IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Ali, et al, Vol. 9 , Issue 1 , Pp. 15-31, (2019) Original Research
DOI: http://doi.org/10.28969/IJEIR.v9.i1.r2 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Received: 26-May-2019 Accepted: 01-Nov-2019 Published: 14-Nov-2019

The New Approach of Hyaluronic Binding Assay in Relevance to Sperm Activation by Direct Swim-up Technique in Iraqi Infertile Men

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Abstract

Hyaluronic acid (HA) is a polysaccharide, that composed in the extracellular matrix of the cumulus cells, and considered to take function in choosing mature spermatozoa. The Sperm- hyaluronic binding assay (HBA) is significant diagnostic instrument for suspected male infertility when examining the semen. The HBA slide supplies the ratio of mature binding sperm in the specimen. To evaluate sperm parameters, and to evaluate the (HBA Score percentage (%) before and after activation. Twenty-five infertile couples a sharer in this current study through their attendance to the Al Nahrain University. Males undergoing seminal fluid analysis were done according to (WHO 2010, and WHO 1999). Sperm parameters assessed and calculate the HBA Score% pre-and-post- direct swim-up technique, the results were statistically tested. In Post- activation there was considerable increment noticed in the progressive motility and morphology of spermatozoa, likewise significant improvement in the (HBA score %). Furthermore, considerable reduction in the concentration, agglutination of spermatozoa and round cells count, when compared to pre-activation. The mean of binding of spermatozoa to hyaluronan pre-activation (direct swim-up technique) was less than normal limit for normozoospermic males. However, it significantly improves post-activation.

Keywords: Hyaluronic Acid, Bound Sperm, direct swim-up technique, normozoospermia.

1. Introduction

A common definition of infertility is the disability of the couple to conceive after more than twelve months of exposure to pregnancy (Bhattacharya, et al. [1]). The factors that affect male fertility are many such as genetic disorders, physical, and mental stress, obesity, malnutrition, smoking, drugs, sexually transmitted diseases, accessory gland infections, germ cell malignancies, ejaculation disorders, and varicocele (Irfan, et al. [2]). Accuracy of diagnosis is the most important factor to solve the infertility problem (De La Rochebrochard, et al. [3]). So that, routine semen analysis has two main features: the overall number of spermatozoon which refers the sperm output through the testes and the patency of the post-testicular duct system and overall volume of fluid formed via accessory glands which reflect the secretory action of these glands (WHO [4]). Seminal fluid analysis is not the only fertility indicator and there are other factors that play roles in spite of normality of seminal fluid (Jarow, et al. [5]). Curative option in many conditions for males and females' infertility is Assisted Reproductive Technologies (ART's) (McLachlan and Cook [6]). The preparation techniques of spermatozoa considered as pivotal constituent of the ART's (Mahfouz, et al. [7]). The widely employed for sperm cell selection in ART's include; Migration, Filtration, Density gradient Centrifugation or Combination of these techniques (Avendano and Oehninger [8]). World Health Organization (WHO) (WHO 2010 guidelines) registered the lowest reference border to semen analysis. Volume = 1.5 mL; concentration = 15 million spermatozoa/mL; total number, (39 million spermatozoa per ejaculate),

morphology (4% normal forms); vitality (58% live), progressive motility (32%), (progressive + non progressive motility), 40% (Cooper, et al. [9]). Spermatozoa of human beings are unable to in-vivo fertilization while bathed in the seminal plasma of the ejaculate. Once removed from seminal plasma, sperm should be bear to maturational changes through which they obtain the capability to fertilize the mature oocyte. This procedure, define capacitation. The active migration selects for spermatozoa that are progressively motile, of more normal morphology, facilitates initiation of capacitation, and quite potentially, contributes to the identification of cohort of spermatozoa that are candidates for fertilization (Gardner and Carlos [10]). The most generally applied ways for separation of sperm cells from seminal plasma are density gradient centrifugation (DGC) and Swim-up techniques (Hamza, et al. [11]). The swim-up technique appears to be the most common and cheapest method for selecting of the viable sperm for most reproductive techniques. Meanwhile, it is still the ideal process for males with normal semen parameters (Kadhim, et al. [12]). It is depending on self-migration of motile sperm (Smith, et al. [13]). Several variations of the swim-up procedure are possible. The seminal plasma can be overlaid directly with culture medium and the sperm allowed to swim from the seminal plasma into the culture medium. On the other hand, the semen sample may be diluted and centrifuged or the semen sample may be centrifuged without prior dilution of the seminal plasma and overlaid with the medium for the swim-up procedure (Gardner, et al. [14]). Hyaluronan was detected via 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 (Carrell and Aston [15]). Hyaluronic acid is the main component of the cumulus oophorus; it plays a part in the natural choice of mature spermetozoa through in-vivo fertilization (Gardner, et al. [16]). The plasma membrane of the spermatozoa remolding in spermiogenesis, with the formulation of the zone pellucida receptors, also receptors for HA will be formed. These receptors refer to the linking of sperm cells to the HA and to the zone pellucida. Then, this idea was conceived that choosing of spermatozoa that display zona pellucida linking through hyaluronan mediated sperm cell selection (Huszar, et al. [17]). Formulation of zona pellucida binding situation and the hyaluronan binding situations happen via the last stages of spermatogenesis associated with cytoplasmic extrusion and nuclear histone-protamine alteration. Subsequently, only mature sperm able to join the hyaluronan (Park, et al. [18]). Recognizing the sperm HA interaction by supervising that in the existence of the HA, spermatozoa of the human being showed increment in tail cross-beat frequency (Huszar, et al. [19] , Sbracia, et al. [20]). The incapability of sperm cells to link to hyaluronan involves many aspects of immaturity: they keep cytoplasm [21](Huszar, et al. [17]), they display higher frequency of irregular morphology (Prinosilova, et al. [21]) and they have lower genomic safety than hyaluronan binders (Yagci, et al. [22]). Sperm motility is stimulated upon binding hyaluronan (Hamamah, et al. [23], Lin, et al. [24]).

2. Materials and Methods

A prospective study conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, AL-Nahrain University through the period from December, 2017 to April, 2019.

2.1 Patients

25 normozoospermic males involved in this study (semen parameters are normal). The collection of semen samples and semen analysis was done according to (WHO 2010), and assessment of the binding of the spermatozoa to the hyaluronic acid. Then the sample underwent to direct swim-up technique, also they were subjected to a history of age, kind of infertility and infertility duration.

2.2 Semen Analysis

The sample of the seminal fluid was accumulating post 3-5 days of sexual abstinence into neat, dry and antiseptic disposable Petri-dish by masturbation in a special and quiet room close to the semen analysis lab. The container should be categorized with the following notification, name, age, abstinence period and time of sample accumulation. The specimens were put in an incubator at 37 ºC for 30-60 minutes to allow liquefaction. The liquefied semen is then closely blended for a few seconds, and then the sample was examined via macroscopic and microscopic examination.

2.3 Hyaluronic Binding Assay (HBA% Score)

is a diagnostic test and is considered as a component of the basic analysis of semen for the testing of male infertility and constituents of analyses of either raw or processed semen for limiting the suitable course of IVF therapy of infertility. The HBA-Slide has two identical hyaluronan-coated assay chamber

with two CELL-VU gridded coverslip. This test was carried out at room temperature. Constant volume of liquefied semen (7-10 µL) is throw down onto HBA Slide and covered with 0.1 × 0.1 mm coverslip, taking care to avoid air bubble formation. The coverslip supplies a grid of one hundred squares, within a viewing circle, placement of the coverslip should have done with no delay as uneven distribution of binding may occur, affecting results. the slide will be incubated for at least 10 minutes and not more than 20 minutes. In 10 minutes all the sperm will link to the hyaluronan layer. Post 20 minutes' weak sperm may begin to miscarry motility. Bound motile sperm will stop progressive movement but keep quick tail beating. Dead and non-motile sperm show no tail movement. Non-binding motile sperm swim around clearly. Count the numbers of motile bound and unbound sperm in the same number of grid squares. The percentage of sperm linking to the hyaluronan layer is studied as follows

$$\textbf{\textit{Bound Sperms}}\ (\%) = \frac{\textbf{Bound Motile Sperms}}{\textbf{Bound} + \textbf{Unbound Motile Sperms}} \boldsymbol{x100}$$

Hyaluronan binding indicates normal maturity and physiological function of sperm cells in the sample. The HBA Score of equal to or more than 65% was used as the key cutoff (Worrilow, et al. [25]).

2.4 Direct Swim-up Technique

is the processing of spermatozoa taking place in a test tube, culture dish, or elsewhere outside a living organism, used for semen samples with an average motility. This procedure was completed via adding (1 ml) of liquefied semen to the test tube have (1 ml) of media was called FertiCult flushing (semen layered down a flushing medium), then incubate at 37 degrees for (30-60)

minutes. Evaluate of the parameters of spermatozoa was done by a drop of 10 µL was suction from the test tube, put on the slide with a coverslip and tested under the microscope (400X) (Soderlund and Lundin [26]). Statistical analysis was done using SPSS (statistical package for social sciences) version 20. For analysis, basic characteristics and hormones profile were analyzed using an independent sample t-test. Chi-square test was used to reveal the significant comparison among percentages of the fertilization rate, and embryo grading in this study.

3. Results

The current study illustrated that the percentages of infertile couples attended this institute, according to the type of infertility. The infertile couples with primary infertility were (68%), while infertile couples with secondary infertility were (32%). Mean age groups for men involved in this study (30.160±1.344) with domain from 20 for 39 years and the mean duration of infertility (4.680 ±0.531) with domain from 2 for 13 years. Sperm parameters for a normozoospermic group of males pre-processing and post-processing according to criteria WHO (2010). Pre-activation parameters included the mean of sperm concentration was (53.840±2.327), progressive sperm motility (%) was (46.400±1.874), non-progressive sperm motility (%) was (20.480±1.945), immotile sperm (%) was (33.120±1.902) and the mean of morphologically normal sperm was (41.600±1.539). Sperm parameters after sperm activation technique; the mean of sperm concentration (26.480±1.699), progressive sperm motility (%) was (84.720±1.745), non-progressive sperm motility was (3.440±0.988), immotile sperm (%) (10.560±0.868) and mean of the morphologically

Table (1): Sperm parameters and HBA –Score for normozoospermic males before and after processing

Sperm parameters		Before swim-up	After swim-up	P-value
Sperm concentration (millions/mL)		53.840 ±2.327	26.480 ±1.699	0.030
Sperm motility (%)		66.880 ±1.902	88.160 ±1.178	0.106
Sperm Activity Grade (%)	**Progressive sperm motility (%)**	46.400 ±1.874	84.720 ±1.745	0.027
	Non- progressive motility (%)	20.480 ±1.945	3.440 ±0.988	0.0001
	Immotile sperm (%)	33.120 ±1.902	10.560 ±0.898	0.013
Morphologically Normal Sperm (%)		41.600 ±1.539	63.040 ±2.226	0.031
Sperm agglutination (%)		6.200 ±0.969	0.000 ±0.000	0.0001
Round cells count (cells/hpf/HPF)		4.560 ±0.802	0.600 ±0.216	0.0001
HBA-Score		61.120 ±1.894	83.320 ±1.022	0.030

- Data are Mean ± SEM.
- SEM= Standard error of the mean.
- Number of males= 25.

normal sperm (63.040± 2.226). The outcome of sperm parameters after activation technique for normozoospermic males was observed and display significant decrement for the concentration of spermatozoa, non- progressive sperm movement, immotile sperm when using the direct swim-up technique, while progressive movement (%) and morphologically normal sperm were observed significant increase (P-value<0.05). In regard to results of agglutination and round cell count, decrement (P-value < 0.05) was observed post-processing. The mean and standard error of hyaluronic binding assay score (HBA) before and after processing were (61.120±1.894, and 83.320±1.022; respectively) so that significantly increase was observed (Table 1).

4. Discussion

Semen analysis is the first and most essential step of the infertility evaluation (Male Infertility Best Practice Policy Committee of American Urological Association, and Practice Committee of the American Society for Reproductive Medicine [27]). The advantage of processing of spermatozoa in ART's, to obtain highest spermatozoa recovery and in vitro treatment of spermotozoa to improve their function like motility and improve or keep the functional capacity for successful fertilization through supplementation of protective media (Astarto, et al. [28]). In this study, swim-up is more popular than other techniques in this Institute. This in vitro sperm activation does not demand specific experience and conserve substance; it is, subsequently, more workable for the laborer and less costly (Kadhim, et al. [29]). The present

study explains that highly decrement ($P < 0.05$) in the mean of spermatozoa concentration post-activation, is due to the failure of the defunct and immotile spermatozoa to swim up and wandering from pellet to the upper layer of culture medium. Advancement in the proportion of spermatozoa progressive movement post-activation as a results of quick movement of normal sperm cells from seminal plasma into layer of media, and thus elicited from effect of some components of semen plasma such as leukocytes, others leading to keep the sperm cell out of stress and reactive oxygen species product that fundamental for DNA damage (Hindal, et al. [29]). This is regarded as a normal response for sperm activity after removal of seminal plasma since it contains dead sperm, leukocytes, debris and epithelial cells that produce many oxygen radicals that can negatively affect the sperm functions (Sharma, et al. [31]). In the early years of assisted reproductive techniques, the concentrate on gain motile sperm cell, in later years, the concentrate changed to the deposition of functional sperm, requirement instructed by the monitoring that functional sperm parameters are connected with the results of fertilization (Mossa, et al. [32]). The sperm- HBA assay is depended on the concept that a low level of sperm linking to hyaluronan explains a low proportion of mature sperm in the sample and subsequently prophesy infertility. The hyaluronan-binding sperm cell is respective in the interaction with the oocyte complex and hyaluronan binding is likewise related to high genomic safety (Yagci, et al. [22]), which recover the goodness of the paternal contribution to the zygote. Consequently, hyaluronan-binding distinguishes high and low

functional safety and fertilizing potency. Since spermatozoa are silent cells, it is highly soon after that modern HA receptor proteins have been created and added to the sperm membrane through culture. Sperm cell culturing and processing could have enhanced elimination and/or rebuilding of cover proteins that are normally sitting on the sperm head to settle down membranes up to the beginning of capacitation (De Jonge [33], Nixon, et al. [34]).

5. Conclusions

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

Acknowledgment

The researchers are thankful for the coworkers.

Funding

The work was funded by the researchers themselves. Application of HBA Slide which was used for the first time in the High Institute for Infertility Diagnosis and ART's/Al-Nahrain University.

Author Contribution

This research was done by Dr. Ezdehar N. Ali as a part of her Ph.D. thesis under the supervision of Assist. Prof. Dr. Hayder A. L. Mossa (corresponding author) and Prof. Dr. Ula Al-Kawaz.

Conflict of Interest

Conflict of interest declared none.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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Biography

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She was born in Thi-Qar in 1969. She received the M.B.Ch.B.- College of Medicine - University of Basra – 1992, the D.G.O.- College of Medicine - University of Basra 2004, the M.Sc. in Applied Embryology the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, in 2015. She worked as a gynecologist and obstetrician in 2004-2013. She is a Member of the Iraqi Society of Fertility. Currently she is a lecturer in the College of Medicine at the University of Thi-Qar - Department of Obstetrics and Gynecology.

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How to cite:

Ali EN, Mossa HAL, Al-Kawaz UMR. The New Approach of Hyaluronic Binding Assay in Relevance to Sperm Activation by Direct Swim-up Technique in Iraqi Infertile Men; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2019); 9(1):15-31. Doi: http://doi.org/10.28969/IJEIR.v9.i1.r2