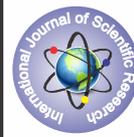


Effect of Morphological abnormalities in male infertility



Biological Science

KEYWORDS: Male infertility, HOS test, sperm membrane, abnormal sperm form.

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ABSTRACT

The quality of sperm has direct influence on fertilization and developmental competence of embryo; therefore we have attempted to review evaluations of criteria of human sperm and their abnormalities. All sperm observed in alcoholic acidic silver nitrate stained samples are classified as normal or abnormal. A functional membrane is requisite for the fertilizing ability of spermatozoa, as it plays integral role in sperm capacitation, acrosome reaction, and binding of spermatozoa to egg surface. The hypo osmotic swelling (HOS) test evaluates the functional integrity of the sperm plasma membrane and also serves as a useful indicator of fertility potential of sperm. This article can be considered as guideline for the manual sperm qualities. The present study aimed to evaluate the relationship of sperm morphology and membrane function with sperm parameters and it also holds special significance in providing information regarding the functional and qualitative aspects of the human sperm. The membrane function and hypo-osmotic swelling showed significant alteration in infertile cases.

Introduction

Sperm morphology is regarded as a significant prognostic factor for fertilization and pregnancy (Franken, 1998)[5]. Abnormal sperm morphology is one of the common factors of male infertility. Morphological changes are considered to be a potential target in contraceptive development. There is therefore, an urgent need to analyze the morphological alteration of human spermatozoa in greater detail. Up to 40% of infertile couples have difficulty in conceiving due to "malefactor" subsequently, meaning one of the sperm parameters are abnormal. Infertility probably affects at least one couple in six and according to Hull et al. (1985)[7] the commonest single defined cause is sperm dysfunction. Recent evidence has suggested that human semen quality is deteriorating by as much as 3% per year, leading to increased male factor infertility. While defective sperm function may now be termed as the most common cause of infertility, it was also recently recognized as being most difficult to evaluate and treat. Part of this difficulty was due to our incomplete understanding of the factors contributing to normal and abnormal sperm function leading to male infertility. There are two major classifications of morphology assessment. The WHO system has normal value >30% normal forms (WHO, 1992)[16]. The method employing strict criteria for normal shapes, which was developed by Kruger, has normal range of >15% normally shaped (Kruger et al., 1986:1988)[10,11]. Semen samples with reduced incidence of normal forms typically have reduced motility and a reduced capacity to penetrate the cervical mucus, to penetrate zona-free hamster eggs and fertilize the human oocytes (Liu and Baker, 1992)[12]. The hypo-osmotic swelling (HOS) test measures sperm membrane integrity by examining its ability to swell when exposed to hypo-osmotic media and has been claimed to be relevant to fertilizing ability (Campana et al., 1995)[3]. This test is based on the assumption that an undamaged sperm tail membrane permits passage of fluid into the cytoplasmic swelling and curling of tail. The HOS test is used as sperm function test known as vitality test. According to WHO (1999)[17], this test is easy to score and gives additional information on the integrity and compliance of the cell membrane of the sperm tail. Jayendra et al. (1984) [8] has put forward the sperm hypo-osmotic swelling test (HOS test) as an indicator of normal membrane integrity. The test is also closely correlated with the hamster-oocyte penetration test (Avery et al., 1988; Mendoza et al., 1988)[1,13]. Hence we have focused on in these investigations which assess the sperm membrane function and Morphology.

MATERIAL AND METHOD

Semen samples were analyzed from selected cases referred with complaints of unexplained infertility and failure of sperm fertilizing ability, to evaluate for various structural and functional parameters of human spermatozoa, related to impair fertilizing potential.

Detailed investigation on the functional aspects of the spermatozoa in semen of cases of unexplained infertility from Ahmedabad and its vicinity was therefore carried out in two groups according to the age range of 20-30 (Group II) and 31-40 (Group III). The hypo-osmotic swelling test described by Jayendran et al. (1984)[6] was used to evaluate the functional integrity of the membranes of the spermatozoa. Spermatozoa "swell" when placed in hypo-osmotic solution due to the influx of water molecules, resulting in expansion of the sperm membranes. When placed again in iso-osmotic conditions, the spermatozoa regain their original form, indicating normal functional integrity and selective permeability of the membranes. In each sample, approximately 100 spermatozoa were scored and the percentage of swollen spermatozoa were determined as follows: No. of swollen spermatozoa / Total No. of spermatozoa in the same field × 100. A modified alcoholic acidic silver nitrate staining technique (Chinoy et al., 1992)[4] was used to obtain differential staining patterns in spermatozoa from normal and infertile semen. The technique was modified based on the aqueous silver staining procedure described by Bongso (1983)[2] and the method of Chinoy et al. (1992)[4] used, resulted in increased staining intensity with clear morphological differentiation of the spermatozoa.

Results

Semen analysis was carried out on freshly collected semen samples of men of proven fertility (Group I, normal - control) and various groups of men with clinical history of unexplained infertility (Group IIA and Group IIB of age range 20-30 years) and (Group III A and III B of age range 31-40 years). The hypo-osmotic test analysis was aimed at determining functional alteration in the spermatozoa in order to evaluate and determine the semen quality. The table I & II result shows significant decrease ($p < 0.01$) was observed in percent swollen in spermatozoa in group IIA & Group IIIA (cases of unexplained infertility with sperm counts compared to normal). There was a highly significant decline ($p < 0.001$) in percentage of spermatozoa showing swelling in sperm membranes in semen of cases of Group IIB and Group III (samples with low sperm density). (Each group was compared to control of matching age group) (tables I and table II). Regarding abnormal sperm morphology, the various abnormal sperm types were scored in stained semen smears and expressed as percent abnormal forms. An insignificant alteration was observed in percentage of sperm anomalies semen sample from individuals of group IIA, while a highly significant increase ($p < 0.01$) was observed in the percent abnormal sperm types of samples from individuals of group IIB as compared to control Group (Table -I). It was observed that samples from individuals of Group III A and IIIB of age group 30 to 40 years, showed a highly increase in the morphologically abnormal spermatozoa, ($p < 0.001$) as compared to normal Group IA (

table I).

TABLE I: SHOWING PERCENT SWOLLEN (HOS) AND PERCENT ABNORMAL SPERM MORPHOLOGY IN SEMEN SAMPLE OF GROUP II MALES (CAUSES OF UNEXPLAINED INFERTILITY OF AGE RANGE 20-30 YEARS)

Group	Parameter	
	Percent swollen spermatozoa(HOS) test %	Abnormal Sperm morphology(%)
Group I Normal n=40	74 ± 12	18.2 ± 1.7
Group II-A (Normospermia) (n=62)	38.5± 5.3**	21.3 ± 0.9+
Group II -B (moderate oligozoospermia) n=46	23.6 ± 9.7**	28.6 ± 3.8**

Values are Mean ± S.E. +Not Significant **p<0.001

TABLE II: SHOWING PERCENT SWOLLEN (HOS -TEST) AND PERCENT ABNORMAL SPERM MORPHOLOGY IN SEMEN SAMPLE OF GROUP III MALES. (CAUSES OF UNEXPLAINED INFERTILITY OF AGE RANGE 31-40 YEARS)

Group	Parameter	
	Percent Swollen Spermatozoa (HOS) test %	Abnormal Sperm Morphology(%)
Group I Normal n=40	71 ± 150.8	18.7 ± 0.4
Group II-A (Normospermia) (n=53)	9.8±0.5*	11.7±0.6**
Group III-B (moderate oligozoospermia) n=59	5.5±0.04**	14.9±0.3**

Values are Mean ±S.E. *p<0.01 **p<0.001

Discussion

The evaluation of sperm viability with the use of a Supra-vital stain is currently accepted as vital tool in evaluating the functional status of sperm. The present investigation revealed that there was a decline in the percent viability correlated with a decline in percentage of Spermatozoa responding to hypo-osmotic stress in samples Collected from all the cases of primary infertility investigated (Groups II A, II B, III A and III B). (This observation indicated the loss of membrane selective semi-permeability and provides further confirmation of altered membrane function which are comparable to the findings of Palma et al. (1988)[12] who have similarly reported reduced hypo osmotic swelling in infertile semen. The decrease in percent viability thus indicates altered membrane function, which may in turn affect normal sperm metabolism. According to Jayendran et al. (1984)[6], there is a good correlation between the percentage of sperm in semen, which shows controlled swelling, and their ability to penetrate and fertilize human oocyte in vitro. The decline observed in all the cases of unexplained infertility studied, indicates a loss of membrane selective semi-permeability. Campana et al. (1995)[3] also reported that the hypo-osmotic swelling (HOS) test measures sperm membrane infertility by examining its ability to swell when exposed to hypo-osmotic media, and has been claimed to be relevant to the sperm fertilizing ability. According to WHO (1992)[13], the HOS test is simple to perform and easy to score and gives information on the integrity and the compliance of the cell membranes of the sperm axonema. Schrader et al. (1986)[15] have similarly obtained a moderate correlation (n=0.52) between sperm viability and the HOS test, since supra-Vital staining evaluates structural integrity while HOS test is indicative of the physiological function of sperm membrane it is clearly evident from the study that in samples from Group II A and III A where sperm concentrations were not significantly altered, the membrane function, viability and hypo-osmotic swelling showed significant alteration.

As emphasized by Kubo-Irie et al. (2005)[9], the morphological analysis of the spermatozoa from fertile and infertile men is generally carried out to clarify the relationship between sperm morphology and sperm fertilizing potential. Morphology refers to the shape and appearance of spermatozoa, taking into account whether or not the sperm head, neck, mid-piece and tail are free of defects. During analysis, sperm are characterized according to specific rates of the criteria, the most common being the WHO criteria for morphology. The WHO system has a normal value of sperm morphology greater than 30% normal forms (WHO, 1992), which was followed in this study. Teratozoospermia (>40% of sperm scored are of abnormal forms) is a rare condition as also observed in our study on unexplained infertility cases. In this pathological condition of teratozoospermia the patient is likely to remain infertile, because of the screening of the semen done at different levels by the female tract leading to less number of spermatozoa, available for fertilization. And this number may not be sufficient enough to dissolve the corona radiate and pierce the zona pellucida. According to Hafez (1977)[8], morphological abnormalities in spermatozoa may be due to infection, trauma, other testicular stress or hormonal imbalance in men.

In present study, the percent abnormal spermatozoa were scored. Semen sample from men of Group IIA showed an insignificant increase in the percent abnormal sperm types, whereas other Groups II B, III A and III B showed a highly significant increase in percent sperm abnormalities as compared to the control group. Thus it could be observed that spermatozoa from Group IIA samples showed no significant change in sperm density and morphology, however the function of the sperm membrane was significantly altered, possibly contributing to impaired sperm fertilizing potential. It is known that the fertilization of ovum by abnormally shaped spermatozoa is one of the causes for infertility. Liu and Baker (1992)[12] reported that semen samples with reduced incidence of normal forms typically have reduced motility and a reduced capacity to penetrate the cervical mucus, to penetrate zone free hamster eggs or fertilize the human oocytes. In our study as well, samples of Group II B, III A and III B showed lower sperm count, motility and increased percentage of abnormal spermatozoa, which was a possible contributing factor to reduced sperm function.

Conclusion

Morphology of spermatozoa was altered in semen of the cases investigated in all the Groups. A higher percentage of abnormal forms, correlated with low motility and motility and loss of nuclear integrity, were scored in semen of infertile individuals, which probably resulted in poor penetrating ability of spermatozoa. Abnormal forms in human ejaculate are related to malformations of nucleus, acrosomal changes, and defects in organelles during Spermatogenesis and improper differentiation and maturation in epididymis and in corroboration with these results, significant alteration was also obtained in the Sperm function tests for nuclear membrane integrity and sperm maturation. The frequency of abnormal forms was greater in oligospermic cases of unexplained infertility of our study.

Low sperm viability and altered sperm membrane function (HOS test) may contribute to loss of fertilizing potential in spermatozoa of infertile semen. Alteration in sperm viability, its membrane, acrosomal and nuclear integrity, sperm morphology probably contributes to lowered sperm penetrating and fertilizing ability and poor fertility in these cases. Impaired fertilization in these cases may therefore be due to loss of sperm viability and membrane integrity. The measurement or evaluation of sperm morphology remains therefore a very important tool in the diagnosis of a male's fertility potential and in the clinical decision making for the treatment of patients with infertility problems.

References:

1. Avery, S., Bolton, W., Mason, B.A. and Mill, S.C. (1988) : The use of the hypo-osmotic sperm swelling test as an indicator of IVF outcome. 4th. Annual Meeting of the European Society of Human Reproduction and Embryology. Barcelona. July 3-6.

- Human Reproduction.3(1):94.
2. Bongso,T.A.(1983): Comparative silver staining patterns of water buffalo, goat and pig spermatozoa. Arch. Androl.,11: 13-17.
 3. Camapan A.,deAgostinin,A. and Bischof, P. (1995): Evaluation of infertility. Human .Repro. Update, 1, 6, 586-606.
 4. Chinoy N.J, Ranga G.M., Highland .N., D'Souza, K.J. and Sequeria,E., (1992): A modified method for the differential staining of spermatozoa using alcoholic acidic silver nitrate. Int.J> Fertility,37(4):232-236.
 5. Franken, R.D.(1998): The clinical significance of sperm –zona pellucida binding. Front Biosci. 3: 247-s2535)
 6. Hafez ,E.S.E,1977: Technique of human Androology. Vol.I.north Holand Publishing Company, New York
 7. Hull,M.G.R, Glazener,C.M.A. and Kelly, N.S.(1985) : population study of causes, treatment and outcome of infertility. Br.Med.S;291:1693-7.
 8. Jayendran,R.S., Van der Ven,H.H., Perez-Pel aez, B.G.,Crabo,B.G.and Zaneveld,L. J. (1984): Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J.Reprod.Fertil. 70:219-228.
 9. Kubo – Irie M.,matsumiya, k. Iwamoto (2005):Morphological abnormalities in the spermatozoa of fertile and infertile men.
 10. kruger ,T.F, menkveld, R. and stander,F.S.H (1986): sperm morphologic features as a prognostic factor in viro fertilization (IVF).Fert.steril.46: 1118-1123.
 11. kruger,T.F, Acosta,a.A., Simmons, k.F., Swanson, R.J., Matta, J.F. and Oehninger , S. (1988): Predictive value of abnormal sperm morphology in vitro fertilization .Fert.Steril.49:117-119.
 12. Liu D.Y. and Becker H.W.G. (1992):Tests of human sperm function and fertilization in vitro.Fertil. Steril.,58:465-83.
 13. Mendoza R., Corcostegyi, B.,Aparicio, Tejada, L., Ramon.o. Carbonero, J. L., and Beniton J.A.(1988)
 14. Palma A, Mendoza.C.and carreras ,A(1988);Semen quality and the hyposomatic swelling test in normal and oligozoospermic men.4th Annual Meetings of the European Society of Human reproduction and embryology, Barcelona,Spain,July 3-6.Human Reproduction 3(Suppl).102, Abstr.No. 325.
 15. Schrader, S.M., Platek, S.F., Zaneveld, L.J.D., Perezpelaez, M. and Jayendran, R.S. (1986): Sperm viability. A comparison of analytical methods, Andrologia. 18 (5): 530-538.
 16. World Health Organization (1992): WHO Laboratory Manual for the Examination of Human Semen and Semen –Cervical mucus interaction Cambridge University Press, Cambridge J., 291,1693-1697.
 17. World Health Organization(1999):WHO): WHO Laboratory Manual for the Examination of Human Semen and Semen Cervical mucus interaction.4th ed. Cambridge, United kingdom: University Press;199.Submitted on September 8, 2000;accepted on March 1,2001.