Relationship between potency of hypo osmotic swelling test and antisperm antibody assay for fertile and infertile men in Thi-Qar governorate

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Abstract:

Background:
The sperm plasma membrane activity and absence of antisperm antibodies in infertile patients ejaculate significantly associated with fertilization potential of human spermatozoa. The sperm viability detected by hypoosmotic swelling test (HOST) and autoimmune infertility detected by antisperm antibody assay presented diagnostic and prognostic tools for male infertility factors.

Objective:
This study was devised to compare the results of hypoosmotic swelling test and antisperm antibody assay for normozoospermic men and infertile patients affected by autoimmune infertility.

Methods:
Thirty semen samples were collected by masturbation and prepared by direct layering technique. Hypo-osmotic swelling test was performed by mixing 0.1 ml of semen with 0.9 ml of a 150 mOsm/ L NaCl as a hypo-osmotic solution and direct immunobead assay were used to determine the presence of Antisperm Antibodies (ASA) bound on sperm surface. However, sperm concentration, sperm motility, and normal sperm morphology were evaluated according to World Health Organization (WHO) criteria and subjected to HOS test and ASA assay. The sperm prepared and incubated for 30 minute in 5% CO_2 at 37ºC after *in vitro* sperm preparation.

Results:
The results of the present study reported that the percentage of HOS test score in antisperm antibodies positive sperm samples (52.30 ± 5.2) was significantly lower than that noticed in antisperm antibodies negative (70.30 ± 3.4) sperm samples.

Conclusion:
It was concluded that the use of HOS test as a simple and diagnostic and prognostic tools for identify male infertility. Further studies are suggested to assess the effect of ASA on sperm plasma functional integrity after ICSI and *in vitro* fertilization and embryo transfers (IVF-ET).

Key words: Male infertility, hypo osmotic swelling test, antisperm antibody assay.

Introduction

The hypo-osmotic swelling (HOS) test originated as a laboratory index of the functional integrity of sperm plasma membrane (1). The HOS-test measures the ability of sperm plasma membrane to transport water when exposed to hypo-osmotic solutions, thus inducing cell swelling and plasma membrane stretching. If water transport does not occur, it can be assumed that the sperm membrane is functionally inactive and that it cannot be functional during the fertilization process (2). Since sperm plasma membranes may be physically intact but functionally inactive, the HOS test gives additional information on the sperm functional status (3).

In this regard the World Health Organization (WHO, 1999) has advised its use as an additional test in the routine semen analysis (4). However, the clinical usefulness of semen samples with low HOS test scores are associated with normal fertilization but low pregnancy rates during IVF techniques (5). Recently, Check's group reported that it is not clear if sperm from subjects affected by antisperm autoimmunity have some plasma membrane alteration leading to low HOS test scores or if the antisperm antibodies directly affect the sperm plasma membrane functionality (6). For that reason, the low HOS-test scores were found to be associated with lower pregnancy rates and fertilization potential of human spermatozoa (7). The men with low HOS test (<50%) rarely achieved a pregnancy with intercourse or conventional intra-uterine insemination (IUI) or even IVF-ET. (8) The defects give the impression to be related to a toxic factor attached to the sperm membrane. Some of these sperm may attach to the zona pellucida and transfer the toxic factor to the oocyte and eventually the embryo, and the defective embryo membrane may prevent proper implantation. Thus, it is not the single sperm fertilizes the egg that is problem; instead, it the supernumerary sperm attached to the zona pellucida (9).

The presence of antisperm antibodies (ASA) has been associated with decreased fertility ability *in vivo* as well as *in vitro* (10). Since approximately 15% of the male population have ASA, it would be beneficial to have a procedure capable of eluting of antibodies from the sperm and altering the sperm membrane configuration (11). One adverse effect of ASA on human spermatozoa may be inhibition of sperm progression through cervical mucus as demonstrated by a poor post-coital test by reduce percentage of sperm positive for both IgA and IgG (12). The possible mechanism was that the culture medium supplemented with human serum albumin (HSA) absorbs the antibody or antigen complex from the sperm membrane (13). The mechanism seems to be related not so much to impaired fertilization but to inhibition of implantation rates. The defect may be present in male with normal or subnormal semen specimens (14). Therefore, the compartment of males with antisperm antibodies (ASAs) has a higher frequency of low HOS-test scores. The possibility exists that ASAs may impair the functional integrity of sperm plasma membrane (15).

The Intra-uterine insemination (IUI) may be an effective therapy for sperm that have an impaired motility (16). Sometimes antibody on the sperm surface cause poor recovery of motile spermatozoa after sperm preparation technique, since agglutinated and poorly progressive spermatozoa are not recovered (17). Conversely, the reduced sperm motility may be related with an intrinsic sperm defect with the simultaneous presence of ASA (18). As a result, the presence of ASA may be etiologic; although the male ejaculate is normally devoid of complement, injury to the male ejaculatory system may have cause complement to leak into it outside (19). This study was designed to see if human sperm hypo-osmotic swelling test and plasma membrane functional integrity are adversely affected by antisperm antibodies attached to sperm surface.

2. Subjects, Materials and Methods

2.1. Subjects

Thirty infertile men 15 for normozoospermic men and 15 for infertile patients affected by autoimmune infertility were obtained from Al-Hussein Teaching Hospital/ Thi-Qar Health directorate/laboratory section. The selection of infertile patients was based on the physical examination and each infertile patient was to have semen samples including the HOS-test and immunobead assay for antisperm antibodies (ASA).

2.2. Semen preparation technique

The semen was prepared using a direct layering technique. However, 1ml of prepared IVF culture medium (Medi-Cult Company, Denmark) was added to the test tube, and then 1ml of the liquefied semen was layered beneath a culture medium. After incubation for 30 minute in 5% CO_2 at 37°C, 10µl of the mixture was aspirated by Pasture pipette and examined under light microscope at 40X magnification for assessment parameters of sperm parameters according to WHO criteria (1999).

2.3. Hypo-osmotic swelling test

The HOS test was performed after examination of standard semen parameters by mixing 0.1ml of semen with 1.9 ml of a 150 mOsm/ kg NaCl as a hypo-osmotic solution. The mixture was incubated for 30 minute at 37°C in 5% CO_2. Then, 10 µl of the mixture was placed on a slide and mounted with a cover and examined immediately at a magnification of 40X objective under a light microscope. A total of 100 spermatozoa were counted in at least ten different fields, and sperm tails were classified into seven distinct subtype of coiling in various regions. The percentage of HOS reacted spermatozoa (with coiled and swollen tail) and non-reacted spermatozoa (with straight or non swollen tails) were calculated according to WHO criteria (1999).

2.4. Antisperm antibodies assay (ASAs)

A direct immunobead assay (IBT) was performed for each semen samples. The percentage of sperm with ASA was noted. Therefore, the washed sperm were mixed with IgG or IgA beads and read microscopically for the percentage and attachment sites of sperm binding to the head. At least three beads had to be attached to be considered positive. A level of ≥ 50% was considered positive and ≥ 20% to 49% weakly positive according to WHO criteria (1999).

2.5. Statistical analysis

Statistical analysis was performed with the SPSS version 12.00 by the Statistical Package for Social Sciences software to compare difference between pairs of groups. P-value < 0.05 was used as a level of statistically significance.

3. Results

Thirty infertile subjects affected by autoimmune infertility (15 patients) with mean age 31.35 ± 0.66 years and duration of infertility 4.65 ± 0.22 years and normozoospermic men (15 patients) with mean age 34.12 ± 0.33 and duration of infertility 3.51 ± 0.11 were involved in this study. The result of the present study demonstrated that the percentage of sperm HOS test score for infertile subjects affected by autoimmune infertility (52.30 ± 5.2) significantly lower than those without antisperm antibodies (70.30 ± 3.4) after *in vitro* sperm activation and sperm processing. However, it was noticed a highly significant (P<0.001) differences in sperm parameters were assessed post *in vitro* sperm activation for both groups as compared to pre-activation. In the meantime, significant (P<0.001) and markedly reduction in sperm concentration were observed for *in vitro* post-activation.

Table (1): Standard semen parameters and hypo-osmotic swelling test for infertile patients affected with antisperm antibodies (ASAs positive).

Semen parameters	Pre-activation	Post-activation
Sperm concentration ($\times 10^6$ sperm/ml)	51.6 ± 2.1	37.2 ± 1.3 *
Sperm motility (%)	37.2 ± 7.0	45.5 ± 1.1 *
Normal sperm morphology (%)	41.5 ± 2.1	48.7 ± 0.2*
HOS-test (%)	43.1 ± 1. 2	52.30 ± 5.2 *

Values are Mean ±S.E.M
Total No. of patients=15

*: means significantly (P<0.001) difference between pre-activation and post-activation

Table (2): Standard semen parameters and hypo-osmotic swelling test for normozoospermic men (ASAs negative).

Semen parameters	Pre-activation	Post-activation
Sperm concentration ($\times 10^6$ sperm/ml)	63.1 ± 4.2	42.4 ± 8.0 *
Sperm motility (%)	54.6 ± 7.4	61.2 ± 6.3*
Normal sperm morphology (%)	47.2 ± 6.1	58.4 ± 3.6 *
HOS-test (%)	61.5 ± 1.4	70.30 ± 3.4 *

Values are Mean ±S.E.M
Total No. of patients=15
*: means significantly (P<0.001) difference between pre-activation and post-activatio

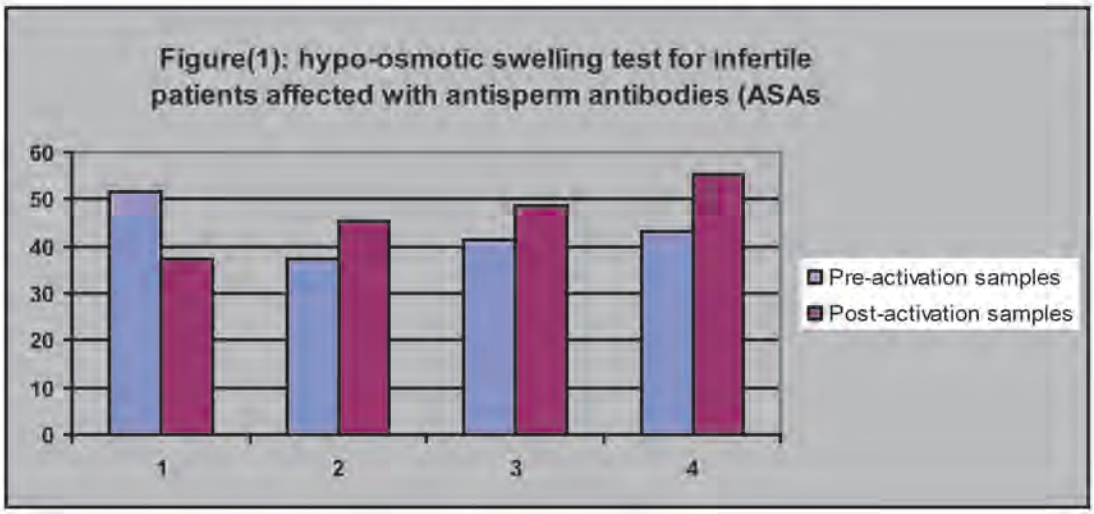

Figure (1): Standard semen parameters and hypo-osmotic swelling test for infertile patients affected with antisperm antibodies (ASAs positive).

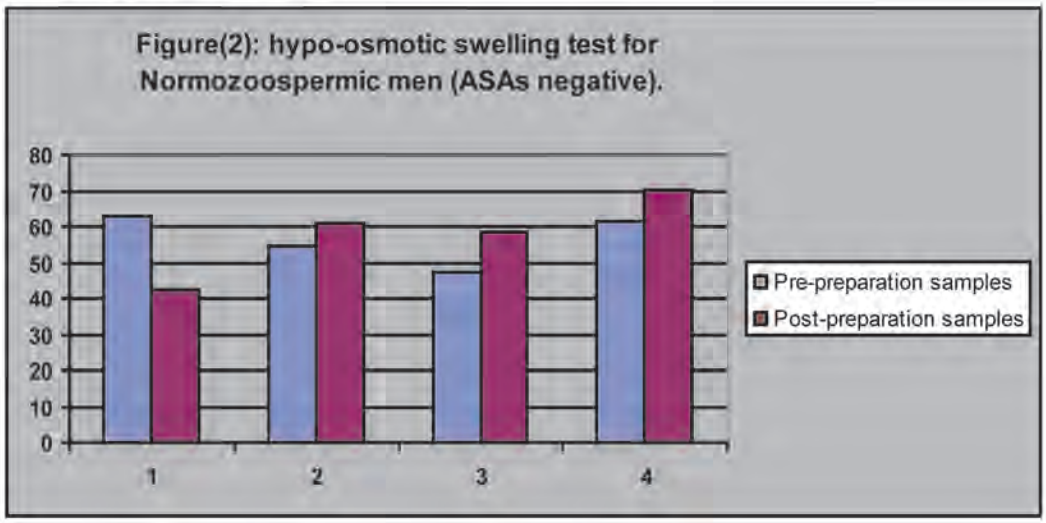

Figure (2): Standard semen parameters and hypo-osmotic swelling test for normozoospermic men (ASAs negative).

Discussion

The HOS-test is an important laboratory process during semen analysis for male infertility assessment and measures the functional integrity of sperm plasma membrane (20). The functional integrity of sperm plasma membrane is an important factor in sperm metabolism, capacitation, acrosome reaction, and the binding of spermatozoa to the egg surface. However, the sperm swelling induce when exposed to the hypo-osmotic solutions due to enter of water within sperm cytoplasm (21).

The sperm plasma membrane can be considered functionally active, thus suggesting the normal functionality of the plasma membrane of these swollen sperm. On this basis, it can be assumed that a dead sperm has a functionally inactive plasma membrane so that it does not swell when exposed to hypo- osmotic solution. In contrast, a live sperm has a physically intact plasma membrane but one that could be functionally inactive, thus not swelling when exposed to hypo-osmotic solutions (22). It is accepted that sperm samples from fertile subjects have normal HOS test scores and that those from infertile subjects with low HOS test scores show low pregnancy rates during assisted reproductive techniques (23).

The antisperm antibodies may fairly modify sperm plasma membrane integrity leading to low HOS test score (24). The present data demonstrated that sperm with ASA bound to their plasma membranes showed low HOS test scores, and this non-specific alteration of the plasma membrane permeability or fluidity may participate in determination of infertility leading to low fertilizing potential of spermatozoa. The potential clarification for low HOS test score in ASA positive sperm samples is that ASA modify water permeability. In this regard it has been demonstrated that water transport across cell plasma membranes utilizes specific water channels named aquaporins (25). Therefore, it is possible that ASA bound to sperm surface may block these water channels, thus altering

sperm water permeability. The antibody cross-linking could prevent plasma membrane distensibility, thus reducing sperm swelling when exposed to hypo-osmotic medium (26).

The role of sperm plasma membrane permeability to water in regulating important sperm functions (27). Though, when human sperm exposure to hypo- osmotic medium activates an influx of water within sperm cytoplasm. This water influx induces a sperm volume increase and plasma membrane stretching, leading to the opening of osmosensitive calcium channels, calcium influx within sperm cytoplasm and activation of acrosome reaction (28). Conversely, the osmosensitivity of sperm acrosome reaction in man and role of external osmolarity in regulation of mammalian sperm functions are well known (29).

The effects of ASA for autoimmune infertility in reducing plasma membrane water permeability and reduced HOS test score could induce also a reduction of the sperm responsiveness to the putative hypo-osmotic stimuli fundamental for sperm activation during the fertilization process, as suggested by the low osmolarity of female genital tract secretions with respect to that of semen (30). Indeed this hypothesis was demonstrated to be true since sperm with ASA bound to their surface show a marked reduction of [Ca+2] rise and acrosome reaction percentage increase induced by sperm exposure to hypo-osmotic medium, as evidenced in sperm from normozoospermic subjects without ASA (31).

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