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Determination of the relationship between percentage of hypo-osmotic swelling test scores and antisperm antibody assay in infertile patients after IUI technique

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Abstract

This study was devised to compare the results of hypo-osmotic swelling (HOS) test and antisperm antibody assay (ASA) for normozoospermic men and infertile patients affected by autoimmune infertility. Thirty semen samples were collected by masturbation and prepared by direct layering technique for IUI procedure.

Hypo-osmotic swelling test was performed by mixing 0.1 ml of semen with 1.0 ml of a 150 mOsm/ L NaCl as a hypo-osmotic solution and direct immunobead assay were used to determine the presence of ASA bound on the sperm surface. However, sperm concentration, sperm motility, progressive sperm motility, normal sperm morphology were evaluated according to standard 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.

The results reported that the percentage of HOS test score in ASA positive (55.20 ± 7.1) sperm samples was significantly lower than that noticed in ASA negative (73.32 ± 4.8) sperm samples (P<000.1). As a result, eight clinical pregnancy rates were accounts for normozoospermic men without ASA and normal HOS test after *in vitro* sperm activation and IUI procedure. Therefore, it was concluded that the use of HOS test as a simple and dependable test to identify successful pregnancy after intra-uterine insemination. Further studies are suggested to assess the effect of ASA on sperm plasma functional integrity after ICSI and IVF-ET.

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).

The low HOS-test scores were found to be associated with lower pregnancy rates and fertilization potential of human spermatozoa (3). Men with low HOS test (<50%) rarely achieved pregnancy with intercourse or conventional intra-uterine insemination (IUI) or even IVF-ET. (4) 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.

The presence of ASA has been associated with decreased fertility ability *in vivo* as well as *in vitro* (5). 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 (6). 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 (7). The possible mechanism was that the culture medium supplemented with human serum albumin (HSA) absorbs the antibody or antigen complex from the sperm membrane (8). The possibility exists that ASA may impair the functional integrity of sperm plasma membrane (9).

The Intra-uterine insemination (IUI) may be an effective therapy for sperm that have an impaired motility (10). 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 (11). Conversely, the reduced sperm motility may be related with an intrinsic sperm defect with the simultaneous presence of ASA (12). 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

form outside (13). The study was designed to see if human sperm HOS test and plasma membrane functional integrity are adversely affected by ASA attached to sperm surface.

2. Materials and Methods

2.1. Subjects

Thirty infertile patients (15 for normozoospermic men) with mean age 34.12 ± 0.33 and duration of infertility 3.51 ± 0.11 and (15 affected by autoimmune infertility) with mean age 31.35 ± 0.66 and duration of infertility 4.65 ± 0.22 were obtained from IVF Institute of Embryo Research and Infertility Treatment/Al-Nahrain University. The selection of infertile patients was based on physical examination and each patient was to have the baseline semen samples including the HOS test and immunobead assay for ASA.

2.2. Semen preparation for IUI procedure

The semen was prepared for IUI 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 37ºC, 10µl. of the mixture was aspirated by pasture pipette and examined under light microscope at 400X magnification for assessment parameters of sperm function.

2.3. Hypo-osmotic swelling (HOS) test

The HOS test was performed after examination of standard semen parameters by mixing 0.1 ml of semen with 1.0 ml of a 150 mOsm/ L NaCl as a hypo-osmotic solution. The mixture was incubated for 30 minute at 37 ºC 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.

2.4. Antisperm antibodies assay (ASA)

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. However, 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.

2.5. Timing of IUI and ovarian hyper-stimulation syndrome (OHSS)

Sonographic examination of follicular size was starting beginning 16 day from expected menses. Intrauterine insemination (IUI) was performed by threading a very thin flexible rubber catheter through the cervix and injected washed sperm into the uterus and the female were given clomiphene citrate (50 mg) two times daily for 5 days (cyclic day; 2-6 day), then recombinant FSH (Gonal-F; 75IU; Serono; Italy) for another 5 days (cyclic day; 7-11). In addition, the vaginal ultrasounographic demonstration was performed for four times (7, 9, 11 and 13 day). At least, when one ovarian follicle reaches ≥18 mm average diameters associated with a serum LH of at least 200pg/ml, Human chorionic gonadotropin (hCG; 10000IU; Serono; Italy) was injected, and later IUI was done after 36 hours and no more than 48 hours from the initiation of LH surge and 12-24 hours from the peak. The onset of LH surge was defined as a doubling of the level from the proceeding day as long as the rise continued the next day and the peak LH surge generally attained at least a fivefold rise over the baseline for the hormones.

2.6. 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.

5. Results

The result of the present study demonstrated that the percentage of sperm HOS test score for infertile subjects affected by autoimmune infertility (55.20 ± 7.1) significantly (P<0.001) lower than those without antisperm antibodies (73.32 ± 4.8) after *in vitro* sperm activation for IUI technique (Table 1, 2). However, a highly significant (P<0.001) differences in sperm functions 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. In contrast, a highly significant (P<0.001) differences in the percentage of sperm motility (%), normal sperm morphology (%), and HOS test scores were recorded. Generally, in the present study, the best results for clinical pregnancy rates were observed for infertile patients without antisperm antibodies (8 ongoing pregnancies) after IUI techniques. It was advisable, clinical pregnancies were achieved for infertile couples where their male have normal percentages of the sperm HOS test scores as compared to male partners

have abnormal HOS test scores with antisperm antibodies, who have no clinical pregnancy rates were achieved in the present study.

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 score (%)	43.1 ± 1. 2	55.20 ± 7.1 *

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	73.32 ± 4.8 *

Values are Mean ±S.E.M

Total No. of patients=15

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

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 (14). The functional integrity of sperm plasma membrane is an important factor in sperm metabolism, capacitation, acrosome reaction, and binding of spermatozoa to the egg surface. The sperm swelling is induced when exposed to hypo-osmotic solutions due to entrance of water within sperm cytoplasm (15).

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 (16). 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 (17).

The antisperm antibodies may fairly modify sperm plasma membrane integrity leading to low HOS test score (18). The present data demonstrate that sperm with ASA bound to their plasma membranes show low HOS test scores, and this non-specific alteration of the plasma membrane permeability or fluidity may participate in the determination of infertility due to ASA leading to low fertilizing potential of human 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 (19). Therefore, it is possible that ASA bound to sperm surface may nonspecifically or specifically 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 (20).

Sperm plasma membrane permeability to water has role in regulating important sperm functions (21). Though, when human sperm exposed 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 (22). The osmosensitivity of sperm acrosome reaction in man and role of external osmolarity in regulation of mammalian sperm functions are well known (23). 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 (24). 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 (25).

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تحديد مستوى العلاقة بين فحص كفاءة انتفاخ الغشاء البلازمي للنطفة البشرية تحت الضغط الازموزي الواطىء مع فحص الاجسام المناعية المضادة للنطف بعد اجراء عملية التلقيح الاصطناعي داخل الرحم لمرضى العقم

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الخلاصة

تهدف هذه الدراسة الى مقارنة نتائج فحص كفاءة انتفاخ الغشاء البلازمي للنطفة البشرية تحت الضغط الازموزي الواطىء مع فحص الاجسام المناعية المضادة للنطف للمرضى المصابين وغير المصابين بعقم المناعة الذاتية للرجال. تم اختيار المرضى وعددهم ثلاثون مريضاً وفق فحوصات السائل المنوي وموزعون بواقع مجموعتين بطريقة التقنية الطباقية المباشرة لغرض التلقيح الاصطناعي داخل الرحم.

أن فحص انتفاخ الغشاء البلازمي للنطفة تم تحضيره بمزج (0.1ml) من السائل المنوي مع من (1.0ml) من محلول الاختبار كذلك تم اعتماد اختبار ارتباط الاجسام المناعية المباشر بسطح الحيمن لتحديد مدى تواجد هذه الاجسام من خلال تقنية الوميض المناعي على راس النطفة البشرية. تم اعتماد اختبارات كفاءة النطف كتركيز النطف, وحركة النطف, والحركة التقدمية للنطف, والنسبة المئوية للنطف السوية حسب مقررات منظمة الصحة العالمية مع اجراء فحص كفاءة انتفاخ الغشاء البلازمي و فحص الاجسام المناعية المضادة للنطف. تم تحضيره في ظروف قياسية (5% CO_2 at 37ºC) بعد اجراء عملية التنشيط. أظهرت نتائج السائل المنوي ان نسبة فحص كفاءة انتفاخ الغشاء البلازمي للنطفة يكون واطىء مع وجود فرقاً معنوياً عالياً للمرضى المصابين بوجود الاجسام المضادة للنطف مقارنة بالمرضى غير المصابين. تم الحصول على ثمان حالات حمل اثناء اجراء عملية التلقيح الاصطناعي داخل الرحم من المرضى الذين ليس لديهم اجسام مناعية مضادة للنطف في عينة السائل المنوي العائدة لهم ونسبة فحص كفاءة انتفاخ الغشاء البلازمي للنطفة كانت عالية. نستنتج من خلال هذه الدراسة ان فحص كفاءة انتفاخ الغشاء البلازمي للنطفة البشرية هو فحص بسيط ومعتمد لغرض تحديد نسبة نجاح الحمل بعد اجراء عملية التلقيح الاصطناعي. هنالك توصية باجراء دراسة لمعرفة مدى ثاثير الاجسام المناعية على الغشاء البلازمي للنطفة بعد اجراء عملية الاخصاب الخارجي وعملية حقن الحيمن في سايتوبلازم خلية البيضة.