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RESEARCH ARTICLE

A NEW SPERM PREPARATION TECHNIQUE BY COMBINATION OF DENSITY GRADIENT CENTRIFUGATION AND GLASS WOOL FILTRATION TECHNIQUES VERSUS EACH ONE ALONE FOR INFERTILE MEN WITH ASTHENOZOOSPERMIA

Ezdehar N. Ali , Ula M. R. Al-Kawaz*, Muhammad-Baqir M-R. Fakhrildin.
High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad-IRAQ.

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Asthenozoospermia, Density gradient centrifugation technique, Glass wool filtration technique, Max pure technique (combination technique) and *in vitro* sperm activation.

Corresponding Author
Ula M. R. Al-Kawaz.

Abstract

Background:- A variety of techniques have been developed to separate motile and morphologically normal spermatozoa from other constituents of the ejaculate to optimize successful assisted reproductive techniques.
Objectives:- To compare the asthenozoospermic semen outcomes of three *in vitro* sperm activation (ISA) techniques and evaluate the efficacy, namely; Density gradient centrifugation (DGC) technique, Glass wool filtration technique and Max pure technique(combination of DGC and GWF techniques).
Methods:- Forty three infertile men with asthenozoospermia were involved in this study. Sperm parameters assessed according to WHO (2010 and 1999). Post- activation of each sample divided into three aliquots, the first one using DGC, the second using GWF, and last one using Max pure technique.
Results:- A significant increase ($P<0.05$) of sperm motility, progressive sperm motility and normal sperm morphology when using Max pure technique as compared to DGC and GWF techniques. Also, there was a significant increase ($P<0.05$) for the same parameters when using DGC technique as compared to the GWF technique.
Conclusions:- Using Max pure technique for semen sample with decreased in the sperm motility were superior to that of DGC and GWF techniques.

Introduction:-

Infertility is defined as failure of a couple to conceive after twelve months of regular intercourse without the use of contraception in women less than 35 years of age and after six months of regular intercourse without the use of contraception in women of 35 years and older[(1).] It affects approximately 15% of couples worldwide and negatively influences the quality of life in those couples who are affected [(2)].

Assisted reproductive technologies (ART's) have become the treatment of choice in many cases for male and female infertility. The quality of semen samples is one of the factors determining the successful assisted reproduction[(3)]. Therefore, the ideal sperm preparation technique is to achieve the largest number of morphologically normal, motile spermatozoa in a small volume of physiological culture media free from seminal plasma, leukocytes and bacteria [(4)]. With the advancement in the techniques of assisted reproduction, the need to improve sperm processing methods and provision of actively motile spermatozoa has increased tremendously [(5)]. However, DGC technique consistently produces semen samples of the highest quality required for the intrauterine insemination (IUI) and for *in vitro* fertilization (IVF) which explains why it is the preferred sperm processing method [(6,7,8)]. The DGC technique that

separates spermatozoa is based on their density. Thus, at the end of the centrifugation, each spermatozoon is located at the gradient level that matches its density[9].

The advantage of this DGC method is that it can be used in cases with a low sperm concentration and motility [10]. While the disadvantages of this method include, the risk of contamination with endotoxins and the production of good interphases between layers can take some time [11].

The principle of GWF technique is rested on the self-propelled movement of the spermatozoa and filtration effect of the glass wool fibers [12]. A major advantage of this approach is the selection of normally chromatin- condensed spermatozoa, a parameter considered as predictive for fertilization ability *in vitro*. The GWF technique is very simple but it is a more expensive procedure[13]. Some debris is usually still present in the sample after the glass wool filtration [11].

Asthenozoospermia is one of the major causes of infertility or reduced fertility in men [14]. Asthenozoospermia, is defined as 'total motility' (progressive + non-progressive) less than 40% or progressive motility less than 32% (WHO, 2010) [15].

Sperm motility is a critical indicator of semen quality and fertility potential because the sperm motility is required for the penetration of cervical mucus, transport through the female genital tract and penetration through the corona radiate and zona pellucid before oocyte fertilization [16].

Materials and Methods:-

Forty three infertile men with asthenozoospermia participated in this study during their attendance to the infertility clinics at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University and the Infertility unit of Al -Hussein Teaching Hospital -Thi-Qar. Semen samples were collected and semen analysis was done according to WHO (2010) and (1999). Each semen sample was divided into three aliquots. The first one using the density gradient centrifugation technique, the second one using glass wool filtration technique, while the third one using Max pure technique(combination technique), then sperm parameters were assessed for these three techniques and the results were statistically analyzed.

Max Pure Technique (combination technique):-

As a new sperm preparation technique was performed as the following, adding 1mL of 80% of Sil-Select Plus gradient as a first layer solution in a test tube followed by 1mL of 40% of Sil-Select Plus gradient as a second layer solution then liquefied semen sample was added on the second layer. This test tube was carefully put in centrifuge at 2600 rpm for 15 minutes. Supernatant discarded and 1mL of Ferticult Flushing medium added to the pellet. Shaking the sample then left for 8-10 minutes in an incubator, after that the semen suspension placed gently over the wet glass wool syringe and allowed to filter by gravity. A drop of 10µL was aspirated, put on a slide with cover slip and examined under the microscope at 400X objective to assess the sperm parameters as recommended by WHO (2010) and (1999).

Statistical Analysis:-

The data were statistically analyzed using Statistical package for social sciences (SPSS) version 20 software. Sperm parameters, pre and post activation assay were analyzed using (one way ANOVA).

Results:-

Table 1: Sperm parameters for infertile men with asthenozoospermia (no.43: 43%), pre- and post-*in vitro* sperm activation techniques. The present study showed a significant decrease ($P < 0.05$) in the certain sperm parameters (sperm concentration, sperm agglutination and round cells count) post-ISA when using these three techniques as compared to pre- activation. While, a significant increase ($P < 0.05$) in the other certain sperm parameters (sperm motility, progressive sperm motility and normal sperm morphology) post- ISA when using these three activation techniques as compared to pre-activation. The same table showed a significant decrease ($P < 0.05$) for the concentration of sperm when using Max pure technique as compared with the GWF technique. In contrast, this table (1) showed significant increase ($P < 0.05$) in the certain sperm parameters (sperm motility, progressive sperm motility and normal sperm morphology) when using Max pure technique as compared with the DGC and GWF techniques. While, a significant increase ($P < 0.05$) for the same sperm parameters when using DGC technique as

compared to GWF technique. This table showed non- significant difference (P>0.05) for the sperm agglutination and round cells count among these three activation techniques.

Table 1: Sperm parameters for asthenozoospermic infertile men pre- and post- *in vitro* sperm activation.

Sperm parameters		Pre- activation	Post- activation		
			Density gradient	Glass wool	Max pure
Sperm concentration (millions/mL)		40.348 a ±2.326	18.976 bc ±1.085	22.465 b ±1.224	15.139 c ±0.998
Sperm motility (%)		51.558 d ±1.331	84.790 b ±0.548	81.139 c ±0.565	90.093 a ±0.439
Sperm grade activity (%)	Progressive sperm motility (%)	22.325 d ±0.737	71.023 b ±0.458	64.511 c ±0.492	80.046 a ±0.499
	Non Progressive sperm motility (%)	29.232 a ±0.967	13.697 c ±0.341	16.627 b ±0.379	10.046 d ±0.310
	Immotile sperm (%)	48.441 a ±1.319	15.279 c ±0.567	18.860 b ±0.565	9.907 d ±0.439
Normal sperm morphology (%)		38.255 d ±0.603	68.767 b ±0.403	63.465 c ±0.467	75.255 a ±0.504
Sperm agglutination (%)		9.279 a ±1.316	0.093 b ±0.064	0.00 b ±0.00	0.00 b ±0.00
Round cells count (HPF)		6.441 a ±0.535	0.00 b ±0.00	0.581 b ±0.142	0.00 b ±0.00

- Means with different superscripts within each row are significant different (P<0.05)in which(a)is the highest value while (d) is the lowest value
- Means with similar superscripts within each row are non -significant different (P>0.05)
- Data are mean ± S.E
- Number = 43

Discussion:-

Sperm preparation techniques are a vital component of assisted reproductive technologies [17]. Meanwhile, improvement in the sperm parameters enhanced the sperm fertilizing capacity and outcomes of ART's [18, 19] .

Importantly, the idea of sperm separation techniques is to treat the spermatozoa *in vitro* in order to improve their functionality i.e. motility and supply a protective environment with the purpose to maintain or improve their functional capacity for successful fertilization. An improvement in the percentages of sperm motility and progressive sperm motility is regarded as normal response for sperm activity after removal of seminal plasma since it contain dead sperm, leukocytes, epithelial cells, debris and microbial contamination that produce many oxygen radicals that can negatively influence the sperm functions[20] . The current study clarified that significant increase (P<0.05) for the progressive sperm motility after ISA when using Max pure technique as compared to the DGC and GWF techniques. Also, this study clarified that a significant increase (P<0.05) for the same parameter post-activation when using the DGC technique as compared to the GWF technique. The present study clarified that a significant increase (P<0.05) in the normal sperm morphology when using Max pure technique as compared to the DGC and GWF techniques. Significant increase (P<0.05) for the same parameter when using DGC technique as compared to GWF technique. Sperm morphology is considered as a sensitive indicator of overall testicular health, because the sperm morphological characteristics are determined during spermatogenesis [21]. Also plays a crucial role in the diagnosis of male fertility potential and it has demonstrated a predictive value for fertilization and pregnancy outcomes in IVF [22].

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