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Patterns of semen parameters in male partners of infertile couples attending rural medical college

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Abstract
Background: Infertility is a condition with psychological, economic, medical implications resulting in trauma, stress, particularly in a social set-up, with a strong emphasis on child-bearing. The prevalence of infertility in the general population is 8-12% worldwide. Of this, the male factor is responsible for 40-50%.Semen analysis is an indispensable diagnostic tool in the evaluation of the male partners of infertile couples.
Materials and Method: Seminal fluid from the male partners was analysed in the clinical laboratory, JIIU'S IIMSR Medical College, Warudi, Jalna during June 2018 to June 2019 using the World Health Organization (WHO) 2010 criteria for human semen characteristics. Parameters like semen volume, liquefaction time, sperm concentration, motility, vitality, morphology were evaluated.
Results: Total 122 males were evaluated in the study. Mean age of presentation was 28.95 yrs. 76 cases [**62.30%**] found to have abnormal semen analysis and 46 [37.70%] with normal pattern. Abnormalities in sperm no. were seen as most common abnormality 40 patients [32.79%], followed by abnormality in motility 20 patients [16.39%]. Normozoospermia [48.36%], Asthenozoospermia [14.75%], Teratozoospermia [2.46%], Asthenozoospermia with teratozoospermia [1.64%], Oligozoospermia [9.02%], Oligoasthenozoospermia [6.56%], Oligozoospermia with teratozoospermia[3.28%], Oligoastheno-teratozoospermia [2.46%], Azoospermia [11.47%]
Conclusion: Though semen analysis has limited role in evaluation of male factor infertility but still it is indispensable and cost effective tool in evaluation of male factor infertility, particularly in rural population.

Keywords: Oligozoospermia, asthenozoospermia, azoospermia, teratozoospermia, IUI, TESE, ICSI, IVF

Introduction
Infertility is defined as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. According to World Health Organization (WHO), the term primary infertility is used when a woman has never conceived. Secondary infertility occurs when couples have achieved a live birth and are unable to do so again [2].
According to the Indian Society of Assisted Reproduction, infertility currently affects about 10 to 14 % of the Indian population, with higher rates in urban areas where one out of six couples is impacted [3]. As per the WHO, the overall prevalence of primary infertility in India ranges between 3.9% and 16.8 % which varies from state to state and across the tribes and castes within same religion [4]. Worldwide, prevalence of infertility is approximately 8-12% [5].
Male infertility refers to a male's inability to result pregnancy in a fertile female. Approximately 40–50% infertility cases are due to "male factor" infertility. Various causes are attributed for infertility and it is proved that infertility is due to many factors, in both male and female. Obesity, Occupational exposure [Specially industrial workers], Alcohol, Smoking, exposure to electronic devices like cell phone, laptop etc., stress, wearing tight fitting under trousers, exposure to therapeutic drugs [like spironolactone, cimetidine etc.], are considered as risk factors of male infertility. Some of the causes of male infertility are varicocele, endocrinal disorders, male reproductive tract infections, ejaculatory disorders, anti-sperm antibodies, genetic mutation and chromosomal aberration, idiopathic male infertility [no cause identified] [4, 6].
Semen analysis is an imperfect tool but remains the cornerstone to investigate male

infertility. It provides insight into the process of sperm production-count and sperm quality (motility and morphology). It is important that while the results may correlate with "fertility," the assay is not a direct measure of fertility. Semen analysis can predict fertility in men with azoospermia, severe asthenozoospermia and globozoospermia. In other cases, semen analyses have a limited role in the evaluation of infertility because female factors can influence fecundity in most couples. It is clear that examination of the semen and the spermatozoa in the ejaculate cannot assess: 1) the process of capacitation of the spermatozoa in the female reproductive tract, 2) the acquisition of sperm surface proteins that are required for zona binding and penetration, and 3) the ability to fertilize the egg [4, 7, 8]

Semen analysis is an indispensable diagnostic tool in the evaluation of fertility potential of the male partners of infertile couple with a sensitivity of 89.6%. The semen analysis functions as both a screening test for the presence of male factor infertility and as the cornerstone for male factor evaluation. History and physical examination, endocrine tests, imaging, testicular biopsy, genetic evaluation are all essential for further evaluation of an abnormal semen analysis, and required for thoughtful formulation of an accurate diagnosis and directed treatment strategy [4, 9, 10].

In rural areas the access to reproductive health care specifically for infertility can be very limited. Present study evaluate the pattern of semen parameters, using the current WHO 2010 criteria, in male partners of infertile couples attending the Obstetrics and Gynaecology Department to identify the contribution of male factors to overall infertility problem in rural area.

Aims & Objectives

1. To determine the proportion of male partners of infertile couples in rural population with normal and abnormal semen parameters.
2. To determine the nature of semen quality abnormality seen among males partners of infertile couples.

Materials and Methods

Approval of the institutional ethical committee and permission from Head of Institution was obtained.

This descriptive cross sectional study was conducted in department of Pathology during June 2018 to June 2019. Approximately 122 male partners of infertile couples who presented in department of Gynaecology and Obstetrics of a Rural Medical College were evaluated. Consent for study was taken from these male partners of infertile couples

Inclusion criteria

Male partners of infertile couples who have failed to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.

Exclusion criteria

1. Male partners of women, who did not present with infertility,
2. Partners of those women with less than 12 month history of infertility,
3. Men who refuse to do semen analysis and do not give consent.
4. Couples not having regular intercourse,
5. Couple with female factor infertility were excluded.

History was asked to all males participants regarding occupation, exposure, alcohol consumption, smoking, past medical history, etc. Complete confidentiality was maintained.

The male partners were counselled and given instructions on how to collect the semen samples. Semen samples collected after 3-5 days of abstinence by masturbation in sterile screw capped plastic universal containers, without spillage, in a private room in laboratory premises was accepted for analysis. Name, age, the period of abstinence, the date and time of collection, any difficulties in producing the sample, and the interval between collection and the start of the semen analysis was noted.

All samples were processed and analysed within one hour of collection by the pathologist as per 2010 WHO Guidelines for Human Semen Analysis [11], in terms of volume, total sperm count, sperm motility and sperm morphology. Volume was measured by graduated pipette. Sperm count was done using Improved Neubauer haemocytometer. Sperm motility was determined in wet cover-slip preparation with 10 µl of semen and using 22 mm X 22 mm coverslips. Approximately 200 spermatozoa were assessed to determine percentage of different motile categories. Diff-Quick stain or Papanicolaou stained air dried and alcohol fixed thin smears of semen were observed for sperm morphology.

Semen analysis parameters were considered abnormal if less than WHO lower reference limits [2010] [11] which are as follows-

Semen volume -- 1.5 ml

Total motility (Progressive + Non- progressive) -- 40%

Progressive motility-- 32%

Sperm concentration -- 15×10^6 spermatozoa per ml

Normal morphological forms -- 4%

All findings were tabulated and appropriate statistical analysis was done.

Result and observations

Total 122 male partners of infertile couples were evaluated in study.

Table 1: Agewise distribution of Semen characteristics

Age (in yrs)	Normal Semen		Abnormal Semen		
	No.	%	No.	%	TOTAL
21-25	19	41.3	20	26.3	39
26-30	9	19.6	36	47.4	45
31-35	13	28.3	18	23.7	31
36-40	3	6.5	2	2.6	5
>40	2	4.3	0	0	2
Total	46	100	76	100	122

Maximum no. patients [**73.68%**] with abnormal semen analysis were seen below 30 yrs. of age with peak in 26-30 yrs.

Table 2: Distribution of seminal fluid abnormalities

Abnormalities	No. of cases
A. Physical characteristics	**10**
1.Abnormal liquefaction	2
2.Increased viscosity	5
3.Low volume	3
B. Microscopic abnormalities	**66**
1.Sperm concentration	40
a. low sperm concentration	26
b. Azoospermia	14
2. Sperm motility	20
3.Sperm morphology	3
4.Sperm agglutination	2
5.Leukocytospermia	1
Total	76

Out of 122 patients, 76 patients [62.30%] found to have abnormal semen analysis and 46 [**37.70%**] patients having normal semen analysis.

Table 3: Relation of Sperm concentration with abnormal liquefaction

Liquefaction time	Normal sperm concentration	Low sperm concentration	Azoospermia	Total
Increased	2	00	00	2
Normal	80	26	14	120
Total	82	26	14	122

2 cases [1.64%] out of 122 cases showed liquefaction time > 1hr.

Table 4: Relation of Sperm concentration with Viscosity

Viscosity	Normal sperm concentration	Low sperm concentration	Azoospermia	Total
Increased	5	1	1	7
Normal	77	25	13	115
Total	82	26	14	122

7 cases out of 122[**5.74%**] cases showed increased viscosity, forming thread greater than 2 cms. Out of 7 cases, 1 case associated with oligozoospermia, 1 case with azoospermia, 5 cases with normal sperm count.

Table 5: Relation of Sperm concentration with sperm volume

Seminal volume	Normal sperm concentration	Low sperm concentration	Azoospermia	Total
<1.5 ml	3	4	5	12
>1.5 ml	79	22	9	110
Total	82	26	14	122

12 cases [**9.84%**] out of 122 cases showed seminal volume less than 1.5 ml, out of which 3 cases associated with normal sperm concentration, 4 cases were oligozoospermic, 5 cases were azoospermic.

Table 6: Relation of Sperm concentration with pH

Seminal pH	Normal sperm concentration	Low sperm concentration	Azoospermia	Total
< 7	0	0	4	4
>7	82	26	10	118
Total	82	26	14	122

pH less than 7 were found in 4 cases **[3.28%]** out of 122 cases, all were azoospermic.

Table 7: Relation of sperm concentration with motility and morphology

Abnormalities	Normal sperm concentration	Low sperm concentration	Total
Asthenozoospermia	18	8	26
Teratozoospermia	3	4	7
Asthenozoospermia with Teratozoosprmia	2	3	5
Normal motility and morphology	59	11	70
Total	82	26	108

Abnormalities of sperm no., morphology and motility were analysed in 108 cases out of 122 cases, excluding 14 cases of azoospermia.

Table 8: Relation of Sperm concentration with seminal fructose

Fructose	Normal sperm concentration	Low sperm concentration	Azoospermia	Total
Present	82	26	10	118
Absent	00	00	4	4

Total	82	26	14	122

4 cases **[3.28%]** out of 122 cases, showed absent fructose in semen by qualitative fructose test, all of them were azoospermic.

Table 9: Relation of Sperm concentration with sperm agglutination

Sperm agglutination	Normal sperm concentration	Low sperm concentration	Azoospermia	Total
Present	2	0	0	2
Absent	80	26	14	120
Total	82	26	14	122

2 cases [**1.64%**] out of 122 cases showed motile sperms sticking together [**sperm agglutination**]

Table 10: Relation of Sperm concentration with Round cell concentration

Round cells	Normal sperm concentration	Low sperm concentration	Azoospermia	Total
Leukocytes	1	0	0	1
Immature germ cells	00	2	3	5
Total	1	2	3	6

6 cases **[4.92%]** out of 122 cases showed round cells in semen with concentration >1x10^{6}ml.Out of 122 cases,1 case showed leukocytospermia, remaining 5 cases showed immature germ cells out of which 2 were oligozoospermic,3 were azoospermic.

Discussion

Infertility is a condition with psychological, economic, medical implications resulting in trauma, stress, particularly in a social set-up, with a strong emphasis on child-bearing. Semen analysis is an indispensable diagnostic tool in the evaluation of the male partners of infertile couples. There are very few studies in rural areas as far as infertility is concerned.

This descriptive cross sectional study was conducted in department of Pathology during June 2018 to June 2019 in clinical laboratory of JIIU'S IIMSR MEDICAL COLLEGE WARUDI JALNA. Total 122 males evaluated in study. Mean age of presentation was 28.95 yrs.90 cases [73.77%] presented with duration of infertility less than 5 yrs. and 32 cases [26.23%] with duration of infertility > 5 yrs. 107 [87.70%] cases presented with primary infertility while 15 [12.30%] cases presented with secondary infertility.

From table 1, 76 cases [62.30%] found to have abnormal semen analysis and 46[37.70%] cases with normal semen analysis. Maximum no. cases [73.68%] with abnormal semen analysis were seen below 30 yrs. of age with peak in 26-30 yrs.

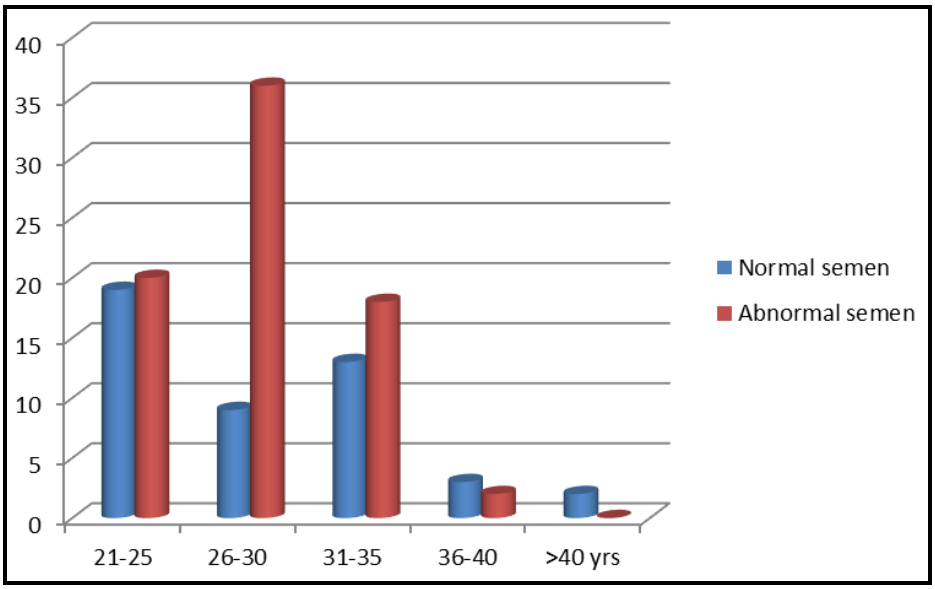

Fig 1: Agewise distribution of semen parameters.

Analysis Present study is consistent with Kambala GM *et al.* [12] and C. Ramya *et al.* [13] in which maximum number of cases with abnormal semen analysis were below 30 yrs. age.

From table 2, abnormalities in sperm no. were seen as most common abnormality 40 cases [32. 79%], followed by abnormality in motility 20 cases [16.39%].

From table 3, 4 abnormal liquefaction was seen in 2 cases. One case liquefied after 1hr, Other case liquefied after 2 hrs.7 cases showed hyper-viscosity with thread greater than 2 cms. Out of 7 cases, 1 case associated with oligozoospermia, 1 case with azoospermia, 5 cases with normal sperm count. Freshly ejaculated semen is a grey-opalescent, semisolid coagulum that should spontaneously liquefy within 15 to 60 minutes. Substances in the seminal vesicle are responsible for coagulation, whereas liquefaction depends upon proteolytic enzymes derived from the prostate. Increased semen viscosity and abnormal liquefaction may be attributed to accessory gland infection, increased levels of leukocytes, and inflammation, as well as dysfunction of the sex glands or even the immune system.

Motility is affected by seminal hyper viscosity, hence fertility may affect. In vitro treatment before proceeding to seminal analysis includes treatment by bromelain or forcing semen through hypodermic needle. In our Institute we follow hypodermic needle method. Sperm washing and IUI may be offered as treatment [10, 11].

From table 5, twelve cases [9.84%] had seminal volume less than 1.5 ml, out of which 3 cases associated with normozoospermia, 4 cases with oligozoospermia and 5 cases with azoospermia. The volume of the ejaculate is contributed mainly by the seminal vesicles and prostate gland, with a small amount from the bulbourethral glands and epididymis. Precise measurement of volume is essential in any evaluation of semen, because it allows the total number of spermatozoa and non-sperm cells in the ejaculate to be calculated. In men with ejaculatory duct obstruction, the seminal vesicle contribution to the ejaculate is lost, resulting in low volume (<1.5 mL), fructose-negative, acidic ejaculate. Low semen volume, normal pH and positive fructose may indicate degree of retrograde ejaculation or hypogonadism. For diagnosis of retrograde ejaculation, immediate post ejaculatory urine sample should be tested [10, 11]. Present study is consistent with Bhaduri *et al.* [14] 7.45% cases and Jairajpuri *et al.* [15] 5% cases having volume less than 1.5ml.

From table 6, 4 cases with pH<7, were found to have low seminal volume and Azoospermia.

From table 7, Abnormalities of sperm no., morphology and motility can be divided as:-

Table 11: Classification of microscopic abnormal semen analysis

Abnormal parameters	No. of cases	%
Normozoospemia	59	48.36
Ashenozoospermia	18	14.75
Teratozoospermia	3	2.46
Asthenozoospermia with teratozoospermia	2	1.64
Oligozoospermia	11	9.02
Oligoasthenozoospermia	8	6.56
Oligozoospermia with teratozoospermia	4	3.28
Oligoastheno-teratozoospermia	3	2.46
Azoospermia	14	11.47
Total	122	100

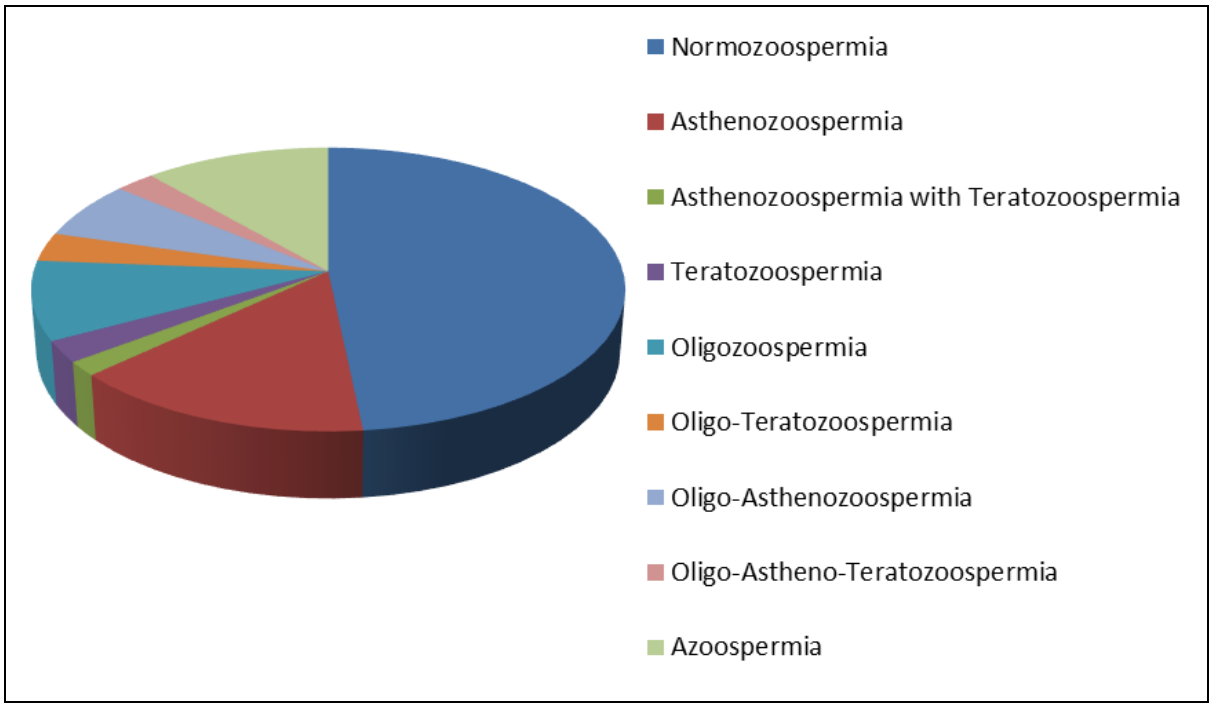

Fig 2: Semen Parameters

A] Abnormal motility:-Total 31 cases [25.41%] out of 122 cases, associated with progressive motility less than 32% divided as

Asthenozoospermia 18 cases,
Astheno with Teratozoospermia- 2 cases,
Oligoasthenozoospermia 8 cases
Oligoastheno teratozoospermia 3 cases

Causes of abnormal sperm motility are low sperm production, anti-sperm antibodies, epididymis or ejaculatory duct obstruction, infection, genetic causes like primary cilliary dyskinesia, immotile cilia syndrome, necrozoospermia etc. Men with persistently abnormal motility may be at higher risk of having abnormal sperm DNA fragmentation, they need to be evaluated further. [10, 16]

Vitality test with eosin Y 2% was done in 31 cases, 7 cases showed reduced vitality.

Table 12. Abnormal motility

cases	Abnormality	Motility	Vitality
1.	Asthenozoospermia	8%	20 %
2.	Asthenozoospermia	15%	25%
3	Oligoasthenozoospermia	5%	8%
4.	Oligoasthenozoospermia	2%	2%
5.	Oligoasthenozoospermia	10%	22%
6.	**Oligoasthenozoospermia**	**0**	**0**
7.	Oligoasthenoteratozoospermia	3%	10%

1 case out of 7 cases, showed no motility [complete asthenozoospermia] with no live sperms [necrozoospermia]. Cases with complete asthenozoospermia need to be differentiated from cases with some motile spermatozoa as rate of fertilisation and pregnancy are low in cases of

complete asthenozoospermia, even if advanced procedure such as ICSI is used. TESE may be used as procedure to retrieve sperm for ICSI in cases of necrozoospermia [16].
Present study is consistent with Ramya *et al.* [13] with abnormal motility in 23.2% cases. Jairajpuri *et al.* [15] with abnormal motility 22.1%.

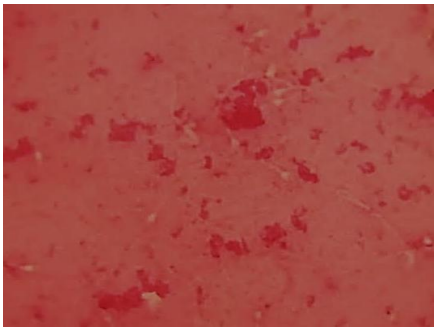

Fig 3: Vitality- live sperms

B] **Abnormal morphology**:-Total 12 cases [**9.8%]** with abnormal morphology were seen, they are divided as

Teratozoospermia:-	3 cases
Asthenozoospermia with teratozoospermia:-	2 cases
Oligo teratozoospermia:-	4 cases
Oligoastheno teratozoospermia:-	3 cases

Most common abnormalities were head abnormalities with 2 cases showed morphology with round head 90% [globozoospermia], followed by mid-piece, then tail abnormalities.
Normal sperm morphology has importance because only sperms having normal morphology can bind zona pellucidum of oocyte. When IVF used as treatment, morphology is important. While in ICSI, it is not of much concern [10].
Other studies like Peter *et al*[17] study shows teratozoospermia in 6.5% cases.

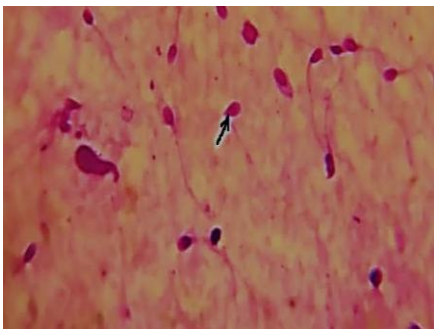

Fig 4: Bent neck with pyramidal head

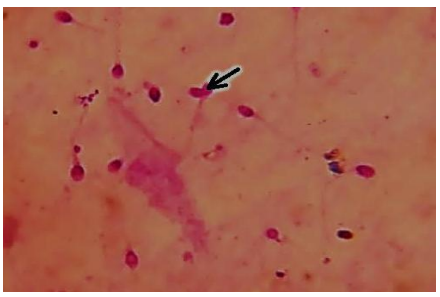

Fig 5: Double head

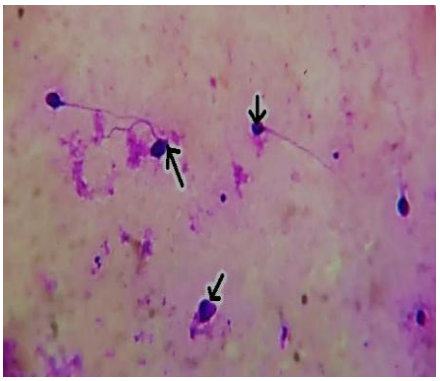

Fig 6: Bent neck, round head, large head with thick mid-piece.

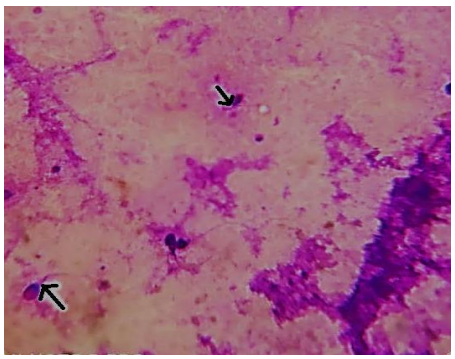

Fig 7: Coiled tail and large abnormal head.

C] Abnormal sperm concentration:- Total 40 cases [32.79%] cases showed abnormal sperm concentration with low sperm concentration [<15 million] in 26 cases [21.31%] and Azoospermia in 14 cases [11.475%].

1. Cases with low sperm concentration can be divided as

Oligozoospermia:-	11
Oligoasthenozoospermia:-	8
Oligo teratozoospermia**:-**	4
Oligoastheno teratozoospermia:-	3

Other studies like Bhaduri *et al.* [14] shows oligozoospermia in 19.5% cases, Jairajpuri *et al.* [15] shows oligozoospermia 17% cases. Hormonal analysis like FSH, LH, Testosterone, genetic evaluation should be done in cases of severe oligozoospermia. Hormonal treatment for specific hormonal disorder, IUI with washed pallet of sperms for mild oligozoospermia,
IVF and ICSI for severe cases are the treatment options available [9, 10].

2. Cases with no sperms in semen [Azoospermia]:- Total 14 cases [11.475%] of azoospermia were seen present study. All are confirmed as azoospermia when no sperms seen after centrifugation. Out of total 14 cases of Azoospermia, 4 cases presented with low volume, acidic PH, absent fructose, findings indicating obstructive aetiology. [Table 5, 6, 8] Hormonal analysis should be done in all cases to differentiate obstructive from non-obstructive azoospermia, as microsurgical treatment can be offered to such patients and if surgery is not feasible, TESE with ICSI is treatment of choice. Non-obstructive azoospermia should be further divided into hypergonadotrophic and hypogonadotrophic hypogonadism. In hypogonadotrophic hypogonadism, hormonal treatment is the option available. While in

hypergonadotrophic hypogonadism TESE with ICSI may be offered as treatment. [10, 18, 19] Present study is consistent with Bhaduri *et al*. [14] 12% cases, Onyebuchi A.K *et al*. [19] with 11% cases. Other studies like Jairajpuri *et al*. [15] with azoospermia in 9% cases, Ramya *et al*. [13] with azoospermia in 8.6% cases.

3. Normozoospermia:- There were 59 cases with sperm concentration, sperm motility, sperm morphology within normal limits. As the couple with female infertility already excluded, these patients need to be evaluated further for functional tests like Human sperm–oocyte interaction tests, Human zona pellucida binding tests, assessment of the acrosome reaction, Zona-free hamster oocyte penetration test, anti-sperm antibody with sperm mucous penetration testing, lastly assessment of sperm chromatin.

From table 9, 2 cases presented with sperm agglutination, with grade II agglutination and mixed type of agglutination with sperm concentration 50 and 60 million/ml and progressive motility 40% and 45% respectively. Sperm agglutination should be differentiated from non-specific sperm aggregation. Agglutination specifically refers to motile spermatozoa sticking to each other, head-to-head, tail-to-tail or in a mixed way. These 2 cases should be further evaluated for anti-sperm antibodies to rule out immunogenic cause [11].

From table 10, round cells seen in 6 cases out of 122 cases. Round cells can be divided as leukocytes and immature germ cells. Stained smears with Giemsa stain were evaluated. Out of 6 cases, 1case presented with sperm concentration 30 million with 40 % progressive motility and 18-20 round cells /HPF with 70% polymorphs on Giemsa stained smear. Semen culture should be advised to rule out any infection. Leukocyte-dependent damage to spermatozoa depends on the total leukocyte number in the ejaculate and the number of leukocytes relative to the number of spermatozoa. Leukocytes can impair sperm motility and DNA integrity through an oxidative attack, thus responsible for infertility even though normal total sperm count. It may indicate trial for antibiotic therapy. [11] In remaining 5 out 6 cases predominant round cells were immature germ cells including spermatocytes and spermatid. 2 cases were oligozoospermic and 3 cases were azoospermic. Reporting of immature germ cells may give clue to diagnosis as it indicates testicular dysfunction. It may indicate maturation arrest shredding spermatogonia, spermatocyte, spermatids in semen. It also differentiate obstructive azoospermia from non-obstructive azoospermia, as immature germ cells in semen present in non-obstructive azoospermia [20, 21].

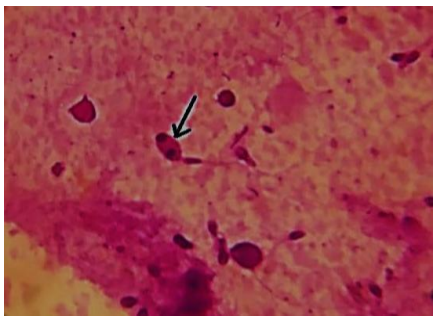

Fig 8: Dividing Spermatid

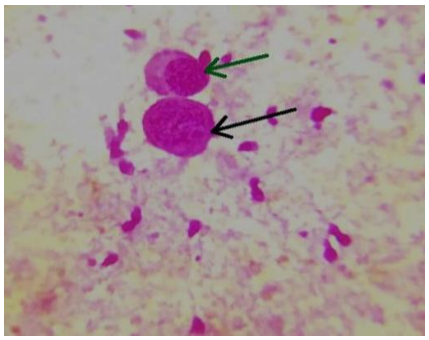

Fig 9: Green arrow spermatid. Black arrow Spermatocyte

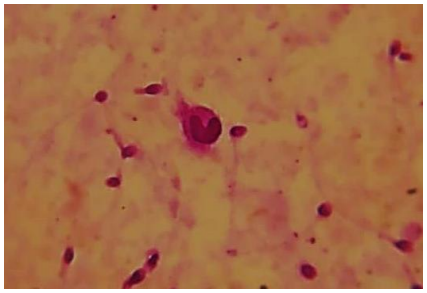

Fig 10: Monocyte

Conclusion

Though semen analysis has limited role in evaluation of male factor infertility but still it is indispensable and cost effective tool in evaluation of male factor infertility, particularly in rural population**.** Each parameter in semen analysis should not be neglected and parameters should be reported according to guidelines set by W.H.O, as further evaluation and treatment modalities depends on parameters of semen analysis reported by pathologist.

References

1. Zegers-Hochschild F, Adamson GD, De Mouzon J, Ishihara O, Mansour R, Nygren K *et al.* ICMART and WHO international committee for monitoring assisted reproductive technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. *Fertil Steril.* 2009; 92(5):1520-4.
2. Benksim A**,** Elkhoudri N, Ait Addi R, Baali A, Cherkaoui M**.** Difference between Primary and Secondary Infertility in Morocco: Frequencies and Associated Factors. Int J Fertil Steril. 2018; 12(2):142-46. doi:10.22074/ijfs.2018.5188. (Accessed on 31 sept.2019)
3. Lal N. India's Hidden Infertility Struggles. *The diplomat.* May 30,2018
4. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015; 8(4):191-96.
5. Singh K, Kumari R, Ranjan A, Bharti G. Analysis of causes and clinical pattern of infertility in couples coming to a tertiary care centre in Bihar, India. Int J Reprod Contracept Obstet Gynecol. 2017; 6(6):2279-83
6. Mahat RK, Arora M, Bhale DV, Holkar S, Kumar S, Yadav T. Risk Factors and Causes of Male Infertility-A Review. Biochem Anal Biochem. 5(2):271. doi:10.4172/2161-1009.1000271(Accessed on 5 oct.2019)
7. Patel AS, Leong JY, Ramasamy R. Prediction of male

infertility by the World Health Organization laboratory manual for assessment of semen analysis: A systematic review. Arab Journal of Urology. 2019; 16(1):96-102, DOI: 10.1016/j.aju.2017.10.005(Accessed 10 Oct.)
8. Wang C, Swerdloff RS. Limitations of Semen Analysis as a Test of Male Fertility and Anticipated Needs from Newer Tests. *Fertil Steril*. 2014; 102(6):1502-07. doi:10.1016/j.fertnstert.2014.10.021 (Accessed on 12 Oct. 2019)
9. Karavolos S, Stewart J, Evbuomwan I, McEleny K, Aird I. Assessment of the infertile male. *The Obstetrician & Gynaecologist.* 2013; 15:1-9. DOI: 10.1111/j.1744-4667. 2012. 00145.x (Accessed on 13 Oct.2019)
10. Interpretation of the Semen Analysis and Initial Male Factor Management Stahl P J, Stember DS, Schlegel PN. Clinical Obstetrics And Gynecology. 2011; 54(4):656-65.
11. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen,5th ed. Geneva: World Health Organization, 2010.
12. Kambala GM, Kalakonda M, Undavalli VK. Pattern of Semen Analysis: An Insight into Male Infertility at a Tertiary Care Hospital. IOSR-JDMS. 2018; 17(2):30-33.
13. Ramya C, Renuka IV, Premalatha P, Madhavi K. Patterns of semen analysis in male partners of infertile couples at a tertiary care hospital. Indian Journal of Pathology and Oncology, 2017; 4(4):536-39.
14. Bhaduri N, Sarkar AP, Dewasi N, Ghosh TK. Abnormalities in semen analysis among male partners of infertile couples: a study in a tertiary care level hospital of West Bengal, India. Int J Reprod Contracept Obstet Gynecol. 2015; 4(1):100-102.
15. Jairajpuri ZS, Rana S, Ali M, Pujani M, Jetley S. Patterns of Semen Analysis: Experiences of a Laboratory Catering to Semi Urban Population of Delhi. Bangladesh Journal of Medical Science. 2017; 16(2):314-19.
16. Ortega C, Verheyen G, Raick D, Camus M, Devroey P, Tournaye H. Absolute asthenozoospermia and ICSI: what are the options? Human Reproduction Update. 2011; 17(5):684-92.
17. Peter AO, Temi AP, Olufemi AP, Simidele OM, Adeniran AS, Amos IA. Pattern of Semen Parameters and Factors Associated with Infertility in Male Partners of Infertile Couples in Nigeria. Andrology (Los Angel). 2016; 5(1):1http://dx.doi.org/10.4172/2167-0250.1000161(Accessed on 13 Oct.2019)
18. Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Male Reproduction and Urology. The management of infertility due to obstructive azoospermia. Fertil Steril. 2008; 90:S121–4.
19. Kumar R. Medical management of non-obstructive azoospermia. Clinics. 2013; 68(S1):75-79. DOI: 10.6061/clinics/2013(Sup01)08 (Accessed on 15 Oct.2019).
20. Onyebuchi AK, Ifeoma EC, Mamah J, Iwe B, Afogou E, Obi VO. Seminal fluid analysis of male partners of infertile couples in Abakaliki, Ebonyi State, Nigeria. Tropical Journal of Obstetrics and Gynaecology. 2018; 35(3):261-65.
21. Balasubramaniam S, Pawar VR. Significance of Differentiation of Round Cell Count in Semen in Infertile Men: A Cost‐effective Study. Journal of Datta Meghe Institute of Medical Sciences University. 2018; 13(4):190-94.
22. Gune AR, Humbarwadi RS, Patil PS, Patil AD. Immature germ cells in semen - correlation with total sperm count and sperm motility. Journal of Cytology. 2013; 30(3):185-89.