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Correlation of Parameters of Semen Analysis in Oligospermia in Infertility

Dr. Sumaiyya Firdous[1], Dr. Zeenath Begum[2], Dr. Suresh Patil[3], Dr. Syeda Heena Kauser[4]

[1]Postgraduate student Dept. of Pathology Faculty of medical sciences, Khaja Banda Nawaz University

[2]Professor and HOD, Dept. of Pathology Faculty of medical sciences, Khaja Banda Nawaz University

[3]Dept. of surgery Kalaburagi scanning and diagnostic center

[4]Assistant Professor Dept. of Pathology Faculty of medical sciences

Corresponding Author: Dr. Syeda Heena Kauser

ABSTRACT

Background: Various semen parameters including morphology, vitality, motility, maturation, pH, count, agglutination and biochemical parameters altogether determine the overall quality of semen. Common factors associated with oligospermia are diseases (genetic, infectious, hormonal and obstructive), smoking, environmental factors such as radiation, heavy metal poisoning, overheating of testicles.

Objective: Semen analysis in infertile males of various age group with possible correlation of parameters in relation to oligospermia.

Materials and methods: This cross-sectional descriptive observational study included 150 semen sample received from various infertility clinics, obstetric gynaecology clinics, In vitro fertilization centers, and from individuals with infertility problems. The study was conducted from July2021-February2022. Parameters studied included volume, liquefaction time, pH, count, motility, morphology, immature forms, agglutination, leucocytes, and fructose.

Results: Out of 150 cases, majority belonged to the age group of 31-40years, mean age was 33.14 years. Seventy-three cases (48.7%) were normal, six (4%) showed azoospermia, 71 (47.3%) showed oligospermia. Parameters correlated positively with oligospermia are age (0.008 Pearson correlation, 0.946 p value, not significant), pH (0.194 Pearson correlation, 0.106 p value, not significant), liquefaction time (0.106 Pearson correlation, 0.378 p value, not significant). Parameters correlated negatively with oligospermia are volume (-0.58 Pearson correlation, 0.633 p value, not significant) and motility (-2.77 Pearson correlation, 0.19 p value, significant)

Conclusion: The study showed a positive correlation of oligospermia with age, pH, liquefaction time and negative correlation with volume and motility. The study also showed a significant association between oligospermia and motility, morphology, agglutination and fructose.

KEYWORDS: semen analysis; oligospermia; sperm morphology; motility; volume; pH; agglutination; fructose

INTRODUCTION

Semen analysis is an important investigation with respect to male fertility and genital tract patency and to enable appropriate treatment for subfertility and to monitor treatment response.[1]

As per WHO estimates 60 to 80 million couples worldwide currently suffer from infertility. It varies across the region of the world and is estimated to affect 8 to 12% of the couples worldwide.[1] According to National Centre for Health Statistics the absolute number of impaired fecundities increased by about 2.7 million women, from 4.56 million in 1982 to 7.26 million in 2002, then fell slightly to 6.71 million in 2006 to 2010.[2] Moreover, the fertility rate in men younger than age 30 years has also decreased worldwide by 15%.[3]

As per WHO, the overall prevalence of primary infertility ranges between 3.9% and 16.8%.[1] It was reported that 40% of infertility cases were related to men, 40% of women and 20% of both the sexes. Males with sperm parameters below the WHO normal values are considered to have male factor infertility. The most significant of these are low sperm concentration (oligospermia), poor sperm motility and abnormal sperm morphology. Other factors associated with infertility include semen volume and other seminal markers of epididymal, prostatic and seminal vesicle function. As high as 90% of male infertility problems are related to count and there is a positive association between abnormal semen parameters and sperm count.[4]

Various semen parameters including morphology, vitality, motility, maturation, pH, count, viscosity, agglutination and biochemical parameters altogether determine the overall quality of semen. The problem with sperm count, motility, and morphology can be due

to pre-testicular, testicular, and post-testicular factors. There is a significant overlap between male infertility and spermatogenic function as measured by seminal fluid analysis. The majority of men with male infertility have oligospermia or azoospermia but some infertile men have normal sperm counts.[5] Hence, the study was carried out to know the reason of infertility even with normal sperm count.

MATERIALS AND METHODS

After 3 days of abstinence, the sample was obtained by masturbation, collected in a clean, dry, sterile, and leakproof wide-mouthed plastic container. Minimum sample required for analysis is 1.5 ml and it is examined within 1 hour of collection.
Parameters studied include volume, liquefaction time, pH, count, motility, morphology, immature forms, agglutination, leucocytes, fructose as per WHO laboratory manual for the examination and processing of human semen (6th edition)[1].

Volume was measured with a graduated disposable pipette. As per WHO, the normal volume of ejaculate after 2-7 days of sexual abstinence is about 2-6 ml. Normal volume is 1.5ml. Volume less than 0.5ml is considered Hypospermia and more than 6ml is considered as hyperspermia. Hypospermia can be due to improper collection, hypogonadism, retrograde ejaculation, obstruction of lower urinary. Hyperspermia can be due to prolonged abstinence or excessive secretion from the accessory sex glands.

pH was estimated by pH strip used for urine analysis.
Count was carried out by Neubaur's chamber. The normal total sperm count is 15 million spermatozoa per ml. Azoospermia is the absence of sperm in seminal plasma. Oligospermia is low sperm count that is <15 million sperms/ml.[1]
Motility is assessed as per WHO guidelines. A four-category system for grading motility is recommended by WHO:
1. Rapidly progressive (25 µm/s) – spermatozoa moving actively, either linearly or in a large circle, covering a distance, from the starting point to the end point, of at least 25 µm (or ½ tail length) in one second;
2. Slowly progressive (5 to < 25 µm/s) – spermatozoa moving actively, either linearly or in a large circle, covering a distance, from the starting point to the end point, of 5 to < 25 µm (or at least one head length to less than ½ tail length) in one second;
3. Non-progressive (< 5 µm/s) – all other patterns of active tail movements with an absence of progression – i.e. swimming in small circles, the flagellar force displacing the head less than 5 µm (one head length), from the starting point to the end point; and
4. Immotile – no active tail movements.

Morphology was studied using the Diff-Quick stain and as per WHO guidelines.
Normally, > 30% of spermatozoa should show normal morphology.[1]
Normal spermatozoon consists of three main components: head, neck, and tail. Tail is further subdivided into midpiece, main (principle) piece, and end piece.
Abnormal morphology includes defects in head, neck and tail.
Defects in head include large heads, small heads, tapered heads, pyriform heads, round heads, amorphous heads, vacuolated heads (> 20% of the head area occupied by vacuoles), small acrosomes, double heads.
Defects in the neck includes bent neck and tail forming an angle >90° to the long axis of head.
Defects in middle piece: asymmetric insertion of midpiece into head, thick or irregular midpiece, abnormally thin midpiece
Defects in tail: bent tails, short tails, coiled tails, irregular tails, multiple tails, tails with irregular width.[1]
Other parameters were also studied like aggregation, agglutination and fructose test was done using Selwinoff reagent.
Semen analysis of all samples was done and correlation of oligospermia with volume, liquefaction time, pH, count, motility, morphology, immature forms, agglutination, leucocytes, fructose was carried out to know the relation between the parameters and results.

IEC approval: Obtained from IEC- Khaja Banda Nawaz University- Faculty of Medical Sciences. Letter number: KBNU-FM/IEC/2022-23/129

Inclusion Criteria: All semen sample received from various infertility clinics, obstetric gynaecology clinics, In vitro fertilization centres as well as by the individual coming with self-references with infertility problems in Khaja Banda Nawaz Teaching and General Hospital and Kalaburagi Scanning and Diagnostic Centre, over a period of 8months from July2021-February2022.

Exclusion Criteria: Abstinence less than 3 days and more than three days. (In shorter period of abstinence sperm count is lower and in longer period of abstinence sperm motility is reduced).
Sample size estimation: 150 semen sample were received from various infertility clinics, obstetric gynaecology clinics, In vitro fertilization centres as well as by the individual coming with self-references with infertility problems for a period of 8months from July2021-February2022.

Data were summarized as mean ± SD (standard deviation). Fertile and infertile groups were studied. SPSS software was used for statistical analysis. For linear association, Chi Square test was used and exact measures of association were done.

RESULTS

Age

The majority of the sample belonged to males of age group 31-40 years. The mean age was 33.14 years. Two cases were included in Others-category as they didn't fit in any age group. Out of these two, one case was of 58 years and the other was of 17 years. Both the cases came with self-reference and as it is a retrospective study, we could not get much details of these patients.

Age (Years)	No. Of cases
20-30	57
31-40	84
41-50	7
Others	2 (17 and 58 years)

Volume:

Age (Years)	No. Of cases	Volume (ml)	
		>1.5 ml Normospermia	<1.5 ml Hypospermia
20-30	57	44	13
31-40	84	66	18
41-50	7	5	2
Others	2	0	2

Out of 35 cases of hypospermia, 15 cases (42.86%) were of oligospermia, 6 cases (17.14%) were of azoospermia and 14 cases (40%) had normal sperm count.

Sperm count:

Age (Years)	No. Of cases	Total count		Result		
		Normal	Reduced	Azoospermia	Oligospermia	Normal
20-30	57	27	28	2	28	27
31-40	84	43	38	3	38	43
41-50	7	3	4	0	4	3
Others	2	0	1 (17years)	1 (58years)	1	0

Out of 150 cases, 71cases (47.3%) were of oligospermia. 6 cases (4%) were of azoospermia. 73 cases (48.7%) were normal.

Motility:

Age (Years)	No. Of cases	Motility		
		Total Motility >42%	Rapid Progressive >25%	Slow Progressive <25%
20-30	57	26	14	14
31-40	84	43	19	19
41-50	7	3	3	1
Others	2	0	0	1(17Y)

Out of 150 cases,71 cases (47.3%) were of reduced sperm motility 79 cases (52.6%) were normal.

pH:

Age (Years)	No. Of cases	Ph	
		6.5-7.5	7.5-9
20-30	57	32	25
31-40	84	44	40
41-50	7	3	4
Others	2	1 (17Y)	1 (58Y)

Out of 150 cases, 80cases (53.3%) were of acidic pH (6.5 -7.5) and 70 cases (46.6%) were of alkaline pH (7.52 -9).

Agglutination:

Age (Years)	No. Of cases	Agglutination	
		Present	Absent
20-30	57	7	50
31-40	84	6	78
41-50	7	1	6
Others	2	0	2

Out of 150 cases, in 14 cases (9.3%) cases agglutination was seen and in 136 cases (90.7%) no agglutination was observed.

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Liquefaction time:

Age (Years)	No. Of cases	Liquefaction time	
		Normal	Delayed
20-30	57	51	6
31-40	84	77	7
41-50	7	6	1
Others	2	0	0

Out of 150 cases, in 14 cases (9.3%) delayed liquefaction time was observed. In 134 cases (89.3%) Liquefaction time was normal.

Morphology:

Age (Years)	No. Of cases	Morphology	
		Normal	Abnormal
20-30	57	37	18
31-40	84	53	28
41-50	7	3	4
Others	2	0	1

Out of 79 cases of normal count, in 69 cases (88%) normal morphology is seen. In 10 cases (12%) abnormal morphology is seen. In 6 cases (60%) tail defects, 2 cases (20%) neck defects, 1 case (10%) head defects and in 1 case (10%) excess residual cytoplasm was seen.

Out of 71 cases of oligospermia, in 41 cases (57.8%) abnormal morphology is seen. In 30 cases (42.2%) abnormal morphology is seen. In 17 cases (56.6%) tail defects, 6 cases (20%) neck defects, 5 cases (16.7%) head defects and in 2 cases (6.7%) excess residual cytoplasm was seen.

Other parameters: (Leukocytes, Immature forms, Fructose)

Age (Years)	No. Of cases	Leucocyte		Immature forms		Fructose	
		Present	Absent	Present	Absent	Positive	Negative
20-30	57	1	56	1	56	55	2
31-40	84	0	0	0	0	0	0
41-50	7	0	0	0	0	0	0
Others	2	0	0	0	0	0	0

Out of 150 cases, leukocytes, casts and crystals were seen in 1 case (0.6%), immature forms were seen in 1 case (0.6%) and in 2 cases (1.3%) fructose was absent.

Correlation of various parameters with oligospermia:

Correlations

		Age	Volume	pH	Liquefaction	Oligo	motility
Age	Pearson Correlation	1	.007	-.094	-.136	**.008**	-.042
	Sig. (2-tailed)		.954	.434	.259	**.946**	.725
	N	71	71	71	71	**71**	71
Volume	Pearson Correlation	.007	1	-.268*	.014	**-.058**	.355**
	Sig. (2-tailed)	.954		.024	.905	**.633**	.002
	N	71	71	71	71	**71**	71
ph	Pearson Correlation	-.094	-.268*	1	.204	**.194**	-.365**
	Sig. (2-tailed)	.434	.024		.088	**.106**	.002
	N	71	71	71	71	**71**	71
Liquefaction	Pearson Correlation	-.136	.014	.204	1	**.106**	-.117
	Sig. (2-tailed)	.259	.905	.088		**.378**	.331
	N	71	71	71	71	**71**	71
motility	Pearson Correlation	-.042	.355**	-.365**	-.117	**-.277***	1
	Sig. (2-tailed)	.725	.002	.002	.331	**.019**	
	N	71	71	71	71	**71**	71

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

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Parameters	Oligospermia	
	Correlation	Statistical significance
Age	Positively correlated	No
Volume	Negatively correlated	No
pH	Positively correlated	No
Liquefaction time	Positively correlated	No
Motility	Negatively correlated	Yes

Association of oligospermia with other parameters with linear relationship:

Morphology	Normal	Abnormal	P value	Statistical significance
Normospermia	63	10	<0.0000001	Yes
Oligospermia	30	41		

Agglutination	Present	Absent	P value	Statistical significance
Normospermia	4	75	0.03314	Yes
Oligospermia	10	61		

Leucocytes	Present	Absent	P value	Statistical significance
Normospermia	0	79	0.2367	No
Oligospermia	1	70		

Immature forms	Present	Absent	P value	Statistical significance
Normospermia	0	79	0.2367	No
Oligospermia	1	70		

Fructose	Positive	Negative	P value	Statistical significance
Normospermia	79	0	0.1112	Yes
Oligospermia	69	2		

DISCUSSION

As per WHO normal total count of sperm is 39 million per ejaculate. Reduced count (< 15 million sperm per ml) is called as oligospermia and complete absence of sperms in the seminal fluid is called as Azoospermia.[1]

Out of 150 cases, 71cases (47.3%) were of oligospermia. 6 cases (4%) were of azoospermia. 73 cases (48.7%) were normal. and This study showed increase in cases of oligospermia which is similar to the study done by Naina K et al[6]. Majority of the cases belonged to the age group of 31 to 40 years. ~~Figure.1 And Figure.2.~~

Out of 150 cases, 6 cases (4%) were of azoospermia. This was similar to the study done by Mahdi et al.[7]

Out of 71 cases of oligospermia, 15 (21.1%) cases had hypospermia. Volume showed a negative correlation with oligospermia. Mahdi et al[7] had similar findings in their study.

Out of 71 cases of oligospermia, in 38 cases (53.5%) acidic pH was observed and in 33 cases (46.5%) alkaline pH was observed. No significant correlation was seen between pH and total count. However, reduced motility is seen in acidic pH. This was similar to the study done by Dhumal et al[8]. The seminal plasma is derived primarily (50-80%) from seminal vesicles with a smaller fraction (13-30%) contributed by the prostate. These secretions determine the pH of the ejaculate predominantly. Normal pH ranges between 7.2 to 8. Advancing age and infection of seminal vesicles and prostate make the pH more basic. Thus, measuring pH is important to know the cause of infertility.[8]

Out of 71 cases of oligospermia, in 5 cases (7%) liquefaction time was delayed. Delayed liquefaction can be due to chronic prostatitis. No significant correlation was seen in oligospermia and delayed liquefaction time. However, it can decrease the fertility potential of the couple by inhibiting release of sperm from the coagulated ejaculate. Liquefaction is the process were Semen gets liquefied after ejaculation to facilitate the ascent of spermatozoa after redeposition in the posterior fornix during intercourse. According to the WHO, normal liquefaction time is 15-30 minutes.[9] If liquefaction is not complete after 60 minutes, this should be included in the final report.[1]

Out of 71 cases of oligospermia, in 10 cases (14%) agglutination was detected. Though the follow up was not done for all cases, two cases came with positive antisperm antibodies. Agglutination specifically refers to motile spermatozoa sticking to each other. The most common cause of sperm agglutination is the presence of sperm antibodies which stick together. It is also very important to identify the true agglutination (Figure.3). Any motile spermatozoa that stick to each other by their heads, tails or mid-pieces should be noted. Motile spermatozoa stuck to cells or debris or immotile spermatozoa stuck to each other (Aggregation Figure.4) should not be scored as agglutination.[1]

Correlation of Parameters of Semen Analysis in Oligospermia in Infertility

Out of 71 cases of oligospermia, 57 cases (80%) were having >42% of total motility. Out of which 29 cases (50.8%) of them were having rapidly progressive motility of >25%, and 28 cases (49.1%) were having rapidly progressive motility of <25% motility. 7cases (10%) were having 20% total motility. In which all of them were having rapidly progressive motility of >25%. Remaining 7cases (10%) were having 10% total motility. In which all of them were having rapidly progressive motility of <10%. Maximum cases of oligospermia were having a smaller number of progressive motile sperms. This was similar to the study done by Mahdi et al.[7]

Out of 71 cases of oligospermia, leukocytes were observed in 1 case (1.4%) and fructose was negative in 2 cases (2.8%). Chronic inflammatory conditions of the male genital tract play an important role in fertility disorders. Ejaculate characteristics of the patient affected by male genital tract infection or inflammation are the presence of leukocytes over one million per ml and increased viscosity, whereas the most frequent clinical symptoms reported by the patient is chronic pelvic pain. Male genital tract infection or inflammation may impair fertility by damaging spermatozoa through a direct effect of inflammatory mediators or reactive oxygen species (ROS) produced by inflammatory cells or by alluring male genital tract micro environment.[1]

Abnormal morphology includes defects in the head, middle piece, and tail. Qualitative defects are seen even in cases with normal sperm counts. However, majority of abnormal morphology was seen in cases of oligospermia. This was similar to the study done by Goyal R et al[10] and other studies in the literature. .

Out of 71 cases of oligospermia, fructose was negative in 2 cases (2.8%). Fructose is the energy source of sperm and is secreted by seminal vesicles. Absence of fructose indicates obstruction proximal to seminal vesicle or lack of seminal vesicle.[11] Follow up was done in these two cases. The two patients underwent surgical treatment and found to be fertile later.

Limitations of the study

1.No further follow up was conducted due to non-cooperation from patient's side revealing infertility issues.

2.Reactive oxygen species study was an important tool in identifying impact of correlation of various parameters of oligospermia which was not carried out in this study due to poor economic conditions of candidates.

CONCLUSION

The study carried out is one of a kind. All parameters have not been correlated with oligospermia in a single study earlier. In this study various parameters of semen as per WHO 2020 are studied in infertile males having a history of no conception in a year and more of unprotected sex or by definition of WHO 2020. The study has positive correlation of decreased cut-off values of various parameters as per WHO in sub-fertile males. The study also revealed increasing trends of oligospermia in recent times which could be because of awareness among men that the male factor is equally responsible for infertility, as compared to previous days when the females were blamed for infertility. The increasing number of oligospermic infertile necessitates more study on this particular area. Various parameters should be correlated and advice to be included as a conclusion which facilitates management. According to WHO-2020, the results of one single ejaculate examination are enough to decide subsequent steps of an infertility investigation of the man.[1]

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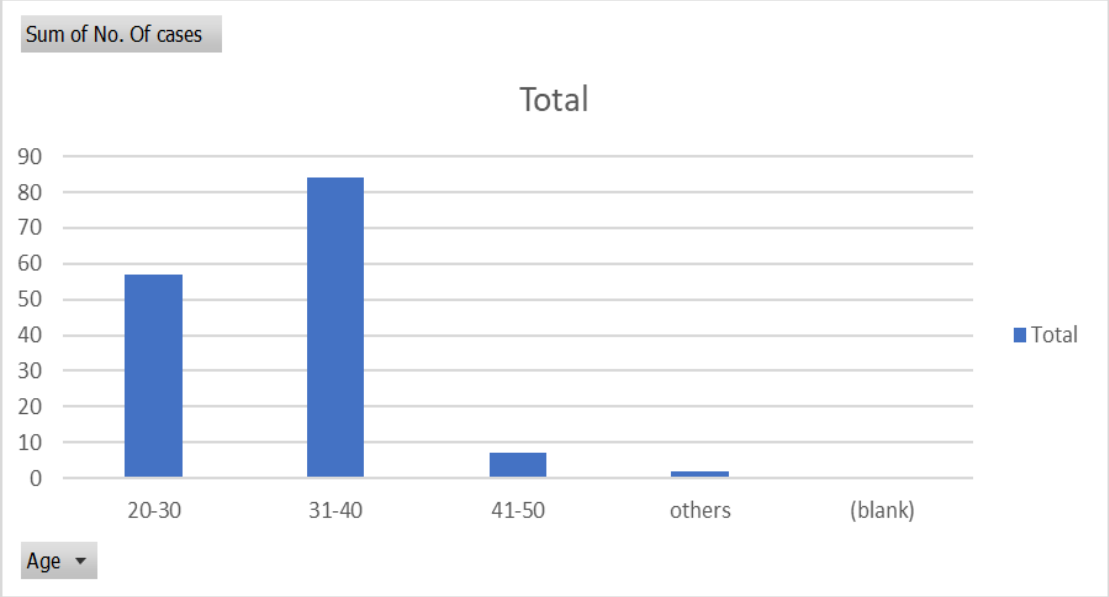

Figure. 1

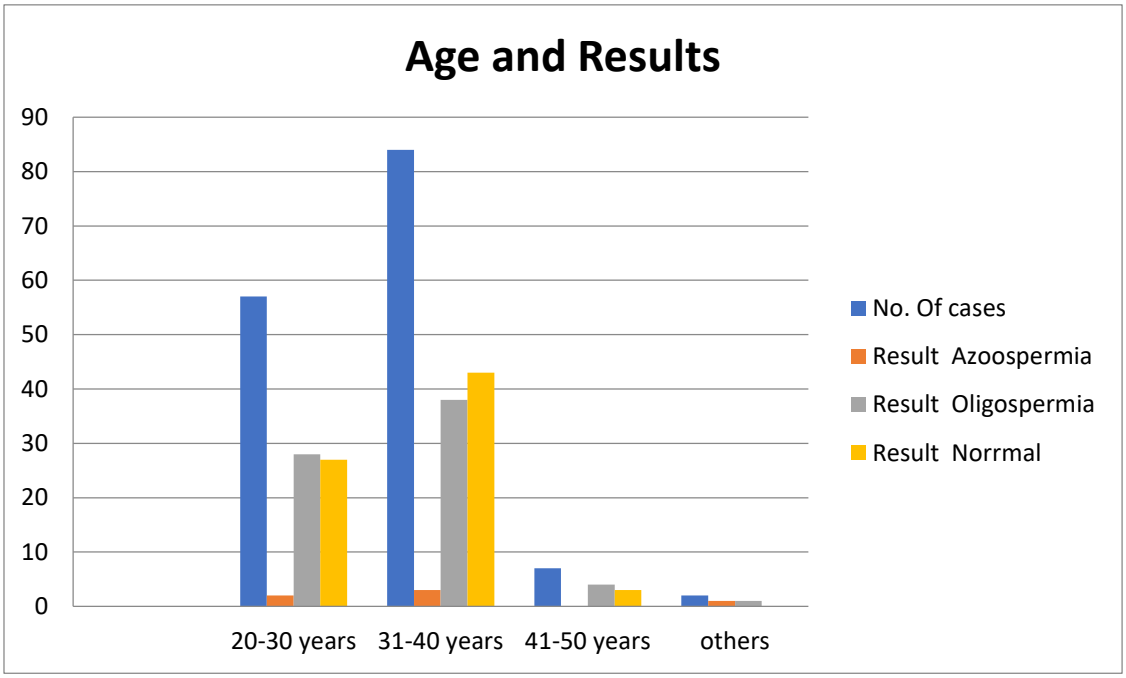

Figure. 2

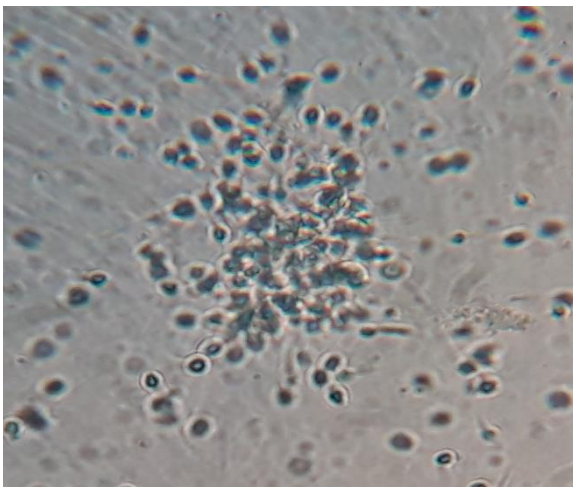

Figure.3. True Agglutination- motile sperms are attached with each other. (10x40X)

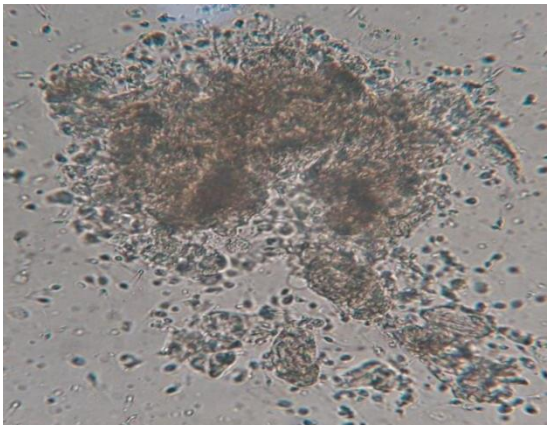

Figure.4. Aggregation immotile sperms are attached with each other and cells or cellular debris. (10x40X)

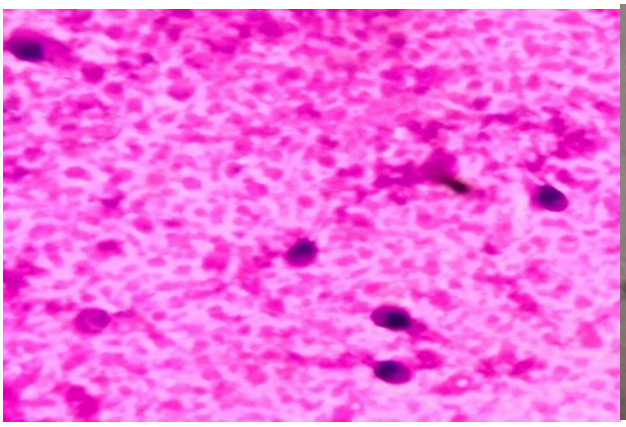

Figure.5. Normal morphology (Diff-Quick stain 10x40X)

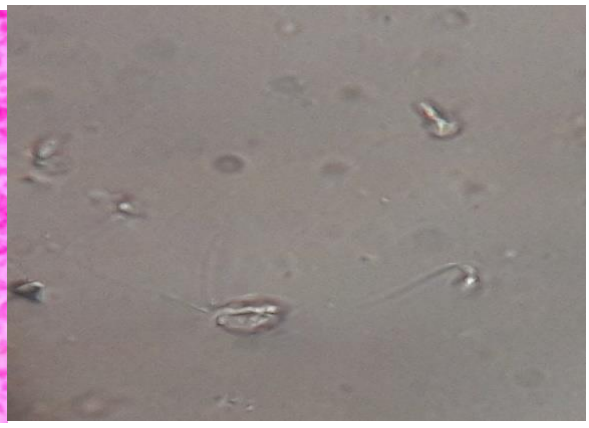

Figure.6. Abnormal morphology showing double tail and excess residual cytoplasm in the head (Wet mount 10x40X)