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Comparative study of testicular sperm maturation in vitro using Ham's F10,Earle's and Ferticult culture media

M. Badawy Abdel-Naser MD,
M.A. Farid MD, M. El Bahrawy, MD
Dept of Dermatology, Venereology and Andrology, Ain Shams University,
Cairo, Egypt

Abstract :

Testicular sperm extraction (TESE) from azoospermic males followed by intracytoplasmic sperm injection (ICSI) is a recent advance in treatment of male infertility. This study describes the total number and motility changes in vitro of freshly extracted testicular sperm and compares between Ham's F-10, Earle's, and Ferticult culture media. Testicular biopsies were obtained from azoospermic patients (n=11) and processed to obtain a cell suspension that was incubated in equal portions in each culture medium supplemented with 10% human albumin for 5 days. The number of total and motile spermatozoa was evaluated on day 0 and daily afterwards for 5 days. Our results revealed that all patients were suffering from obstructive azoospermia with mean age of 39 ± 3.36 and duration of infertility of 9.25 + 2.98 years. The total number of spermatozoa showed no significant changes in all days of incubation and between all used media ($p>0.05$). However, the number of motile spermatozoa was significantly higher in Ferticult medium when compared with Ham's F10 and Earle's media ($p<0.05$). In all used media, spermatozoa become motile within 24 hours of culture and the motility was maintained for the 4th day before a decline to the near 0 level is observed on the 5th day of incubation. Furthermore, the number of progressively motile sperm significantly peaked on day 3 of incubation in all media but the best significant yield was observed in Ferticult medium ($p<0.05$). No significant correlation was found with age of patients or duration of infertility ($p> 0.05$). From our study we recommend that TESE is carried out approximately 3 days before oocyte retrieval and that Ferticult medium is the medium of choice to use in in vitro cultivation for obtaining the most mature of testicular tissue sperm to be used for IVFrelated procedures, e.g. ICSI.

Key words : *Obstructive azoospermia- testicular sperm extraction-sperm maturation in vitro - culture media.*

Address of correspondence :
Dr. M. Badawy Abdel-Naser
Al Obeid General Hospital,
Dahran Str. Al Hasa,
P.O. Box 11017 ñ Pin code 31982 Saudi Arabia
Tel: (++9663) 593111
Fax:(++9663) 5929372

Introduction

Testicular sperm extraction (TESE) or aspiration of sperm from the epididymis e.g., percutaneous epididymal sperm aspiration (PESA) or microsurgical epididymal sperm aspiration (MESA) and its use in intracytoplasmic sperm injection (ICSI) to achieve fertilization and pregnancy for azoospermia has become one of the main treatment options in the recent years [1]. Nevertheless the fertilization rates obtained with testicular germ cells seem to be lower than with ejaculated spermatozoa [5]. The lower fertilization rate in this case has been attributed to poor motility and immaturity of the extracted sperm. It has been shown that in vitro cultivation of testicular spermatozoa for few days greatly improves the progressive motility but the information available on in vitro culture of testicular spermatozoa is still insufficient [6]. Furthermore, the optimum conditions for obtaining fully mature sperm have not been fully established.

In this study, we evaluated the total number and number of motile testicular spermatozoa cultivated in vitro and we compared the results obtained using three different culture media, namely Ham's F10, Earle's and Ferticult.

Patients, material and methods :

Eleven patients complaining of primary infertility with azoospermia (semen analysis repeated 3 times) were included in this study. Each patient was subjected to full history taking, clinical examination and FSH level determination. Cases with atrophic or small tests were excluded Testicular biopsy was done from all patients under local anesthesia as previously described [7] and each was divided into two pieces. One portion was processed for histopathology, whereas the other portion was homogenized using a pair of sterile scissors and scalpel blade.

The homogenate was repeatedly forced through a 25G needle with a 5 cc plastic syringe to obtain individual cell suspension [8]. The homogenate tissue was washed twice with 5 ml of phosphate buffer saline, centrifuging each time at 500g for 10 min.

The tissue pellet was resuspended and equally distributed in 1 ml of each culture medium (supplemented with 10% human albumin) in 24 well tissue culture plates (Becton & Dickinson, USA). The used culture media were Ham's F10 (Seromed, Germany),

Earle's (Seromed, Germany) and Ferticult (Ferti-Pro, Belgium).

The cultures were incubated for 5 days at 370 C in 5% CO2 humidified air mixture. Each culture system was examined on the same day of incubation, i.e. day 0 and then daily afterwards for 5 days using an inverted phase contrast microscope (Reichert, Germany) equipped with Hoffmann modulation contrast. In each day, two independent observers using the high power. Magnification (HPF X400) counted the total sperm and number of progressively motile sperm (equivalent to grades a and b according to WHO criteria)[9] . The maximum, minimum, the mean and standard deviation (SD) were calculated. Student's t and ANOVA tests were used for statistical analysis and p values of ≤ 0.05 were considered significant.

Results :

All patients included in this study were suffering from primary infertility of several years' duration. The mean age was 39.75 ± 3.86 (range 36-45 years) and the mean duration of infertility was 9.25 ± 2.98 (range 5-12 years). General examination revealed no abnormality and genital examination revealed normal size testicles, palpable vasa and absent varicocele. Repeated semen analysis including centrifugation revealed absence of sperm and spermatogenic cells.

Hormonal assay revealed normal range of FSH. In all patients, histopathology revealed normal spermatogenesis thus verifying the diagnosis of obstructive azoospermia.

Evaluation of the total number of sperm revealed no significant difference between the 3 culture media used ($p > 0.05$). In addition, no significant change in the number of sperm on all days of incubation was found ($P > 0.05$) (table 1). Also, no significant difference between the 3 media regarding the number of motile sperm was detected on day 0 of incubation ($p > 0.05$). On the other hand, there was a significant increase in the number of progressively motile sperm in all culture media in day 1 through day 5 ($P < 0.05$) except in Ham's F10 medium, which was insignificant in day 5 ($P > 0.05$). Ferticult medium showed significantly higher number of motile sperm when compared with Ham's F-10 and Earle's media in day 1 through day 5 ($p < 0.05$).

Similarly, number of motile sperm was significantly higher in Earle's medium when compared with Ham's F10 but only in day 3 and 4 of incubation ($p < 0.05$). When the number of motile sperm was compared throughout the 5 days of incubation, all culture media showed the peak motility on the 3rd day of incubation, however, Ferticult medium showed significantly superior number of motile sperm over Ham's F10 and Earle's media ($p < 0.05$) (table 2, fig.,1). No significant correlation with either the age of patients or duration of infertility was found ($p > 0.05$).

Discussion :

Testicular sperm extraction (TESE) followed by ICSI is now a commonly used treatment modality for inducing pregnancy in cases of obstructive azoospermia that can not be corrected or failed to be corrected by surgical microanastmosis [2,10].

PESA and MESA are still in use but obtaining a viable sperm is not always feasible and repeated PESA may induce fibrosis of the epididymis with less chance of obtaining sperm [10,11]. Unfortunately; the results of pregnancy by TESE are still low [5].

Several factors may be incriminated for this low success rate and immaturation of injected sperm comes on the top of the list [13]. In this work, we showed that culture of testicular sperm for few days greatly enhances sperm motility. Since acquisition of progressive motility is regarded as an indication of sperm maturation, our results, therefore, provide clear evidence that testicular sperm can acquire maturation in in vitro culture. [8, 14]

All culture systems supported sperm motility in vitro from day 1 through day 5 of incubation. However, the culture system using Ferticult medium was found to be superior over Ham's F10 and Earle's media as it gave the best yield of motile sperm.

The number of motile sperm peaked on the 3rd day of incubation in all culture media.

A similar study performed by Edrisinghe et al showed that testicular sperm motility improved markedly on the 3rd day of culture and peaked around day 5 [6]. The difference between the two studies is not known but could be due to the different culture media used. Nevertheless, it is anticipated that selection of the most motil esperm would increase the success rate of ICSI. Therefore, assessment of the fertility potential of testicular sperm in Ferticult medium is required to address this issue.

It has been suggested that the improvement of motility of testicular sperm homogenate be due to cells other than sperm cells plating out to form

monolayers, which provide co-culture effects benefiting sperm maturation process [6].

At this moment it is not known whether the improvement of sperm motility observed in our study and that of others [6,8] is due to direct effect on sperm or due to effect on co-cultured cells. In vitro cultured sperm swim away from the testicular tissue and cellular debris. This makes it easier to avoid injecting immature cells or cellular debris, which may contain DNA into the ooplasm [8]. Interestingly, sperm can be recovered from the testes in some patients who are clinically diagnosed as having non-obstructive azoospermia, which justifies a diagnostic TESE in functional azoospermia regardless of the FSH level [15].

Another important point to consider is the emotional and financial implications of unsuccessful sperm recovery in the management of the symptom of infertility [16]. Therefore, we believe that the result of the present study provide necessary information that are helpful in planning of sperm collection and preparation prior to oocyte collection for IVF related procedures.

The results of this work did not show any increase in the total number of sperm in any of the used media. Indeed, the issue of increasing the total number of sperm in vitro culture is less important in obstructive than in non-obstructive azoospermia. obstructive azoospermia, as shown in our study and studies of other groups [6,8,17], spermatozoa are always obtained by TESE and it is the maturity that will improve the success rate of ICSI. Similarly, neither the age of patients nor the duration of infertility has a significant impact on sperm maturation in vitro.

We conclude from our study that testicular spermatozoa from men with obstructive azoospermia can be easily collected from the testicles and maintained in vitro for up to 5 days. For the best yield of motile sperm, we recommend the use of Ferticult medium in which number of motile sperm peaked in the 3rd day of incubation.

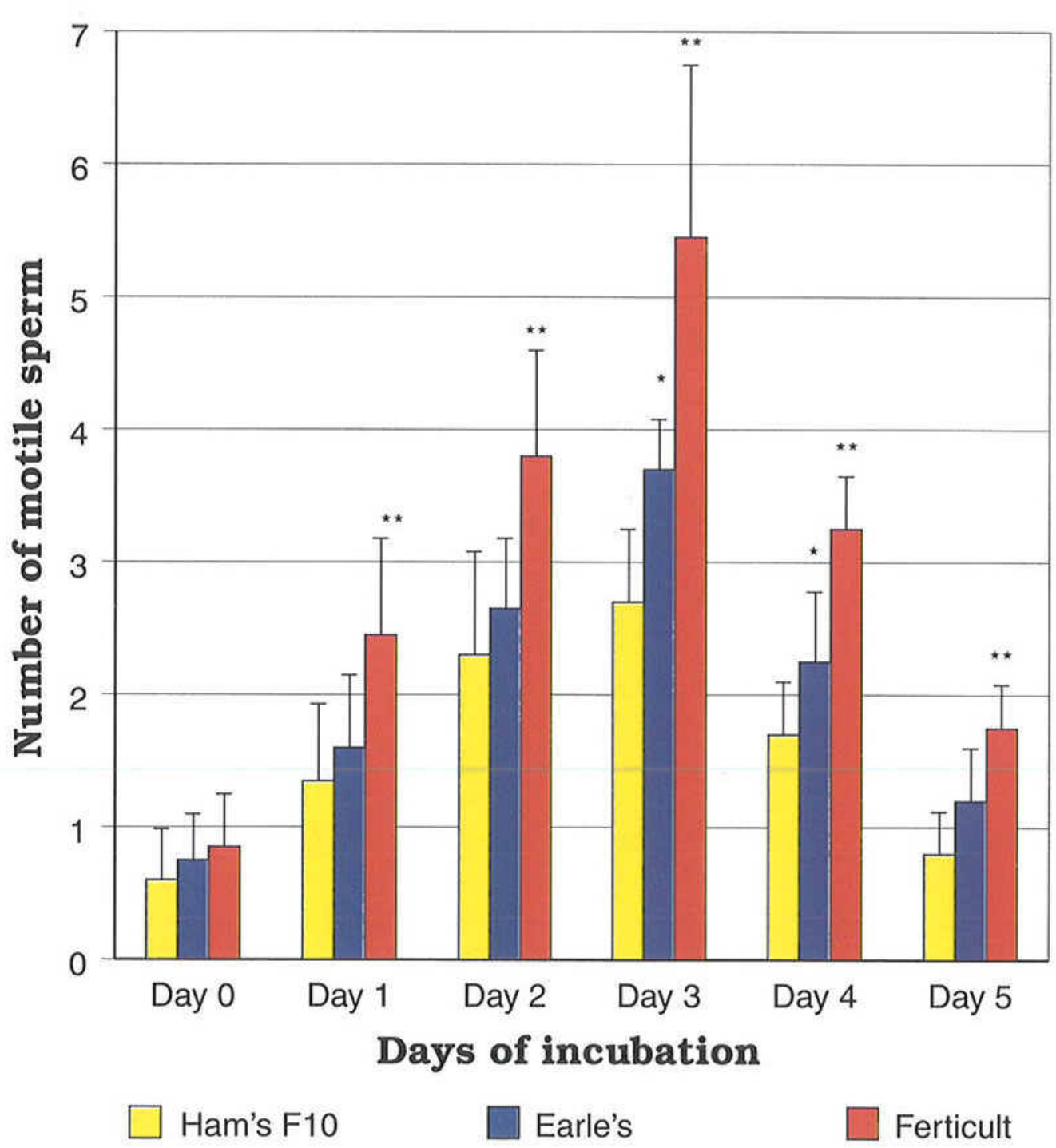

*Fig. 1. A histogram showing the changes in the number of motile sperm in vitro. Ferticult medium yielded the best number from day 1 to day 5 with a peak number on day 3. **p vs. Earle's and Ham's F10 ($p<0.05$). * p vs . Ham's F-10 ($p<0.05$).*

Table 1.

Day of incubation	Ham's F-10	Earle's	Ferticult
Day 0	7.52 ± 2.11 (6-10.5)	8.12 ± 2.7 (4.5-11)	8.1 ± 2.99 (5.5 -11)
Day 1	7.9 ± 2.24 (5.5-9.6)	8.6 ± 3.2 (6-13)	8.85 ± 3.4 (5-13)
Day 2	8.65 ± 2.53 (6-11.5)	9.1 ± 2.55 (7-11.5)	8.83 ± 3.4 (6-12)
Day 3	8.8 ± 2.28 (6-11.1)	8.95 ± 2.3 (6.5-11)	8.65 ± 3.01 (5.5-11)
Day 4	7.27 ± 1.89 (5-8.5)	8.67 ± 2.56 (6.5 - 11)	8.72 ± 2.31 (7.1 -11)
Day 5	7.75 ± 1.85 (6-9.5)	8.02 ± 2.21 (6-10.5)	8.27 ± 2.65 (6-11)

Table. 1Total number of spermatozoa in the used culture media. No significant difference between any of these values is detected ($p>0.05$).

Table 2

Day of incubation	Ham's F-10	Earle's	Ferticult
Day 0	0.55 ± 0.43 (0-1)	0.72 ± 0.35 (0.5-1)	0.82 ± 0.4 (0.5-1)
Day 1	1.35 ± 0.53 (1-2)	1.61 ± 0.5 (1-2)	2.47 ± 0.67 (1.5-3) **
Day 2	2.39 ± 0.66 (1.5-3)	2.65 ± 0.49 (2-3)	3.85 ± 0.74 (3-4.5) **
Day 3	2.73 ± 0.49 (2.5-3.5)	3.72 ± 0.35 (3.5-4)*	5.47 ± 1.39 (4-7)**
Day 4	1.72 ± 0.35 (1.5-2)	2.3 ± 0.45 (1.5-2.5)*	3.22±0.37(2.5 - 3.5)**
Day 5	0.79 ± 0.32 (0.5-1)	1.22 ± 0.35 (1-1.5)	1.77+0.29 (1.5-2) **

*Table 2. Number of Motile sperms in culture media. Ferticult medium shows significantly higher numbers over Ham's F-10 and Earle's media from day 1 through day 5 ($p<0.05$). * p vs. Ham's F-10 ($p<0.05$). Similarly Earle's medium shows significantly higher number over Ham's F10 but only on day 3 and 4 ($p<0.05$). ** p vs. Earle's and Ham's F-10 ($p<0.05$) * p vs . Ham's F-10 ($p<0.05$)*

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