Indian J Physiol Pharmacol 2001; 45 (3) : 373–377

THE ROLE OF HYPO-OSMOTIC SWELLING TEST IN RECURRENT ABORTIONS

SIMANTINI S. PATANKAR, ATUL M. DESHKAR*, MANISH V. SAWANE, NILAM V. MISHRA, ASHOK H. KALE AND GEETA B. GOSAVI

Department of Physiology, Govt. Medical College, Nagpur – 440 010

(Received on January 21, 2001)

Abstract : The role of male factors in abortions has not been studied extensively. We undertook this study to determine if there was any relationship between hypo-osmotic swelling test score and recurrent abortions. This was a cross-sectional case control study conducted at the semen analysis laboratory at a tertiary level referral centre.

The male partners of 25 patients who had three or more first trimester abortions of unknown etiology were chosen as cases. Controls were 26 unmarried volunteers with unknown fertility potential to represent the entire population.

The conventional seminal parameters were studied according to WHO guidelines. The HOS test score were obtained by the method advocated by Jayendran et al. There were no statistically significant differences in mean sperm count, percentage motility and morphology among the cases and controls. We found a low hypo-osmotic swelling test score among the cases [55.7±1.197 (SEM)] than the controls [69.3±1.143 (SEM)] which was statistically significant [P<0.001].

The normal spermatozoal membrane is the prerequisite for the specialized cell-to-cell communications and cell-to-cell binding. In spite of apparently normal semen analysis, subtle membrane defects in the spermatozoa, which could be the cause of defective membrane functions in the embryo effecting miscarriages, can be elucidated by the hypo-osmotic swelling test.

Key words : hypo-osmotic swelling test recurrent abortions sperm function test

INTRODUCTION

Recurrent abortions have a multifactorial etiology (1). Spontaneous recurrent abortions is defined as three or more consecutive abortions (2, 3). Abortions have an incidence of 10%(2). Various etiological factors have been demonstrated

*Corresponding Author

like genetic [50–60%] endocrinological [10–15%], chorioamniotic separation [5–10%], anatomical defects [15%], infection [15%], immunological [3–5%], nutritional and unknown reasons including sperm factors [3%] (4). Yet in spite of advanced investigations the etiology remains idiopathic (2). The role of male factors in recurrent abortions remains largely unknown barring a few cytogenetic abnormalities (2). Good quality sperms yield good quality fetus. Any deviation from the normal or sub-standard sperms will affect its motility, penetration and decondensation, ultimately hampering the conception, implantation and nidation, as one of the limiting steps in the success of fertility is the quality of the fertilized ovum (2). Sperm morphology abnormalities do not seem to be involved in recurrent abortions (5, 6) and the functional parameters have not been studied extensively. Hypo-osmotic swelling test is the qualitative indicator of the sperm fertility potential routinely carried out in most of the Advanced Reproductive Technology Centers (7). It assesses the functional integrity of the spermatozoal membrane (8). The hypo-osmotic swelling test was claimed to assess an independent functional characteristic of human spermatozoa bearing relevance to their fertilizing capacity (9). Very few researchers have studied an association between altered hypo-osmotic swelling test and recurrent abortions (10, 11, 12). Hence we have tried to find out any association between altered hypo-osmotic swelling test score and recurrent abortions.

METHOD

This study has been carried out in the semen analysis laboratory of the Department of Physiology, Govt. Medical College, Nagpur. Semen samples were collected from 25 partners of the patients who had history of recurrent abortion of unknown etiology. The control group was formed by randomly selecting 30 healthy unmarried volunteers of unknown fertility potential, as they represented the entire population, rather than choosing known fertile or infertile males. An exhaustive history was taken of both partners regarding age, previous illness, tuberculosis, diabetes mellitus, hypertension, sexually transmitted disease, vaginal discharge, sickling, smoking, and any other relevant family or personal history.

These patients were investigated for complete blood count, hemoglobin electrophoresis, blood sugar, urine albumin and sugar, erythrocyte sedimentation rate, VDRL, Montoux and MMR.

Four days of sexual abstinence was advised and the sample was collected by masturbation. The subjects were provided sterile nonspermicidal containers from the laboratory for the collection of the semen samples.

After 30 minutes, which is the time required for the liquefaction, routine semen analysis was carried out according to WHO standard criteria (13). Hypo-osmotic swelling test was carried out by diluting

0.1 ml of the semen sample with 1 ml of hypo-osmotic solution. The hypo-osmotic solution was prepared by mixing the 1.351 g of fructose with 0.735 g of sodium citrate in 100 ml of distilled water. The sample was incubated at 37° C for half an hour. A slide was prepared by taking one drop of incubated solution and mounting it with a cover slip. This was observed immediately under high power microscope [400 X magnification] (8). The hypo-osmotic swelling test positive sperms i.e. sperms with curled tails were counted in different high power fields, and thus the percentage of hypo-osmotic swelling test positive sperms was calculated. The percentage of initial number of sperms with curled tail was subtracted from the later and thus the final hypo-osmotic swelling test score was obtained.

RESULTS

Of the 30 volunteers, who were considered in the study as the control group, 2 were azoospermic, 5 were oligozoospermic, and 3 had reduced motility. The 2 azoospermics were not included in the study as also 2 with previous history of tuberculosis, and thus remaining 26 subjects formed the control group. The average age of the control group was 24 years. This was significantly lower than the cases, as the group was taken from the unmarried healthy volunteers. Male partners of 25 females with history of recurrent miscarriage were included in the study as cases.

We observed a higher mean sperm density in the control group, but statistically the difference was not significant [$P<0.05$]. All other conventional semen parameters like percentage motility, and percentage normal sperm morphology were statistically similar in cases and controls.

The mean hypo-osmotic swelling test score for control group was 69.3 % ± 1.1432 [standard error of mean] while that for cases it was 55.7 % ± 1.1977 [standard error of mean]. This difference of mean between these two groups was statistically highly significant [student 't' test; $P<0.001$] (see Table I).

TABLE I: Comparison of age and different seminal parameters in controls and cases.

Parameters	*Controls N = 26*	*Cases N = 25*
Age	24±0.642	29.2±0.725
Sperm count (millions/ml)	68.9±8.092	62.3±7.153
Percentage motility	70±2.053	67.1±2.212
Percentage of sperms with normal morphology	63.8±2.603	61±1.791
Hypo-osmotic swelling test score in percentage	69.3±1.143	55.7±1.197

DISCUSSION

Even up to the recent times the semen analysis was done in the same age old fashion, and it is only from the last few years that the steps have been taken adopting advanced techniques. The role of male factors in recurrent abortion remains largely unexplored as only a few studies have been conducted on the subject (10, 11, 12). The spermatozoal factors account for about 3% of all recurrent abortions (2). It has been shown in various studies that inspite of apparently normal semen parameters, the male factor can be responsible for early pregnancy loss (10, 12). This may be a consequence of functional spermatozoal abnormalities resulting in sub standard embryo (12).

Although, minor chromosomal abnormalities do not result in gross morphological defects of the sperm (14), the functional defects in the spermatozoa may be a result of such chromosomal abnormalities causing subtle alteration in the membrane constitution. These defects can be detected by hypo-osmotic swelling test, as it is the test of functional integrity of the cell membrane.

The hypo-osmotic swelling test can detect minute alterations in the constitution of the sperm membrane. When the cell wall is functionally intact it acts as the semipermeable membrane. In the hypo-osmotic swelling test, as the fluid in the vicinity of the sperm is hypo-osmotic in relation to the intracellular fluid, there occurs entry of the hypo-osmotic solution inside the sperm membrane causing the tail of the sperm to swell. This swollen tail curls around itself showing a typical "curling." The curling is thus the sequelae to the swollen tail. Curling indicates that the sperm membrane is functionally intact. This test was first advocated by Jayendran et. al (8). And now a days is considered as one of the integral parts of the infertility protocol in most of the Advanced Reproductive Technology Centers. Hypo-osmotic swelling test is proving to be a pioneer in the armamentarium against male infertility and in future will be indelible in selecting the sperms for ICSI [Intra-cytoplasmic sperm injection] and IUI [Intrauterine insemination] (15, 16). In our study we observed a significantly low hypo-osmotic swelling test scores in the male partners of females with history of recurrent miscarriage.

Both the groups were comparable except for the age. The mean age for the control group was significantly lower, as the controls were the bachelor volunteers, but there is no association between hypo-osmotic swelling test score and age of the subject (9, 17, 18). The normal spermatozoal membrane is the prerequisite for the specialized cell-to-cell communications and cell-to-cell binding. Studies have shown that cell-to-cell binding precedes the penetration of the ovum by the sperm (19). Thus any subtle defects in the membrane will not only hamper the process of fertilization, but also will be responsible for the subsequent abnormal membrane function in the embryo. The abnormal membrane function of the embryo may result in abnormal implantation, thus predisposing to early pregnancy complications (12).

Few studies have shown that in some animals (20) and human (21) paternally derived proteins are expressed in the embryo at the preimplantation stage indicating a role of male factor in

implantation. The same minor cytogenetic abnormalities, which result in sperm membrane defects, may be responsible for abnormal expression of these paternally derived proteins.

Further research needs to be undertaken to evaluate and explain the occurrence of low hypo-osmotic swelling test score in recurrent abortions with the help of spermatozoal chromosomal and electron microscopic studies.

REFERENCES

1. Warburton D, Fraser FC. Spontaneous abortions risks in man: Data from reproductive histories collected in a medical genetics unit. *Am J Hum Genet* 1964; 16: 1.
2. Cunningham FG, MacDonald PC et al. Williams Obstretics 19th ed: 661–690.
3. Hatasaka HH. Recurrent miscarriage: Epidemiological factors, definitions and incidence. *Clinic Obst Gynecol* 1994; 37: 625–624.
4. Fernando Aries. Practical guide to high risk pregnancy and delivery 2nd ed: 53–70.
5. Hill JA, Abbott AF, Politch JA. Sperm morphology and recurrent abortion. *Fertil Steril* 1994; 64: 776–778.
6. Sbracia M, Cozza G, Grasso JA, Mastrone M, Scarpellini F. Semen parameters and sperm morphology in men in unexplained recurrent spontaneous abortion, before and during a 3 year follow-up period. *Hum Reprod* 1996; 11: 117–20.
7. Zeyneloglu-HB, Baltaci-V, Ege-S, Haberal-A, Batioglu-S. Detection of chromosomal abnormalities by fluorescent in situ hybridization in immotile viable spermatozoa dertermined by hypo-osmotic swelling test. *Hum Repord* 2000 Apr, 15(4): 853–856.
8. Jayendran RS, Van der Ven HH, Perz- Paleaz M, Crabo BG, Zaneveld LJD. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other sperm characteristics. *J Reprod Fertil* 1984; 70: 19–29.
9. Van den Saffele J, Vermeulen L, Schoonjans F, Comhaire FH. Evaluation of the hypo-osmotic swelling test in relation with advanced methods of semen analysis. *Andrologia* 1992; 24: 213–217.
10. Biljan MM, Buckett WM, Taylor CT, Luckas MJM, Aird IA, Kingsland CR, et al. Effect of abnormal hypo-osmotic swelling test on fertilization rate and pregnancy outcome in IVF cycles. *Fertil Steril* 1996; 66: 412–416.
11. Buckett WM, Luckas MJM, Aird IA, Farquharson RG, Kingsland CR, Lewis-Jones DI. The hypo-osmotic swelling test in recurrent miscarriage. *Fertil Steril* 1997; 68: 506–509.
12. Check JH, Stumpo L, Lurie D, Benfer K, Callan C. A comparative prospective study using matched samples to determine the influence of subnormal hypo-osmotic swelling test score of spermatozoa on subsequent fertilization and pregnancy rates following in vitro fertilization. *Hum Reprod* 1995; 10: 1197–1200.
13. World Health Organization. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. 2nd ed. New York: Cambridge University Press, 1992.
14. Martin RH, Rademaker AW. The relationship between chromosomal abnormalities and sperm morphology in humans. *Mutat Res* 1988; 207: 159–164.
15. Katsoff-D, Check JH. Two methods of achiving pregnancies despite subnormal hypo-osmotic swelling test score. *Fertil-Stril* 1997 Sep; 68(3): 549–551.
16. Datta S, Giri A, Datta AK. Role of hypo-osmotic swelling test in assisted reproduction. *J Indian Med Assoc* 1996; 94(12): 440–442.
17. Smith R, Madariage M, Bustos-Obregon E. Reappraisal of the hypo-osmotic swelling test in relation with advanced methods of semen analysis. *Int J Androl* 1992; 15: 5-13.
18. Avery S, Bolton VN, Mason BA. An evaluation of the hypo-osmotic swelling test as a predictor of fertilizing capacity *in vitro*. *Int J Androl* 1990; 13: 93–99.
19. Denker HW. Implantation: A cell biological paradox. *J Exp Zool* 1993; 266: 541–558.
20. Matsumoto K, Anzai M, Nakagata N, Takahashi A, Takahashi Y, Miyata K. Onset of paternal gene activation in early mouse embryos fertilized with transgenic mouse sperm. *Mol Reprod Dev* 1994; 39: 136–140.
21. Daniels R, Kinis T, Serhal P, Monk M. Expression of the myotonin protein kinase gene in preimplantation human embryos. *Hum Mol Genet* 1995; 4: 389–393.