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The New Approach of Hyaluronic Binding Assay in Relevance to Sperm Activation by Direct Swim-Up Technique in Infertile Men with Asthenozoospermia

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Abstract

Background:-.

Asthenozoospermia is one of the main causes of infertility or diminished fertility in men , Hyaluronic acid (HA) is a complex bioactive agent , which is fully represented in the female reproductive tract. The oocyte of human being is enclosed by (HA), which acts as a physical selector of spermatozoa. Human sperm cells that Assay (HBA Score%) is an important diagnostic tool for suspicious male infertility in the analysis of semen. In a matter of minutes , provides the mature binding spermatozoa in the sample .Direct swim-up technique is the oldest and most frequently used activation technique.

To assess the sperm parameters and the percentage of binding spermatozoa to HA pre- and post-*in vitro* sperm activation for asthenozoospermic men.

Twenty –two asthenozoospermia men were collaborate in this study. Assessmet of sperm parameters and HBA Score% pre- and post - direct swim up technique and the results were statistically analyzed.

Significant increment (P-value<0.05) was noticed within progressive motility, morphology of spermatozoa and a significant improvement in (HBA- Score) post - activation.

Keywords:

Asthenozoospermia , hyaluronic acid, bound sperm , hyaluronic binding assay –score , and direct swim-up technique .

Medical College , Thi-Qar University, Al-Nasiriyah city , Iraq. High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq. apparent (HA) receptors and bind to hyaluronan possess normal shape, least DNA fragmentation and low frequency of chromosomal aneuploidies. The Sperm- Hyaluronic Binding

1.Introduction: -

Infertility is consider as a complex disorder and a unique medical event because it includes a couple, rather than a single individual with considerable medical, psychosocial, and economic problems [(1)]. There are many factors that influence male fertility like genetic disorder, germ cell malignancies, ejaculation disorders , varicocele , obesity, physical and mental stress, malnutrition, smoking, drugs, cryptorchidism, autoimmunity disease , and sexually transmitted diseases [(2)]. Routine semen analysis is still the basis in the laboratory workup of the infertile male , likewise the microscopically noticeable properties of the spermatozoa such as concentration ,motility, and morphology are important[(3)].

Asthenozoospermia, is defined as 'total motility' (progressive + non-progressive) less than 40% or progressive motility less than 32% (WHO, 2010)[(4)]. Motility of spermatozoa is a definitive indicator for the quality of semen sample and fertility potency because it is wanted for the permeation of cervical mucus, transport during the female genital tract and penetration the corona radiate with zona pellucid prior to fertilization of the oocyte[(5)].

Hyaluroninc acid (HA) or hyaluronan was revealed by Karl Meyer in the 1930s, as polymer of disaccharides consists of D-glucuronic acid and D-N-acetylglucosamine, attached via B-1,4 and B-1,3 glycosidic bonds[(6)].

Remolding of sperm plasma membrane at spermiogenesis, along with the formation of the zona pellucida receptors, receptors for HA are also formed . The common origin of the zona pellucida and HA receptors on sperm suggest that sperm will bind to HA as well bind to the zona pellucida. [(7)]. The formation of zona binding sites and HA binding sites occur during the late stages of spermatogenesis naturally accompanying with cytoplasmic extrusion and nuclear histone-protamine replacement. Thus, only mature sperm can bind to HA[(8)], human sperm exhibited a substantially increased tail cross-beat frequency [(9,10).]

The basic introduction of any sperm preparation technique is to eject any factors harmful to fertilization, block factors that cause uterine contractions such as prostaglandins, this achieved by speedy separation and effective separation of the seminal plasma from spermatozoa [(11)]. Sperm selection techniques which utilized for assisted reproductive technologies (ART's) take place in one of the following categories: Sperm migration, filtration, density gradient centrifugation or a combination of these methods[(12)].The simplest *in vitro* sperm activation procedure to get a highly motile sperm population that is free of poorly motile ,immotile ,and dead spermatozoa ,round cells, and other contaminations is a direct swim-up technique[(4)].

.Patients, Materials, and Methods :-

A cross-sectional analytic study done in prospective pattern conducted in High Institute of Infertility Diagnosis and Assisted Reproductive Technology / AL-Nahrian University during the period from January-2019 to May- 2019. Twenty-two infertile men (asthenozoospermic men were progressive motility < 32% and < 27%) have been enrolled in

2 this study.

2.1. Semen Analysis

Seminal fluid sample was collected directly into a clean, dry and sterile disposable at three to five days post sexual abstinence. Petri-dish by masturbation in a private and quiet room adjacent to the semen analysis

laboratory. The container must be labeled with name , age, abstinence period and time of sample collection. The specimens were placed in an incubator at 37 ºC for 30-60 minutes to allow liquefaction. The liquefied semen is then carefully mixed for few seconds, and then the specimen was examined by macroscopic and microscopic examination.

2.2. Hyaluronic Binding Assay (HBA) Test

The HBA –Slide has two corresponding hyaluronan- coated assay chamber with two CELL-VU gridded cover slip. This diagnostic test was achieved at room temperature. A fixed volume of liquefied semen (7-10µL) is delivered onto HBA Slide and covered with 0.1 ×0.1 mm cover slip**,** taking care to avoid air bubble formation. The cover slip provides a grid of 100 squares, within a viewing circle. Incubate the slide for at least 10 minutes and not more than 20 minutes .Bound motile sperm will cease progressive movement but retain active tail beating .Dead and non-motile sperm show no tail movement . Non-binding motile sperm swim about freely. The numbers of motile bound sperm and unbound sperm in the same number of grid squares will be counting. The percentage of sperm binding to the hyaluronan layer is calculated as follows:

$$\text{Bound Sperms (\%)} = \frac{\text{Bound Motile Sperms}}{\text{Bound + Unbound Motile Sperms}} \times 100$$

Hyaluronan binding refer to normal maturity and physiological function of sperm cell in a semen sample. The HBA-Score of equal or more than 65% was used as the key cutoff [13] .

2.3. Direct Swim- up Technique

This technique was performed by adding (1ml) of liquefied semen to the falcon tube containing (1mL) of FertiCult Flushing medium (semen layered beneath a culture medium), then incubate at 37degree for (30-60) minutes in air incubator. The next step is the collection of the supernatant , which contain the progressive motile spermatozoa, that's migrate from the semen layer into the culture medium. Evaluation of the sperm parameters was achieved by a drop of 10µL was aspirated, put on a slide with a cover slip and examined under the microscope at 400X objective [14].

The study was approved by the Ethical Approval Committee.

Statistical analysis was carried out by using SPSS (statistical package for social sciences) version 20. For analysis, Sperm parameters, and HBA-Score were analyzed using independent sample t-test. The P-value was considered significant ($P \leq 0.05$).

3.Results: -

Sperm parameters and sperm-hyaluronic binding assay for asthenozoospermic men pre-activation and post- *in vitro* sperm activation as showed in table (1). The infertile couples with primary infertility were (36%), while with secondary infertility group were(64 %). The largest group of men within age group (35-39) years , account (32%). According to the duration of infertility, the largest group was with duration of infertility (6-9) years were (45%) .

Pre-activation sperm parameters include the mean of sperm concentration was (41.454±1.052), progressive sperm motility was (29.772±0.173), and the mean of morphologically normal sperm was (34.727±0.379).

Post- *in vitro* sperm activation technique , parameters include the mean of sperm concentration (19.818±0.820), progressive sperm motility was (76.636±1.676), and the mean of the morphologically normal sperm was (52.636±1.161). In regarded to the mean of sperm agglutination was (0.000±0.000) and round cells count (0.090±0.090).

The outcome of sperm parameters in the post- activation exhibit a significant decrease ($P < 0.05$) for the non-progressive sperm motility and immotile sperm, while progressive sperm motility and morphologically normal sperm were observed a significant increase ($P<0.05$) result. In regard to the results of sperm agglutination and round cells count , significant decrease ($P < 0.05$).

The mean and standard error of HBA-Score pre-activation and post-*in vitro* sperm activation technique were (55.590±1.080 , and 76.681±0.645; respectively). So that

Sperm parameters		Pre-swim up	Post-swim up	*P*-value
Sperm concentration (millions/mL)		**41.454 ±1.052**	**19.818 ±0.820**	**0.063**
Sperm motility (%)		**59.636 ±2.668**	**86.363 ±1.839**	**0.014**
Sperm grade activity (%)	**Progressive sperm motility (%)**	**29.772 ±0.173**	**76.636 ±1.676**	**0.0001**
	Non- progressive motility (%)	**29.863 ±2.720**	**9.727 ±1.723**	**0.003**
	Immotile sperm (%)	**40.363 ±2.668**	**13.318 ±1.797**	**0.011**
***Normal sperm morphology (%)**		**34.727 ±0.379**	**52.636 ±1.161**	**0.004**
Sperm agglutination (%)		**5.863 ±1.145**	**0.000 ±0.000**	**0.0001**
Round cells count (HPF)		**3.272 ±0.614**	**0.090 ±0.090**	**0.0001**
HBA-Score		**55.590 ±1.080**	**76.681 ±0.645**	**0.025**

significantly increase were (p- value=0.025).

Table -1: Sperm parameters and HBA-Score for asthenozoospermic men pre-and post-*in vitro* sperm activation, according to WHO (2010), and WHO (1999).

- *WHO (1999)
- Data are Mean ± SEM.
- SEM= Standard error of mean.
- Number of men= 22.

4.Discussion: -

Infertility is a complicated and stressful state for both the patients and the treating physicians, male infertility continues to be a clinical challenge of increasing importance. Fertility of male is impaired by fewer motile spermatozoa coming into proximity with the oocyte and/or by lack of spermatozoa function, such sperm-zona interaction, acrosome reactivity or egg penetration [15].

The most important factor to solve the infertility problems through the precision of diagnosis [16] . The initial and most essential step to evaluate the male infertility is the seminal fluid analysis [17]. The necessary functional characteristic of ejaculated human spermatozoa that governs their capability to penetrate into, and migrate through cervical mucus , cumulus-corona complex and zona pellucida is the progressive motility[6].

The typical sperm preparation technique is to obtain the largest number of morphologically normal, motile spermatozoa in a small volume of physiological culture media free from seminal plasma, leukocytes and bacteria [18].

In this study, direct swim up technique was applied for *in vitro* sperm activation ,it is more popular than other techniques in this Institute and country, does not require particular expertise and less expensive [19].

The actual sperm selection techniques, like density gradient centrifugation and swim-up, depend on sedimentation or migration of the sperm and on the embryologist's morphological assessment. Apoptosis, DNA integrity, membrane maturation and ultrastructure are important sperm characteristics , but they are not targeted by these techniques. The methods inspected are based on surface charge (electrophoresis and zeta potential), ultramorphology (high magnification), apoptosis (magnetic cell sorting and glass wool), or membrane maturity (hyaluronic acid binding) [20].

The major component of the cumulus matrix after LH surge is hyaluronic acid ; therefore, HA, and the capacity to bind to it, plays an important function in the physical selection of the spermatozoon that will fertilize the oocyte. The membrane of mature spermatozoon which show the HA receptors reflects normal spermatogenesis and various upstream maturational events that affect DNA chain integrity [21].

The concept of sperm –hyaluronic binding assay is based on that a low level of sperm binding to hyaluronan demonstrates a low ratio of mature sperm in the semen sample and subsequently predicts infertility. Hyaluronan -binding sperm, in contrast, are respective in the interaction with the oocyte complex and likewise associated with high genomic integrity [22], which improves the quality of the paternal contribution to the zygote. Consequently, hyaluronan-binding distinguish high and low functional

integrity and fertilizing potency .Since HA is a physiological component of the cervix, cumulus cells and follicular fluid, it should pose no additional safety risks when used for sperm selection [23].

5.Conclusions:-

The mean binding of spermatozoa to hyaluronan pre-activation (direct swim-up technique) was less than the normal limit for asthenozoospermic men. However, it significantly improves post-activation.

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Application of HBA Slide which was used for the first time in the High Institute for Infertility Diagnosis and ART's/Al-Nahrain University

References: -

1- Cousineau TM and Domar AD. Psychological impact of infertility. Best Pract Res Clin Obstet Gynaecol. 2007; 21(2):293-308
2- Irfan M, Shabbir A, Raja GK, Kiyani AR, and Ismail M. Sperm disorders and aetiologies of male infertility in Pakistan: Meta-analysis and review. Austin Journal of Reproductive Medicine and Fertility.2015; 2(6):1034.
3- Douglas T. Carrell and Kenneth I. Aston (eds.),Spermatogensis:Methods and Protocols, Methods in Molecular Biology .2013;vol.927:121
4- WHO (2010) Laboratory manual for the examination and processing of human semen. World Health Organization , Department of Reproductive Health and Research, Geneva. 5th edition .
5- Hadwan MH, Almashhedy LA and Alsalman AS. The key role of zinc in enhancement of total antioxidant levels in spermatozoa of patients with asthenozoospermia. Am J Mol Cell Bio. 2013; 1: 52-61.
6- Carrell DT, Aston KI, Spermatogensis: Methods and Protocols, Methods in Molecular Biology. Humana Press, 1st edition, 2013;264. Doi: http://dx.doi.org/10.1007/978-1-62703-038-0 [Springer]
7- Huszar G, Ozenci CC, Cayli S, Zavaczki Z, Hansch E, and Vigue L. Hyaluronic acid binding by human sperm indicates cellular maturity, viability and unreacted acrosomal status. Fertil Steril,2003; 79(Suppl 3): 1616–24.
8- Park CY , Uhm SJ, Song SJ, Kim KS, Hong SB, Chung KS, Park C, and Lee HT . Increase of ICSI efficiency with hyaluronic acid binding sperm for low aneuploidy frequency in pig .Theriogenology,2005; 64: 1158-69
9- -Huszar G, Willetts M, and Corrales M. Hyaluronic acid(sperm select) improves retention of sperm motility and velocity in normospermic and oligospermic specimens. Fertil Steril 1990; 54: 1127–34.
10- Sbracia M, Grasso J, Sayme N, Stronk J, and Huszar G. cryop reserved/thawed human spermatozoa Hum Reprod 1997; 12: 1949–54.
11- Karamahmutoglu H, Erdem A, Erdem M, Mutlu M, Bozkurt N, Oktem M, Ercan DD, and Gumuslu S. The gradient technique improves success rates in intrauterine insemination cycles of unexplained subfertile couples when compared to swim up technique; a prospective randomized study. J Assist Reprod Genet.2014; 31(9): 1139–45.
12- Avendano C and Oehninger S. DNA fragmentation in morphologically normal spermatozoa: How much should we be concerned in the ICSI era? J Androl. 2011; 32(4):356-63.
13- Worrilow, K.C. Eid S, Woodhouse D, Witmyer J, Khoury C, and Liebermann J.Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI); significant improvement in clinical outcomes-multicenter, double blinded and randomized trial, Hum.Repro.2012;28(2):306-14.
14- Soderlund B, and Lundin K. The use of silane-coated silica particles for density gradient centrifugation in in-vitro fertilization. Hum. Reprod. 2000;15 (4): 857-60.
15- Liu DY and Baker HW. Defective sperm - zona pellucida interaction: a major cause of failure of fertilization in clinical *in vitro* fertilization. Hum Reprod. 2000; 15:702-08.
16- De La Rochebrochard E, De Mouzon Thepot F, *et al*. Fathers over 40 and increased failure to conceive: the lessons of *in vitro* fertilization in France. Fertil Steril. 2006; 85(5):1420-4.
17- Male Infertility Best Practice Policy Committee of American Urological Association, Practice Committee of the American Society for Reproductive Medicine. Report on optimal evaluation of the infertile male. Fertil Steril. 2004; 82 (1):123–30.

18- Van Voorhis BJ. Outcomes from assisted reproductive technology. Obstet Gynecol. 2006,107(1):183-200.
19- Kadhim A.A., Mossa A.L., and Abbood M.S. A comparison of new sperm preparation technique by glass wool filtration combined with pentoxifylline with other techniques in asthenozoospermic men. International Journal of Advanced Research, 2017 ,5(4):1178–82.
20- Fruchter R. Shalev E., and Weiss A. Clinical benefit using sperm hyaluronic acid binding technique in ICSI cycles: a systematic review and meta-analysis; 2016, vol.32.Issue 3 :286-98 Published :December 23,2015 DOI: https ://doi.org/10.1016/j.rbmo.2015.12.001
21- -Prinosilova P. Kruger T. Sati L. Ozkavukcu S. Vigue L. Kovanci E.and Huszar G.: Selectivity of hyaluronic acid binding for spermatozoa with normal Tygerberg strict morphology. *Reprod. Biomed. Online.* 2009; 18: 177-83
22- Yagci,A,W. Mark.J.Stronk and G.Huszar.Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity :an acridine orange fluorescence study.J. Androl. 2010; 31(6):566-72.
23- David K, Ariel W, Colin M, and Zeev S.: Textbook of Asisted Reproductive Techniques; 5th edition vol. 1: Labrotory Perspectives, 2018: 119

النهج الجديد لفحص ارتباط الهيالورونيك فيما يتعلق بتنشيط الحيوانات المنوية من خلال تقنية السباحة المباشرة للرجال العقيمين (وهن الحيوانات المنوية)

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الخلاصة:

يُعد وهن الحيوانات المنوية أحد الأسباب الرئيسية للعقم أو انخفاض الخصوبة عند الرجال.
حمض الهيالورونيك هو عامل نشط حيويًا معقدًا، والذي يتم تمثيله بالكامل في الجهاز التناسلي الأنثوي. يحيط حمض الهيالورونيك بالبويضات البشرية، والذي يعمل كمحدد فيزيائي للحيوانات المنوية. تمتلك خلايا الحيوانات المنوية البشرية التي تظهر مستقبلات حمض الهيالورونيك وترتبط به شكلًا طبيعيًا، وأقل تفتتًا للحمض النووي وتكرارًا منخفضًا لاختلال الصيغة الصبغية.
يعتبر فحص الحيوانات المنوية - الهيالورونيك: أداة تشخيصية مهمة لعقم الذكور في تحليل السائل المنوي في غضون دقائق . يحدد سلايد - الهيالورونيك نسبة الحيوانات المنوية الناضجة المرتبطة في العينة.
تقنية السباحة المباشرة هي أقدم التقنيات وأكثرها استخدامًا.
أجريت هذه الدراسة لتقييم المعايير الوظيفية للحيوانات المنوية ونسبة ارتباطها بحمض الهيالورونيك قبل وبعد التنشيط.
اشترك في هذه الدراسة اثنان و عشرون رجلاً مصابين بالعقم يعانون من وهن الحيوانات المنوية خلال مراجعتهم الى عيادة العقم في المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب-جامعة النهرين
جمعت عينات المني وتم تنشيطه باستخدام تقنية السباحة المباشرة وتقييم المعايير الوظيفية للحيوانات المنوية مع تقييم نسبة ارتباطها بحمض الهيالورونيك قبل وبعد التنشيط و تم تحليل النتائج احصائيا.
لوحظت زيادة ذات دلالة معنوية (P-value<0.05) في الحركة التقدمية والحيوانات المنوية ذات الشكل الطبيعي وكذلك في نسبة ارتباطها مع حمض الهيالورونيك بعد التنشيط.