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DIAGNOSTIC UTILITY OF DIFF-QUIK STAINING IN ROUTINE SPERM MORPHOLOGY ASSESSMENT

Pathology

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ABSTRACT

Background: Infertility is defined as failure of a couple to conceive after 1 year of regular sexual intercourse. The prevalence of infertility in the general population is 15-20%. Of this the male factor is responsible for 20-40%. The analysis of semen remains the preliminary investigation for males in the workup of infertility in couples. **Materials & Methods:** This is a prospective study, conducted in upgraded department of Pathology, Osmania General Hospital, from Jan 2025 to June 2026 .Semen of 100 subjects were evaluated and results were analyzed. For assessment of morphology semen sample was centrifuged and smears are prepared on clean glass slides, and stained with Diff-quik stain. **Results:** A total of 100 semen analysis were studied. Out of which 22 cases were Azoospermia, 20 cases were Asthenozoospermia, 18 cases were Oligoasthenozoospermia& 10 cases were Oligozoospermia, remaining 30 cases were Normozoospermia. For assessment of morphology semen sample was centrifuged and smears are prepared on clean glass slides, and stained with Diff-quik stain. **Conclusion:** Routine semen analysis remains the backbone and an indispensable tool in the evaluation of male factor infertility. A normal semen analysis doesn't guarantee the fertilization potential of sperm.

KEYWORDS

Semen Analysis, Male Infertility, Spermatozoa, Diff-Quik Stain.

INTRODUCTION

Infertility is a condition with psychological, economic, medical implications resulting in trauma, stress, particularly in a social set-up like ours, with a strong emphasis onchild − bearing.[1]The prevalence of infertility in the general population is 15%-20%. Of this, the male factor is responsible for 20% − 40%.[2] In Indian couples seeking treatment, the male factor is the cause in approximately 23%.[3] Semen analyses is an indispensable diagnostic tool in the evaluation of the male partners of infertile couples.[1]

According to the International Committee for Monitoring Assisted Reproductive Technology, World Health Organization (WHO), infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. It can also be defined as failure of couple to conceive after 12 months of regular intercourse without the use of contraception in women<35 years; and after six months of regular intercourse without the use of contraception in women ≥ 35 years .[4]

The various factors like genetic disorders, chromosomal disorder, physical and mental stress, smoking, long term medication, autoimmunity, obesity, malnutrition, cryptorchidism, Sexually Transmitted Diseases, life style, malignancies and testicular carcinoma, varicocele and endocrinological disorder were resulted into primary or secondary fertility issues .[5]

The male factor is known to be solely and partially implicated in 20-50% of the cases of infertility.[6]The analysis of semen remainsthe preliminary investigation for males in the workup of infertility in couples.[7]It is a simple test that assesses the formation and maturity of sperm as well as how the sperm interacts with the seminal fluid so it provides insight not only on sperm production (count), but sperm quality (motility, morphology) as well.[8]Semen analysis is a key element in the fertility evaluation of men and permits male reproductive potential to be evaluated in association with possible risk factors.[7]Semen analysis is an easy non invasive test which provides baseline information and a prelude to further investigations.

The present study is an attempt to assess and analyse the semen characteristics of all the males who presented to our hospital, irrespective of primary or secondary infertility .[7]

MATERIALS & METHODS

This is a prospective study conducted in the upgraded Department of Pathology, Osmania General Hospital, Hyderabad, Telangana from 1year 6 months.

Semen collection was done at the hospital in the sterile plastic containers by masturbation after 3 days of abstinence. Informed consent was obtained from all the study participants. The sample was collected and processed using WHO laboratory manual for the examination and processing of semen. Semen analysis was done for volume, viscosity, sperm concentration, motility & morphology.

Semen assessment was performed as soon as the samples were liquified but within 1 hour of collection. Volume of semen was measured, while sperm count was done in the Improved Neubauer counting chamber after an appropriate dilution. Sperm motility was assessed by direct visualisation under the light microscope. For assessment of morphology semen sample was centrifuged and smears are prepared on clean glass slides, and stained with Diff-quik stain.

RESULTS

A total of 100 semen analysis were studied. Of these 100, 22 cases were azoospermia, 20 cases were Asthenozoospermia, 18 cases were Oligoasthenozoospermia and 10 cases were Oligozoospermia. Remaining 30 cases were Normozoospermia

Table 1: Age Distribution

Age in Years	Number of semen analysis	Percentage
20 – 30 years	66	66
31 – 40 years	30	30
41 – 50 years	4	4
Total	100	

Table 2: Quantity Of Semen

Volume in ml	Number	Percentage
< 2 ml	20	20
2 – 4 ml	46	46
4.1 – >/= 6 ml	34	34
Total	100	

Table 3: Sperm Count

Sperm count in million/ml	Number	Percentage
</= 20	44	44
21 – 40	20	20
41 – 50	8	8
51 – 60	4	4
61 – 70	0	

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71 – 80	10	10
>80	14	14
Total	100	

Table 4: Motility of Sperms

Active Motility	Number	Percentage
</= 25	54	54
26 – 50	24	24
51 – 75	22	22
76 – 100	0	
Total	100	

Table 5: Showing Morphology

Abnormal forms	Number	Percentage
Present	80	80
Absent	20	20
Total	100	

Table 6: Presence of Pus Cells

Pus cells	Number	Percentage
Present	88	88
Absent	12	12
Total	100	

Table 7: Subjects With Abnormality

Abnormality	Number	Percentage
Oligo	10	10
Oligo-Astheno	18	18
Astheno	20	20
Azoo	22	22
Aspermia	0	
Terato	0	
No abnormality	30	30
Total	100	

Table 8: Correlation Between Varicocele and Sperm Morphology Abnormality

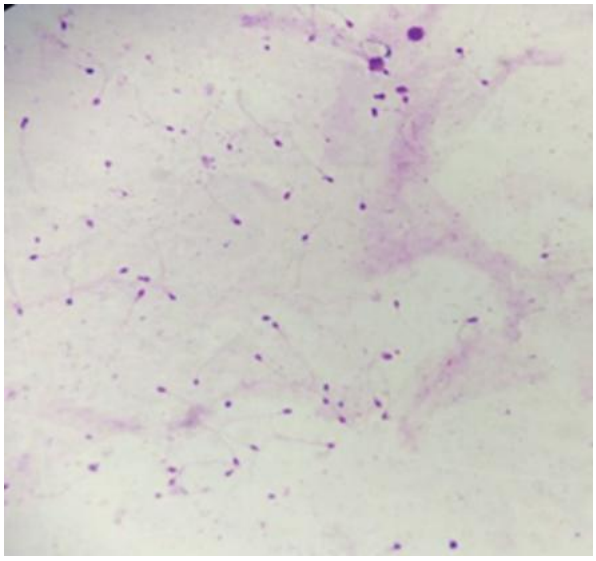
Abnormality	Patients with Varicocele
Oligo	4
Astheno	2
Oligo-Astheno	4
Total	10

Table 9: Marital Life

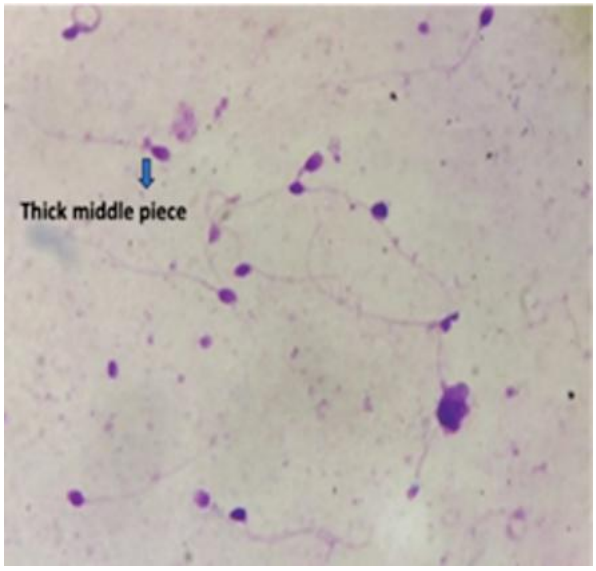
Duration of active married life in years	Number	Percentage
</= 5	46	46
>5 – 10	30	30
Unmarried	24	24
Total	100	

Table 10: Marital Status

Marital status	Number	Percentage
Married	76	76
Unmarried	24	24
Total	100	



DIFF QUIK 40X



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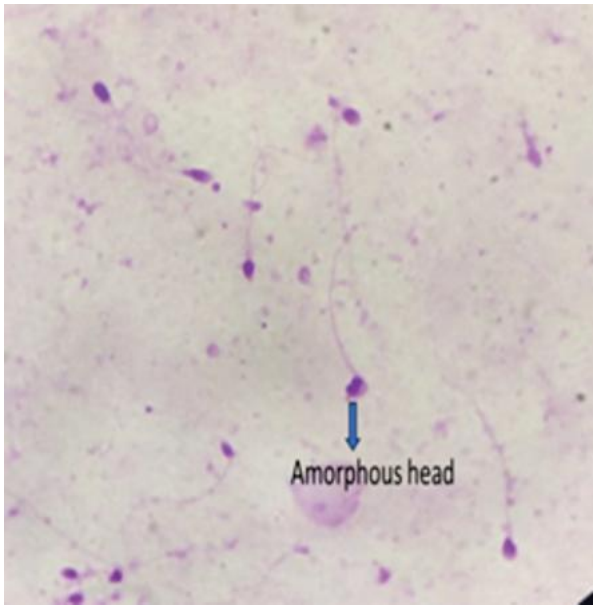

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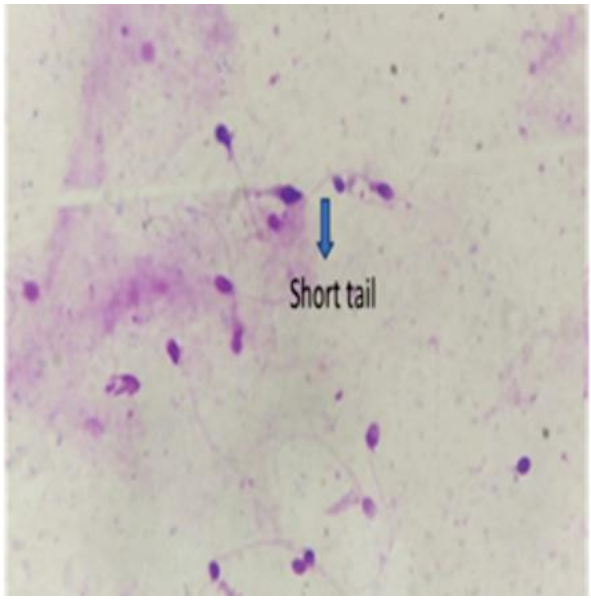
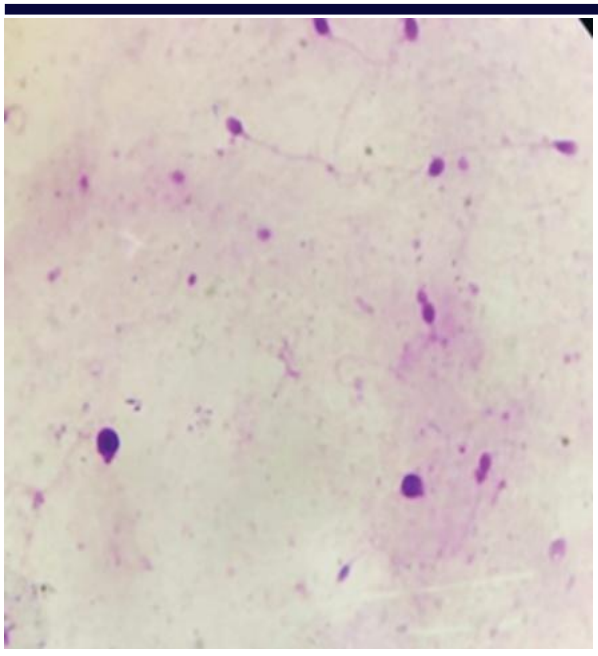


DIFF QUIK 10X MATURE SPERMS



DIFF QUIK 10X IMMATURE SPERMS

DISCUSSION

In this study, the maximum number of cases are in the age range 20-30 years similar to the study Jairajpuri ZS et al.,[7]

Majority of cases i.e., 46 cases (%) had duration of infertility below 5 years and 30 cases (%) between 5-10 years of infertility as compared to a study by Jajoo et al.,[9] who found 62% had duration of infertility below 5 years, 32% between 5-10 years and there were 6% with more than 10 years of infertility.

As far as semen volume is concerned, 20% cases had volume <2ml, 46% had volumebetween 2-4 ml and 34% had volume 4-6 ml. According to the study by Jain et al.,[1] 28% males had volume < 2ml, 70% had volume between 2–4 ml and only 1(2%) had volume between 4–6 ml.

In a study at bangalore by Joshi et al.,[10] 6% cases had semen volume of less than 2ml.

The factors affecting the quality of the semen smear (WHO 2010)[11]:

- Volume of the semen and the sperm concentration: The degree of overlapping of sperms decreases with the number of sperms
- The angle of the spreader: The more acute the angle, the thinner the smear
- Smearing speed: The more rapid the movement, the thicker the smear.

In this study majority cases (44%) had sperm count < 20 million per ml, 20% had sperm count of 21-40 million per ml as compared to Jajoo et al.,[9]25% had sperm count of < 20 million per ml. In this study, 78% cases had <50% motile sperms/ HPF, out of which 54% cases had <25% motile sperms.

The germ cells were identified with the following morphological criteria[12]:

- Spermatogonia are cells which are smaller than spermatocytes and larger than spermatids, have thin rim of cytoplasm, high N: C ratio, and single nucleus which is round to oval, 6–7 µm in diameter, darkly stained, has evenly distributed finely granular chromatin, and has one or two nucleoli
- Primary spermatocytes are larger cells, with large centrally located spherical nucleus, 8–9 µm in diameter, coarse or thread-like/woolly nuclear chromatin, and irregular nuclear outline
- Secondary spermatocytes are small round cells, with a central spherical dark nucleus, 6–7 µm in diameter (sometimes two nuclei); bi-nucleation is common
- Spermatids are small cells measuring approximately 4–5 µm in diameter with scant cytoplasm, small, dark round to oval nucleus, sometimes two in number; may be confused with lymphocytes
- Spermatozoa are cells which have a head, a neck, and a tail
- Sertoli cells have indistinct cytoplasmic borders, oval nucleus with smooth outline, vesicular chromatin, and prominent large nucleolus
- The cytoplasm of the immature germ cells decreases with the maturation of the cells[13]. Diff-quik is a widely used stain for evaluating sperm morphology, particularly for assessing the presence of normal and abnormal sperm forms.

CONCLUSION

Diff-Quik stain is easy to use, convenient, and rapid.. Morphological interpretation can be done with this stains even in nonliquefied samples. Diff-Quik stain can be used in the detection of inflammatory cells and immature germ cells. Total sperm count is independent of the round cell count or the presence of inflammatory cells/ immature germ cells. DQ did not demand much expertise, simple, andconvenient as the procedure required less than a minute.

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