ORIGINAL ARTICLE

FREQUENCY OF POLYZOOSPERMIA AND TERATOZOOSPERMIA IN INFERTILE MEN

Muhammad Shoaib Khan[1], Irshad Ali[2], Aziz Marjan Khattak[3], Asmat Ullah[3], Mamtaz Ali Khan[4], Arshad Javed[1]

[1]PMRC Research Centre and Department of Pathology, Hayatabad Medical Complex, Peshawar, [2]Department of Chemistry, Gomal University, [3]Department of Pathology, Gomal Medical College, D.I.Khan and

[4]Department of Paediatrics Federal Government General Services Hospital, Islamabad, Pakistan

ABSTRACT

Background: This study was aimed to see the frequency of polyzoospermia and teratozoospermia, among men with history of infertility, presenting at National Institute of Health, Islamabad.

Material and Methods: Seven hundred and ninety married men presenting with a complaint of infertility underwent their semen analysis. They were categorized on the basis of sperm count and morphology. The sperm count was performed by Neubauer haemocytometer, while morphology assessed after staining the slides as described by the WHO methodology.

Results: The 790 men were classified as having azoospermia (203), oligozoospermia (353), polyzoospermia (13) and normozoospermia (221). While on the basis of morphology as teratozoospermia (37) and normozoospermia (550). The frequency of polyzoospermia in our study was 1.65% and teratozoospermia 6.30%.

Conclusion: The study highlights the importance of both qualitative and quantitative analysis of sperms i.e. count and morphology in order to accurately diagnose the causes of infertility. It shows low frequency of polyzoospermia and teratozoospermia in infertile men.

Key words: Polyzoospermia, Teratozoospermia, Male infertility, Pakistan

INTRODUCTION

Fertility is defined as the ability of a man or woman to reproduce, while infertility denotes lack of fertility, or an involuntary reduction in the ability to produce children. Similarly, sterility is defined as the total inability to reproduce.[1] The definitions of these words are different from those of other clinical conditions, as normal fertility is associated with a variable period of time for pregnancy to occur.[2] Therefore, infertility too has an element of time in its definition. Infertility is defined as failure to conceive after one year of regular unprotected intercourse with the same partner.[3] Couples who do not conceive in more than one year and has a sperm count <20 million/ml should be regarded as subfertile.[4]

Semen analysis is a cornerstone in the clinical workup of the infertile man. Since first published in 1980, the WHO Manual for Andrology Laboratories[5,6] has gained worldwide acceptance as a source of standard methodology for human semen analysis. Complete semen analysis is one of the most valuable tests that plays critical role in Andrology. The introduction of computer assisted methods of human semen analysis in vitro fertilization and other auxiliary methodologies have greatly enhanced the expectation of infertile couples in having a baby. However, routine semen and hormonal analysis play a significant role especially in developing countries.[7]

Total absence of spermatozoa in the semen samples is called azoospermia.[5,8] Azoospermia is defined as complete absence of spermatozoa from seminal fluid, accounting for about 40% of the causes of male infertility.[9] A low sperm concentration less than 20 million/ml is referred as oligozoospermia and is responsible for subfertility, which is caused either by hormonal disturbances or obstructive lesions. Oligozoospermia can be classified into mild (10-20 million/ml), moderate (5-10 million/ml) and severe (1-5 million/ml).[10] It is said that 50% of fertile men have their sperm count 20-40 million/ml.[11] Similarly, the condition of a high concentration of spermatozoa (more than 250 million/ml) is termed as polyzoospermia and is not considered to be a normal semen concentration,[12, 13,5] however, it is a rare condition. Excess of spermatozoa also acts as a limiting factor. In such a case, capacitation slows down and both the induced and spontaneous acrosomal reactions are significantly lowered, resulting in capacitated sperms failing to undergo induced acroso-

mal reaction.[14] This constitutes one of the causes of reduced fertility.[15] Even a reasonable concentration of spermatozoa within the ejaculate may result in failure of spermatozoa to penetrate the female genital tract and fertilize the ovum, unless they possess the ability to progress towards the ovum. Thus impaired sperm motility (asthenozoospermia) and sperm agglutination[16] are also limiting factors resulting in infertility.

Sperm morphology is assessed routinely as part of standard laboratory analysis in the diagnosis of male infertility. This practice has its origin in the work of MacLeod & Gold (1951) which showed that sperm morphology was significantly different in fertile as compared to infertile men. Despite standardization, human semen evaluation continues to be influenced by subjectiveness of the investigator and a lack of objective measurement for sperm morphology continues to be a problem.

WHO describes normal spermatozoa as an oval head shape with regular outline and acrosomal cap covering more than one-third of the head surface. The head length is 3-5 μm, width 2-3 μm; length/width ratio 1.5-2. The mid piece is 7-8 μm long, straight and regular in outline, slender, less than 1/3 the width of the head. The tail is at least 45 μm in length, slender, uncoiled and regular in outline.[5]

In keeping with the move to a more strict definition of sperm morphology, WHO has redefined what they consider to be a normal spermatozoon and subsequently set an 'empirical reference value of 30% normal forms and above as normal'.[6] A normal spermatozoon has an oval head shape with regular outline and a well-defined acrosomal region covering 40-70% of head; vacuoles occupy less than 20% of the head area. The head length is 4.5-5 μm, width 2.5-3.5 μm and length/width ratio 1.5-1.75, no cytoplasmic droplets more than 1/3 of the size of a normal sperm head. All borderline forms are considered abnormal.

MATERIAL AND METHODS

The present study was carried out in the Department of Reproductive Physiology /Health, Public Health Laboratories Division, National Institute of Health, Islamabad. 790 subjects were received, referred to Reproductive Physiology /Health, National Institute of Health, Islamabad for their semen analysis in 3 years of study from 2002 to 2005.

The collection and analysis of semen were done by properly standardized procedures as mentioned in WHO Laboratory Manual.[9] After taking history, proper instructions regarding the collection of semen were followed.[17]

Sperm count: The sperm count was determined by haemocytometeric method. This was done by an improved Neubauer heamocytometer counting chamber. In this procedure 1:20 dilution was made from a well-mixed sample by diluting it with the semen diluting fluid. A drop of diluted semen was transferred to Neubauer heamocytometer counting chamber with cover slip. The counting chamber was allowed to stand for 15-20 minutes for the spermatozoa to settle. The Neubauer counting chamber has a grid, which contains a number of large squares (1-5). The central square 5 is subdivided into 25 smaller squares of which four corner squares are designated 5a, 5b, 5c, 5d and central small square is 5e. The large square 5 holds a volume of 0.1 mm^3 of fluid.

The number of spermatozoa per ml of diluted semen, applied to heamocytometer was obtained by multiplying the number of spermatozoa counted in square 5 by the multiplication factor 10,000. The concentration of sperm in the original semen samples was obtained by multiplying the above number by dilution factor i.e. sperm count = number of sperm counted in square 5 x multiplication factor (10,000) x dilution factor (20).[17]

Sperm morphology: Basic semen evaluation is performed after liquefaction of the specimen. Two morphology slides were prepared for each patient .The smear was fixed in 95% v/v ethanol for 5-10 minutes and allowed to air dry. The smear was washed with sodium bicarbonate formalin (semen diluting) solution. The smear was rinsed several times with water. Then it was covered with diluted (1 in 20) carbol fuchsin for 3 minutes. The stain was washed off with water and then counter stained with diluted (1 in 20) Loeffler's methylene blue for two minutes. The stain was washed off with water again and allowed to air dry and then mounted with the mounting media.

The preparation was examined for normal and abnormal spermatozoa by using the 20x and 40x objectives. Abnormalities were confirmed by using 100x objectives. At least two hundred (200) sperms were counted to estimate the percentage of normal morphology.[28] The abnormal morphology was broadly categorized into three groups of head, middle piece and tail. The head abnormalities were further categorized into pin, round, small/triangle, large, bi-head and amorphous. The middle piece was categorized into swollen with cytoplasmic appendages, depressed and absent while the tail abnormalities were categorized into the small, large, curled and bi-tail.

RESULTS

Results of the study are tabulated in Table 1, 2 & 3. Table-1 shows the overall distribution of patients on the basis of concentration and morphology. The 790 patients were classified on the basis of sperm concentration as azoospermia (203), oligozoosper-

Table-1: Distribution of patients on the basis of concentration and morphology.

On Concentration			On Morphology		
Group	Number of patients	Percentage	Group	Number of patients	Percentage
Azoospermia	203	25.70%	Teratozoospermia	37	6.30%
Oligozoospermia	353	44.68%	Normozoospermia	550	93.70%
Normozoospermia	221	27.97%			
Polyzoospermia	13	1.65%			
Total	790	100.00%	Total	587	100.00%

Table-2: Main morphological abnormalities in teratozoospermic patients.

Condition	Head defect%	Neck defect %	Tail defect %
Teratozoospermia(37)	67.12 ± 5.9	12.33 ± 4.06	20.55 ± 5.9

Values are mean ± SEM

mia (353), polyzoospermia (13) and normozoospermia (221), while on the basis of morphology as teratozoospermia (37) and normozoospermia (550). The frequency of polyzoospermia was (1.65%) and teratozoospermia (6.30%).

Table-2 depicts the main morphological abnormalities in teratozoospermic patients. It has been seen that head defect (67.12 ±5.9%) is the major abnormality in teratozoospermic patients.

Table-3 Showing the common parameters in polyzoospermic patients.

Table-3: Semen characteristics of polyzoopsremic patients. (n=37)

Semen parameter	Values
Age (years)	32.33 ± 00.98
Abstinence period (days)	7.00 ± 00.00
Volume (ml)	1.47 ± 00.21
pH	8.35 ± 00.15
Time of liquefaction (minutes)	15.00 ± 00.57
Sperm concentration (million per ml)	563.33 ± 56.24
Active motility (%)	53.33 ± 07.58
Total motility (%)	70.00 ± 08.16

Values are mean ± SEM

DISCUSSION

Globally, the male is considered to be a factor in nearly one third couples affected by infertility [2]. It is only in the past few decades that male factor has been recognized as a significant cause of infertility. An important point is that male infertility is not an entity but reflects a variety of different pathogenetic mechanisms.[19]

The frequency of polyzoospermia, as indicated in our study is only 1.65%, while in Mexico is 13%[20] in Germany 1.75%,[21] in South Africa 5%,[22] 2% in the United States[12] and 13.84% in the former East Germany.[23] The highest sperm count reported in this study is 660 million/ml, but an earlier study carried out in the United States[24] has reported counts even up to 1.75 billion/ml. Another group in Pakistan, working on *in vitro* fertilization methods, did not come across patients with more than 600 million sperms/ml.[25]

In polyzoospermic men, capacitation slows down, both the induced and spontaneous acrosomal reactions are significantly lowered and even capacitated sperms fail to undergo induced acrosomal reaction.[14] It has also been reported that several degrees of acrosomal membrane alterations are observed in semen of polyzoospermic patients.[26] Functionally defective acrosomes hinder fertilization,[27] as they are incapable of penetrating the outer investments of the oocyte, which is one of the reasons for reduced fertility.[28]

Although only 13 out of 790 patients examined in this study were found to have their sperm concentrations in excess of 250 million/ml, this condition needs attention and must be taken into consideration for assessment of the male partner of an in-

fertile couple because it drastically reduces the fertility potential. Due to extremely low frequency of polyzoospermia in the population, the information gained presently is merely of preliminary nature. The incidence and characteristics of polyzoospermia need to be investigated further.

The association between semen morphology and male infertility has been known for more than 40 years. Having reviewed the literature, it seems clear that strict morphology has a clinical relevance, being an excellent biomarker of sperm fertilizing capacity, in vivo and in vitro, independent of motility and concentration.[29] Sperm morphology evaluation is considered to be a highly subjective procedure because, unlike the haematopoietic cells, it is difficult to classify sperm morphology because of the existence of large variety of abnormal forms found in the semen of infertile and fertile men, in which, only certain types of sperm abnormality are of clinical interest.[30]

It has been reported that physical sperm aberrations may occur during either production of sperm or during the storage of sperms in the epididymus. In cases of teratozoospermia, one should exclude the presence of monomorphic genetic syndromes such as globozoospermia, microcephaly and short tail spermatozoa.[31] In epididymal dysfunction and in frequent ejaculations, the increased number of immature spermatozoa has been reported. Similarly, large number of spermatozoa with tapered head, cytoplasmic droplets and bent tail are found in cases of varicocele,[32] whereas in our study we found 37(6.30%) of patients with teratozoospermia, mainly with head defect abnormalities, which is an agreement with previous study.[32]

Sperm morphological properties, determined with strict criteria, are important factors for sperm ability to penetrate the mucus barrier at the uterine cervix before reaching the site of fertilization, but sperm morphology is only one among other parameters determining the complex phenomenon of sperm-mucus interaction.[33] Cigarette smoking is associated with reduced semen quality, with a significant decrease in sperm density (-15.3%), total sperm count (-17.5%), total number of motile sperm (-16.6%), and citrate concentration (-22.4%). The percentage of normal forms was significantly reduced in smokers, and sperm vitality, ejaculate volume, and fructose concentration were slightly but non-significantly affected.[34]

WHO recommends that each laboratory recruit fertile men, in order to investigate and determine the real cut off values for normality in that laboratory,[35] as these are difficult to recruit, therefore only a few laboratories are actually performing this specialized analysis.[36]

CONCLUSION

This study highlights the importance of both qualitative and quantitative analysis of sperms i.e. count and morphology in order to accurately diagnose the causes of infertility. It shows low frequency of polyzoospermia and teratozoospermia in infertile men.

REFERENCES

1 Nieschlag E. (1997). Scope and goals of Andrology. In: Andrology - male reproductive health and dysfunction. Nieschlag E and Behre HM. (eds). Springer Verlag, Berlin, Federal Republic of Germany. pp. 1-8.

2 Seibel MM. (1990). Workup of the infertile couple. In: Infertility A Comprehensive Text, Seibel MM. (ed). Appleton & Lange, Norwalk, Connecticut. USA. pp. 1-21.

3 Martini MC. Infertility. In: Pernoll M,l. ed. (1991). Current obstetric and gynecologic diagnosis and treatment. Reproductive endocrinology and infertility. 7th ed. Norwalk (CT): Appleton & Lange. 1025-1036.

4 Wong WY, Thomas CM, Merkus JMWM, Zielhuis GA. Male factor sub-fertility: Possible causes and the impact of nutritional factors. Fertility and sterility 2000; 73: 435-442.

5 WHO. (1987). WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. Cambridge University Press, Cambridge. pp. 1-67.

6 WHO. (1992). WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press, Cambridge. pp. 1-107.

7 Sheriff DS. Analysis of semen in constantly changing social context of medline. Arch Androl 1995; 34: 125-32.

8 Amelar RD. (1966). The semen analysis. In: Infertility in men. F. A. Davis Company, Philadelphia, U.S.A. pp. 30-53.

9 Rehan N. Azoospermia. Pak J Med Res 1991; 30: 72-75.

10 Subhan F, Tahir F, Ahmed R, Khan Z. Oligospermia and its relation with hormonal profile. JPMA 1995; 45: 246-247.

11 Rikhasor RM, Rathi SL, Jalbani MH, Pirzada ZA. Semen analysis of infertile men and plasma levels of LH, FSH, and Testosterone in oligospermia. Medical Channel 2001; 7: 30-32.

12 Glezerman M, Bernstein D, Zakut C, Misgav N, Insler V. Polyzoospermia: a definite pathologic entity. Fertil Steril 1982; 38: 605-608.

13 Damjanov I. (1993). Clinical evaluation of the infertile couple. In: Pathology of infertility. Mosby-Year Book, Inc. St. Louis, Missouri, USA. pp. 7-42.

14 Kholkute SD, Meherji P, Puri CP. Capacitation and the acrosome reaction in sperm from men with various semen profiles monitored by a chlortetracycline fluorescence assay. Int J Androl 1992; 15: 43-53.

15 Schill WB, Feifel M. Low acrosin activity in polyzoospermia. Andrologia 1984; 16: 589-591.

16 Badenoch DF, Moore HDM, Holt WV, Evans PR, Sidhu BS, Evans SJW. Sperm motility, velocity and migration. Br. J. Urol 1990; 65: 204-208.

17 Aitken RJ, Aribarg A, Gopal Krishnan K, Hamilton DW, Katz DF, Wang C, Vanlook PFA. (1980) Collection and examination of human semen. WHO laboratory manual for the examination of human semen and sperm cervical mucus interaction. Cambridge University Press.

18 Monica: Microbiological test In: Monica Cheesbrough. (2000). District Laboratory Practice in Tropical countries Part 2 ed, Cambridge University Press. pp. 130-132.

19 Shakkeback VE, Giwercman A, de-krester D. Pathogenesis and management of male infertility. Lancet 1994; 343: 1473-1479.

20 Merino G, Carranza-Lira S. Semen characteristics, endocrine profiles, and testicular biopsies of infertile men of different ages. Arch Androl 1995; 35: 219-224.

21 Henkel R, Muller C, Miska W, Schill WB, Kleinstein J, Gips H. Acrosin activity of human spermatozoa by means of a simple gelatinolytic technique: a method useful for IVF. J Androl 1995; 16: 272-277.

22 Bornman MS, Schulenburg GW, Boomker D, Chauke TR, Reif S. Observations in infertile African males at an Andrology clinic in South Africa. Arch Androl 1994; 33: 101-104.

23 Krieg K, Gerlach R, Rost D. Impulszytophotometrische Spermauntersuchungen zum Nachweis von Fertilitatsstorungen. [Impulse-cytophotometric studies of spermatozoa for the demonstration of fertility disorders]. Zentralbl. Gynakol 1977; 99: 1217-1224.

24 Amelar RD, Dubin L, Quigley MM, Schoenfeld C. Successful management of infertility due to polyzoospermia. Fertil Steril 1979; 31: 521-524.

25 Rabbani KJ. Infertility: the male factor. First national symposium on current trends in developmental and reproductive biology, held at the Pakistan Academy of Sciences, Islamabad, Pakistan. 22-23 November 1997.

26 Schill WB. Determination of active, non-zymogen acrosin, pro-acrosin and total acrosin in different andrological patients. Arch Dermatol Res 1990; 282: 335-342.

27 Schill WB, Topfer-Petersen E, Heissler E. The sperm acrosome: functional and clinical aspects. Hum Reprod 1988; 3: 139-145.

28 Calamera JC, Giovenco P, Brugo S, Dondero F, Nicholson RF. Adenosine 5 triphosphate (ATP) content and acrosin activity in polyzoospermic subjects. Andrologia 1987; 19: 460-463.

29 Topfer-Petersen E, Volcker C, Heissler E, Schill WB. Absence of acrosome reaction in polyzoospermia. Andrologia (Spec No) 1987; 19: 225-228.

30 Ombelet W, Menkveld R, Kruger TF, Steeno O. Sperm morphology assessment: Historical review in relation to fertility. Hum Reprod Update 1995; 1: 543-557.

31 Compana A, deAgostini A, Biscchof P, et al. Evaluation of infertility. Hum Reprod Update 1995; 1: 586-606.

32 Rrumbullaku L, Boci R, Dedja A, Dautaj K. Sperm morphology in infertile men with varicocele. Ist Balkan symposium of Andrology June 12-14, 1998, Alexandroupolis, Greece.

33 Eggert-Kruse W, Reimann-Andersen J, Rohr G, Pohl S, Tilgen W, Runnebaum B. Clinical relevance of sperm morphology assessment using strict criteria and relationship with sperm-mucus interaction in vivo and in vitro. Fertil Steril 1995; 63: 612-624.

34 Kunzle R, Mueller MD, Hanggi W, Birkhauser MH, Drescher H, Bersinger NA. Semen quality of male smokers and nonsmokers in infertile couples. Fertil Steril 2003; 79: 287-291.

35 Ombelet W, Wouters E, Boels L, Cox A, Janssen M, Spiessens C, Vereecken A, Bosmans E, Steeno O. Sperm morphology assessment: Diagnostic potential and comparative analysis of strict or WHO criteria in a fertile and a subfertile population. Int J Androl 1997; 20: 367-372.

36 Barrat CLR, Naeeni M, Clements S, Cooke ID. Clinical value of sperm morphology for in vivo fertility. Comparison between World Health Organization criteria of 1987 and 1992. Hum Reprod 1995; 10: 587-593.

Address for Correspondence:

Muhammad Shoaib Khan
PMRC Research Centre
Hayatabad Postgraduate Medical Complex
Peshawar, Pakistan