Original article:

Patterns of Semen Analysis: Experiences of a Laboratory Catering to Semi Urban Population of Delhi

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Abstract:

Background:Infertilityis defined as failure of a couple to conceive after one year of regular sexual intercourse. It remains both prevalent and problematic among couples worldwide. The analysis of semen remains the preliminary investigation for males in the workup of infertility in couples.It is a key element in the fertility evaluation of men and permits male reproductive potential to be evaluated in association with possible risk factors. ***Aim:*** To assess and analyse the semen characteristics of all the males who presented to our hospital, irrespective of primary or secondary infertility. ***Material and Methods:*** This is an observational study conducted in the Department of Pathology, Hamdard Institute of Medical Sciences, Delhi; from Jan 2012 to Oct 2013. Semen of 139 subjects were evaluated and results analyzed ***Results:*** The present study included 139 subjects whose age ranged from 22 years to 48 years with a mean age of 29.42 years. Patients were divided into oligospermia[17%], normospermia[16%] and azoospermia[9%]. 42.5% of the cases had sperm counts in the range of 51-80million/ml. Asthenospermia was seen in 22.1% of the cases.An age specific comparative analysis of the mean sperm counts, total motility and normal morphology revealed a decline in the aaverage values of these parameters with age. ***Conclusions:*** Routine semen analysis remains the backbone of the evaluation of the male factor infertility, but it is important to acknowledge the limitation of semen analysis with respect to collection, processing, evaluation, biological variation of the parameters and lack of information on sperm function. A normal semen analysis does not guarantee the fertilization potential of sperm.

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Introduction:

Semen quality is usually considered to be a measure of male fertility, and changes in semen quality are an indication of reproductive potential. Infertility on the otherhand is defined as failure of a couple to conceive after one year of regular sexual intercourse. It remains both prevalent and problematic among couples worldwide. The male factor is known to be solely and partially implicated in 20-50% of the cases of infertility.[1] The fertility potential of any male can be predicted through the evaluation of his semen. The analysisof semen remains the preliminary investigation for males in the workup of infertility in couples.In about one third of these couples, a male factor is the primary cause and in another one quarter, both the male and female partner contribute to the infertility.[1] Males and females individually attribute 40%each with a combined cause of 20%to the infertility factor.[2] Inspite of the many limitations of semen analysis it is still performed in laboratories all over the world. It is a simple test that assesses the formation and maturity of sperm as well as how the sperm interacts with the seminal fluid so it provides insight not only

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on sperm production (count), but sperm quality (motility, morphology) as well.[3]Semen analysis is a key element in the fertility evaluation of men and permits male reproductive potential to be evaluated in association with possible risk factors. Currently, routine semen analysis remains the backbone of the evaluation of male factor infertility, along with detailed medical history and thorough physical examination. This relies on the fact that the semen parameters such as sperm concentration, motility, and morphology are significantly associated with conception.[4]Semen quality is usually considered to be a proxy measure of male fertility, and changes in semen quality can occur after exposure to toxic agents, or from host factor effects such as age.[5] Abnormal sperm morphology and insufficient sperm motility remains a significant contributor to overall infertility.[6]In our society infertility of a couple is mainly attributed to the female, and males are hesitant to undergo counselling or tests which may question their virility. In this scenario semen analysis is an easy non invasive test which provides baseline information and a prelude to further investigations. The present study is an attempt to assess and analyse the semen characteristics of all the males who presented to our hospital, irrespective of primary or secondary infertility.

Material and Methods:

This is an observational study conducted in the Department of Pathology, Hamdard Institute of Medical Sciences, Delhi; from Jan 2012 to Oct 2013. The study population comprised of 139 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.[5]

Semen assessment was performed as soon as the samples were liquefied but within 1 hour of collection. Seminal volume was measured while sperm count was done in the haemocytometer (Improved Neubauer counting chamber) after an appropriate dilution. The cases with nil sperms were re-evaluated on three occasions before labelling them as azoospermia. Sperm motility was assessed by direct visualization under the microscope. Smears made on clean slides were fixed and stained, morphology was analyzed on light microscopy. For the assessment of morphology, the semen sample was centrifuged and smears prepared and stained with Papanicoloau and hematoxylin and eosin stains.

Ethical consideration: This study was approved by ethical committee of Hamdard Institute of Medical Science, Delhi

Result:

The present study included 139 subjects whose age ranged from 22 years to 48 years. The maximum number of cases were in the age range 20-30 years accounting for 56.1%(n=78)of the cases, (Figure I) the mean age being 29.42 years. 41and 20 cases were seen in the 30-40 years and 40-50years age group.

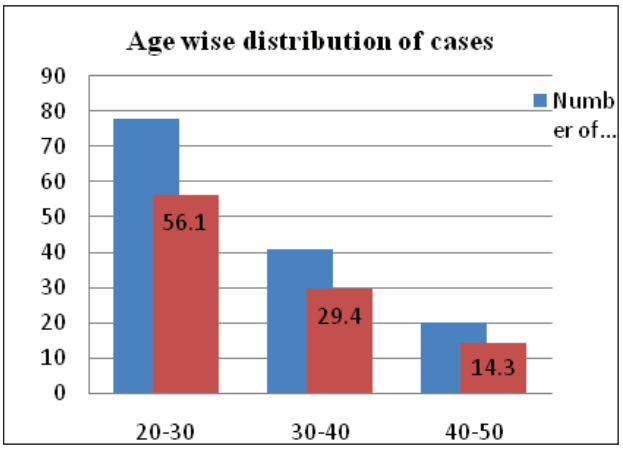

Figure I: Age wise distribution of the individuals

Keeping in view the latest WHO recommendations,1.5ml or more was taken as normal volume. Out of the 139 subjects in the current study, with a mean value of 1.9ml, 7 individuals (5.0%) had an ejaculate volume less than 1.5 ml.132 individuals (94.9%) had an ejaculate volume of 1.5 ml and above(Table 1). The age range of these 132 cases was between 22-34 years.(mean 27.5 years). Two individuals (aged 25yrs and 31 yrs)had an ejaculate of 0.5ml with all dead sperms.

Table 1: Distribution of cases according to volume of semen (n=139)

Volume of Semen (in ml)	Number of males(%)
< 1.0	2 (1.4%)
1-1.4	5(3.5%)
1.5-1.9	25(17.9%)
2.0 or >2.0	107(76.9%)

It is well known that sperm concentration are predictors of fertility potential.The sperm counts

in the present study ranged from 0-100 million per ml out of this17.2% (24) of the cases had a sperm concentration of less than 15 million/ml and a total of

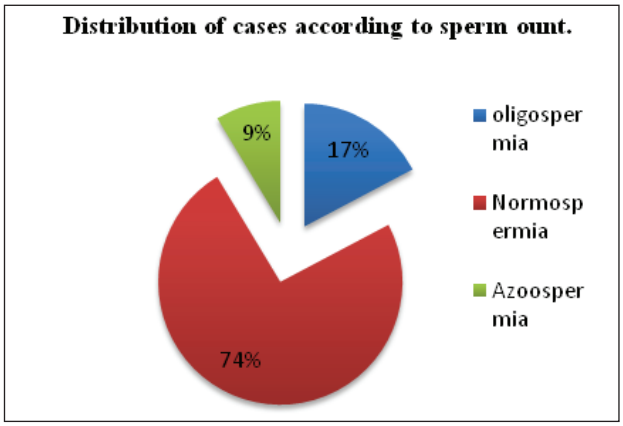

Figure 2: Distribution of cases on the basis of sperm count

74.1% of the analysed population was in the normal range. Azoospermia, that is no sperms in the ejaculate, was seen in 12 (8.6%) individuals(Figure 2)

These cases were also ditributed based on age group and sperm count . Oligospermia were cases with counts less than 15 million/ml, Normospermia were those with sperm counts above 15million/ml while azoospermia had no sperms.

On exclusion of the 12 azoospermic cases, the 127 subjects left were distributed according to the range of sperm count as depicted in the Table 2

Table 2: Distribution of cases according to sperm count (in millions, n=127)

Sperm count (in millions/ml)	Number of males (%)
0-14	24 (18.9%)
15-20	15 (11.8%)
21-50	24 (18.9%)
51-80	54 (42.5%)
81-100	10 (7.9%)

Besides sperm concentration, sperm motility is also an important parameter and determinant of male fertility. It is important to realize that sperm motility should be analysed as early as possible and must be measured within 60 minutes of collection. According to the latest WHO criteria[7]a total sperm motility of 40% with progressive movement of >32% is taken as cut off value , individuals above this are taken to be normal. In the present study, 77.9% of the cases were above the reference motility and all these cases had > 32% of sperms with progressive movement.

Table 3: Distribution of cases on the basis of total motility (n=127)

Total Motility	No. of Cases
>40%	99
<40%	28

22.1% of the cases were less motile i.e motility <40% (asthenospermia)and 92.8% of these had progressive sperm movement <32% .Three cases out of the 28 cases in our study had 100% dead sperms at the time of evaluation

The morphology was assessed on fixed stained smears of the semen samples in the current study population 8.6% (12 individuals) had no sperms in the ejaculate (azoospermia). 98.43% were in the normal range with a mean normal morphology of 66.5%.One individual had a count of 5 millions/ml with 99% abnormal morphology. Any defects of head, neck, mid piece and tail were considered as abnormal morphology.

Table 4: Agewise representation of average sperm parameters

Age range	Average sperm count millions/ml	Average Total motility (%)	Average Morphologically normal form (%)
20-25 yrs	57.4	61.8	71.3
26-30 yrs	60.8	62.5	70.4
31-35 yrs	49.2	56.3	63.2
36-40 yrs	50.1	48.2	64.4
41-45 yrs	49.8	45.1	59.3
46-5 yrs	20.3	36.6	40.3

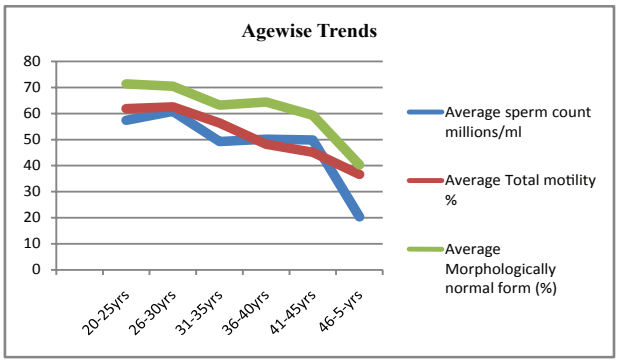

Figure 3: Age wise trends of average sperm parameters

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.

Discussion:

Semen analysis is an extremely common, yet most under interpreted test carried out in evaluation of infertility of a couple. In spite of reservations in the society, the test is being undertaken widely and is often the first and in most instances the only test for males. In our society, females have often been the target of society for infertility, however, advancing knowledge and development of assisted reproductive techniques have proven males to be an equal contributor to this problem.

Our study was an observational study with a population of 139 individuals having a mean age of 29.42 yearst with a maximum number of cases were in the age range 20-30years. A relatively higher mean age of 36.8years and 34 years has been reported by other authors.[8,9] However, a mean age of 30years in concordance with our finding was observed by Jajoo S. et al.[2] Male fertility usually peaks at around 35 years of age and declines after 45 years of age.[10] The changes associated with aging are moderate, but significant, although the capacity to fertilize is maintained.[11]

Ejaculated seminal volume is a parameter that reflects abnormalities in accessory sex glands fluid synthesis i.e seminal vesicle It can also be indicative of a physical obstruction somewhere in the reproductive tract or in cases of incomplete retrograde ejaculation.[5] According to the latest WHO recommendations, the lower reference value for semen volume is 1.5ml,[7] with reference to this 5.03% of our study population had low volume while the remaining was within the normal range. More so, had the WHO (1999) [12] criteria of 2ml and above been applied, only 107 males (76.9%) would have been considered normal similar to the Punjab study by Fauzia et.al, 74.24% of the population had normal semen volume.[5] Jajoo S. et al on the other hand had 22% males with semen volume < 2 ml,[2] this is similar to our result of 23 % subjects with volume < 2 ml.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. Low semen volume is characteristic of obstruction of the ejaculatory or congenital absence of the vas deferens, however low semen volume can also result due to problems in collection.[7] High semen volume may on the other hand reflect exudation in cases of active inflammation of accessory organs.[7]

Sperm concentration are often proposed to be predictors of fertility potential.In recent years there have been reports of declining sperm concentration in men around the world.[9] The new WHO 2010 guidelines has taken lower reference limit of 15 million/ml with values above these taken as normal.[7] Oligospermia (sperm counts <15 million/ml) in the present study was seen in 17% of the cases while higher rates of 23.2%[13], 32%[14] and 25.6%[15] are reported altough lower values of 11.11% have also been observed.[5] It has been suggested by authors that low sperm counts are among the most common cause of male infertility.[6,13] Association of oligospermic semens with increased morphological abnormalities has been suggested by Butt et.al.[5] However no such correlation was seen in our study, this could be attributed to a smaller sample size in the present study. Azoospermia, defined as absence of spermatozoa in the ejaculation was seen in 9% of the cases which was lower compared to those seen in other studies such as 14.8%[5], 12.3%,[16] 28.6%[17] The problem of azoospermia is thought to be associated with sperm production or sperm transport.[5] 74% (103) of the analyzed subjects had normal sperm counts, in concordance with 73.9% reported by Butt et.al.[5] however these values are much higher than other reports such as 20%[17], 36.7%[6] and 51.8%.[16] 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.[4]

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.[5] 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.[7] In previous editions the total progressive motility for normal range was 50% and above; which included 25% with rapid progressive motility .In the present study 77.9% of the cases were above the reference motility and all these cases had > 32% of sperms with progressive movement. This was in concordance with 76% reported by Emma-Okon et.al.[16] however a lower value of 62.02% has been notedby Alemnji.[8] Asthenozoospermia (or "asthenospermia") is the medical term for reduced sperm motility. 22.1% of our samples had asthenospermia similar to 24%[16]

, 25%[5]and a higher value of 35.2% [13]reported by other authors. Complete asthenozoospermia, a condition with 100% immotile spermatozoa in the ejaculate is reported at a frequency of 1 of 5000 men. [18] Three cases in our study had100% dead sperms at the time of evaluation. As stated earlier sperm motility is an essential requisite and a predictor of fertility, it comes with sperm maturation in their passage through the epididymis. Motility is also a determinant of how efficiently the sperms penetrate the cervical mucus transport through the female genital tract, and penetration through the corona radiate and zonapellucida before oocyte fertilization.

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 referencelimit for normal sperm morphology is 4% as per the latest WHO guidelines.[7]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.[19] Morphological changes (teratozoospermia) that were considered where defects in the head, neck, midpiece and tail, the details of the type of defects were not recorded in our study. Teratozoospermiahas a deleterious effect on the rate of fertilization.[20]The mean normal morphology in our study was 66.5% in concordance to 65% reported to Butt et.al[5] although much lower value of 31% has also been reported.[2] The percentage of normal forms has been linked to exposure to toxic substances, chronic diseases and directly with the man's fertility potential, therefore, while being altered, it will provide information that may be useful while preventing further systemic diseases.[20] Our study was limited by availability of proper environmental and other relevant history along with details of morphological defects.

The lower reference value limits as per WHO guidelines represents the 5th percentile, although these values are useful for comparison with values obtained from the patient being assessed, it is important not only to compare the patient results with the lower reference limit but also with the 50th percentile, which represents the value beneath which 50% of the reference population of fertile men falls.[4] Reproductive quiescence in women is seen around 50 years of age, this is in contrast to men, whose aging follows a more gradual time course. Still, men commonly do not experience complete reproductive senescence and maintain spermatogenesis well into old age.[21] However, increasing in age significantly influences semen parameters required for healthy male fertility.[21] Age related changes on the seminal parameters were also evaluated in our 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 another study.[21]

Conclusion:

Routine semen analysis remains the backbone of the evaluation of the male factor infertility, but it is important to acknowledge the limitation of semen analysis with respect to collection, processing, evaluation, biological variation of the parameters and lack of information on sperm function.The semen parameters such as sperm concentration, motility, and morphology are known to be significantly related to conception, however due to limitations as above, a normal semen analysis does not guarantee the fertilization potential of sperm. The present study represented a predominantly normal semen analysis of the study population. It was however limited due to small sample size and lack of comprehensive history. There is a need to undertake a study with a much larger sample size complemented by adequate history and re-enforced conventional semen analysis to include more parameters besides sperm count, motility and morphology and get a more viable statistical data.This will be of immense assistance to reproductive health sciences in managing men with fertility issues.

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