A Comparison Between the Effects of Global Sperm Washing® and FertiCult Flushing ™ Media on Certain Sperm Function Parameters of Asthenozoospermic Men

مقارنة بين تاثيري الوسطيين الزرعيين Global Sperm Washing® و FertiCult Flushing ™ على معايير النطف الوظيفية الرئيسية للرجال المصابين بوهن النطف

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Abstract

The World Health Organization (WHO) and many studies considered the infertility as a disease and so many couples complaining from unsuccessful assisted reproductive technologies procedures to overcome their problem. One of the reasons of this dilemma is the sperm preparation method when no optimum result obtained even by using any of media found globally. However Global sperm washing®, and FertiCult flushing™ media were proved their capability to obtain good results of certain sperm function parameters. Nevertheless, the studies that compare between these media were rare. Therefore, this study aimed to compare between Global sperm washing medium®, FertiCult flushing ™ media that used for sperm washing before using the partner sperm for ART procedure. After detecting asthenozoospermia in sixty semen samples, they were divided into two groups according to medium used for sperm activation *in vitro* Global sperm washing medium ® (n=31) and FertiCult flushing medium™ (n=29) groups.The semen analysis was done after 3-5 days of abstinence as recommended by the manual of WHO (1999). Certain sperm function parameters were recorded. Semen fluid samples were treated with sperm activation media (Global sperm washing medium and FertiCult flushing medium TM) by using direct swim-up technique for *in vitro* sperm activation test. A significant (P<0.05) improvement was noticed between the two media regarding active sperm motility grades A and B when using FertiCult flushing medium™ compared to Global sperm washing medium®. Whereas no significant (P>0.05) differences were detected between the two media regarding sperm motility grades C and D. There was no significant (P>0.05) differences in morphologically normal sperm following *in vitro* activation by using the two media. It is concluded that FertiCult flushing medium™ was better than Global sperm washing medium® in improving active sperm motility of asthenozoospermic men which can be utilized in future for successful of assisted reproduction.

Key words: Global sperm washing medium ®, FertiCult flushing medium TM, asthenozoospermia

الملخص

اعتبرت منظمة الصحة العالمية العقم كمرض ، وان الكثير من الأزواج تذمر من اجراءات المعالجة وعدم نجاح عمليات التقنيات المساعدة على الإنجاب للتغلب على مشاكلهم. وان أحد أسباب هذه المعضلة هو طرق إعداد وتحضير النطف التي تفسر عدم الحصول على أفضل النتائج حتى باستخدام أفضل أنواع الاوساط الزرعية الموجودة على مستوى العالم. ومع ذلك فان الأوساط الزرعية المعروفة باسم FertiCult flushing medium ™ و Global sperm washing medium® اثبتت فعاليتها وقابليتها بالحصول على نتائج مشجعة. بالرغم من ذلك فان الدراسات التي قارنت بين هذه الاوساط الزرعية نادرة، لذلك هدفت هذه الدراسة مقارنة بين أوساط غسل وتحضير النطف FertiCult flushing medium ™ و Global sperm washing medium® لتهيئتها قبل استخدامها في التقنيات المساعدة على الإنجاب. فحصت 60 عينة من السائل المنوي التي قسمت إلى مجموعتين وفقا للوسط الزرعي الذي تم استخدامه: مجموعة مصابة بوهن النطف (العدد=31) عوملت بالوسط الزرعي FertiCult flushing medium ™ مجموعة مصابة بوهن النطف (العدد =29) عوملت بالوسط الزرعي Global sperm washing medium ®. فحص وحلل السائل المنوي بعد اعتماد 3-5 أيام من الامتناع عن الجماع حسب توصيات منظمة الصحة العالمية (1999). واستخدمت طريقة فحص السباحة المباشرة لتنشيط النطف في الزجاج. بينت النتائج وجود فرق معنوي (P <0.05) بين الوسطين FertiCult flushing ™ media و Global sperm washing ® فيما يتعلق بالحركة النشطة للنطف من الدرجة A والدرجة B عند استخدام وسط (FertiCult flushing medium™) لتنشيط النطف بالمقارنة بوسط (Global

sperm wash medium®)، في حين لم يلاحظ وجود فروق معنوية (P> 0.05) بين الوسطين فيما يتعلق بحركة النطف الدرجة C و D. وكذلك لم تكن هناك فروق معنوية (P> 0.05) في شكلياء النطف الطبيعية بعد تنشيطها في الزجاج باستخدام الوسطين. نستنتج من الدراسة الحالية أن وسط (FertiCult flushing medium ™) أفضل بكثير من وسط Global sperm washing medium® في تنشيط الحركة الفعالة للمرضى المصابين بوهن النطف والتي يمكن ان تستخدم مستقبلا لانجاح اي تقنية ضمن التقنيات المساعدة على الانجاب.

الكلمات الدالة: وهن النطف، وسط Global sperm washing medium® ، ووسط FertiCult flushing™

Introduction

Many people assume that infertility is a" man or woman's "problem, but many cases of infertility are a result of a man factor too. Male problems may be a contributing factor in 30 to 50% of couples suffering infertility [1]. There are many factors causing male infertility. The treatment of infertility has undergone phenomenal development and become a highly specialized field involving a multitude of interventions known collectively as assisted reproductive techniques [2]. The successful rate and pregnancy rate of artificial insemination in husband (AIH) varies considerably, and researchers do their best to stand on the causes of low success rate of AIH to find the best procedures that improve male fertility, which through the semen must be present in sperm motile, normal morphology, and be able to penetrate an ovum [3].

Sperm preparation and washing are usually performed by different media such as Sage®, *In Vitro* life®, Global®, FertiCult™, MediCult® and others to wash any semen sample for intrauterine insemination (IUI) and other assisted reproductive technologies (ARTs) procedures. Sperm washing achieves two very important goals. First, it removes most of the seminal plasma from the semen that would otherwise not react well if directly inseminated into the uterus. Second, it concentrates the sperm density into a small volume that is suitable for any assisted reproduction technologies. In most cases, sperm washing may also enhance the forward progression of the sperm or the percent motility [4]. This study was designed to compare between the effects of two cultures media to improve sperm function parameters for sperm preparation and *in vitro* activation of patient's semen samples.

Materials and Methods

One hundred semen samples were collected from men consulting in Infertility Unit at the Biotechnology Research Center and High Institute of Infertility diagnosis & ART at Al Nahrain University for *in vitro* Sperm Activation Test (SAT) through the period from September 2015 to March 2016. Following seminal fluid analysis the asthenozoospermic samples were divided into two groups according to culture medium used for *in vitro* activation, Global sperm washing medium ® (n=31) and FertiCult flushing medium ™ (n=29) groups .The semen analysis was accomplished after 3-5 days of abstinence as recommended by WHO guidelines [5].Certain sperm function parameters were recorded namely; sperm concentration million/ml, sperm motility (%) and morphologically normal sperm (%) [5]. All semen samples were activated with sperm preparation media (Global sperm washing ® and FertiCult flushing ™ media) by using direct swim-up technique for sperm activation test.

The direct swim-up technique was performed either by layering culture medium over the liquefied semen or by layering liquefied semen under the culture medium [6,7]. One ml of liquefied semen samples layering under 1ml of the culture media (Global® or FertiCult™) and evaluated after 30 minutes of incubation at 37° C

Statistical analysis

Crude data were collected and analyzed using SPSS (Statistical program for social studies, Version 17, Illinois, USA) for descriptive statistics involving means and standard deviation of mean (SD) .Paired *t*– test was used to detect the significant differences of before and after activation between the two groups. P value less than (0.05) was statistically considered as significant [8].

Results and Discussion

In Table (1), A significant (P<0.05) decrease was found in sperm concentration (million/ml) of asthenozoospermic men after activation *in vitro* by Global sperm washing® medium compared to before activation. There was a significant (P<0.05) improvement in active sperm motility grade A after activation (16.87 ± 11.02) compared to before activation (4.70 ± 6.65). The sperm motility grade B was significantly (P<0.05) increased when the semen sample of asthenozoospermic men was activated *in vitro* by Global® medium (47.48 ± 20.32) compared to before activation (27.41 ± 13.12). There was a significant (P<0.05)

decrement in sperm motility grades C and D following *in vitro* activation compared to before activation. The percentage of morphologically normal sperm (MNS) was significantly (P<0.05) higher after activation (55.64±11.16) than that of before activation (37.87 ± 8.87).

Table (1): Certain sperm function parameters of asthenozoospermic men before and after *in vitro* activation by Global sperm washing® medium for 30 minutes incubation using direct swim-up technique

Certain sperm function parameters		*In vitro* activation by Global medium		*P* – Value
		Before Activation	After Activation	
Sperm Concentration (million/ml)		48.16 ± 23.16	22.48 ± 11.95	P < 0.05
Sperm Motility (%)	Grade A (%)	4.70 ± 6.65	16.87 ± 11.02	P < 0.05
	Grade B (%)	27.41 ± 13.12	47.48 ± 20.32	P < 0.05
	Grade C (%)	24.87 ± 7.34	20 ± 7.52	P < 0.05
	Grade D (%)	42.67 ± 18.82	13.87 ± 15.90	P < 0.05
Morphologically normal sperm (%)		37.87 ± 8.87	55.64 ± 11.16	P < 0.05

Values were expressed as Mean ± SD.
Patients No. = 29

Table (2), showed that there was a significant (P<0.05) decrease in sperm concentration (million/ml) of asthenozoospermic men following activation *in vitro* compared to before activation. A significant (P<0.05) increase was noticed in active sperm motility grade A after activation (33.07 ± 13.49) compared to before activation (8.07 ± 9.26). The sperm motility grade B was significantly (P<0.05) improved following *in vitro* activation of the semen sample of asthenozoospermic men (33.46 ± 11.80) compared to before activation (22.73 ± 12.28). There was a significant (P<0.05) reduction in sperm motility grades C and D after *in vitro* activation compared to before activation. The percentage of morphologically normal sperm (MNS) was significantly (P<0.05) increased after activation (58.07 ± 8.0) compared to before activation (40.11 ± 7.17).
The comparison between the effects of Global® and FertiCult® media was shown in Table (3).There was a significant (P<0.05) decrement in sperm concentration after using Global ® medium (22.48 ± 11.05) compared to FertiCult® medium (29.53 ± 15.47). A significant (P<0.05) differences was found between the two media regarding active sperm motility grade A (Global medium=16.87 ± 11.02 and FertiCult=33.07 ± 13.49). The Sperm motility grade B was improved significantly (P<0.05) when using Global® medium compared to FertiCult® Medium. Whereas no significant (P>0.05) differences were observed between the two media regarding sperm motility grades C and D. There were no significant (P>0.05) differences in MNS following *in vitro* activation by using the two media (Global® medium=55.64 ± 11.16 and FertiCult® medium= 58.07 ± 8.0).

Table (2): Certain sperm function parameters of asthenozoospermic men before and after *in vitro* activation by FertiCult flushing medium™ for 30 minutes incubation using direct swim-up technique

Certain Sperm Function Parameters		*In vitro* activation by FertiCult medium		*P* – Value
		Before Activation	After activation	
Sperm Concentration (million/ml)		51.76 ± 25.48	29.53 ± 15.47	P < 0.05
Sperm Motility (%)	Grade A (%)	8.07 ± 9.26	33.07 ± 13.49	P < 0.05
	Grade B (%)	22.73 ± 12.28	33.46 ± 11.80	P < 0.05
	Grade C (%)	30.96 ± 10.63	21.15 ± 9.42	P < 0.05
	Grade D (%)	38.46 ± 19.90	13.26 ± 9.92	P < 0.05
Morphologically normal sperm (%)		40.11 ± 7.17	58.07 ± 8.0	P < 0.05

Values were expressed as Mean ± SD
Patients No. = 31

Table (3): Comparison between the effects of Global sperm washing ® and FertiCult flushing TM media on certain sperm function parameters of asthenozoospermic men.

Sperm Function Parameters		*In vitro* activation		*P* – Value
		Global medium*	FertiCult medium**	
Sperm Concentration (million/ml)		22.48 ± 11.95	29.53 ± 15.47$	P < 0.05
Sperm Motility (%)	Grade A (%)	16.87 ± 11.02	33.07 ± 13.49$	P < 0.05
	Grade B (%)	47.48 ± 20.32β	33.46 ± 11.80	P < 0.05
	Grade C (%)	20 ± 7.52	21.15 ± 9.42	P > 0.05
	Grade D (%)	13.87 ± 15.90	13.26 ± 9.92	P > 0.05
Morphologically normal sperm (%)		55.64 ± 11.16	58.07 ± 8.0	P > 0.05

Values were expressed as Mean ± SD.
***Patients No. = 31.**
****Patients No. = 29**

The significant reduction in sperm concentration after activation *in vitro* was observed in both cultures media, may be resulted from inability of the dead and poor grade activity sperm to move up and travel from the lower layer to the upper layer of culture medium. Similar results were recorded by other studies [9,10]. There was an improvement in the percentage of sperm motility (grades A & B) and percentage of morphologically normal sperm after activation. This finding may be due to the fast movement of normal spermatozoa from the lower layer of culture medium as both media constitute of Ca^{++} and energy supplements to enhance the motility in addition to the impact of some seminal plasma components like leukocytes, and other decapacitation factors which in turn keeping the sperm out of stress factor and reactive oxygen species production that responsible for impaired sperm motility and DNA damage [11,12,13]. This in turn leads to significant reduction of abnormal, non – progressive and immotile sperm (grades C & D) respectively [14,15].

The comparison between the effects of two media showed significant differences in certain sperm function parameters. An increment in sperm concentration and grade A active sperm motility was noticed by using FertiCult medium ™ for washing and activating spermatozoa *in vitro*, while a level of elevation in grade B active sperm motility was noticed when using Global sperm washing® medium for *in vitro* sperm activation. This in turn may be related to the composition of FertiCult flushing medium **™**, which contains combination of HEPES, bicarbonate, physiologic salts, glucose, lactate and human serum albumin (4.00g/liter) [16]. HEPES alone in the media as a buffering system increased the buffering capacity and the stability of the pH in the range of (7.2 to 7.6). This allows the media to be better resist fluctuations in the pH resulting from changes of cellular metabolism therefore no CO_2 incubation is required to avoid reduction of pH less than 7.0 [17,18]. Thus, it is concluded from current study that FertiCult sperm washing medium ™ has better effect than Global sperm washing ® medium on certain sperm function parameters of asthenozoospermic men.

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