A STUDY OF SPERM MORPHOLOGY IN A PAKISTANI POPULATION

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Background: The aim of this study was to determine the sperm morphology of proven fertile males and to compare the same with that of infertile males. **Method:** This study was carried out at International Medical College Rawalpindi and its attached Railway hospital and Islamabad Clinic Serving Infertile Couples Islamabad, from June 2005 to July 2006. 50 healthy fertile males were selected and their semen morphology was determined according to Tygerberg's strict criteria, while another 50 infertile males were recruited as controls **Results:** Proven fertile group showed significantly higher morphologically normal forms of sperms (3.04 ± 1.63) than the infertile group. **Conclusion:** Sperm morphology assessed by strict criteria is of value in the in-vivo situation to identify a group with greater chance of having an infertility problem and strict criteria sperm morphology analysis should be used to minimize variations in intra and inter-individual and inter-laboratory sperm morphology assessment.

Key Words: Sperm morphology, Strict criteria, Fertile males, Semen parameters.

INTRODUCTION

Male factor contributes about 30 to 40 % to infertility.[1] Over the last decade or so, clinicians have tried to identify male partners in couples having significantly lower chance of fertilization in vitro[2] or in intrauterine insemination (IUI) programmes[3,4]. It has been found that in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) performed for male factor has been shown to have significantly higher chances of conception than when performed for female factor[5].

The estimation of sperm concentration, motility and morphology is the mainstay of the assessment of male reproductive health[6]. Sperm morphology however, is the single indicator most widely discussed and debated in the literature over the years and is perhaps the most significant variable, whether estimated using strict criteria or by more traditional methods. A recent study highlighted the importance of sperm morphology and indicated that the effect of normal sperm on time to pregnancy may be independent of sperm concentration[7].

Although it is clear that the evaluation of a single sperm feature or function may not provide enough power for prediction of the outcome of fertilization or implantation due to the complexity and multiplicity of events leading to sperm–oocyte interaction and conception. But still, sperm morphology assessed by strict criteria[8] has been shown by multiple authors to have a high predictive value not only for the outcome of advanced assisted reproductive technologies like IVF and gamete intra-Fallopian transfer (GIFT) but also for those of intrauterine insemination and in-vivo reproduction[9,10]. Limited data is available from Pakistan about success rates with assisted reproductive techniques.[11]

According to the WHO data from assisted reproductive technology programmes, the strict criteria sperm morphology suggests that most normal and fertile ejaculate contains >15%[6] sperms with normal morphology. It was also shown in a structured review that the majority of authors used the strict criteria to judge sperm morphology[2]. It was indicated that a threshold of 5% normal forms was of clinical relevance in IVF programmes as there was significant difference in the total pregnancy rate in the group with less than 5% compared to the group with more than 5% normal forms. The 5% threshold was also found to be of value in an IUI programme in a recent publication[12].

Each spermatozoon is an intricate motile cell and consists of three major parts i.e. the head, the neck and mid-piece and the tail. The head is oval in shape 4-5μm in length and 2.5-3.5μm in width and has a well-defined acrosomal region comprising 40 – 70% of the head, the mid-piece projects for the center of the base of the head and is 5-7μm in length and 1μm in width, whereas tail continues with the mid-piece and projects at its center, it is 45-50μm in length and slightly thinner than the mid-piece and tapering down the last 10μm[6]. For a spermatozoon to be considered normal the size and shape must be within normal limits[13]. The aim of the present study was to determine the sperm morphology assessed by the strict criteria of proven fertile males and compare this with that of infertile males.

MATERIAL AND METHODS

This was a cross-sectional comparative study comparing a fertile group with an infertile group. It took place at Islamic international medical college and its attached Railway hospital, Rawalpindi and Islamabad clinic serving infertile couples, Islamabad, form June 2005 to July 2006. The sampling technique was convenience non probability. Husbands of fifty

pregnant women attending the antenatal clinic at Railway hospital, Rawalpindi were asked to participate in the study whose semen were collected for analysis. Another fifty infertile men were recruited into the study as a control group, as they consulted at the Islamabad clinic serving infertile couples, Islamabad. Proforma was completed and an informed consent was obtained. Inclusion criteria for the proven fertile males were the pregnancy achieved within one year of marriage with successful coituses. For the infertile males the inclusion criteria was failure to achieve pregnancy without the use of assisted reproductive techniques, with no infertility factors in the female partner. The exclusion criteria was secondary infertility, tuberculosis, mumps, orchitis, any chronic debilitating illness, vericoceole, sexually transmitted diseases, any drug affecting male fertility e.g. beta-blockers, anti-neoplastic agents etc.

The semen samples were obtained after 3 to 4 days of sexual abstinence at the laboratory and the subjects were given clearly written and oral instructions. Sperm morphology was according to strict criteria according to which all borderline forms are considered abnormal. A stained slide of sperm from ejaculate[14] was prepared after liquefaction. A clean dry glass slide was labelled with patient's number and a 5 - 10µl drop of ejaculate was placed on the slide and a smear was made using edge of another glass slide or a cover slip. Care was taken not to prepare thick smear. The smear was dried in air and fixed by spraying ethyl alcohol. The slide was dipped in the Giemsa stain for 3 – 5 minutes and washed under running tap water and then dried in air. Sperm morphology was assessed under oil (Immersion oil) at x100 magnification of microscope using ocular micrometer [ocular micrometer should be calibrated with stage micrometer to measure the exact size]. The sperm head, mid-piece and tail was brought over the micrometer to measure the exact size. 100 sperms were counted at random measuring carefully their head, mid-piece and tail size. At least two observations were taken.

Results were entered into SPSS version 10. Descriptive statistics were used to calculate means and standard deviations for numerical data. These were compared using t-tests at a confidence level of 95%. Frequencies were calculated for categorical data. These were compared using chi-square tests.

RESULTS

The results of this study are summarized in Tables 1 to 3 and in Figures 1 and 2. Table-1 shows Mean ± SD of weight and age of the proven fertile and infertile groups. The difference is significant in both of these (**p**<0.000). When the ages of the subjects in both the groups were compared, the infertile group was found to be statistically older then the proven fertile group, i.e., (36.60 versus 31.32 years). However, the minimum age for the proven fertile males was 20 years and maximum was 49 years, as against 27 and 51 years respectively for the infertile males group. Table-2 gives distribution of the subjects in upper, middle and lower classes of the two groups. The difference between the two groups is significant (p<0.000) with infertile group predominantly comprising of upper and middle class and the proven fertile comprising mainly the lower class, reflecting that it is mainly the affluent class which resorts to and can afford the expensive assisted reproductive techniques.

Table-1: Demographic Data of Proven Fertile and Infertile Group

Group	Weight (Kilograms)	Age (Years)
Proven Fertile (n=50), (Mean ± SD)	74.26 ± 6.49	31.32 ± 6.10
Infertile (n=50), (Mean ± SD)	81.58 ± 4.03	36.60 ± 6.28
P-Value	< 0.000*	< 0.000*

* P = Significant

Table-2: Socio-economic Status of Proven Fertile and Infertile Group

Group	Upper Class	Middle Class	Lower Class
Proven Fertile (n=50)	8	18	24
Infertile (n=50)	23	26	01
P-Value	< 0.000*		

* P = Significant

Table-3 presents Mean ± SD percentage of Morphologically Normal Sperms in proven fertile and infertile group, which is significantly higher in the proven fertile males as compared to the infertile males (**p**<0.000). The percentage of morphologically normal sperms ranges from 0 to 8% in the proven male group and from 0 to 3% in the infertile group.

Table-3: Percentage of Morphologically Normal Sperms of Proven Fertile and Infertile Group

Group	Percentage of Morphologically Normal Sperms
Proven Fertile (n=50), (Mean ± SD)	3.04 ± 1.63
Infertile (n=50), (Mean ± SD)	0.92 ± 0.72
P-Value	<0.000*

* P = Significant

Figure-1 shows the simple bar charts of the number of proven fertile males in different ages.

Figure-2 gives the simple bar charts of number of infertile males in different ages.

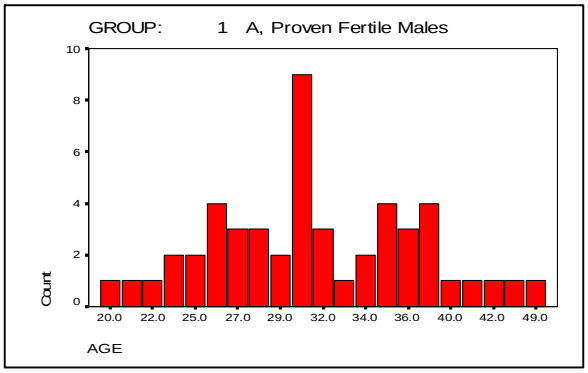

Figure-1: Proven Fertile Males in Different Ages (in years)

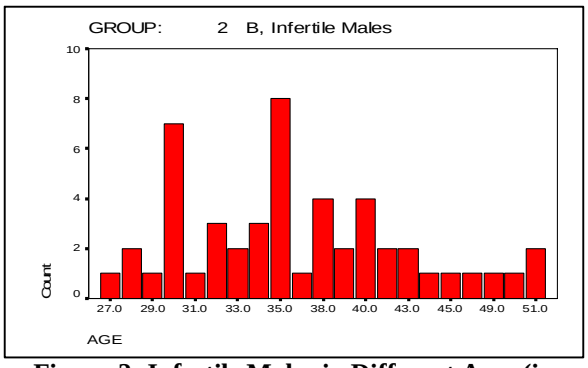

Figure-2: Infertile Males in Different Ages (in years)

DISCUSSION

Semen analysis is used in clinical practice to evaluate fertility potential of the males. However, the role of traditional semen analysis and semen parameters including sperm morphology as a prognostic factor of a male's fertility potential is a matter of on-going debate[15-19]. For the in-vivo situation in particular, there is deficient information on normal and minimal values on sperm morphology, sperm concentration and motility, for the establishment of a male's fertility potential[16], because the fertile population has very infrequently been studied[7].

Inconsistency between different methods of sperm morphology assessment has been identified by Ombelet et al[20] and others [21,22] who suggested that the semen analysis methodologies should be standardized. To be of clinical value, the methods used for semen analysis should be standardized and threshold values for fertility and infertility should be calculated for various parameters used in standard semen analysis. Since there are so many different methods for semen evaluation, especially sperm morphology that it would be difficult to standardize the methods used for semen analysis. The two classifications most widely accepted are the WHO (1987 & 1992) and the Tygerberg strict criteria[6,9].

Van Zyl et al[23] were the first to show the faster than linear decline in fertilization rate, when the proportion of normal forms dropped to <4%. It was found that a definite cut-off point could be established at <4% morphologically normal spermatozoa with an in-vivo pregnancy rate of 11.5% and a pregnancy rate of 21.5% for the group of men with 4–9% normal spermatozoa. Eggert-Kruse et al [24] found a higher in vivo pregnancy rate for higher percentage normal forms at thresholds 4, 7 and 14% using strict criteria for morphology assessment. It was found that, under in-vivo conditions, the pregnancy rate was significantly higher when semen samples had a better sperm morphology, the lowest thresholds being at >4% of strictly normal forms with a pregnancy rate of 21.5%. Therefore, it was suggested that the cut-off value for strict criteria sperm morphology may be in a range of 3–4% morphologically normal spermatozoa[16].

Zinaman et al [25] confirmed the value of sperm morphology (strict criteria) by demonstrating a clear-cut fall in pregnancy rate when normal morphology dropped below 8% and sperm concentration below 30×10^6/ml. In the IUI analysis, motility[12], total motile sperm count[26] and concentration[4] also played a role in some of the studies. However, sperm morphology had a high predictive power, and in fact was found to have the best performance of the different semen parameters[15,27]. Gunalp et al[28] found morphology (strict criteria) and progressive motility to have an almost identical predictive power and calculated a lower threshold of 5% for sperm morphology by screening the population with the positive predictive value as indicator. Assuming 50% prevalence of infertility in their study population, Menkveld et al [16] calculated an adjusted cutoff point of 3% using strict criteria. In a study by Haugen et al[29]the percentages of normal spermatozoa (i.e. percentage with normal morphology according to WHO strict criteria) calculated were 3 by using 5th percentile of the fertile population.

The mean value of morphologically normal spermatozoon in our study was found to be 3% in the proven fertile males, which is consistent with the results of Haugen et al[29] and the cutoff point calculated by the Menkveld et al[16].

The variation in intra and inter-individual and inter-laboratory sperm morphology assessment[13,30] could be solved by using Tygerberg strict criteria and applying continuous quality control programs as it was found out that consistent reading

could be achieved[19]. Previous WHO thresholds of 50% and 30% for sperm morphology were only empiric values and not based on any clinical trials. Therefore, most authors hardly found them to be of any clinical signifance[31,32].

High cost of assisted reproduction demands that the males with good or reasonable fertility potential under in vivo conditions should be identified on the basis of semen quality and males with a poor fertility potential should be identified and sent to assisted reproduction programs. It is more ethical to diagnose infertile males falsely as fertile (false negative, on the basis of a semen analysis result above the cut-off values), than to diagnose fertile males as infertile[16] (false positive, on basis of a semen analysis result below the cut-off values). This approach will prevent over-treatment of potential fertile males, for instance referring the couple for ICSI treatment in cases where IVF might have been employed and also social problems and stress among the couples. The data from the current study and also from the literature reviewed indicate that cut-off values for morphologically normal sperms as applicable to in-vivo fertilization are substantially lower than those proposed by the WHO manuals. To conclude, it is suggested that sperm morphology assessed by strict criteria is of value in the in-vivo situation to identify a group ith greater chance of having an infertility problem. It also suggested that strict criteria sperm morphology analysis should be used to minimize variations in intra and inter-individual and inter-laboratory sperm morphology assessment.

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