Effects of short term storage of culture medium on the extraction of actively motile spermatozoa

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Summary : **The present study analyzed the effect of short term storage of Ham's F-10 medium on the outcome of actively motile spermatozoa. The medium was used for the extraction of motile spermatozoa by swim-up technique from the fresh semen samples of the patients reported for the IUI treatment. The enhancement of sperm motility was assessed for a total period of four weeks using the same set of medium. The influence of medium on the extraction of motile spermatozoa declined with increase in the age of the medium. This finding suggests that, the use of medium two weeks after preparation is less effective for sperm preparation than the fresh medium.**

Introduction

Culture medium provides an ideal environment which is essential for fertilization. The specifically formulated culture media will artificially enhance the probability by conception in vitro by stimulating specific aspects of sperm function. Distinctive changes in motility patterns occur in the fertilization medium (Yanagimachi 1970). Success in assisted reproduction is dependent on maintenance of suitable culture conditions for gametes. A number of different culture conditions have been used with success for assisted reproduction technologies (ART). Culture media used in ART have ranged from simple buffered, balanced salt solution (Trounson et al 1984) to more complex media supplemented with aminoacids and various co-factors (Sokoloski et al 1984). Sperm motility is an important factor in determining fertilization rate in man (Mahadevan et al 1984). Extraction of rapidly motile sperm is being done by swim-up technique using various culture media. Ham's F-10 medium is routinely used in many centres. A good success rate will be attained by using the medium in the recommended life span. Shelf life recommendations for these formulations range from one week (Purdy 1982) to a maximum of 30 days or more from the time of media preparation. Prolonged storage/use of medium is not recommended for ART programmes since it may affect the result. However, there is no report on the influence of this medium used within its shelf life on the outcome of actively motile spermatozoa. Therefore, this study is an attempt to evaluate the quality of the medium for short period of one month. The influence of medium on the enhancement of motility was taken as end point.

Materials and methods

Patient selection

This study was carried out at the Manipal Assisted Reproduction Centre, Kasturba Hospital, Manipal. A total of 396 patients reported for the IUI treatment over a period of six months were selected using following criteria:

1. Total amount of spermatozoa > 50 X 10^6
2. Percent motility above 50
3. Normal values of other semen parameters (WHO 1992)

Preparation of medium

Ham's F-10 culture medium (cat. No. 10-401 26) was obtained from ICN Biomedicals Inc., USA was used throughout this study. The medium was prepared in tissue culture grade, 18mW water. After dissolving the base powder, 124 mg/L magnesium sulphate, 300 mg/L calcium lactate, 1.68 g/L sodium bicarbonate, 0.508 g/L potassium carbonate were added to the medium and the pH was adjusted between 7.4 and 7.6. The osmolality was adjusted to 280 m Osm/Kg. The medium was fil-

tered using Nalgene filters, stored in sterile bottles at 4°C. The media prepared were used for sperm preparation for a period of one month. Results obtained every week were grouped and compared.

Semen collection and swim-up

Fresh semen samples were collected by masturbation in sterile containers and routine analysis was performed. After liquefaction, motility was analyzed according to the WHO guidelines (WHO, 1992). Semen was processed for swim-up. Briefly, the specimen was mixed with equal volume of Ham's F-10 medium, centrifuged twice at 300 g for 10 minutes. The pellet was then overlaid with few drops of medium and incubated with 5% CO_2 in air, in a humidified atmosphere at 37°C for one hour.

The rapid and linear progressive motility (grade III) after swim-up was assessed and compared with motile spermatozoa seen before swim-up. The data were expressed as percent enhancement (PE) of motility. The statistical analysis was carried out using Analysis of One-way Variance (ANOVA).

Results

A total of 396 samples were proceeded using Ham's F-10 medium prepared in five different batches. A maximum enhancement of motility was observed within first week of medium preparation. A gradual decline in PE was seen in subsequent weeks. However, the PE between I and II week was not statistically significant. An average of 10-35% reduction in PE was observed between early (I & II week) and later (IV week) period (Table-I).

Discussion

Until now in all studies, the effect of long term storage on the sperm quality was evaluated. In the original paper of Ham (1963), medium stored by either refrigeration or freezing of various ingredient stocks, has given satisfactory results after mixing and stored at +4°C for a short period, but no specific period is mentioned as optimal. Ham's F-10 medium stored at 4°C for 3 months provided adequate support for fertilization and early in vitro devel-

Table I

Effect of medium ageing on the sperm motility (percent enhancement ± SEM)

Medium	Weeks			
	I	II	III	IV
1.	50.94±5.71	61.11±7.52	51.59±7.56	51.68±8.05
	N=21	N=24	N=26	N=21
2.	65.37±5.6	66.81±6.12	60.39±2.27	43.10±3.18[a,b,c]
	N=14	N=8	N=16	N=12
3.	88.23±7.79	83.57±4.46	63.93±5.8[b]	51.09±3.72[a,b]
	N=12	N=27	N=16	N=16
4.	65.28±4.9	53.56±6.54	46.85±6.88	46.89±3.30
	N=17	N=29	N=15	N=8
5.	57.44±5.32	53.2±4.59	45.18±4.98	43.96±3.75
	N=29	N=38	N=19	N=28

a,b,c = compared to respective I, II & III week values (Banferroni $p<0.05$)

opment to the four-cell stages of human embryogenesis (Bell et al 1995). Many authors have used Ham's F-10 in their studies did not discuss the medium longevity (Jones et al 1986; Wood et al 1992; Damewood 1990). Dandekar et al (1984) recommended that Ham's F-10 may be used within 4 weeks. However, development of one cell embryo to the blastocyst stage was not affected by the medium stored up to 6 months at 4°C (De Silva, 1993). But no supporting data are available in this context.

The shelf life of powdered Ham's F-10 prepared by GIBCO has been suggested to be 3 years from the date of manufacture when sealed and maintained at +4°C. The determination of this expiration date is based on the ability of the medium to support the growth of a mouse melanoma cell line. But this model is not acceptable for our conditions since gametes are more sensitive than transformed cells to the various factors like toxic substances, growth factors, temperature, pH etc. The determination of medium shelf life is based on the stability of the chemicals used. Penicillin and sodium pyruvate are the labile components in the medium (Wales et al 1971). Prolonged storage reduces the activity of these compounds in the medium. However, Weathersbee et al (1995), reported only 20% decline in the amino acid and vitamin contents after a long term storage of 18 months of modified Ham's F-10 medium.

The successful outcome of therapeutic insemination can be influenced by several factors including a good sperm preparation.

In the present study, the enhancement of sperm motility was evaluated for a period of 4 weeks. The influence of medium on the extraction of motile spermatozoa was not similar throughout the period used. In the fresh prepared media, the PE was found to be maximum. No significant decline in the enhancement of motility was seen in first two weeks. However, a significant decline in PE was noticed in later periods. The decline in the ability of the medium to extract motile populations in the later period may be due to inactivation of unstable components of the medium.

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