Original Research Article

A study of Semen analyses in male infertility cases at a tertiary care center in Konkan region, Maharashtra over five years

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Abstract

Background: An analytical study on routine semen analyses or seminogram is under-reported in clinical pathology even though the rates of male infertility are increasing nowadays due to compounding factors like late marriage, stressful life. We conducted a pioneer Konkan study on semen analyses in male infertility cases. **Aims:** Routine semen analyses of male patients referred with infertility will be studied based on clinical history, semen data, physical and chemical evaluation, sperm data, other cells in semen fluid with respect to WHO, 2010 guidelines. In few known infertile males, based on routine semen analyses, In-utero-insemination (IUI) was done, that requires pre-wash and post-wash handling and reporting of the semen. The quality of post-wash sample was analysed with respect to pre-wash results. **Material and Methods:** 120 male patients referred for infertility check up with inability to conceive with wife for 12 months after unprotected intercourse were studied over five years period from 1st January 2014 to 31st July 2019. **Results:** Maximum male cases (44.17%) who came for semen analyses were in the age group of 25-30 years of age. Maximum male cases had 1-3 years of marital status (55%). 91.67% cases had no living children before testing. Only nine cases had one living child while one case had two living children, before semen analysis. Maximum cases had abstinence before semen analyses of more than three days (67.5%). Miscarriage status of wife in men who came for routine semen analyses was noted in seven out of 120 cases. **Conclusion:** The pioneer Konkan study on semen analyses will help to evaluate and treat the causes for male infertility.

Key Words: Semen analyses, IUI

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INTRODUCTION

Semen (seminal fluid) is a fluid that contains living human sperms which is produced by male reproductive organ to fertilize the female ova in copulation.[1] In 1667, for the first time, Leuwenhoek examined his own semen ejaculate under the microscope to see live human sperm cells in a drop of semen.[2] Since centuries, the female partner was generally blamed for infertility but, the Greeks were aware of male infertility. Various causes are attributed for infertility and it is proved that infertility is due to many factors, in both males and females.[3] Infertility is defined as the inability of a couple to achieve conception during one year of marriage.[2] Infertility globally affects approximately 10-15% of couples. In men who smoke, researchers found a 13% decrease in sperm motility. These DNA damaged sperms may lead to problems with fertilization, embryo development, embryo implantation, and increased miscarriage rates. Male smokers may also have abnormal hormone levels, which can affect fertility. In 40% infertility cases, male factors

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are alone to blame. In rest of the 40%, female factors are alone responsible for infertility. 20% infertility cases are due to combined male and female factors.[2] Semen analysis is a reliable diagnostic method to analyse the male partners of infertile couples. Evaluation of the semen sample parameters may show causes of infertility in couple and it will be helpful in further treatment.[2] Age plays important role in fertility in male. Fertility in males reaches peak at an age of 35 years and decreases slowly after the age of 45 years.[3] A study conducted on the basis of the quality of semen is also a major factor to evaluate male partners in couples. We analysed the distribution of number of the semen analyses, the changing semen data and sperm parameters depending on the aetiology and also the cause of these results in male partners of infertile couples in Konkan region.

Aims and Objectives

1. Routine semen analyses of male patients referred with infertility will be studied based on clinical history, semen data, physical and chemical evaluation, sperm data, other cells in semen fluid.
2. The distribution of number of the semen analyses, the changing semen data, and sperm parameters depending on the etiology and also the cause of these results in male partners of infertile couples will be studied. Semen reporting for male infertility will be based on WHO 2010, 5th guidelines.
3. In few known infertile males, based on routine semen analyses, In-utero-insemination (IUI) was done, that requires pre-wash and post-wash handling and reporting of the semen. The quality of post-wash sample was analysed with respect to pre-wash results.

The study will help to evaluate and treat the causes for male infertility.

MATERIALS AND METHODS

Type of study and study period: This was a pioneer, prospective observational study done on semen analysis in a rural tertiary care hospital in Konkan region over five years' period from 1st January 2014 to 31st July 2019.

Sample Size: 120

Ethics approval: The institutional medical ethics committee approved this study.

Inclusion criteria:

1. Male patient who is giving consent for semen analysis.
2. Male patients referred for infertility check up with inability to conceive with wife for 12 months after unprotected intercourse.
3. Males who came for IUI procedure.

Exclusion criteria: Male patient who is not giving consent for semen analysis.

Study Method: Masturbation

Requirements for collection: Sterile Container

Figure 1: Sterile container With Semen Sample

Macroscopic Examination of semen sample:

Volume, colour, odour, viscosity: noted.

Liquefaction time: Normal viscosity: small discrete drop. Abnormal viscosity: drop from thread >2cm. Mechanical liquefaction was done, in case; sample did not liquefy for > 45 mins.

pH: Dip pH paper in sample. Check pH in indicator strip.

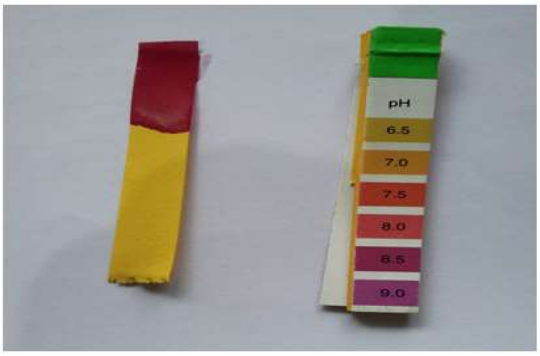

Figure 2: pH Paper Strip- Indicator strip denoting pH 8.5

Microscopic Examination:

Sperm motility (wet mount): All motile and non-motile sperms are counted in randomly chosen fields in a wet preparation under 40× objective. Result is expressed as a percentage of motile spermatozoa observed according to WHO 2010 guidelines.[1]

Sperm count: The sperm count is done after liquefaction in a counting chamber following dilution and the total number of spermatozoa is reported in millions/ml (10^6/ml).

-Dilution: (sodium bicarbonate-formalin diluting fluid)

In 1 drop of semen sample, add 19 drops of semen diluting fluid (1:20). Mix well. Incubate for 5 mins at room temperature.

-Neubauer's chamber: Neubauer`s chamber is thick glass plate with the size of glass slide of 30x70mm.

Figure 3: Neubauer's chamber with coverslip and processed semen sample for sperm count

-Charging the Neubauer's chamber:

Use flat surface like table or a workbench to place the delicate glass chamber.

Put the coverslip glass on the Neubauer's chamber.

With a pipette, carefully draw up around 10 ml of the dilution.

Place the pipette tip against the edge of the cover slip and slowly expel the liquid until the counter chamber area covering the coverslip is full. Allow semen to settle for 5 mins.

Counting: Count the cells in the corner four large squares.

N= Number of cell counted in 4 squares.

-Latest' Grades Of Sperm Motility In Semen Analysis (WHO 2010)[1]:

Grade IV: Fast and forward progression in straight direction.

Grade III: Move forward, slower speed or in curved direction.

Grade II: Slow but poorly defined direction.

Grade I: Move but fail to progress forward

Grade 0: No movement

Sperm Morphology by Pap smear: Smears are prepared from semen sample for this procedure. After this, wet fixation is done of smears instantly. Fix smear with methanol in Coplin jar or by biofix spray (30min). Later Papanicolaou (Pap) staining was done to look for sperm morphology. At least **200** spermatozoa should be counted under **oil immersion.** Percentages of normal and abnormal spermatozoa should be recorded. Normally, > 4% of spermatozoa should show normal morphology (WHO, 2010)[1]. The defects in morphology that are associated with infertility in males include defective mid-piece (causes reduced motility), an incomplete or absent acrosome (causes inability to penetrate the ovum), and giant head (defective DNA condensation).

Terminology in semen analysis:[1]

Normozoospermia: All semen parameters - normal.

Hypospermia: Low semen volume

Hyperspermia: High semen volume

Oligozoospermia: Sperm concentration <15 million/mL.

Azoospermia: Absence of sperms in seminal fluid.

Aspermia: Absence of ejaculate.

Asthenozoospermia: Reduced sperm motility; percentage of progressively motile sperms below lower reference limit (<32%) and/or percentage of total motile sperms below lower reference limit (<40%).

Tetrazoospermia: Spermatozoa with reduced proportion of normal morphology (or increased proportion of abnormal forms).

Oligoasthenoteratozoospermia: All sperm variables are abnormal.

Necrozoospermia: All sperms are non-motile or non-viable.

Leukocytospermia / Leucospermia: >1 million white blood cells/ml of semen.

Biochemical Test:

Fructose test: (Resorcinol Method) : Fructose is the energy source for sperm motility. **A positive fructose is considered normal.** Azoospermia and fructose negative results may indicate an absence of seminal vesicles / vas deferens in the area of seminal vesicles / obstruction of seminal vesicles. The procedure for determining the amount of fructose in semen involves heating semen in a strong acid in the presence of resorcinol to develop brown colour. **Faint or no Brownish colour change** indicates absence of fructose in sample.

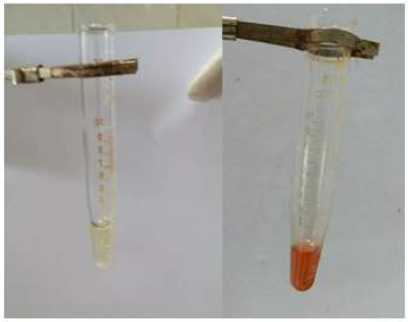

Figure 4: *Left image-Before, Right image-* After the reaction: Fructose positive Resorcinol test reaction with brown colour indicating presence of fructose

IUI PROCEDURE (SOP):

Patient may come two days before the IUI procedure for paying the charges for IUI procedure. If husband is present at that time, instruction on semen collection may be given at the time of paying charges for IUI.

Patient (husband) will come for semen analysis for IUI on the prescribed date, earliest by 8:30 am. If patient has already paid charges, preservative media for semen preparation may be kept in incubator at 8 am on the day

of IUI to save time. If semen takes more than 45 mins for liquefaction, it should be liquefied: either mechanically (with pipette) or chemically (adding the same sperm wash media /puresperm-2). Semen sample after collection should be instantly kept in incubator at 37^0 C for 15 mins only. Bring all components of the kit and semen sample to 37^0 C before use. this will avoid shock to the spermatozoa, IUI sample should ideally be prepared within 2 hours after semen collection. Transfer 1 ml of PURE SPERM-1 (density gradient) solution to the conical centrifuge tube (falcon tube). Analyze the prewash motility and count with diluting fluid on Neubauer`s chamber (1:20 dilution, depending on pathologist opinion). Also provide wet mount of the same applying the coverslip. Gently layer 2-3 ml, of semen sample using sterile pipette over the pure sperm solution-1 in the conical tube. Centrifuge at 2000 rpm at 15-20 minutes depending on the viscosity of the specimen. Using transfer pipette, discard the supernatant. Supernatant contains seminal fluid and debris. Supernatant should be discarded without disturbing the sperm pellet. Add 3ml of PURE SPERM -2 (wash solution) to the sperm pellet and re-suspend the pellet by gently tapping the tube with fingers. Carefully discard the supernatant. Resuspend the pellet with 0.4 ml 0f PURE SPERM-2. Analyze post wash motility and count with semen diluting fluid on Neubauer`s chamber (1:20 dilution, depending on pathologist opinion). Also provide wet mount of the same applying the coverslip. Then sample is ready to use. Final IUI prewash and post wash report of the male patient is given to the concerned patient with his processed final post wash sample. Sample is provided either to the male patient or his wife. This sample is used up instantly by the gynaecologist for fertility treatment procedure on the female patient.

RESULTS

Table 1: Age distribution of men who came for routine semen analyses

Age (years)	No of cases	
	No.	%
21-25	7	5.85
26-30	53	44.17
31-35	39	32.5
36-40	14	11.66
41-45	4	3.33
46-50	2	1.66
51-55	1	0.83
Total	120	100

Table 2: Semen data parameter: Volume status of men who came for routine semen analysis w.r.t 1.5 ml cut off of semen volume

Volume (ml)		<1.5 ml	>1.5 ml	Total
No of cases	No	29	91	120
	%	24.16	75.84	100

Table 2 shows that maximum 91 out of 120 cases (75.84%) had semen volume of more than 1.5 ml. According to one lab test manual semen volumes between 2.0 mL and 5 ml are normal.[15] WHO regards 1.5 ml as the lower reference limit.

*Semen data parameter: Colour of semen sample of men who came for routine semen analyses: Maximum 112 cases out of total 120 cases (93.36%) had expected normal color of the semen, that is, whitish grey on collection. Semen normally has a normal whitish-gray colour. It tends to get a yellowish tint as a man ages. Semen color is also influenced by the food we eat.

*Semen data parameter: Liquefaction Time of semen sample of men who came for routine semen analyses: The liquefaction is the process when the gel formed by proteins from the seminal vesicles is broken up and the semen becomes more liquid. It normally takes less than 20 minutes for the sample to change from a thick gel into a liquid, as per WHO, 2010. We used WHO guidelines. Maximum cases had liquefaction time of >30 mins in our study (38.34%), followed by those between 20-30 mins (36.66%) and <20 mins (25% cases). The cases with normal liquefaction time were 25%. We did not follow the NICE guidelines. In the NICE guidelines, a liquefaction time within 60 minutes is regarded as within normal ranges.[4]

Table 3: Semen data parameter: pH of semen sample of men who came for routine semen analyses

pH of semen sample		<7.2	7.2-7.8 (WHO)	>7.8	Total
No of cases	No	08	17	95	120
	%	6.61	14.19	79.20	100

According to one lab test manual normal pH range is 7.1-8.0, [5] **WHO criteria specify normal pH as 7.2-7.8.**[6] Semen with normal pH comprised 79.20% of cases. Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. pH less than 7.2 comprised 6.61% of cases. A basic ejaculate (higher pH value) may indicate an infection.[6] pH value more than 7.8 comprised 79.2% of cases. A pH value that is outside of the WHO normal range is harmful to sperm and affects their ability to penetrate the egg.[5] ***Fructose level** in the semen may be analysed to determine the amount of energy available to the semen for moving.[5] Quantitatively, WHO specifies a normal level of 13 µmol of fructose per semen sample. Absence of fructose may indicate a problem with the seminal vesicles.[6] It was absent in 5% of our cases. ***Semen data parameter: Pus cells in semen (leucospermia)** indicate infection, either directly or via urethra (associated urinary tract infection).Leucospermic cases (>3/hpf) were 88 cases out of 120 cases (73.33%). Negligible pus cells in semen were seen in 26.67% of cases. ***Sperm data parameter: Sperm count** of men who came for routine semen analyses: Over 15 million sperm per millilitres is considered normal, according to the WHO in 2010.[7] A lower sperm count is considered oligozoospermia. Oligozoospermic cases were 45% in our study while normal sperm count was noted in 55% of the cases. Aspermia (absence of semen) or azoospermia (absence of sperms) cases were not seen in our study.

Table 4: Sperm data parameter: Sperm motility of men who came for routine semen analyses

Motility (%)		No of cases	
		No	%
Progressive sperm motility (Grades 3+4)	<32	61	50.83
	>32	59	49.17
	Total	120	100
Total sperm motility (Grades 2+3+4)	<40	32	26.66
	>40	88	73.34
	Total	120	100

The World Health Organization has a value of 40% total sperm motility and 32% progressive sperm motility and this must be measured within 60 minutes of collection. The reduced total sperm motility and reduced progressive sperm motility was **26.66% and 50.83% respectively** in our study, amongst 120 cases (Table 4). WHO also has a parameter of vitality judged with Eosin-Nogrosin stain, with a lower reference limit of 60% live spermatozoa, which was not done in our study.[7]

Table 5: Sperm data parameter: Sperm motility indicator of men who came for routine semen analyses

Motility indicator	No of cases	
	No	%
Both Normal (total sperm motility and progressive sperm motility)	59	49.17
Both Abnormal (total sperm motility and progressive sperm motility)	32	26.66
One Abnormal (Normal total sperm motility and reduced progressive sperm motility)	29	24.17
Total	120	100

Total sperm motility and progressive sperm motility (Table 5): Both abnormal in 26.66% of cases (asthenozoospermic); Both normal in 49.17% of cases; One abnormal (Normal total sperm motility and reduced progressive sperm motility): 24.17% of cases (asthenozoospermic).

Table No. 6: Sperm data parameter: Sperm morphological abnormalities in 66 cases with normal sperm count (>15 Millions/ml.)

Part of sperm	Abnormalities detected as:	Number of cases with abnormalities (0ut of total 66 cases)	Percentage of sperms showing morphological abnormalities	
			<5 %	5% to 20%
Head	**Small**	57	16	41
	Large	60	9	51
	Round	59	21	38
	Tapered	58	12	46
	Amorphous heads	44	38	6
	Abnormal forms / Others	38	32	6
	Duplicate forms	45	30	15
Middle piece	**Cytoplasmic droplets/ Excess residual cytoplasm**	32	25	7
	Thick Middle piece	61	13	48
	Bent neck defect	40	33	4
Tail	**Coiled tail**	58	22	36

Table 6 shows that sperm head defects are more in number in our study with normal sperm count cases (66 in number). Improper sperm morphology (teratozoospermia) leads to abortion or miscarriage. WHO 2010 says even 4 % normal sperm morphology is good enough for male fertility.

Table 7: Sperm data parameter : Frequency of 66 cases with multiple/single sperm part abnormalities with normal sperm count (>15 Millions/ml.)

Part of sperm with abnormalities	Number of cases with single part/ multiple abnormalities in sperm morphology	
	No	%
Head	2	3.04
Middle piece	3	4.54
Tail	2	3.04
Head, middle piece	1	1.51
Head, tail	0	0
Middle piece, tail	0	0
Head, middle piece, tail	58	87.87
Total	66	100

Frequency of cases with multiple abnormalities was noted in 59/66 cases (89.38%). Only 7 cases had single sperm part abnormality (10.62%). Maximum cases were having multiple sperm part defects. In these 66 cases; the category of Head, middle piece, tail combination defects were 87.87%.

Table 8: Sperm data parameter: Morphological abnormalities in 54 cases with reduced sperm count (<15 Millions/ml)

Part of sperm	Abnormalities detected as:	Number of cases with abnormalities (0ut of total 54 cases)	Percentage of sperms showing morphological abnormalities	
Head	Small	48	8	40
	Large	46	10	36
	Round	41	10	31
	Tapered	40	10	30
	Amorphous heads	25	17	8
	Abnormal forms / Others	16	12	4
	Duplicate forms	30	19	11
Middle piece	Cytoplasmic droplets/ Excess residual cytoplasm	17	9	8
	Thick Middle piece	39	9	30
	Bent neck defect	21	16	5
Tail	Coiled tail	41	15	26

Table 8 shows that sperm head defects are more in number in our study with normal sperm count cases (54 in number). Improper sperm morphology (teratozoospermia) leads to abortion or miscarriage. WHO 2010 says even 4 % normal sperm morphology is good enough for male fertility. Multiple sperm part abnormalities were more common than single sperm part abnormality.

Table 9: Sperm data parameter: Frequency of cases with multiple/single sperm part abnormalities in 54 cases with reduced sperm count (<15 Millions/ml.)

Part of sperm with abnormalities	Number of cases with single part/ multiple abnormalities	
	No	%
Head	6	11.12
Middle piece	0	0
Tail	1	1.85
Head, middle piece	4	7.40
Head, tail	7	12.97
Middle piece, tail	0	0
Head, middle piece, tail	36	66.66
Total	54	100

Table 9 shows that the frequency of cases with multiple abnormalities was noted in 47/54 cases (87.03%). Only 7 of 54 cases had single sperm part abnormality (2.97%). Maximum cases were having multiple sperm part defects. In these 54 cases; the category of Head, middle piece, tail combination defects were 66.66%.

DISCUSSION

Table 10: Comparative table of cases with pH less than 7.2 and fructose test results

Qualitative Result of semen-fructose test	pH	
	<7.2	%
Present	2	25
Absent	6	75
Total	8	100

Fructose level in the semen may be analysed to determine the amount of energy available to the semen for moving.[5] Table 10 shows that fructose tests were negative in 75% of cases wherein pH of semen sample was less than 7.2, indicating problem in the seminal vesicles. Total cases with pH less than 7.2 were 8 cases. This proves the fact that absence of fructose may indicate a problem with the seminal vesicles.[6] Acidic ejaculate (lower pH value : <7.2) may indicate one or both of the seminal vesicles are blocked.

Table 11: Comparative table of cases with pH more than 7.8 and pus cells in semen

Pus cells in Semen sample (cells/ hpf)	No of cases		
	No	%	%
0-2	29	30.52	30.52
2-4	14	14.82	
4-8	32	34.68	
8-12	10	10.52	
12-16	06	6.31	69.48
16-20	01	1.05	
20-24	02	2.10	
Total	**95**	**100**	**100**

A basic ejaculate (higher pH value: >7.8) may indicate an infection.[6] Pus cells in semen (leucospermia) indicate infection, either directly or via urethra (associated urinary tract infection).95 cases had pus cells in semen with high. Out of this, 29 cases (30.52%) had negligible pus cells (0-2/hpf). Rest 66 cases out of 95 cases (69.48%) had >2 pus cells/ hpf in semen with bacteria indicating infection and higher pH: >7.8. 88/120 cases had leucospermia. 50 out of 88 cases had corresponding pus cells in urine (58.82%). 43.18% cases had no pus cells in urine.

Table No. 12: Comparative findings of studies showing pus cells in Semen

Jajoo S, Kalyani KR (2013)[2]			Our study (2019)		
Pus Cells in semen	No of cases		Pus Cells in semen	No of cases	
	No	%		No	%
Present(>3/hpf)	33	33	Present(>3/hpf)	88	73.33
Absent(02/hpf)	67	67	Absent(02/hpf)	32	26.67
Total	**100**	**100**	**Total**	**120**	**100**

Table 12: 88 cases had leucospermia in our study while 33 cases were leucospermic in the other study. This means infected semen samples were more seen in our study due to humid Konkan environment and associated UTI.

Table 13: Comparative table of cases with defective sperm morphology in cases with history of abortion

Part of sperm	Abnormalities detected as:	Number of cases with abnormalities (0ut of total 7 cases with history of abortion)
Head	Small	4
	Large	5
	Round	5
	Tapered	2
	Amorphous heads	2
	Abnormal forms / Others	1
	Duplicate form	2
Middle piece	Cytoplasmic droplets/ Excess residual cytoplasm	1
	Thick Middle piece	3
	Bent neck defect	1
Tail	Coiled tail	3

Table 13 shows that defective sperm morphology was noted in all 7 cases with history of abortion. This means teratozoospermia was noted to occur in abortion cases. Head defects were more common.

Table 14: Comparative findings of studies showing age group distribution

Jajoo S, Kalyani KR (2013)[2]			Our study (2019)		
Age (years)	No of cases No	%	Age (years)	No of cases No	%
<30	56	56	**<30**	38	26.66
>30	44	44	**>30**	82	68.34
Total	100	100	**Total**	120	100

Table no. 14 shows that male infertility cases aged <30 years were 26.66% in our study while Jajoo S, Kalyani KR (2013)[2] recorded 56% cases aged <30 years. Male infertility cases aged > 30years were 68.34% in our study while Jajoo S, Kalyani KR (2013)[2] recorded 44% cases aged >30 years.

Table 15: Comparative findings of studies showing distribution w.r.t. addiction history

Jajoo S, Kalyani KR (2013)[2]			Our study (2019)		
Addiction	No of cases No	%	Addiction	No of cases No	%
Alcohol	33	33	**Alcohol**	20	16.66
Tobacco	42	42	**Tobacco**	46	38.34
Both	25	25	**Both**	24	20
None	-	-	**None**	30	25
Total	100	100	**Total**	120	100

Table no. 15 shows tobacco addiction in infertile men in 38.34% cases in our study, while Jajoo S, Kalyani KR (2013)[2] recorded 42% of the same.Alcoholic addiction in infertile men was seen in 16.66% cases in our study, while Jajoo S, Kalyani KR (2013)[2] recorded 33% of the same.Both Alcoholic and tobacco addiction in infertile men was seen in 20% cases in our study, while Jajoo S, Kalyani KR (2013)[2] recorded 25% of the same.No addiction history in infertile men was seen in 25% cases in our study, while Jajoo S, Kalyani KR (2013)[2] recorded none in this category.

Table 16: Comparative study findings of studies showing blockwise distribution w.r.t. duration of male infertility

S. Samal, *et al.* (2012)[3]							Our study (2019)					
Block	No of cases	Duration of infertility 1-4	5-8	9-12	>12	T	Block	No of cases	Duration of infertility 1-4	5-8	9-12	>12	T
A	No	162	163	78	27	430	A	No	-	-	-	-	0
	%	13.54	12.86	19.25	20.45	14.33		%	-	-	-	-	0
B	No	178	198	114	35	525	B	No	38	11	4	1	54
	%	14.88	15.62	28.14	26.51	17.5		%	23.33	9.16	3.32	0.83	45
C	No	233	240	95	32	600	C	No	-	-	-	-	0
	%	19.48	18.94	21.11	24.24	20		%	-	-	-	-	0
D	No	243	309	81	27	660	D	No	37	15	8	1	61
	%	20.31	24.38	20	20.45	22		%	27.5	12.5	6.66	0.83	50.84
E	No	380	357	37	11	785	E	No	1	2	-	1	4
	%	31.77	28.17	9.13	8.33	26.17		%	0.83	1.67	-	0.83	3.33
F	No	-	-	-	-	0		No	1	-	-	-	1
	%	-	-	-	-	0		%	0.83	-	-	-	0.83
T	No	1196	1267	405	132	3000	T	No	77	28	12	3	120
	%	39.87	42.23	13.5	4.4	100		%	64.17	23.33	10	2.5	100

A-Normozoospermia B- Oligozoospermia C- Azoospermia D-Asthenozoospermia E-Teratoasthenozoospermia F-Oligoasthenoteratozoospermia (OAT) T-Total

Table no 16 shows comparative study findings of studies showing blockwise distribution w.r.t. duration of male infertility. Blocks (A, B, C, D, E, F) are showing abnormalities in semen parameters, as shown below in this table. In our study we had a total 120 male cases of infertility which were studied while S. Samal, *et al.* (2012)[3] recorded 3000 cases. **Normozoospermic condition** in infertile males was seen in total 14.33% cases by S. Samal, *et al.* (2012)[3] and 20.45%

cases had Normozoospermic condition seen in duration of infertility >12 years in study of S. Samal, *et al.* (2012)[3] while no Normozoospermic condition was seen in our study.

Oligospermic condition in infertile male was seen in total 17.5% of their cases while it was 45% in our study. Maximum 23.33% Oligospermic conditions were seen in duration of infertility of 1-4 years in our study. S. Samal, *et al.* (2012)[3] recorded Oligospermic condition in infertile males in 17.5% and 28.14% with infertility duration of 9-12 years and >12 years respectively.

Azoospermic condition in infertile males was seen in total 20% of their cases. 24.24% of these cases had Azoospermic condition seen in duration of infertility >12 years in study of S. Samal, *et al.* (2012)[3]. No Azoospermic condition was seen in our study.

Asthenozoospermia condition in infertile males was seen in total 50.84% in our study and 22% in the other study. 27.5% of these Asthenozoospermia conditions were seen in duration of infertility 1-4 years in our study while S. Samal, *et al.* (2012)[3] reported Asthenozoospermia condition in 24.38% infertile males maximum with infertility duration of 5-8 years.

In our study, **teratoasthenozoospermia condition** in infertile males in total 3.33% of our cases with 1.67% of these cases having infertility duration of 5-8 years. S. Samal, *et al.* (2012)[3] recorded Teratoasthenozoospermia condition in infertile males in total 26.17% cases with 31.77% of these cases having infertility duration of 1-4 years.

Oligoasthenoteratozoospermia condition in infertile males was seen in total 0.83% in our study and 0.83% of these had infertility duration of 1-4 years. S. Samal, *et al.* (2012)[3] recorded no Oligoasthenoteratozoospermia condition in their study.

Table No. 17: Comparative findings of studies showing blockwise distribution w.r.t. age distribution

S. Samal, *et al.* (2012)[3]								Our study (2019)							
Block	No of cases	Age range (Years)					T	Block	No of cases	Age range (Years)					T
		21-25	26-30	31-35	36-40	>40				21-25	26-30	31-35	36-40	>40	
A	No	127	798	734	138	3	1800	A	No	-	-	-	-	-	0
	%	7.05	44.33	40.77	7.66	0.16	61.98		%	-	-	-	-	-	0
B	No	15	122	173	500	36	846	B	No	4	24	18	6	2	54
	%	1.77	14.42	20.44	59.10	4.25	40.40		%	7.40	44.44	33.33	11.11	3.70	45
C	No	-	1	59	94	42	196	C	No	-	-	-	-	-	0
	%	-	0.005	30.10	47.95	21.42	9.36		%	-	-	-	-	-	0
D	No	6	1	16	7	12	42	D	No	3	27	20	7	4	61
	%	14.28	2.38	38.09	16.66	28.57	0.19		%	4.91	44.26	32.78	11.47	6.55	50.83
E	No	-	1	10	1	8	20	E	No	-	1	1	1	1	4
	%		5	50	5	40	0.95		%		25	25	25	25	3.33
F	No	-	-	-	-	-	0		No	-	1	-	-	-	1
	%	-	-	-	-	-	0		%	-	100	-	-	-	0.83
T	No	148	923	992	740	101	2094	T	No	7	53	39	14	7	120
	%	5.1	31.78	34.16	25.48	3.48	100		%	5.84	44.16	32.5	11.66	5.84	100

A-Normozoospermia B-Oligozoospermia C-Azoospermia D-Asthenozoospermia E-Teratoasthenozoospermia F-Oligoasthenoteratozoospermia T-Total

Table no.17 shows the comparative findings of studies showing blockwise distribution w.r.t. age distribution. Block (A, B, C, D, E, F) are showing abnormalities in semen parameters, as shown below in this table. In our study we had total 120 male cases of infertility which were studied while S. Samal, *et al.* (2012)[3] recorded 2094 cases.

Normozoospermic condition in infertile males was seen in total 61.98% cases by S. Samal, *et al.* (2012)[3]. 44.33% cases had normozoospermic condition seen in age group of 26-30 years out of 1800 normozoospermic cases. No normozoospermic condition was seen in our study.

Oligospermic condition in infertile males was seen in total 45% in our study and 40.40% in the other study, which were nearly equal. 44.44% of these Oligospermic conditions were seen in age group of 26-30 years in our study while S. Samal, *et al.* (2012)[3] reported Oligospermic condition in 59.10% infertile males (maximum) with infertility age group of 36-40 years out of 846 cases.

Azoospermic condition in infertile males was seen in total 9.36% of their cases in study of S. Samal, *et al.* (2012)[3]. 47.95% of these cases had Azoospermic condition seen in the group of 35-40 years. No Azoospermic condition was seen in our study.

Asthenozoospermic condition in infertile males was seen in total 50.83% in our study and 0.19% in the other study, which was much more in our study. 44.26% of these Asthenozoospermic conditions were seen in age group of 26-30 years in our study while S. Samal, *et al.* (2012)[3] reported maximum Asthenozoospermic condition in 38.09% infertile males with infertility age group of 31-35 years.

Teratoasthenozoospermic condition in infertile males was seen in total 3.3% in our study and 0.95% in the other study, which was lowest in both studies. Oligoasthenoteratozoospermia (OAT): One case was seen in our study in age range of 26-30 years, comprising 0.83% in our study. No OAT condition was seen in the study of S. Samal, *et al.* (2012)[3].

This table no. 16 shows that **asthenozoospermic cases were more in number followed by oligozoospermic cases in our study with 26-30 years of age group as the most commonly affected age group.** In-utero insemination (IUI) procedure improves the quality of sperm parameters. Though sperm count is low on post-wash count, as dead, immotile sperms get discarded; the sperm data parameters like motility grades, sperm morphology are best for the latter IUI procedure. This post-wash sample is provided to gynaecologist via the male patient for respective wife's infertility treatment which is dependent on her ovulation status. We don't have results of the success of infertility treatment with IUI procedure as it's a gynaecologist's exercise.




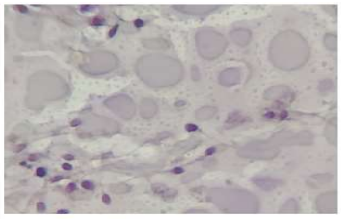

Figure 5 **Figure 6** **Figure 7** **Figure 8**

Figure 5: Wet Mount image: Sperms are looked for motility; **Figure 6:** Neubauer's chamber: Sperms are counted manually as those dots there in 4x field in the corner four squares of the chamber; **Figure 7:** Pap smear: Stained smear with PAP stain for sperm morphology; **Figure 8:** Sperm morphology: Microscopic view (PAP, x400) for sperm morphology

CONCLUSION

Our study shows that the maximum male cases (44.17%) who came for semen analysis were in the age group of 25-30 years of age. Maximum male cases had 1-3 years of marital status (55%).

Maximum 110 cases (91.67%) out of total 120 cases had no living children. Only nine cases had one living child while one case had two living children, before semen analysis.

Maximum cases had abstinence before semen analysis of more than three days (67.5%). In semen analysis, if the duration of abstinence is more, then the sperm motility is affected. If the duration of abstinence is less than 2 days, then sperm count is low. So ideally it should be of three days only.

Miscarriage status of wife in men who came for routine semen analysis was noted in seven out of 120 cases. One abortion was noted in six cases while two abortions were noted in one case. Maximum cases had no abortion (94.17%).

According to WHO, 5th (2010) guidelines, the minimum volume for adequate semen analysis should be 1.5 ml (lower reference limit). Its range is 1.4-1.7 ml to be precise. Maximum cases (60%) had semen volume between 1.6-2.0 ml. Maximum 112 cases out of total 120 cases (93.36%) had expected normal colour of the semen, that is, whitish grey on Semen collection.

Maximum cases had liquefaction time of more than 30 mins (38.34%), followed by those between 20-30 mins (36.66%). Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. pH less

than 7.2 comprised 6.61% of cases. A basic ejaculate (higher pH value) may indicate an infection. pH value more than 7.8 comprised 79.2% of cases. Absence of fructose may indicate a problem with the seminal vesicles. Fructose was absent in 5% of our cases.

Pus cells in semen (leucospermia) indicate infection, either directly or via urethra (associated urinary tract infection). Leucospermic cases were 88 cases out of 120 cases (73.33%). Negligible pus cells in semen were seen in 26.67% of cases. Oligozoospermic cases were 45% in our study. Aspermia (absence of semen) or azoospermia (absence of sperms) cases were not seen in our study. Reduced total sperm motility and reduced progressive sperm motility was 25.83% and 46.67% respectively in our study, amongst 120 cases (asthenozoospermic).

Improper sperm morphology (teratozoospermia) leads to abortion or miscarriage. WHO 2010 says even 4 % normal sperm morphology is good enough for male fertility. On PAP staining, multiple sperm partmorphological abnormalities were more common than single sperm part abnormality in both categories of normal and low sperm count. Defective sperm morphology was noted in all 7 cases who had history of abortion. This means teratozoospermia was noted to occur in abortion cases, in our study.

Pus cells in semen (leucospermia) indicate infection, either directly or via urethra (associated urinary tract infection). Leucospermic cases can have corresponding urinary tract infection as well, which needs to be treated. 43.18% cases had no pus cells in urine. On comparative analysis, Male infertility cases aged <30 years were 26.66% in our study while Jajoo S, Kalyani KR (2013)[2] recorded 56% of cases aged <30 years. Male infertility cases aged >30 years were 68.34% in our study while Jajoo S, Kalyani KR (2013)[2] recorded 44% cases aged >30 years.

Blockwise distribution w.r.t. duration of male infertility: In our study we had a total 120 male cases of infertility which were studied while S. Samal, *et al.* (2012)[3] recorded 3000 cases. Due to varied sample size, the results varied.

Normozoospermic condition in infertile males was seen in total 14.33% cases by S. Samal, *et al.* (2012)[3] while no Normozoospermic condition was seen in our study.

Oligospermic condition in infertile male was seen in total 17.5% of their cases while it was 45% in our study. Maximum 23.33% Oligospermic conditions were seen in duration of infertility of 1-4 years in our study.

Azoospermic condition in infertile males was seen in total 20% of their cases. 24.24% of these cases had Azoospermic condition seen in duration of infertility >12 years in study of S. Samal, *et al.* (2012)[3]. No Azoospermic condition was seen in our study.

Asthenozoospermia condition in infertile males was seen in total 50.84% (maximum) in our study and 22% in the other study. 27.5% of these Asthenozoospermia conditions were seen in duration of infertility of 1-4 years in our study.

Teratoasthenozoospermia condition in infertile males was seen in total 3.33% of our cases with 1.67% of these cases having infertility duration of 5-8 years.

Oligoasthenoteratozoospermia condition in infertile males was seen in total 0.83% in our study and 0.83% of these had infertility duration of 1-4 years.

Asthenozoospermic cases were more in number followed by oligozoospermic cases in our study with 26-30 years of age group as the most commonly affected age group. This finding was unlike the varied findings in the comparative study of S. Samal, *et al.* (2012)[3].

Intra-uterine insemination (IUI) procedure improves the quality of sperm parameters. Though sperm count is low on post-wash count, as dead, immotile sperms get discarded; the sperm data parameters like motility grades, sperm morphology are 'best' in the post-wash processed semen sample. This post-wash sample is provided to gynaecologist via the male patient or his respective wife for her fertility treatment. The success of this fertility treatment also depends on the ovulation status of the treated female.

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