International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Relation of Semen pH with Sperm Count & Motility

Dr. Archi Ghanti[1], Dr. KM Kundavi Shankar[2], Dr. Yamini Asokan[3], Dr. Geetha V[4], Dr. Rashmi GV[5], Dr. Nithya M Naaram[6], Hemaniveda KR[7]

[1, 2, 3, 4, 5, 6, 7]Institute of Reproductive Medicine & Women's Health, Madras Medical Mission Hospital, 4A, Dr JJ Nagar, Mogappair East, Chennai-600037, India

[1]Corresponding Author Email: *archi.gbk.275[at]gmail.com*
Contact no.- (+91)8918654743

Abstract: ***Introduction: Seminal pH is one of the most important parameters that predict semen quality. The aim of our study was to determine a relation of Semen pH with Sperm Count andmotility in men presenting with subfertility. Methodology: This was a retrospective observational study done at theInstitute of Reproductive Medicine (IRM), Madras Medical Mission Hospital during October 2021- September 2022, for all male patients who came to our OPD for evaluation of subfertility & underwent semen analysis for the 1st time. Our sample size was 306. Out of 306 patients, subjects were classified in 3 groups: Group 1 (pH 7.2-8) had 231 patients (75%), Group 2 (pH<7.2) had 39 patients (13%), & Group 3 (pH>8) had 36 patients (12%). Results & Analysis: Sperm count, total motility, progressive motility & slowly progressive motility were positively associated with pH group 1(pH 7.2-8). Although sperm parameters were suboptimal in both group 2 (pH<7.2)& group 3 (pH>8) compared to group 1 (pH 7.2-8), but total motility & slow progressive motility were relatively better in group 2 (pH<7.2). Conclusion: This study suggested that optimal pH is necessary for normal semen parameters (count, concentration & motility). This study found that semen parameters deteriorate when pH goes below 7.2 & beyond 8, suggesting that pH range of 7.2 – 8 is where sperm parameters remain within their normal limits.***

Keywords: Sperm, Semen pH, sperm count, sperm motility

1. Introduction

Sperm is the only human cell that is supposed to perform its function outside the male body. The microenvironment of sperm, i.e. seminal plasma, is very important – it is a mixture of secretions from testes, epididymis & accessory sex glands. Seminal plasma contains bicarbonate ion, multiple inorganic ions, organic acids, sugars, lipids, steroids, amino acids, polyamines, nitrogenous bases and proteins[1]. So seminal plasma has a very high buffering capacity, which is designed to maintain an optimal pH normally. Therefore, the pH of the seminal fluid may play a significant role not only in maintaining the viability and quality of the sperm, but also in ensuring fertilization.[1]

Wang et al recently published an article on the '*Evolution of the WHO Semen processing manual from the first (1980) to the sixth edition (2021)*'.[2] World Health Organization (WHO) *Laboratory Manual for the Examination and Processing of Human Semen* was first published in 1980 to standardize the procedures for the examination of human semen.[2] This WHO semen manual has undergone five revisions and is constantly updated in response to the growing global needs in andrology and reproductive medicine and to standardize procedures for the examination of human semen.[2]

The first edition, published in 1980, was called Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. It was based on 33 participants, and its main objective was to provide laboratory procedures for semen analysis that are standardized, precise, reproducible, sensitive, and validated. The second edition of WHO Lab manual (1987) first defined the criteria for "normality" of semen samples.[2] This edition stated the upper limit of normal human pH to be 7.8.[2] The third edition of WHO manual, published in 1992, introduced quality control of semen analysis. It stated normal values for pH in liquefied semen to be 7.2 - 8.0 according to Haugen et al.[3] The fourth edition was published in 1999, and it stated the normal pH of semen to be 7.2 or more.[4]

WHO published the Laboratory Manual for the Examination and Processing of Human Semen (5th edition) in 2010. It mentioned "normal" semen pH to be 7.2 to 8. The reference in 5th edition was derived from fertile men whose partners had time to conceive < 12 months, - 1959 men from 8 countries across 4 continents.[5] But after publication of 5th edition, there were concerns regarding reference ranges because of voluntary nature of inclusion of population cohorts and over & under representation of some areas of World. The 6th edition (July, 2021) aims to amend the problem by incorporating 1789 new fertile men (total 3589 subjects–1800 subjects from 5th edition + 1789 new), from areas which were under-represented previously. WHO laboratory parameters manual for human semen, 6th edition (2021) considers seminal pH≥7.2 to be considered normal.[6]

A lot of studies have been done to understand the effects of various semen& systemic parameters on sperm characteristics. But the effect of semen pH on sperm parameters is an understudied aspect. So, we designed a study to understand whether & to what extent semen pH affects sperm parameters like sperm count & motility. This may in turn help to establish optimal pH levels in our current clinical setting.

2. Aim of study

To determine relation of Semen pH levels with Sperm Count and Motility in males presenting with subfertility

3. Review of Literature

Zhou et al [1] conducted a study to find how semen pH affects sperm motility & capacitation. They found that sperm motility & intracellular Ca^{2+} concentration were significantly reduced in spermatozoa that were cultured in acidic medium. But motility was not affected in alkaline medium (pH=8.2).

Dhumal SS [7] et al conducted a study to find correlation of semen pH with motility & count. Their study found the mean pH to be 8.4 (±0.3), with range from 6.9 to 9.5. This study found a significant & positive correlation between total motility & pH, volume & pH, sluggish progressive motility & pH, and abstinence & pH. But a negative correlation was noted between pH & total count, rapidly progressive motility, and non-motile spermatozoa.

Natarajamani et al [8] conducted a study among infertile couple attending a tertiary ART centre in south India & found that the Semen pH showed no major correlation with other Semen parameters and also varied greatly from the given WHO standard. They found that the semen pH determined during semen analysis is devoid of much relevance for clinical practice.

Harraway et al [9] found that mean semen pH was 8.2 (±0.3) with range of 7.3 – 9.5, among 1199 semen records between January 1994 & December 1998. And also mean pH value of group of patients whose semen achieved clinical pregnancy after IUI, was 8.3 (±0.3). The mean age of this study population was 36.2 (±6.3) years.

4. Methodology

A **retrospective analytical study** was done at the Institute of Reproductive Medicine (IRM), Madras Medical Mission Hospital, over a period of**1** Year (October 2021- September 2022).All male patients who came to our OPD for evaluation of subfertility & underwent semen analysis for the 1st time, were **included** in our study. Patients with aspermia&those on current/previous medical treatment for male infertility were excluded from our study.We got a sample size of **306**patients after meeting inclusion & exclusion criteria. Semen samples were collected after 2-7 days of sexual abstinence and examined following 30 min of liquefaction at 37°C, in the same laboratory, by the same andrologist. In our study, **pH test strips in the range 6.0-10.0** were used.

We plotted individual values of sperm count, concentration & motility values against sperm pH, and we found **an interesting & repetitive pattern,** that sperm parameters begin to deteriorate when semen pH goes beyond 8. So we decided to divide our data set into **3 such groups** as per pH:- **GROUP I**: pH-7.2-8, **GROUP 2**: pH<7.2 & **GROUP 3**: pH>8.

5. Results & Analysis

We used SPSS version 26.0 for data analysis, and ANOVA, regression analysis & Kruskal-Wallis test for statistical analysis. Our study population was divided into 3 groups: - Group 1 (pH 7.2-8) – 231 patients, Group 2 (pH<7.2) – 39 patients & Group 3 (pH>8) – 36 patients.Population distribution of 3 groups were as follows -

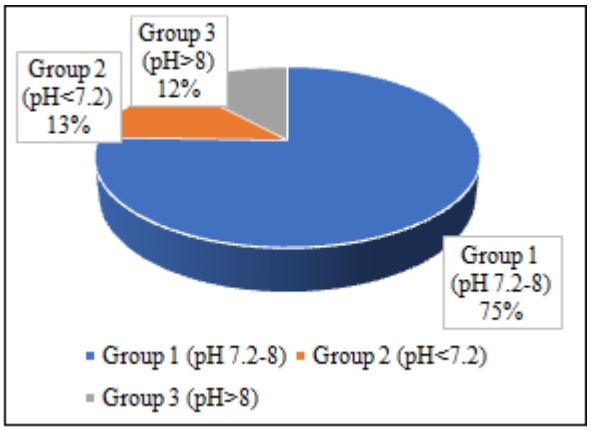

Figure 1: Distribution of study population among pH groups

The demographic parameters among three groups are described below:

Table 1: Demographic & Associated Characteristics amongst Three Groups

GROUP	AGE (Years)	BMI (Kg/m^2)	Abstinence Duration (Days)	Time of Assessment (Minutes)
Group 1 (pH 7.2 – 8)	34.00 ± 2.77	29.06 ±3.44	4.12 ± 1.05	32.00 ± 12.04
Group 2 (pH<7,2)	34.00 ± 3.03	29.73 ± 3.18	4 ± 1.56	31.10 ± 11.06
Group 3 (pH >8)	35.00 ± 3.41	28.36 ± 3.32	4.42 ± 1.79	32.36 ± 12.23

Difference in distribution of demographic parameters among pH groups **was not statistically significant (p>0.05).**
pH of the study subjects was in the **range from 6.4-9** with a **mean of 7.64** and **SD of ± 0.46.**

1) Macroscopic Analysis:

a) **Semen Volume:**

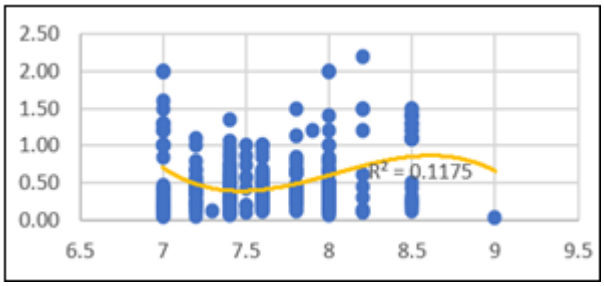

Figure 2: Semen volume vs pH

The mean & SD of semen volume in group 1 (pH 7.2-8), group 2 (pH<7.2) & group 3 (pH>8) were 2.22 ± 1.10, 1.75 ± 0.73 & 2.13 ± 1.97 mL respectively. Semen volume was

not statistically significant between the three groups (p=0.39).

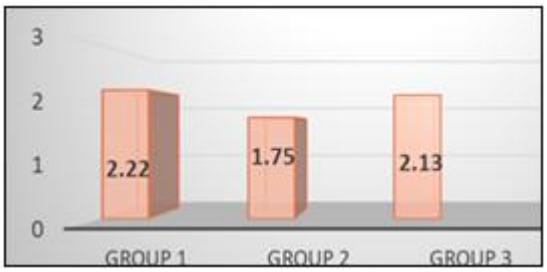

Figure 3: Semen volume distribution across pH groups

2) Microscopic Analysis

a) Sperm Count:

Sperm motility parameters were plotted against semen pH in scatter plot.

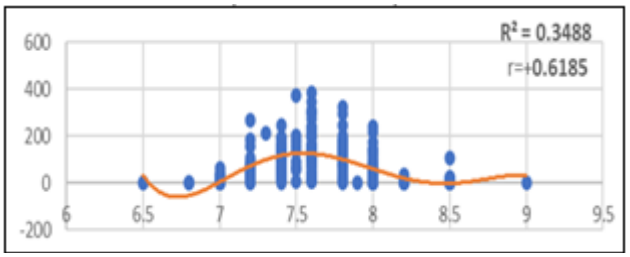

Figure 4: Sperm count vs pH

Sperm count showed a **non-linear regression** line which is **inverted U shaped** (because it **decreased both below pH 7.2 & above 8).** Then sperm count was compared between the three groups. Mean & SD of sperm count in group 1 (pH 7.2-8), group 2 (pH<7.2) & group 3 (pH>8) were 93.39 ± 79.72, 10.52 ± 14.74 & 7.13 ± 18.80 Million/ejaculate. Sperm count was statistically significantly higher in group 1 compared to other groups (p<0.01). But sperm count was not significantly different between group 2 & group 3 (p>0.05).

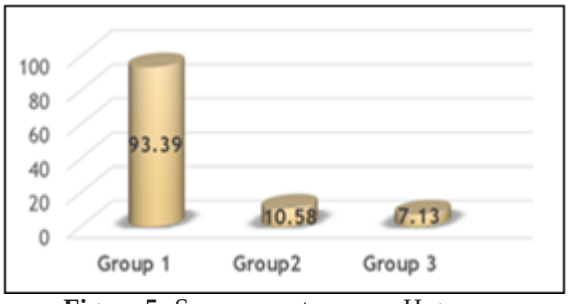

Figure 5: Sperm count across pH groups

Similarly for sperm concentration, the mean & SD in group 1 (pH 7.2-8), group 2 (pH<7.2) & group 3 (pH>8) were 42.61 ± 29.17, 5.9 ± 8.48 & 3.92 ± 11.99 Million/ml respectively.

Sperm concentration showed **higher statistical significance in group 1** compared to other groups (p<0.01). But it was not significantly different between group 2 & 3 (p>0.05).

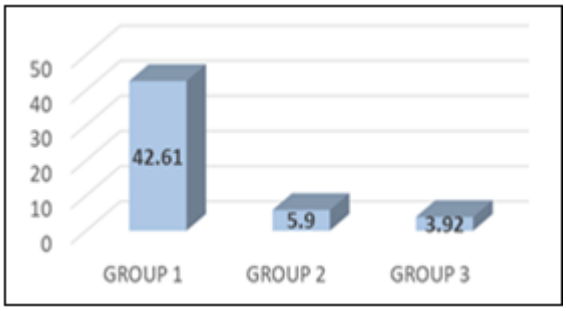

Figure 6: Sperm concentration across pH groups

a) Sperm Motility

Sperm motility parameters were plotted against semen pH in scatter plot.

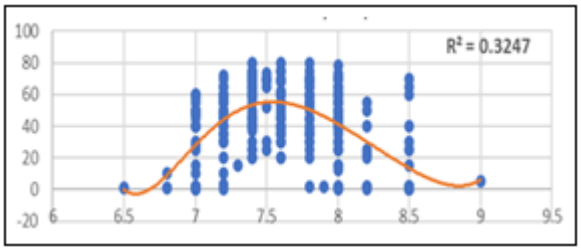

Figure 7: Total motility vs semen pH

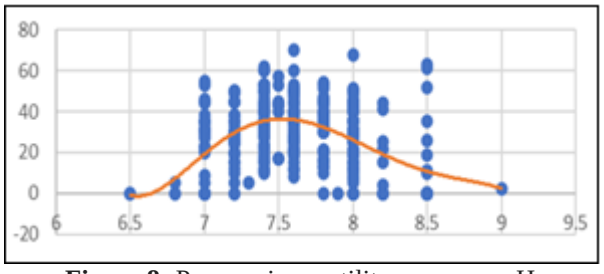

Figure 8: Progressive motility vs semen pH

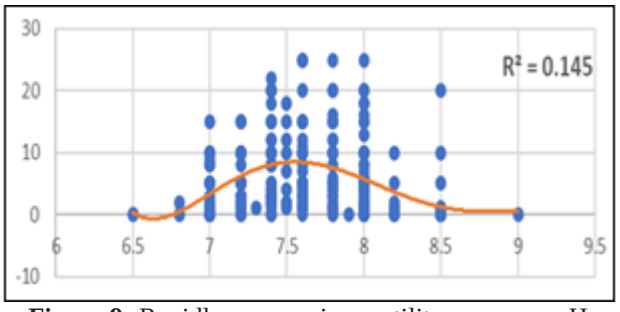

Figure 9: Rapidly progressive motility vs semen pH

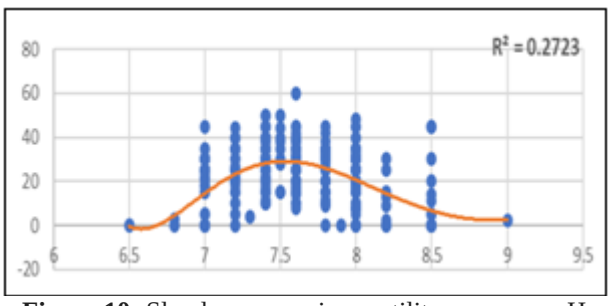

Figure 10: Slowly progressive motility vs semen pH

All plots showed a **non-linear regression** line which is **inverted U shaped**. This is because most of the normal parameters of motility in our study were found in the pH range of 7.2-8 & **decrease both below 7.2 & above 8.**

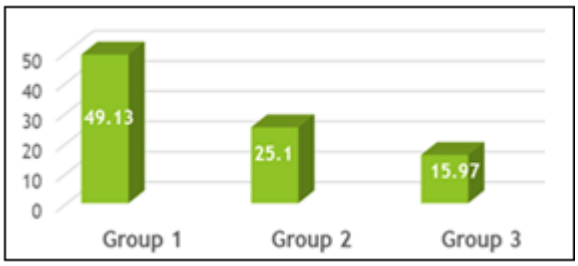

Figure 11: Total motility vs semen pH

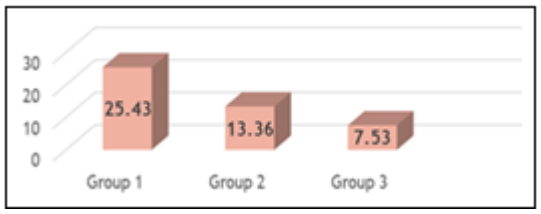

Figure 12: Slow progressive motility vs semen pH

The mean & SD of **total motility** in group 1 (pH 7.2-8), group 2 (pH<7.2) & group 3 (pH>8) were 49.13 ± 19.18 %, 25.10 ± 23.24 %& 15.97 ± 21.53% respectively. Total motility was statistically significantly **higher in group 1** compared to other groups (p<0.01). Total motility was also significantly **higher in group 2 than in group 3 (p<0.05).**

The mean & SD of slow progressive motility in group 1 (pH 7.2-8), group 2 (pH<7.2) & group 3 (pH>8) were 25.43 ± 11.84%, 13.36 ± 13.55%& 7.53 ± 12.74% respectively. Slowly progressive motility was statistically significantly **higher in group 1** compared to other groups (p<0.01). Slowly progressive motility was also significantly **higher in group 2 than in group 3 (p<0.05).**

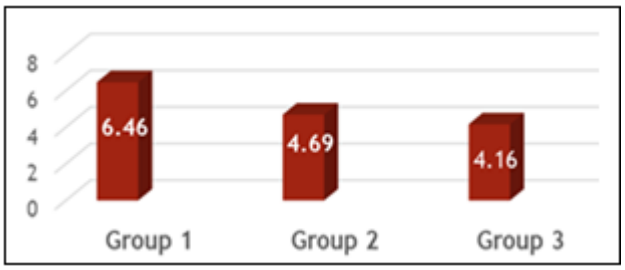

Figure 13: Rapid progressive motility vs semen pH

The mean & SD of rapid progressive motility in group 1 (pH 7.2-8), group 2 (pH<7.2) & group 3 (pH>8) were 6.46 ± 5.22%, 4.69 ± 5.52%& 4.16 ± 6.61% respectively. Percentage of rapid progressive motility showed no statistical significant difference between the three groups (p=0.78).

Table 2: Sperm Parameters amongst different pH groups

PARAMETERS	Group 1(pH 7.2 – 8)	Group 2 (pH<7.2)	Group 3 (pH>8)	p Value
Sperm count (Million/ejaculate)	93.39 ±79.72	10.58 ±14.74	7.13 ±18.80	<0.01
Sperm concentration (Million/mL)	42.61 ±29.17	5.90 ±8.48	3.92 ±11.99	<0.01
Semen volume (mL)	2.22 ±1.10	1.75 ±0.73	2.13 ±1.97	0.39
Total motility (%)	49.13 ±19.18	29.10 ±23.24	15.97 ±21.53	<0.01
Slow progressive motility (%)	25.43 ±11.84	13.36 ±13.55	7.53 ±12.74	<0.01
Rapid progressive motility (%)	6.46 ±5.22	4.69 ±5.52	4.16 ±6.61	0.78

6. Discussion

It was found in our study that sperm count, total motility, progressive motility & slowly progressive motility are **Strongly Positively Associated** with **group 1 (pH group-7.2-8).**

Although sperm parameters are significantly worse in both group 2 (pH range <7.2) & group 3 (pH range >8) compared to group 1 (pH 7.2 – 8),but total motility & slow progressive motility were relatively better in group 2(pH range 6.4 - <7.2) than group 3 (pH range >8)(p<0.05).

An acidic ejaculate with pH less than 7.2 may be an indication of blockage of seminal vesicles, while that with an alkaline pH of above 8.0 is usually associated with infections.[5]Like all living cells, proper functioning of sperms is also dependent on optimal pH in its environment, so that the ion channels on sperm plasma membrane (e.g. CatSper) can function properly and maintain a homeostatic milieu inside sperms& maintain optimal intracellular Ca^{2+} ion concentration.[10]

WHO laboratory manual 2021 (6th edition) [6] has stated that normal semen pH is >7.2 & acidic pH is abnormal & is detrimental to semen parameters. There is no mention of any upper limit of semen pH. But our study has suggested that semen parameters deteriorate when semen pH goes above 8 (Group 3).

Zhou et al [1] found sperm motility & intracellular Ca^{2+} concentration to be significantly reduced in spermatozoa cultured in acidic medium, but not in alkaline medium. The reduced activity of Na/K ATPase pump in acidic pH (<7.2) was responsible for reduced intracellular Ca^{2+} level that reduced motility as well as ability for capacitation. But our study found sperm count & motility are best within pH of 7.2 – 8 & deteriorate both in acidic (pH<7.2) & alkaline (pH>8) media.

The study by **Dhumal SS et al**[7]suggested positive correlation between semen pH & volume, total motility & slowly progressive motility, but negative correlations with rapidly progressive motility & count. Our study found count, total motility & slowly progressive motility to be highest within pH range of 7.2 – 8, whereas semen volume & rapidly progressive motility were not found to be affected by semen pH.

Study by **Natarajamani et al**[8]in South India found that semen pH did not significantly affect sperm parameters, but they found somewhat better outcomes in alkaline than normal and acidic groups but none was statistically significant. They concluded that the semen pH determined during semen analysis does not appear to have much

relevance for clinical practice. They postulated sperm parameters are not influenced by semen pH. Butour study has found best parameters at pH 7.2-8.

The study by **Harraway et al**[9]in Baltimore, USA, found that semen parameters (sperm count, conc, motility) were not different significantly between "normal" & "abnormal" semen pH group, and semen samples with alkaline pH (8.3 ± 0.3)achieved more successful pregnancies after IUI. The mean age of this study population was 36.2 (±6.3) years. This study justified that seminal fluid pH may become more basic with advancing age & with past history of infection, and the mean pH of the entire study population was 8.2 (±0.3) in this study. But our study not only observed sperm parameters to deteriorate after pH goes beyond 8, but also found that sperm parameters in this alkaline pH group is even worse than in acidic (pH<7.2) group.

7. Conclusion

This study suggested that optimal pH is necessary for normal semen parameters (count, concentration& motility). This study found that semen parameters deteriorate when pH goes below 7.2 & beyond 8, suggesting that pH range of 7.2 – 8 is where sperm parameters remain within their normal limits.

The limitations of this study are that it is a retrospective study & of a relatively shorter duration & with a small sample size. Hence, a larger prospective study of longer duration is warranted for further evaluation of the optimal pH range for good sperm quality.

References

[1] Zhou J, Chen L, Li J, Li H, Hong Z, Xie M, Chen S, Yao B. The Semen pH Affects Sperm Motility and Capacitation. PLoS One. 2015 Jul 14;10(7):e0132974. doi: 10.1371/journal.pone.0132974. PMID: 26173069; PMCID: PMC4501804.

[2] Wang C, Mbizvo M, Festin MP, Björndahl L, Toskin I; other Editorial Board Members of the WHO Laboratory Manual for the Examination and Processing of Human Semen. Evolution of the WHO "Semen" processing manual from the first (1980) to the sixth edition (2021). Fertil Steril. 2022 Feb;117(2):237-245. doi: 10.1016/j.fertnstert.2021.11.037. Epub 2022 Jan 5. PMID: 34996596; PMCID: PMC8842884.

[3] Haugen TB, Grotmol T. pH of human semen. Int J Androl. 1998 Apr;21(2):105-8. doi: 10.1046/j.1365-2605.1998.00108.x. PMID: 9675619.

[4] WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction - fourth edition (1999).

[5] *WHO Laboratory Manual for the Examination and Processing of Human Semen* – Fifth edition (2010)

[6] World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen* – Sixth edition (2021)

[7] Dhumal SS, Naik P, Dakshinamurthy S, Sullia K. Semen pH and its correlation with motility and count - A study in subfertile men. JBRA Assist Reprod. 2021 Apr 27;25(2):172-175. doi: 10.5935/1518-0557.20200080. PMID: 33507718; PMCID: PMC8083857.

[8] Natarajamani, Sasikala & Medsci, M & Moorthy, Janani & Tech, M & Subramanian, Mahalakshmi & Manikere, Archana. (2014). Correlation of Semen pH with other Semen Parameters in a Sub fertile male Population Attending a Tertiary ART center in South India. International Journal of Scientific and Research Publications, Volume 4, Issue 8, August 2014 1 ISSN 2250-3153. 4. 1-2250.

[9] Harraway C, Berger NG, Dubin NH. Semen pH in patients with normal versus abnormal sperm characteristics. Am J Obstet Gynecol. 2000 May;182(5):1045-7. doi: 10.1067/mob.2000.106051. PMID: 10819822.

[10] Wang, H., McGoldrick, L.L. & Chung, JJ. Sperm ion channels and transporters in male fertility and infertility. *Nat Rev Urol* **18**, 46–66 (2021). https://doi.org/10.1038/s41585-020-00390-9.