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CORRELATION OF SPERM MORPHOLOGY WITH SEMEN PARAMETERS : A COMPREHENSIVE APPROACH TO MALE INFERTILITY

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ABSTRACT

Background: Assessment of semen parameters is one of the most important steps in evaluation of male factor in infertile couples. Sperm morphology sometimes bears a more important role than count as it has less fertilizing potential and may have abnormal DNA. Therefore, this study is conducted to detect the nature and number of defective sperms and correlate it with other semen parameters. Material and methods: This is a prospective study done in Dept. of Pathology, S.C.B. Medical College and Srusti Hospital, Cuttack over a period of one year from December 2015 to November 2016. All the infertile couples were included in the study. The semen samples were collected from infertile males and examined as per the guidelines of WHO manual-2010 for processing and examination of semen. Results: Total 295 semen samples were collected during the period and examined for different parameters. Majority of patients 81-61.4%) were in 4th decade with most of them (94-51.9%) having sperm count >40million /ml. Predominant sperm defect detected was head defect (39.6%) followed by multiple or mixed type of defects (20.8%).There was an inverse relationship between age with count, motility and sperm defects. Conclusion: This study shows that semen analysis bears immense importance in evaluation of infertility and sperm defects are commonly associated with male factor infertility.

Introduction: Infertility is a huge social burden recently with one in every 15 couples experiencing difficulty in conceiving. Reproduction capacity depends on the fertility status of both male and female partners equally. Among the numerous causes of infertility male factors account for 50% cases. The incidence of infertility worldwide is 1 in every 7 couples [1]. In India the infertility rate is 9% of the reproductive population i.e. 12 to 18 million couples visit infertility clinics annually for treatment [1]. Therefore infertility management is crucial as the prevalence of infertility is increasing with time. To deal with this problem, there are rapid advances with emergence of a lot of sophisticated and expensive newer diagnostic methods now a days. But there is lack of required trained personnel and infrastructure for these methods at the primary health center level. Further these investigations lead to a huge financial burden on the couple as well as society which is beyond the reach of the middle class group even in a developing country like India. In such a scenario, semen analysis is the basic, rapid, cost effective and non-invasive investigation for screening of such a large number of infertile patients.

Defective spermatogenesis and some epididymal pathologies are commonly associated with an increased percentage of spermatozoa with abnormal shapes. The quality of the sperm (morphology) is often more significant than the count. The morphological defects can be specific like head, mid piece and tail defect or can be mixed. Abnormal spermatozoa generally have a lower fertilizing potential, depending on the types of anomalies, and may also have abnormal DNA. Morphological defects have been associated with increased DNA fragmentation, increased incidence of structural chromosomal aberrations, immature chromatin and aneuploidy [2].So the study was conducted to correlate the sperm morphology with semen parameters in infertile population of Odisha.

Materials and methods:

This study was conducted during a period of one year starting from December 2015 to November 2016 in Dept. of Pathology, S.C.B. Medical College, Cuttack and Srusti Hospital, Cuttack. Couples with a complaint of infertility after one year of uninterrupted sexual intercourse were included in the study. Both the partners were investigated for infertility according to standard protocol. Males were subjected to seminal fluid analysis as per WHO 2010 guidelines [2]. The patients were advised to collect semen sample after a minimum of 2 days and a maximum of 7 days of sexual abstinence. The sample was obtained by masturbation into a clean wide mouthed container of glass or plastic. The specimen container was kept at 37 degree Celsius and evaluated within 60 minutes after collection .The patient's age, date and time of collection of sample were noted. First the semen was assessed macroscopically by evaluating volume, color, pH. Semen sample usually liquefies within 15 minutes at room temperature. Continuous gentle mixing was done at room temperature to produce a homogenous sample. If complete liquefaction didn't occur within 60 minutes, it was recorded. The viscosity was evaluated by gently aspirating the semen sample into a wide bore plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. It was recorded as abnormal when the thread exceeded 2 cm. Then the sperm concentration, motility and morphology were assessed microscopically. The concentration was recorded by counting the spermatozoa in improved Neubauer's chamber. Motility was tested by hanging drop preparation and graded. For studying morphology, following liquefaction 10µl of semen was put on a clean glass slide, a smear was made from the semen sample using "feathering" method. The smear was air dried and then stained with Giemsa/pap stain to assess morphology of spermatozoa. Two different examiners counted 200 cells per smear using bright field illumination at final magnification of 1000x and oil immersion. According to WHO criteria, a morphologically normal spermatozoon has an oval head and an acrosome covering 40%–70% of the head area(Fig 1a). A normal spermatozoon has no neck, mid piece, tail abnormalities nor cytoplasmic droplets larger than 50% of the sperm head.

The heads of spermatozoa are stained purple while tail and middle pieces take red or pink colour.100 to 500 spermatozoa are examined for detecting morphologic abnormalities. Head defects include too small, pointed, round, ragged, double heads, pin head, pyriform heads and presence of acidophilic vacuoles (Fig 1b,c). Mid piece may be absent, swollen, bent or bifurcated (Fig1d, 2a) and tail may be double, curled, rudimentary or absent. (Fig 2b,cb,c). There were many mixed defects and immature spermayozoa also

(Fig 2d) . All abnormal spermatozoa can have one to four abnormalities, including head, neck/midpiece and tail defects or presence of cytoplasmic droplets [1]. MPO stain was done to differentiate leucocytes in the semen sample [3].The data was tabulated in the master chart and correlation was made between semen parameters and morphology.

Results: Total number of males examined were 295.These semen samples were 1.5ml in volume and liquefied within 30 minutes. Age range was 25 to 53 years with mean age being 39 years. Majority of patients (181/295-61.4%) belonged to 4th decade (Table-1). Most of the males with sperm concentration and motility in normal range belonged to 4th deacde. Azoospermic cases were 12 in number comprising 4.5% of infertile cases.

172 males (58.3%) had sperm count within normal range. Sperm count decrease with age and majority had less than 15 million count in 5th decade. Motility was reversely proportional to sperm count, with decrease in number of sperms, motility decreased and many had <40 % actively motile sperms. Abnormal sperms were detected in 91 samples (30.8%). But sperm morphology was reversed i.e. majority of males(194=65.8%) in younger age range of 2nd to 4th decades had normal morphology. Majority of males (16/26 patients) with defective sperms were in 5th decades. Predominant morphology defect found in our study was head defect (39.6%) followed by multiple defects (20.8%) (Table 2). Sperm morphology was found to be inversely proportional to sperm concentration. Out of 295 males 176 had abnormal spermatozoa but those males also had normal forms >4% hence were capable of fertility. However, 91(30.8%) patients had normal spermatozoa <4% (abnormal forms >96%) which showed different types of morphologic defects.

Discussion : Semen analyses play critical role in evaluation of infertility . In fact, up to 50% of infertility can be attributed to sperm factors. A semen analysis (seminogram) is a simple, relatively inexpensive, noninvasive and rapid test. It has many components that can be complicated to interpret, but simply stated, the most important parts of a semen analysis are the concentration (number of sperm/mL of ejaculate), motility (percentage of sperm swimming forward), and strict morphology (percentage of sperm that are perfectly shaped. This will facilitate proper fertilization of the egg by the sperm). Sperm count, otherwise known as sperm concentration measures the concentration of sperm in one ml of a man's ejaculate. It should not be confused with total sperm count, which is the sperm count multiplied with volume. According to WHO manual, 2010 for a normal male the concentration should remain above 15 million sperm/mL. The motility should remain above 40% and the normal morphology should remain above 4%[2] . Any or all of these parameters can be abnormal. Assessment of sperm morphology as a component of semen analysis is one of the most important steps in the evaluation of male partner in infertile couples. Several manuals has been published so far by the World Health Organization (WHO) in order to standardize semen analysis procedures and WHO criteria have become widely accepted in sperm morphology examination in laboratories all over the world [4,5].

In this study we have examined 295 infertile couples and subjected the male partners to seminal fluid analysis. Importance was given to these three major parameters like sperm concentration, motility and morphology. Normal spermatozoa have an oval head, 4.0–5.0 µm long and 2.5–3.5 µm wide, measured with an ocular µm. The length-to-width ratio should be 1.50–1.75. A normal spermatozoon should have a well-defined acrosome that covers 40%–70% of the head. The midpiece is thin, less than 1 µm wide, about 1.5 times longer than the head. Cytoplasmic droplets, if present, should not be larger than half of the head width. The tail is thin, uniform, uncoiled and about 45 µm long. According to this classification system, all borderline forms are considered as abnormal [6]. Regarding sperm morphology, the WHO criteria as described in 2010 state that a sample is normal (samples from men whose partners had a pregnancy in the last 12 months) if 4% (or 5th centile) or more of the observed sperm have normal morphology[7,8].

There were 12(4.1%) azoospermic males which was less in comparison to earlier study done by Aleisa NAS [9].In the present study, there was negative correlation of semen parameters with age(Table-1) which is comparable with Chen et al. (2003) who reported an inverse relationship between age and semen parameters when the sample size and the age range were larger[10], but not with Maya et al., 2009[11]. Chen et al. (2004), reported nonsignificant impact of age on semen parameters in a sample size of 306[12],In their study, Ailesa NAS has shown that there is a positive correlation between age and semen parameters of fertile men but negative correlation in subfertile men. Present study reveals negative correlation between age and sperm motility and morphology. There was an inverse correlation between age and sperm motility and semen volume in a study done by Kidd et al. in 2001 suggesting that advanced age was associated with a decrease in semen volume, sperm motility, and sperm morphology but not sperm concentration[13]. Cavalcante et al. (2008) also showed an inverse effect of age on semen volume but not on other semen parameters[14]. Maya et al. (2009) also have shown an inverse relationship between age and the main semen parameters[11]. In contrast, some studies reported no impact of age on semen parameters[12,15]. The contradiction between these studies could be due to differences in the age-range and the sample size, in addition, to other corroborating factors like ethnics, genetics, geographical location and the surrounding environment.

Conclusion: Sperm morphology is recognized as a semen parameter that mostly correlates with the in vivo and in vitro fertilizing ability [16,17]. Especially, morphology is a predictor of success in fertilizing oocytes during in vitro fertilization. Up to 10% of all spermatozoa have observable defects and as such are not suitable for fertilising an oocyte [18].Nevertheless, there is an ongoing debate regarding the reliability of the results of semen analysis . Hence there is necessity for standardization and continuous quality monitoring. The authors believe that, this study will encourage other laboratories to investigate their inter-observer variation and should take initiative for standardization of sperm morphology assessment at the national level.

Legends for images-
Fig 1a-Photomicrograph of normal sperm, Giemsa x400
Fig 1b-Abnormal sperm with round head, without acrosome
Fig 1c-Pin head sperm, Giemsa x400
Fig 1d-Abnormal sperm with bent midpiece
Fig 2a-Bent and double midpiece defect of sperm
Fig 2b- Coiled midpiece and short tail of two separate sperms
Fig 2c-Double tail of abnormal sperm
Fig 2d-Immature spermatozoa

Table 1
Relation of semen parameters with age (n=295)

Age group	Number	Sperm concentration in million/ml			Motility		Morphology in percentage	
		<15	15-40	>40	<40	≥40	<4	≥4
21-30	88(29.8%)	11	13	64	23	65	22	66
31-40	181(61.4)	46	41	94	103	78	53	128
41-50	26(8.8%)	09`	03	14	17	09	16	10
Total	295 (100%)	66 (22.4%)	57 (19.3%)	172 (58.3%)	143 (48.5%)	152 (51.5%)	91 (30.8%)	204 (69.2%)

Table 2
Group wise distribution of semen morphology (n=250)

Group	Type of defect	Number	Percentage
I	Normal	84	33.6
II	Head	74	39.6

III	Mid piece	12	04.8
IV	Tail	28	11.2
V	Mixed	52	20.8

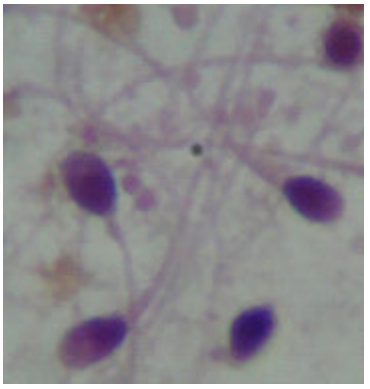

Fig 1

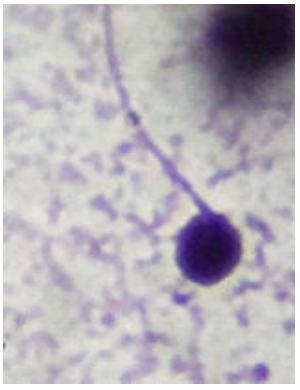

Fig 1b

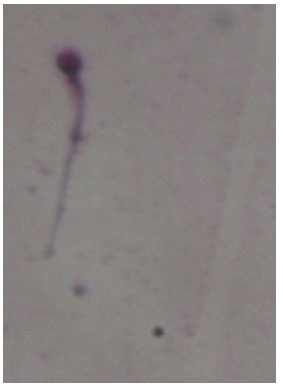

Fig 1c

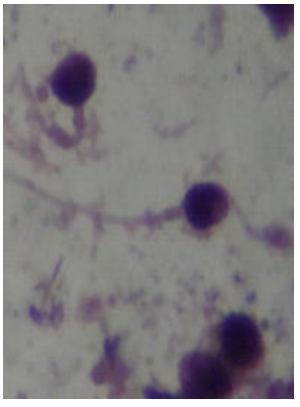

Fig 1d

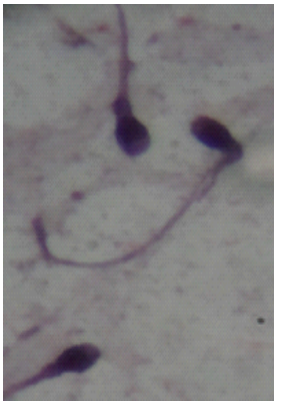

Fig 2a

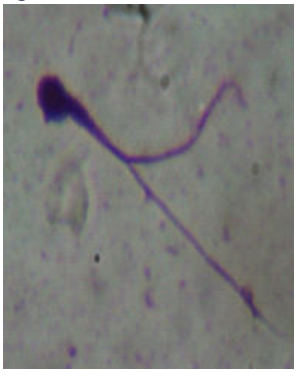

Fig 2b

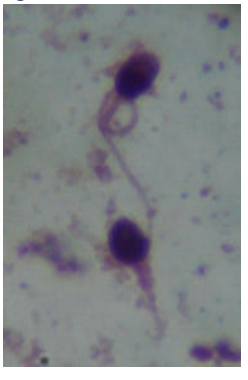

Fig 2c

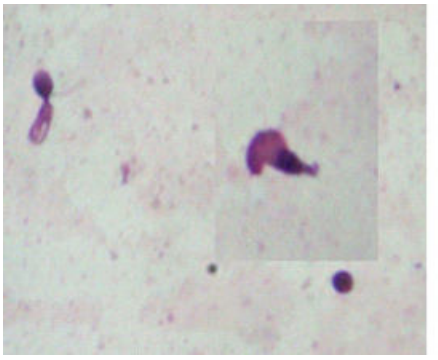

Fig 2d

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