plasma [20]. While some studies observed no significant difference in seminal total calcium concentrations in fertile and infertile men and also in men exhibiting seminal hypo-motility and normal motility [14,21], others studies demonstrated a relationship between high calcium levels and fertility in men [22].

Some studies even indicated that a high calcium ion concentration suppresses sperm motility [3,10]. It is therefore reasonable to suggest that an optimal seminal calcium concentration may be required to promote sperm motility and all steps leading to successful fertilization [22].In addition, estimation of seminal calcium concentration was suggested in normozoospermic infertile men who are astheno-zoospermic [23].

Since no significant difference was observed in the volume and pH of seminal plasma between the hypomotile and normal motility groups, $p < 0.05$, it is plausible to suggest that seminal plasma volume and pH do not have an effect on sperm fertilization potential except when the levels are excessively abnormal.

The neutral pH of 7.0 is where most enzymes in the spermatozoa are best active. Therefore, deviation towards alkalinity or acidity can reduce metabolic rate. It has been suggested that semen pH has little significance for spermatozoa fertility potential unless the levels are excessively abnormal. Acidic ejaculate of pH <7.2 may be associated with blockade of the seminal vesicles and activities of reactive oxygen species, thereby inhibiting sperm motility and function.

While seminal plasma total calcium concentration may not be so relevant in assessing male infertility, it is plausible to suggest estimation of seminal pH, calcium ions and inorganic phosphate in the understanding of male fertility [12]. It is also not preposterous to suggest assessment of seminal citrate; the main regulator of seminal plasma Ca^{2+} ion concentration. Furthermore, in view of other reports from other studies, an optimum Ca^{2+} concentration for sperm motility and function needs to be determined. This can be very helpful in further understanding male infertility and in the preparation of media for preservation of spermatozoa for in vivo fertilization.

Although simple microscopy was used for estimation of sperm motility for this study, in contrast to computer assisted semen analysis (CASA) currently in vogue [17], the results are still valid. The lack of use of CASA for sperm morphology can however be regarded as a limitation of this study.

CONCLUSION

In this study, spermatozoa motility and function were observed to be negatively influenced by reduced seminal plasma Ca^{2+} ions and inorganic phosphate but not total calcium concentration and estimation of inorganic phosphate could be useful in assessing assessor gland function and in cases of infection, it's prognosis. In addition, seminal plasma pH is relevant in dectecting infections as associated with alkaline ejaculate and seminal vesicle obstruction as associated with acidic pH. It is therefore suggested that semen analysis should be complemented with semen functional assays which may include that of pH, calcium ions, inorganic

phosphate and citrate which indirectly measures the ability of one spermatozoon to deliver the correct complement of chromosomes to an ovum.

Spermatozoa must be produced in specific volumes, exhibit normal mobility and shape and pass through the cervical mucus, uterus and ampullae of the oviducts after undergoing capacitation, acrosome reaction (AR), zona pellucida binding and nuclear decondensation. Defects of any of these complex events can result in male infertility.

ACKNOWLEDGEMENT

The authors acknowledge the cooperation of the Department of Obstetrics and Gynaecology University College Hospital, Ibadan, Nigeria in carrying out this study.

REFERENCES

[1] Alonso Marques V, Goulart LR, Feliciano Silva AEO. Variations of protein profiles and calcium and phospholipase A2 concentrations in thawed bovine semen and their relation to acrosome reaction. *Genet. Mol Biol.* 2000; 23 (4): 825-29.
[2] Mortimer D. Practical Laboratory Andrology. OUP, New York, 1994; Pp. 66–69.
[3] Arver S, Sjoberg HE (1982) Calcium fractions in seminal plasma and functional properties of human spermatozoa. *Acta Physiol. Scand.* 116 (2): 159-65.
[4] Magnus O. Brekke I, Abyholm T. Purvis K. Effects of manganese and other divalent cations on progressive motility of human sperm. *Arch. Androl.* 1990; 24(2):159-66.
[5] Haugen TB, Grotmol T. pH of human semen. *Int. J. of Andrology.* 1998; 21(2): 105-08.
[6] Wolters-Everhardt E, Dony JM, Lemmens WA, Doesburg WH, DePont JJ. Buffering capacity of human semen. *Fertil Steril.* 1986; 46(2):114-19.
[7] WHO laboratory manual for the examination of human and sperm –cervical mucus interaction. Cambridge, Cambridge University Press 3rd Edn 1992.
[8] Sorensen MB, Bergdahl 1A, Hjollund NHI, Bonde JPE, Stottenberg M, Enst E Zinc, magnesium and calcium in human seminal fluid: relations to other semen parameters and fertility. *MHR. Basic Sci. Reprod. Med.* 1999; 5(4): 331–37.
[9] Irvine DS, Aiken RJ. The value of adenosine triphosphate (ATP) measurements in assessing the fertilizing ability of human spermatozoa. *Fertil Steril.* 1985; 44,806.
[10] Hong CY, Chiang BN, Turner P. Calcium ion is the key regulator of human sperm function Lancet. 1984; 2: 1449-51.
[11] Alveraz L, Dai L, Friedrich BM, Kashikar N, Gregor I, Pascal R, Kaupp UB. The rate of change in Ca2+ concentration controls sperm chemotaxis. *J Cell Biol.* Online: 2012.
[12] Bassey I.E., Essien O.E., Udoh A.E, Imo I.U, Effiong I.O, Seminal plasma, selenium, magnesium and zinc levels in infertile men. *J Med Sci.* 2013; 13: 483-87.
[13] Abou – Shakra FR, Ward NI, Everard DM. The role of trace elements in male infertility.
[14] Prien DS, Lox DC, Messer RH, Deleon FD. Seminal concentrations of total and ionized calcium from men with normal and decreased motility. *Fertil Steril.* 1990; 54(1): 171-72.
[15] Fakih H, Mcalusky M, DeCherney A, Wallimann T, Huszar G. Enhancement of human sperm motility and velocity in vitro: effects of calcium and creatine phosphate. *Fertil Steril.* 1986; 46: 938.
[16] Bernstein D, Tyler JPP, Driscoll GL. A comparison of WHO and Tygerberg strict criteria for assessing human spermatozoa morphology. *Austr J Med Sci.* 1995;16(3):115-17.
[17] Valsa J, Skandhan Kp, Gusani P, Khan PS, Amith S, Gondalia M. Effect of daily ejaculation on semen quality and calcium and magnesium in seme Rev Int Androl 2013. http://dx.doi.org/10.1016/j androl. 2013. 03.001.
[18] Adamopoulos DA and Deliyiannis V. Seminal plasma pH, calcium and inorganic phosphate concentration in normozoospermic and subfertile men. *Andrologia.* 1983; 115(6): 648–54.
[19] Lee CH, Anderson M,Chien YW. Characterization of in vitro spermicidal activity of chelating agent against human sperm. *J Pharm Sci.* 1996,85(6):649-54
[20] Owen DH, Katz DF. A review of the physical and chemical properties of human semen and the formulation of a semen stimulant. *J. Androl.* 26 (4):459-46. Fertil Steril. 2005; 52: 307.
[21] Umeyama T, Ishikawa H, Takeshima H, Yoshii S, Koiso K. A comparative study of seminal trace elements in fertile and infertile men. *Fertil Steril*. 1986; 46: 494 doi:10.1083/jcb201106096.
[22] Logoglu G, Kendirci A, Ozgunen T. The role of seminal calcium in male infertility. *Medical Journal of IAS.* 1997; 10 (1): 25-27.
[23] Fraser LR. Minimum and maximum extracellular Ca++ requirements during mouse sperm capacitation and fertilization in vitro. *J. Reprod. Fert.* 1987; 81: 77- 89.

PARTICULARS OF CONTRIBUTORS:
1. Senior Lecturer, Department of Chemical Pathology, Institute of Public Health, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.
2. Medical Laboratory Scientist, Department of Chemical Pathology, University College Hospital, Ibadan, Nigeria.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. S.O. Banjoko,
Department of Chemical Pathology, College of Health Sciences, Obafemi Awolowo University Ile-Ife, Nigeria.
Fax: +1 270 717 5845, E-mail: bosunbanjoko@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 12, 2013**
Date of Peer Review: **Jul 01, 2013**
Date of Acceptance: **Sep 17, 2013**
Date of Online Ahead of Print: **Oct 25, 2013**
Date of Publishing: **Nov 10, 2013**