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Original Research Article

Semen analysis and sperm function parameters in patients with infertility in Navi Mumbai and Panvel region

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ABSTRACT

Background: Although semen analysis is routinely used to evaluate male partner in infertile couples, infertility and problems of impaired fecundity have been a concern through ages and is also a significant clinical problem today, which affects 8-12% of couples worldwide. Aim of the study was to study different semen parameters in male factor infertility (MFI) and thus increasing the awareness regarding same.
Methods: This is cross sectional study conducted between period of September 2016 to December 2018. Semen of 150 patients were studied and results were analysed as per recent WHO (2010) criteria.
Results: The present study included 150 patients whose age ranged from 24 to 51 years. Patients were divided into different age groups and sperm count was studied in each group. Abnormal sperm morphology was studied with respect to sperm head, neck, tail defects and combined defects. Sperm deformity index (SDI) and Teratozoospermic index (TZI) were calculated. Other parameters including semen volume, pH, liquefaction time, sperm vitality and motility were also studied which showed significant variations.
Conclusions: Although semen analysis is first and most informative investigation for evaluation of male factor infertility, studying individual semen parameters and sperm function and increasing its awareness in general population especially in developing countries is equally important. Besides, it is necessary to acknowledge its limitation with respect to collection, processing, evaluation and biological variation of samples. Also, a normal semen analysis may not prove successful fertility potential of an individual.

Keywords: Male factor infertility, Morphological defects, Sperm count, Sperm indices

INTRODUCTION

The investigations of infertile couple mainly cater around female partner, however male partner needs to be screened alongside as male related factor is solely responsible in about 20% of cases of infertility and is a contributory factor in another 30% - 40%.[1] Alterations in normal physiology of reproductive organs affecting sperm functions ultimately results in oligozoospermia (low sperm count), teratozoospermia (abnormal morphology), azoospermia (sperm's absence in ejaculation) asthenozoospermia (loss of motility). This results in unsuccessful fertilization. Thus, pathogenesis is multifactorial.[2,3] Also when genderwise fertility or fecundity is looked upon, women are fertile for certain duration in their entire life thus offering a rate limiting step because of the regulated monthly production of ova and other barriers during and around pregnancy. Men on the other hand have no such barriers and are regular producers. This makes them fertile during their entire reproductive life if fecundity is to be tested. Hence, any abnormality in spermatogenesis can affect species renewal and propagation.

Various studies have been published supporting a decline in sperm quality or dismissing the same.[4-8] The decline in the semen quality coincides with an increasing incidence of abnormalities of the male genital tract including testicular cancer and cryptorchidism in various countries.[9]

Hence, semen analysis remains the single most useful and fundamental investigation with a sensitivity of 89.6%.[10] It is a simple test that assesses the formation and maturity along with sperm interactions in seminal fluid. It also provides insight not only on sperm production (count), but the sperm quality (motility, morphology) as well.[11]

Societal pressure and dilemma surrounds the investigation of the male. A complete history and physical examination of the male partner is mandatory in cases of primary or secondary infertility. In most instances, the next step will be to order semen analysis as it is an easy non-invasive test which provides baseline information and a path to further investigations.

The purpose of the study was to

- Evaluate sperm characteristics in patients undergoing infertility evaluation.
- To help decide further modality of treatment.

METHODS

This is cross sectional study conducted between period of September 2016 to December 2018.

Study population

- Infertile couples in which the female partners had normal results on fertility evaluation were included. All of these couples had been unable to conceive for minimum of 12 months
- The women were required to have regular menstrual cycle, normal results on laparoscopy and hysterosalphingography and a luteal-phase endometrial biopsy specimen that was histologically consistent with menstrual dating
- All the men were required to be between the ages of 20 and 55 years at the time of enrollment.

Semen collection and laboratory evaluation

- Written informed consent was obtained from all participants after recruitment
- The study population comprised of 150 male patients referred to the laboratory for semen analysis for primary or secondary infertility. After providing proper instructions to the person regarding semen collection, samples were collected after a minimum of 48 hours but no longer than 7 days of sexual abstinence. Increased sperm concentration is associated with prolonged abstinence while improved motility is associated with shorter period of abstinence but with lower sperm density. The sperm morphology does not vary with length of sexual abstinence.[10]
- Semen samples were collected by masturbation at the laboratory. All semen analyses were performed manually within one hour after the sample was collected
- It included measurements of the volume, pH, liquefaction time and presence of pus cells and viable sperms in the ejaculate (vitality test) and determinations of the sperm count, sperm concentration and the percentage of sperm with evidence of flagellar movement (percentage motility). Sperm deformity index (SDI) and teratozoospermic index (TZI) were also counted. Fructose content of sample was also estimated. Hypo-osmotic swelling test was also performed
- Sperm count was done on 10-microns depth chamber - sperm meter

  (Product code SP/INT/001-A) of Sperm Processor company.

10-microns depth chamber-sperm meter

- Sperm Meter requires no dilution of semen, so only a 'neat semen' is analysed resulting in more accuracy. It is designed to achieve uniform smear of 10 micron thickness which allows free head movements of sperms in all directions. This also avoids overlapping of sperms
- It has a micrometric grid of 100 squares; each of 100 micron. The number of sperms in any 10 squares of the grid, simply implies their concentration in million per ml
- The cases with nil sperms were re-evaluated on three occasions before labeling them as azoospermia. Sperm motility was assessed by direct visualization under the microscope
- Semen smears were fixed and stained at the laboratory by the Papanicolaou method for assessment of sperm morphology by light microscopy. Sperms were classified as having normal or abnormal morphologic features including defective morphology of head, neck or tail according to strict criteria.

Ethical consideration

- The study was conducted in accordance with the principles of Declaration of Helsinki (World Medical Association) and Good Clinical Practice guidelines issued by the ICMR. All the patient's confidentiality was maintained.

Statistical analysis

- All data was entered in Microsoft Excel (MS office version 2010) and tabulated. Data analysis was done using Windows PC based software "MedCalc

Statistical Software" version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). Data for all analysis was done at alpha 0.05 (95% confidence limits)

- Data expression
- The measurement data for the flow (mm) is expressed as means with SD/SEM and median
- Kolmogorov-Smirnov test was used for testing of flow rate data for Normal distribution
- Analyses methods
- Since the data was no-normal, non-parametric test Kruskal-Wallis test (non-parametric ANOVA) was used to analyse the flow rates in the between the three groups with sealer type as the factor.

RESULTS

The present study is cross sectional study conducted between period of September 2016 to December 2018 in association with Dr. Bhalekar's Pathology Laboratory, Navi Mumbai.

The study includes 150 patients with age range from 24 to 51 years. All semen parameters mentioned in table 3 are studied. Patients were divided in three main age groups as, 20-30 years, 30-40 years and 40-60 years. The maximum number of cases were in the age range 30-40 years accounting for 63.3% (n=95/150) of the cases. 30% (n=45/150) cases and 6.7% (n=10/150) cases were seen in the 20-30 years and 40-60 years age groups respectively.

According to the latest WHO recommendations, 1.5 ml or more was taken as normal semen volume. Out of the 150 subjects in the current study 16.7% (n=25/150) had an ejaculate volume less than 1.5 ml. 83.3% (n=125/150) had an ejaculate volume of 1.5 ml and above.

It is well known that sperm concentration is one of the important predictor of fertility potential. The sperm counts in the present study ranged from 0-280 million per ml. Out of this 12% (n=18/150) of the cases had a sperm concentration of less than 15 million/ml and a total of 82% (n=123/150) of the analysed population was in the normal range. Azoospermia, that is no sperms in the ejaculate, was seen in 6% (n=09/150) individuals. Oligozoospermia were cases with counts less than 15 million/ml, normozoospermia were those with sperm counts above 15 million/ml while azoospermia had no sperms (Figure 1).

It is observed that 20.7% (31/150) cases alone constitutes low sperm count category. Also 6.0% (09/150) cases fell in category of sperm count between 15-20 million/ml which forms borderline category. Fertility potential in these cases is equivocal and are to be investigated as per oligozoospermic cases.

Sperm motility being an important parameter and determinant of male fertility it should be analysed as early as possible and must be measured within 60 minutes of collection. According to the latest WHO criteria a total sperm motility of 40% with progressive movement of >32% is taken as cut off value.[2]

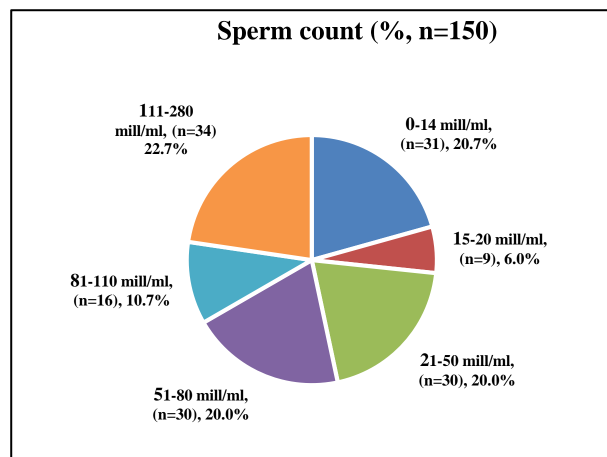


Figure 1: Distribution of cases in different sperm count groups.

Individuals above this are taken to be normal. In the present study, 80.7% (n = 121/150) of the cases were above the reference motility and all these cases had >32% of sperms with progressive movement. 19.3% (n=29/150) of the cases showed non-progressive motility. These cases were also distributed based on age group (Table 1).

Table 1: Sperm motility in different age groups and sperm count groups.

	N	Progressive	Non-progressive
Age group			
20-30 years.	45	36 (80.0%)	09 (20.0%)
30-40 years.	95	79 (83.2%)	16 (16.8%)
40-60 years.	10	06 (60.0%)	04 (40%)
Sperm count			
0-14 mill/ml	31	9 (7.4%)	22 (75.9%)
15-20 mill/ml	9	7 (5.8%)	2 (6.9%)
21-50 mill/ml	30	25 (20.7%)	5 (17.2%)
51-80 mill/ml	30	30 (24.8%)	0 (0.0%)
81-110 mill/ml	16	16 (13.2%)	0 (0.0%)
111-280 mill/ml	34	34 (28.1%)	0 (0.0%)
Total	**150**	**121 (80.7%)**	**29 (19.3%)**

The morphology was assessed on fixed stained smears of the semen samples. In the current study 6% cases had no sperms in the ejaculate (azoospermia).

One individual had a count of 5 million/ml and a borderline case of 6 million/ml sperm count with 99% abnormal morphology. In the study, five cases with count less than 5 million/ml had abnormal morphology of sperms ranging from 40 - 80%.

Any defects of head, neck and tail were considered as abnormal morphology. Maximum morphological defects were identified as head defects which was common finding in all age groups (Table 2) and (Figure 3).

Table 2: Sperm defects in different age groups.

	Age Group								
	20 - 30 years. (n=45)			30 - 40 years. (n=95)			40 - 60 years. (n=10)		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Head defects (%)	42	35	30	54	47	34	35	24	24
Neck defects (%)	12	05	22	12	4	20	14	1	31
Tail defects (%)	08	03	08	10	08	07	11	3	18

Morphologically abnormal spermatozoa often have multiple defects (of the head, midpiece or principal piece, or combinations of these defects). A detailed study of the incidence of morphological abnormalities may be more useful than a simple evaluation of the percentage of morphologically normal and abnormal spermatozoa. Recording the morphologically normal spermatozoa, as well as those with abnormalities of the head, midpiece and principal piece, in a multiple-entry system gives the mean number of abnormalities per spermatozoon assessed. These indices have been correlated with fertility in vivo (TZI) and in vitro (SDI), and may be useful in assessments of certain exposures or pathological conditions.[2]

Two indices were calculated from records of the detailed abnormalities of the head, midpiece and principal piece in a multiple-entry system.[2]

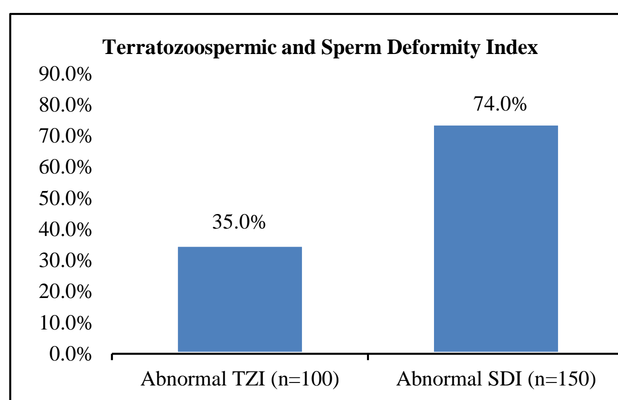
- The teratozoospermia index (TZI)
- The sperm deformity index (SDI)

Calculation of these indices.[2]

Each abnormal spermatozoon is scored for defects of the head, midpiece and principal piece, and for the presence of excess residual cytoplasm (volume more than one third of the sperm head size). In Teratozoospermia Index (TZI), a maximum of four defects per abnormal spermatozoon is counted: one each for head, midpiece, and principal piece and one for excess residual cytoplasm.

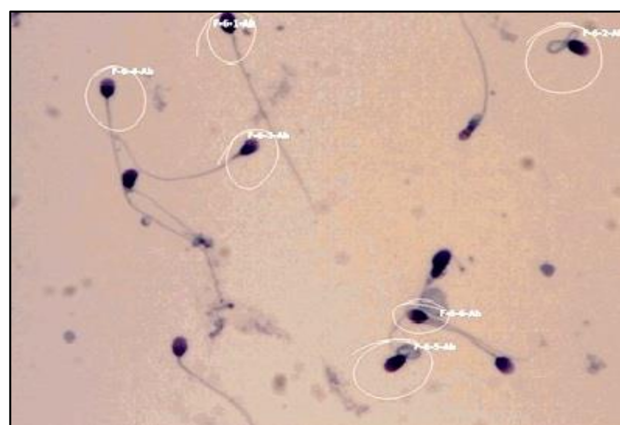
The Sperm Deformity Index is the number of defects divided by the total number of spermatozoa (not only the abnormal spermatozoa). It incorporates several categories of head anomaly but only one for each midpiece and principal piece defect.

WHO lower reference limit for TZI and SDI of >4% was considered as normal, ≥3 to ≤4 % was considered as equivocal and values <3 was counted as abnormal. In present study, TZI* was normal in 43.3% (n = 65/100) cases and SDI was normal in 26% (n = 39/150) cases. TZI* was abnormal in 23.3% (n = 35/100) cases with mean value of 1.1 and SDI was abnormal in 74% (n=111/150) cases with mean value of 0.5 (Figure 2).



*TZI values were available in 100 cases

Figure 2: Percentage of cases with abnormal TZI and SDI.



Abnormal : F-6-3-Ab HD,HD,MD| F-6-4-Ab HD| F-6-5-Ab TD| F-6-6-Ab HD,TD| F-6-1-Ab HD,MD| F-6-2-Ab TD, F- Figure, HD- Head defects, TD- Tail defects, Ab-Abnormal

Figure 3: Vitality test using eosin-nigrosin method.

In present study, fructose was present in all cases, pus cells were present in 29.5% cases, semen volume was

low (hypospermia) in 16.7% cases and liquefaction time was normal in all cases. Cases in which pus cells were found were subjected for culture. If culture was positive, patients were cleared of infection and semen analysis was repeated. Vitality of sperms was assessed by two methods: Eosin Nigrosin method (Figure 3) and Hypo-osmotic swelling (HOS) test.

Vitality test using hypo-osmotic swelling is an alternative to dye exclusion. Spermatozoa with intact membranes swell within 5 minutes in hypo-osmotic medium and all flagellar shapes are stabilized by 30 minutes.[2]

In present study, 17.3% (n = 26) cases showed abnormal hypo-osmotic swelling test. This is useful when staining of spermatozoa must be avoided, e.g. when choosing spermatozoa for ICSI.[2] An age specific comparative analysis of the mean sperm counts, total motility and normal morphology revealed a decline in the average values of these parameters with age (Table 3).

Table 3: Semen parameters in different age groups.

	20-30 years (n=45)	30-40 years (n=95)	40-60 years (n=10)	Total (n=150)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age (years.)	28.00 (1.75)	34.42 (2.97)	44.10 (2.77)	33.14 (4.90)
Semen volume (ml)	2.93 (1.87)	2.75 (1.43)	2.00 (0.97)	31.37 (8.45)
Liquefaction time (min)	32.00 (13.71)	30.58 (4.24)	36.00 (6.58)	75.85 (71.58)
Sperm count (mill/ml)	88.64 (82.86)	71.42 (66.04)	60.30 (66.54)	73.01 (14.00)
Viable sperms (%)	76.13 (11.67)	71.84 (14.25)	70.57 (21.28)	70.18 (11.72)
HOS reactive (%)	72.83 (10.76)	69.23 (11.70)	67.43 (16.11)	22.55 (20.29)
Rapid progressive (%)	22.50 (16.54)	22.46 (21.23)	24.00 (29.33)	34.02 (14.33)
Slow progressive (%)	34.60 (13.03)	33.67 (14.38)	35.29 (21.87)	13.65 (8.55)
Non-progressive (%)	14.88 (10.02)	13.22 (7.49)	12.14 (12.65)	29.76 (14.34)
Immotile (%)	28.03 (11.60)	30.62 (15.51)	28.57 (13.45)	45.53 (27.99)
Normal morphology (%)	50.90 (28.06)	42.35 (27.39)	56.14 (32.23)	53.86 (28.33)
Abnormal morphology (%)	46.98 (28.79)	57.65 (27.39)	43.86 (32.23)	56.43 (29.54)
Defective morphology (%)	48.88 (29.33)	60.58 (28.68)	45.57 (34.93)	49.59 (33.05)
Head defects (%)	42.05 (29.57)	54.04 (34.37)	34.86 (24.45)	12.42 (20.75)
Neck defects (%)	12.35 (21.64)	12.35 (19.69)	13.71 (31.07)	9.11 (8.00)
Tail defects (%)	7.58 (7.89)	9.64 (6.78)	11.00 (18.47)	56.57 (19.20)
Sperm deformity index	0.62 (0.51)	0.78 (0.54)	0.60 (0.57)	0.72 (0.54)
Teratozoospermic index	1.35 (1.12)	1.22 (0.37)	1.20 (0.32)	1.25 (0.68)

DISCUSSION

Childbearing is considered as an essential part of living and yardstick by which women's worth is measured especially in a developing country. So it invites social stigma. It's no longer a private sorrow instead it's coming out of the woodwork due to its social and interpersonal ramifications. Besides, recent researches have proved that problem is not gender specific. Semen analysis remains the cornerstone to investigate male infertility.

"Male factor" infertility (MFI) is considered as a change in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyzes, collected 1 and 4 weeks apart.[12] Most recent WHO (2010) manual for semen analysis have changed nomenclature from "normal" to "reference values" with respect to sperm concentration, motility, morphology and all other semen parameters. Males with sperm parameters below the WHO normal values or reference values are considered to have male factor infertility.[13]

The study was a cross-sectional study with a population of 150 individuals. Maximum number of cases were in the age range 30 - 40years. A relatively higher mean age of 36.8years and 34 years has been reported by other authors.[14,15] However, a mean age of 30 years in concordance with our finding was observed by Jajoo S et al.[16] Male fertility usually peaks at around 35 years of age and declines after 45 years of age.[17] The changes associated with aging are moderate, but significant, although the capacity to fertilize is maintained.[18]

According to the latest WHO recommendations, the lower reference value for semen volume is 1.5 ml[2] with reference to this 16.7% of our study population had low volume while the remaining 83.3% was within the normal range. Precise measurement of volume is essential in any semen analysis as it allows the total count of

spermatozoa and non sperm cells in the ejaculate to be assessed.

Volume is generally assessed in a graduated glass cylinder with a flared top. Ineffective collection may adversely affect volume assessment. In addition, a variable relationship exists between the frequency of emissions, continence and seminal volume.[19,20] Ejaculated seminal volume is a parameter that reflects abnormalities in accessory sex glands fluid synthesis i.e. seminal vesicle which forms a bulk. Low semen volume is characteristic of obstruction of the ejaculatory duct or congenital absence of the vas deferens.[2] High semen volume may on the other hand reflect exudation in cases of active inflammation of accessory organs.[2]

Sperm concentration is often proposed to be predictors of fertility potential. In recent years there have been reports of declining sperm concentration in men around the world.[21,22] The new WHO 2010 guidelines has taken lower reference limit of 15 million/ml with values above these taken as normal.[2] Oligozoospermia (sperm counts <15 million/ml) in the present study was seen in 12% of the cases while higher rates of 23.2%, 32% and 25.6% are reported.[23-25] Although lower values of 11.11% have also been observed.[10]

It has been suggested by authors that low sperm counts are among the most common cause of male infertility.[23] Association of oligozoospermic semens with increased morphological abnormalities has been suggested by Butt et al.[10]

Azoospermia, defined as absence of spermatozoa in the ejaculation was seen in 6% of the cases which was lower compared to those seen in other studies such as 14.8%, 12.3%, 28.6%.[10,26,27] The problem of azoospermia is thought to be associated with sperm production or sperm transport.[10]

82% of the analyzed subjects had normal sperm counts, which was higher than reported by Butt et al.[10] However these values are much higher than other reports such as 20%, 36.7% and 51.8%.[26-28] More so, it may be noted that normal semen counts are a common event in infertile males, where the cause may be other factors such as immune related and marked biological variation.[29]

Assessment of sperm motility is essential as the spermatozoa have to travel in the female genital tract to fertilize the oocyte, a requisite of normal pregnancy.[10] It is a critical parameter which indicates semen quality and fertility potential. As per the WHO 2010 recommendations, samples having 40% motile sperms with 32% showing progressive, motility are considered normal.[2] In the present study 121 (80.7%) cases were above the reference motility and all these cases had >32% of sperms with progressive movement. This was in concordance with 76% as reported by Okon E et al, and higher as compared to value of 62.02% reported by Alemnji.[26,14]

Sperm morphology along with motility and sperm count is also an important contributing factor in male fertility. The total number of morphologically normal spermatozoa in the ejaculate is of biological significance, the lower reference limit for normal sperm morphology is 4% as per the latest WHO guidelines.[2] The WHO criteria for morphology has seen a marked change over the years from 50% and above to as low as 4% in the 5th edition.[30] Sperm morphology is assessed by microscopic examination. Normal sperm contains head, middle piece, and tail. Morphological changes (teratozoospermia) were defects in the head, neck, midpiece and tail of spermatozoa. The details of the type of defects were recorded in present study. Some studies show that these defects have a prognostic bearing as some defects are irreversible, and others which are due to acquired/environmental factors can be reversible.[31,32] Most of the studies describe the association of low sperm count and abnormal morphology. They studied that sperm morphological defects increases with decreasing sperm count.[33] Only few studied the defects with normal sperm counts also.[34] Defects in the head are the most common defects.[35]

Clinical significance of some of these defects described by many authors such as tapering and megaloheads are reversible defects. It is mainly due to ongoing stress or some medication. After stopping the precipitating factor, most of them revert back. These defects increase with decreasing sperm count.[33] Amorphous head is a defect with genetic aberration, so this is a severe form and incapable of fertilization. Globozoospermia small round head with no acrosome are also genetically determined and due to the absence of acrosin unable to bind to zona pellucida. Small head spermatozoa also have very small, abnormally formed acrosome. Large head spermatozoa have severely abnormal megaloheads.[32] Teratozoospermia has a deleterious effect on the rate of fertilization.[36]

Most common morphological defects were observed in head of sperms which is in concordance with Goyal et al.[37] Present study was limited by availability of proper environmental factors and other relevant history and follow up. Some have noted the association of multiple defects with increased chances of spontaneous abortions.[38] In present study, many cases with multiple combined abnormalities was also found. However, the study is limited owing to lack of follow-up.

Semen is normally ejaculated as a liquid, immediately gels, and liquefies within five to 20 minutes. Less than one percent of specimens will fail to liquefy. Such an event makes the analysis difficult, but is not clearly related to infertility. Viscosity is assessed by pouring the specimen from the collection bottle into a graduated cylinder, and grading on a scale of zero (normal) to four

is done. A normal sample is capable of being poured in single, small droplets; grade 4 specimen remains as a solid blob. As with non - liquefaction, high viscosity makes analysis extremely difficult but does not interfere with fertility.[39] In present study, liquefaction time was in normal range in all cases.

The pH of fresh semen will be 7.3-7.7. This will shift to the more alkaline range when the sample is left standing. With further passage of time the accumulation of lactic acid will make the sample more acidic. In present study, pH was in normal range only in 3 cases. Fructose is produced by the seminal vesicles. It will be absent from the semen in three clearly defined circumstances: in a rare form of retrograde ejaculation, when both ejaculatory ducts are obstructed and in azoospermic men with congenital bilateral absence of the vas deferens.[40] In present study, fructose was present in all cases.

Reproductive quiescence in women is seen between 45-55 years of age. This is not the case with men, who age gradually. Still, they commonly do not experience complete reproductive senescence and maintain spermatogenesis well into old age.[13] However, increasing age significantly influences semen parameters required for healthy male fertility.[13] Age related changes on the seminal parameters were also evaluated in present study, it was noted that mean sperm counts, total motility and normal morphology revealed a decline in the average values of these parameters with age, this was in concordance to similar studies conducted in past.[13]

CONCLUSION

Although semen analysis is first and most informative investigation for the evaluation of male factor infertility, studying individual semen parameters and sperm function and increasing its awareness in general population especially in developing countries is equally important. Besides, it is necessary to acknowledge its limitation with respect to collection, processing, evaluation and biological variation of samples. Also, a normal semen analysis may not prove successful fertility potential of an individual. Poor sperm function is usually associated with high proportion of abnormal spermatozoa. Besides morphologically normal-appearing spermatozoa should also be further investigated for normal sperm function and DNA content as morphological normal sperms does not necessarily imply normal sperm function. In addition, male fertility and various semen parameters are seen to decline with age in this part of India.

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