ORIGINAL RESEARCH PAPER

Anatomy

KEY WORDS: Infertility, Semen analysis, Sperm morphology, Geimsa stain, Papanicolaou stain.

COMPARATIVE STUDY OF GEIMSA AND PAPANICOLAOU STAIN EFFICACY FOR DEMONSTRATION OF NUCLEAR AND CYTOPLASMIC STAINING FOR ASSESSMENT OF HUMAN SPERMATOZOA MORPHOLOGY.

Shivani Chawla M.Sc. (Med.) Anatomy, Department of Anatomy S. P. Medical College, Bikaner, Rajasthan, India.

Dr. Kavita Pahuja Professor, Department of Anatomy, S. P. Medical college, Bikaner, Rajasthan, India.

Dr. Jaskaran Singh* Assistant Professor, Dept of Anatomy, S. P. Medical college, Bikaner, Rajasthan, India. *Corresponding Author

ABSTRACT

Introduction: Human sperm show large variation in sperm morphology. The morphology seen with the microscope is not the true morphology of living spermatozoones, but an image we create. This image comprises a number of factors: smearing technique, fixation, staining, mounting and the optics and illumination used. It is of great importance that the preparations (smearing and staining) are of high quality when assessing sperm morphology.

Aim: Our study aimed to assess the efficacy of Geimsa and Papanicolaou staining for demonstration of nuclear and cytoplasmic staining for assessment of human spermatozoa morphology.

Material and Methods: The Present study was conducted Department of Anatomy, S.P.Medical College, Bikaner, Rajasthan, India, to observe and compare of human spermatozoa morphology according to WHO criteria; we studied semen samples of 100 infertile males, collected by Private IVF Lab of Bikaner District, the Geimsa and Papanicolaou staining technique were used.

Results: In comparison of Geimsa and Papanicolaou nuclear staining and cytoplasmic staining, Geimsa stain showed deep staining whereas light staining obtained by Papanicolaou stain. Nuclear membrane integrity was shown smooth by Papanicolaou stain and rough by Geimsa stain with significantly positive correlation (p = 0.0001) and cytoplasmic transparency was high by Papanicolaou stain and low by Geimsa stain

Conclusion :- In Comparison the Papanicolaou Stain shows advantage and is less time consuming as compared to Geimsa stain.

INTRODUCTION :-

The World Health Organisation (WHO) evaluate 60-80 million couples currently experienced from infertility globally. Infertility alter in domain and is evaluate to 8 to 12% of couples globally[1]. The male has been relate to infertility factor as 40%–50%[2]. Male infertility is recognized as commonly due to deficiencies in the semen, and semen quality[3]. The exactment of sperm morphology estimation depends on careful smear preparation, fixation and staining since these procedures can affect the sperm dimensions[4]. The sperm need to be stained for a better estimation of morphology[5]. Papanicoloau stain should be performed for quantification of defects[6]. Papanicolaou gave better results as in stain nuclear chromatin well give good cytoplasmic transparency[7]. Giemsa stain was accomodate to histology from microbiology because of the high-quality staining of chromatin and nuclear membrane of all cells[8].

The present study was aimed to see the comparative efficacy of two stains (Giemsa stain and Papanicolaou stain) for study of nuclear and cytoplasmic staining for assessment of human spermatozoa morphology.

MATERIALS AND METHODS :-

Present study was a comparison study, Department of Anatomy, Sardar Patel Medical College & A.G. Hospital , Bikaner, Rajasthan. Total 100 semen samples were obtained from Private Labs of Bikaner with informed consent of the person.

- Inclusion and exclusion criteria were the healthy subjects are included in this study; subjects who have below the reproductive age group, Subjects who have any history of STDs (like hepatitis-B and HIV) and Subjects who have azoospermia are excluded.

For the manual evaluations, the preparation of slides, making of smears and staining procedures were performed according to standard methods (WHO strict criteria). A small drop of semen used so that a very thin smear results with 5-10 spermatozoa per visual field, at 100x oil immersion magnification. Slides are air-dried and fixed in ethanol before staining. This methodology of preparation of smear were repeated two times, one for the staining of freshly prepared Papanicoloau and one for the staining of freshly prepared Giemsa stain.

STATISTICAL ANALYSIS:-

chi^2 test was performed by using SPSS statistical software to find the statistical difference between normal and abnormal groups. A p value 0.05 is considered to be significant.

RESULTS:-

Table no.1 Distribution of nuclear staining of Human Spermatozoa as light and deep between both the stains.

Nuclear staining	Giemsa stain (%)	Pap stain (%)	P Value
Light Stained	12	59	0.0001
Deep Stained	88	41	

Table no.1, shows that each sample was analyzed for identification of morphology of human spermatozoa and other staining properties of the chosen both the stains. In our observation we have seen that the light nuclear staining is better for sperm morphology detection, because in light nuclear background the deeply stained sperm morphology was seen prominently. In comparison the Papanicolaou stain came out to be a better stain for sperm morphology from the rest of the chromatin matter of the nucleus. For comparison of both the stains for nuclear staining chi square test was performed and the result showed significant difference ***(P = 0.0001)*** between both the stains.

Table no.2 Distribution of nuclear membrane integrity of Human Spermatozoa as smooth and rough between both the stains.

Nuclear membrane integrity	Giemsa stain (%)	Pap stain (%)	P Value
Smooth	23	92	0.0001
Rough	77	8	

Table 2, shows the characteristics of nuclear membrane

integrity rendered by Geimsa stain and Papanicolaou stain. Both the stains preserve nuclear membrane integrity but in different proportion.In our observations we have seen that the preserved nuclear membrane integrity was seen smooth and less preserve nuclear membrane integrity seen rough, but smooth nuclear membrane integrity is better for sperm morphology detection, because in smooth nuclear membrane integrity the sperm morphology was seen easily to it internally. In comparison the Papanicolaou stain came out to be a better stain for sperm morphology differentiation from the rest of the nuclear membrane. For comparison of the both the stains for nuclear membrane integrity chi square test was performed and the results showed significant difference ***(P = 0.0001)*** between both the stains.

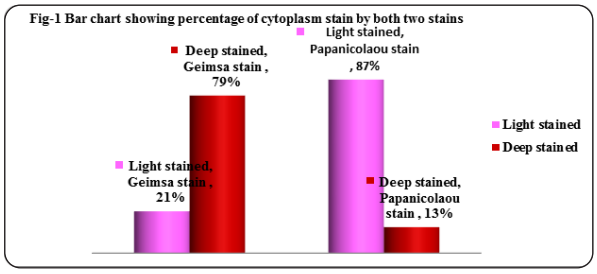

Fig-1, shows the characteristics of cytoplasmic staining performed by Geimsa stain and Papanicolaou stain. Geimsa stain gave violet staining to the cytoplasm and Papanicolaou stain gave eosinophilic colour to the cytoplasm, whereas both the stains gave cytoplasmic staining in different intensity. The Geimsa stain gave 79% of sperm's deep cytoplasm stain and 21% of cells light stain. For the Papanicolaou stain, it gave 13% of sperms deep stain and 87% of sperms light cytoplasm stain.

In our observation we have seen that the light cytoplasm staining is better for sperm morphology detection, because in light cytoplasm background the deeply stained sperm morphology was seen prominently. In comparison the Papanicolaou stain came out to be a better stain for sperm morphology differentiation from the rest of the cytoplasmic organelles. For comparison of both the stains for deep and light cytoplasm staining chi square test was performed and the results showed significant difference (P= 0.0001) between both the stains.

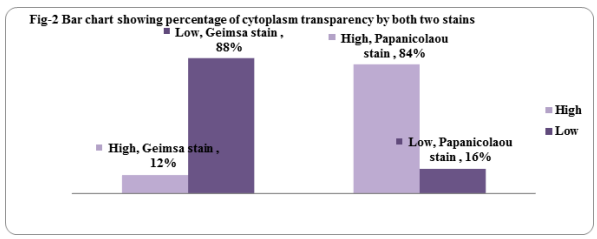

Fig-2, shows the characteristics of cytoplasm transparency performed by Geimsa and Papanicolaou stains. Geimsa stain gave low cytoplasm transparency to cytoplasm and Papanicolaou stain gave high cytoplasm transparency to the sperm cell cytoplasm, whereas both stains gave cytoplasm transparency in different intensity. The Geimsa stain gave 88% of sperm cells low cytoplasm transparency and 12% of sperm cells high cytoplasm transparency. For the Papanicolaou stain, it gave 84% of sperm cells high cytoplasm transparency and 16% of sperm cells low cytoplasm transparency.

In our observations we have seen the high cytoplasm transparency staining for nuclear and sperm morphology detection, because in high cytoplasm transparency the deeply stained sperm morphology was seen more prominently.

In comparison the Papanicolaou stain came out to be a better stain for nuclear and sperm cells differentiation from the rest of the cytoplasmic structures. For comparison of both the stains for high and low cytoplasm staining chi square test was performed and the results showed significant difference **(P = 0.0001)** between both the stains.

DISCUSSION :-

Present study showed both the stains gave violet staining to the nucleus but in different intensity. Geimsa stain gave 88% of the nucleus deep stain and 12% of nucleus light stain. For the Papanicolaou stain, it gave 41% of the nucleus deep stain and 59% of nucleus light stain. We have seen that the light nuclear staining is better for sperm morphology detection. Juan J. Barcia8,studied Giemsa stain was accomodate to histology from microbiology because of the high-quality staining of chromatin and nuclear membrane of all cells, the metachromasia of some cellular components and different qualities of cytoplasmic staining depending on the cell type. Previous studies quoted principle of papanicolaou stain is to clearly distinguish between basophilic and acidophilic cell components and obtain a detailed chromatin pattern9.

In our study both the stains gave cytoplasmic staining and but Geimsa stain gave violet staining to the cytoplasm and Papanicolaou stain gave eosinophilic colour to the cytoplasm, where as both the stains gave cytoplasmic staining in different intensity. The Geimsa stain gave 79% of sperms deep cytoplasm stain and 21% of cells light stain. For the Papanicolaou stain, it gave 13% of sperms deep stain and 87% of sperms light cytoplasm stain. We have seen that the light cytoplasm staining is better for sperm morphology detection, because in light cytoplasm background the deeply stained sperm morphology can be seen prominently. Bhattacharya M7, studied cytoplasmic staining MGG (2.56±0.51) and in evaluating nuclear details PAP (2.80±.422) gave better results, as it stains nuclear chromatin well, gives good differential cytoplasmic counterstaining and produces good cytoplasmic transparency.

When we compare both the stains the Geimsa stain gave 88% of sperm cells low cytoplasm transparency and 12% of sperm cells high cytoplasm transparency. For the Papanicolaou stain, it gave 84% of sperm cells high cytoplasm transparency and 16% of sperm cells low cytoplasm transparency.we have seen that the high cytoplasm transparency staining for nuclear and sperm morphology detection, because in high cytoplasm transparency the deeply stained sperm morphology have to be seen more.

Present study found that highly significant difference between the Geimsa stain and Papanicolaou stain for demonstration of nuclear and cytoplasmic staining for assessment of human spermatozoa morphology.

CONCLUSION :-

We found that Papanicolaou stain for the sperm morphology is better than the usual Geimsa stain because it is more reliable and gives a higher count. Considering the above compared parameters between both the stains the papanicolaou stain excelled in both the efficacy and accuracy, from the Geimsa stain.

REFERENCES :-

1. Paul c. Adamson, krupp et al. Prevalence & correlates of primary infertility among young women in Mysore, India, Indian Journal of Medical Research, 2011 oct , 134 (4) : 440-446.
2. Rajvi H. Mehta, Sanjay Makwana, et al. Prevalences of oligozoospermia and azoospermia in male partners of infertile couples from different parts of India, Asian J Androl 2006; 8 (1): 89–93.
3. Mascarenhas MN, Cheung H, et al. Measuring infertility in populations: Constructing a standard definition for use with demographic and reproductive health surveys. Popul Health Metr 2012; 10: 17.
4. lingappa, et al. Quest for An Ideal, Simple and Cost- Effective Stain for Morphological Assessment of Sperms Journal of Clinical and Diagnostic Research. 2015 Oct, Vol-9.
5. Aksoy, Aktan et al. Assessment of Spermatozoa Morphology under Light Microscopy with Different Histologic Stains and Comparison of

Morphometric Measurements,Int.J.Morphol.,30(4):1544-1550,2012.
6. Smita Singh, Sunita Sharma, et al. Importance of Papanicolaou Staining for Sperm Morphologic Analysis. American Society for Clinical Pathology. Am J Clin Pathol 2011;136:247-25.
7. Bhattacharya M, Sonone. Comparison of Efficacy and Reliability of different Histochemical stains in Oral Exfoliative Cytology: A Qualitative Analysis, International Journal of Oral Health and Medical Research | ISSN 2395-7387 | Sep-oct 2017 | Vol 4.
8. Juan José Barcia, MD. The Giemsa Stain: Its History and Applications. International Journal of Surgical Pathology Volume 15 Number 3 July 2007 292-296.
9. Kalyani Raj., Evolution of Pap Stain, Biomedical Research and Therapy 2016, 3(2):490-500.