

Correlation of Semen pH with other Semen Parameters in a Sub fertile male Population Attending a Tertiary ART center in South India.

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Abstract- Purpose: To determine the clinical relevance of determining the semen pH during semen analysis as per WHO (World Health Organisation) standard criteria among the infertile couple attending a tertiary ART (Assisted Reproductive Technology) Centre in South India.

Methods: Regular Semen analysis as per WHO standards (2010)

Results: The Semen pH value showed no major correlation with other Semen parameters and also varied greatly from the given WHO standard.

Conclusion: The semen pH determined during semen analysis is found to have not much relevance for clinical practice. The clinical relevance of semen pH for Azoospermia patient needs to be revised.

Index Terms- Semen pH, WHO standard Criteria, South Indian Population, other Semen Parameters.

I. INTRODUCTION

Infertility affects up to 15% of reproductive-aged couples worldwide. Statistics show that about one third of infertility problems are related to women, one third to men, and one third are a mixture of both male and female related infertility issues [1].

Male infertility refers to the inability of a male to achieve a pregnancy in a fertile female [2]. Data available over the past twenty years reveal that in approximately 30% of cases pathology is found in the man alone, and in another 20% both the man and woman are abnormal [3]. Therefore, the male factor is at least partly responsible in about 50% of infertile couples [4,5]. Male infertility is caused by various reasons like sperm abnormalities, Varicocele, immunologic factors causing anti-sperm antibody formation, ejaculatory duct obstruction, hormonal imbalance, Azoospermia Factor micro deletions and chromosomal aberrations and environmental factors. The cornerstone of the evaluation of an infertile man is a careful history and physical examination. The history may include details on specific childhood illnesses, a detailed history of exposure to occupational and environmental toxins, cancer chemotherapy, the drug history, illicit drugs and excessive alcohol consumption and previous surgical procedures. This is followed by a blood work for profiling of the hormonal levels. Another important predictive indicator of male fertility is Semen analysis. From a sample of semen routinely obtained through

masturbation into a sterilized container, the physician will be able to assess factors such Volume of the semen sample, Liquefaction time, Semen pH, Viscosity, Sperm count, Sperm morphology, Sperm motility, White blood cell count in semen and Fructose level as per the respective lab standards that help or hinder conception. Various studies show that 30% of men with a normal semen analysis actually have abnormal sperm function [6]. Conversely, men with poor semen analysis results may go on to father children [7].

Semen Analysis is considered to be most accurate while using The World Health Organization Laboratory Manual for Examination of Human Semen and Semen-Cervical Mucus Interactions as the standard criteria. Each factor is briefly described in the laboratory manual stating the lower reference limit and higher reference limit.

Volume of the semen is measured to rule out the possibility of inflammation or blockage. Low semen volume is characteristic of obstruction of the ejaculatory duct or Congenital Bilateral Absence of the Vas Deferens (CBAVD) a condition in which the seminal vesicles are also poorly developed. High semen volume may indicate inflammation of accessory glands [8,9,10,11].

The total number of spermatozoa – “sperm number” and the number of spermatozoa per unit volume of semen – “sperm concentration” is related to both time to pregnancy and pregnancy rates and are predictors to conception [12, 13, 14, 15, 16]. For normal ejaculates, the sperm number is a measure of the capability of the testes to produce spermatozoa and the patency of the male tract while sperm concentration is not a specific measure of testicular [17, 18, 19, 20, 21]. The sperm concentration may be related to fertilization and pregnancy rates though [22]. Based on the sperm number and concentration the male infertility is classified into Oligozoospermia, Aspermia, Hypospermia, Azoospermia, Teratospermia and Asthenozoospermia.

Sperm motility and sperm morphology helps us to identify the potentially fertilizing subpopulation of spermatozoa which may result in viable pregnancy. Based on the sperm motility the sperms are classified into those with progressive motility, non-progressive motility and immotile sperms. The sperm morphology identifies the structural defects of the sperm and is classified into head defects, mid piece defects, tail defects or multiple defects in a sperm.

Semen pH is considered an essential parameter during analysis because abnormal semen pH may indicate any infections

or blockage of seminal vesicles. WHO criteria specify the normal pH range as 7.2 to 8.0. Deviation from this range may be an indication of inflammation of the male accessory sex organs or chronic disease of the prostate gland and seminal vesicles. Prostatic secretion is acidic while seminal vesicles fluid is alkaline. Acidic ejaculate may be associated with the blockage of the seminal vesicles [8,9,10,11]. Alkaline ejaculate is usually associated with infections that impair fertilization invitro and invivo. In general a pH value outside the range is harmful to sperms.

In a study conducted by Haugen TB *et al.* in Norway, it was noticed that the pH range of the Norwegian population was well above the range given by the WHO criteria. From our semen analysis reports, we also observed similar pH range variations, which are discussed in brief. From our study we observed that semen pH has no major relevance to other semen parameters as it had no uniform correlation with all the other semen parameters.

II. MATERIALS AND METHODS

All evaluations are done according to WHO laboratory manual, 2010 standards.

Sample collection: Samples were collected in-house, where a separate facility is present for sample collection. A clean sterile, non toxic and clearly labeled container was given to the patient and the instructions as per WHO standards were given in writing. Samples of those patients with 2-7 days of abstinence were only considered for evaluation.

Initial Macroscopic Evaluation:

Liquefaction and Viscosity: The samples were given a time of 15-60 min for liquefaction. If the sample is highly viscous, mechanical pipetting was done using a wide bore syringe which has a diameter of approximately 1.5mm and if the sample still remained viscous it was diluted using sperm wash media. The sample was mixed well but gently before using it for analysis. A completely liquefied and thoroughly mixed sample is essential to obtain accurate observations.

Volume and Appearance: Volume was measured using a modified graduated cylinder with a wide mouth. 1.5ml of volume sample was regarded as lower reference limit according to WHO standard criteria. The colour was noted manually [24].

pH: The pH was measured using pH paper as per WHO manual. A drop of the thoroughly mixed sample was spread evenly on a strip of the pH paper. The pH strip was compared with the calibration strip as soon as the colour of the impregnated zone became uniform.

Initial Microscopic Investigations:

Sperm Concentration: Concentration of the Sperms was counted using Neubauer chamber. The counting was done according to the directions indicated in the WHO manual – 5th edition.

Sperm Motility: Motility was observed on a glass slide according to the WHO standards. A wet preparation 20µm deep was prepared and the slide was examined with a phase contrast optics (Magnus) at ×200 or ×400 magnification.

Sperm Morphology: A smear of semen was prepared on the glass slide and was air dried. Diff-Quik staining was done

according to the WHO laboratory manual – 5th edition and the morphology was observed in a ×100 oil immersion bright field objective with a ×10 magnification.

Agglutination: Agglutination was also observed from smears according to WHO criteria.

III. RESULTS

During the study period which ranges from the year 2011 to June 2013, we analyzed the semen samples of 150 men who attended the infertility clinic. We found no sample in the normal criteria range and each sample had atleast one abnormal parameter according to WHO standards. The parameters in comparison with the pH values are discussed in detail.

The overall study observed that maximum numbers of samples were in the pH range 8-9 and some samples were in the pH range 7-8. Among the samples obtained from the sub fertile population attending our centre in South India, 150 samples were included in this study. Out of this 54% samples lie in the pH range 8-9, 40% samples in pH range 7-8, 2% lie in the acidic pH range and 4% lie in the alkaline pH range. (Fig.1)

Semen pH vs. Motility of sperms

From the graph (Fig.2, 3) we may observe that the number of samples with more motile sperms are in the pH range 8-9. According to WHO standards the given pH range is 7-8 where only 83% samples have optimum motility whereas in the pH range 8-9 we may observe 94% of the samples with good sperm motility.

Semen pH vs. Morphology of the sperms

When we observe the graph for morphology (Fig. 4, 5, 6, 7) it is obvious and contradictory that almost equal distribution of defects in both the pH range. When the head defects in the pH range are studied, we find 83% defects in pH range 7-8 but in the pH range 8-9 the head defects are 86%. Same is observed with other defects also, where, in mid piece defects 83% is observed in pH range 7-8 and 88% in pH range 8-9. Tail defects also show similar trends with 84% of defects in pH range 7-8 and 89% in pH range 8-9. Multiple defects are of the same intensity with 84% defects in both the pH range 7-8 and 8-9.

Semen pH vs. Volume of the Semen sample

Volume of the semen sample has no defined higher reference criteria, so by taking 1.5 ml as the lower reference value, as per WHO criteria, the following were observed. In the pH range 7-8, 69% of the samples have more than 1.5ml volume of semen whereas in the pH range 8-9 64% samples have normal semen volume. But in the pH range 7-8, 32% has low semen value and 30% samples in pH range 8-9 have low semen value. Here we find no major difference in values among both the pH range.

Semen pH vs. Viscosity of the Semen sample

When the viscosity is compared with the pH we may see that the normal sample number is higher in the pH range 8-9 where only 1.3% samples are highly viscous in this pH range. Whereas 5% of samples are highly viscous in the pH range 7-8. We found no important correlation of pH with infection which is

determined by counting the number of leukocytes and agglutination.

IV. DISCUSSION

Male infertility caused by impaired semen quality is an enormous problem for the infertile couple as well as the Andrologist. Semen ejaculate is contributed by seminal vesicles and prostate gland along with a small amount from bulbourethral glands and epididymis. The pH of the semen reflects a balance between the pH of different accessory gland secretions, mainly alkaline seminal vesicles secretion and the acidic prostatic secretion [25].

The WHO standard criteria have given a lower reference limit of pH 7. In our study conducted on 150 sub fertile men attending an infertility centre in south India, we noticed that the pH range of maximum number of samples vary from pH 8- pH 9. Similar findings were also observed in the Norwegian population in a study conducted by Haugen *et al.* It was also noticed that similar Y chromosome haplotype existed between Norwegians and Dravidians and North Indians [26,27,28].

Semen pH vs. Motility of sperms

To achieve fertilization in vivo it is essential that the sperms are of good quality i.e. more than 40% must be fast progressive motile sperms[25]. When the sperms under this study were observed against their respective semen pH, it was observed that higher numbers of motile sperms were seen in semen samples with pH8- pH9.

Semen pH vs. Morphology of the sperms

It is essential to screen the morphology of the sperms to assess the prognosis of fertility. WHO standard criteria states that, the over all percentage of normal morphology for both infertile and fertile men is likely to be 0% – 30%, with few samples exceeding 25% normal spermatozoa. Sperm morphology includes analysis for head defects, tail defects, midpiece defects and multiple defects. We found no major correlation of pH with sperm morphology.

Semen pH vs. Viscosity of the Semen sample

Increased viscosity is the result of prostatic infection and seminal vasculitis [25]. It is difficult to accurately analyze highly viscous samples. In our study we noticed that as the pH increases the number of viscous samples tends to decrease. It was observed that the samples were less viscous in the pH range 8-9.

Semen pH vs. Agglutination of the sperms

When there is a case of non-specific infection, we may find agglutinates and presence of many leukocytes, predominantly neutrophils, and it is also associated with poor sperm quality. If more leukocytes are found further biochemical assays are suggested, but absence of leukocytes do not rule out the possibility of infection. In our study we noticed that pH does not influence the presence or absence of leukocytes and similarly we found no correlation of pH with agglutination in our study.

Semen pH vs. Volume of the Semen sample

When pH is compared with the semen analysis parameter-volume of the sample, we find no significant correlation. The volume of the semen sample is higher in the samples with pH range 7-8. When we consider the overall study, other semen parameters are not in the standard reference limit as per WHO, in the pH range 7-8. Low semen values may be indications of ejaculatory duct obstruction or congenital bilateral absence of the vas deferens. Whereas high semen volume may be due to active exudation from inflammation of accessory glands. In this case the higher reference limit is not clearly described.

Semen pH and Azoospermia

In the evaluation of patients with Azoospermia, the semen volume and pH are important for determining the deferential. When the volume of semen is low and has acidic pH, it may reflect cases of Congenital Bilateral Absence of the Vas Deferens (CBAVD) and Ejaculatory Duct Obstruction (EDO). If the semen samples have normal volume and alkaline pH then the ejaculatory ducts are patent. In cases where semen has alkaline pH and low volume ejaculate, the semen vesicles are present and functional but need further clinical diagnosis.

WHO standard Laboratory manual comments that if pH is less than 7 in low volume semen sample and low sperm number, there may be ejaculatory duct obstruction or congenital bilateral absence of vas deferens, but high pH values may provide little clinically useful information. From our observations we infer that pH may not play a significant role during semen analysis. We emphasize on more studies to evaluate the clinical importance of pH during semen analysis. We do not suggest that measuring of pH be stopped completely but suggest revising its clinical importance since no interventions are based on semen pH. In Andrology laboratories where Semen Analysis is routinely done for large number of samples, avoiding the pH analysis if it is irrelevant may save a lot of time and manpower which can be efficiently used in relevant analysis. And also an important observation that was found is that the Semen pH value is slightly higher than the mentioned WHO standard criteria, among the South Indian population we observed.

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Overall distribution

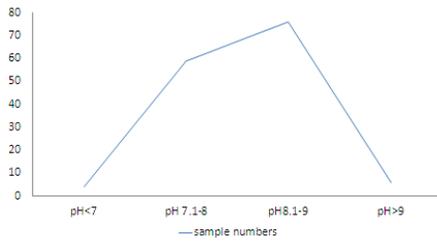


Fig 1: Distribution of all samples according to their pH

pH vs motility

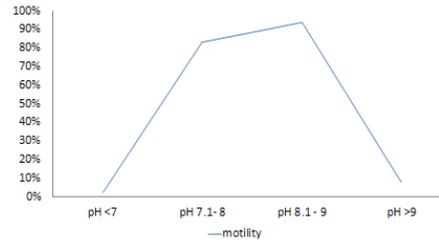


Fig 2: Distribution of samples based on their sperm motility in each pH range

pH Vs immotility

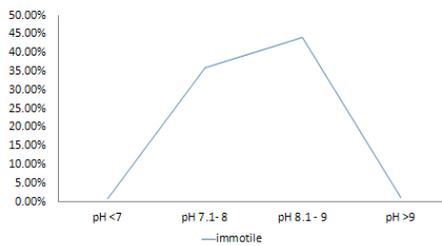


Fig 3: Distribution of samples based on their sperm immotility in each pH range

pH Vs morphology

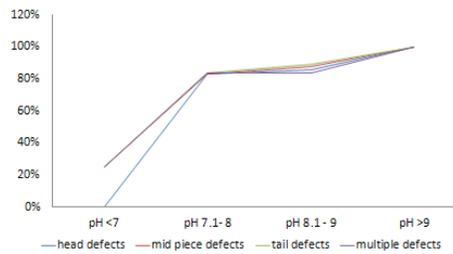


Fig 4: Distribution of samples based on their sperm morphology in each pH range

pH vs Volume of Semen Sample

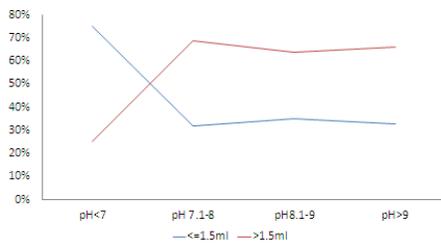


Fig 5: Distribution of samples based on their volume in each pH range

pH Vs Viscosity

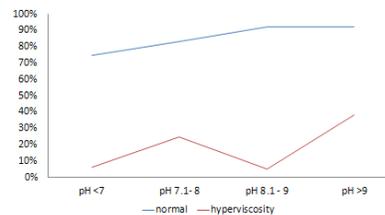


Fig 6: Distribution of samples based on their viscosity in each pH range