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Research Article

Influence of Modified Cryopreservation Protocol on Sperm Retrieval Rate

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Abstract: Desire to leave genetic footprints has been a long cherished dream of mankind. The concept of sperm banking has fulfilled these desires by preserving gametes and genital tissues. Cryo banking of spermatozoa is an essential aspect of fertility preservation. Developments in the field of cryopreservation have proved that frozen sperm is as good as fresh sperm in fertilizing oocytes. Semen banking has major role in fertility preservation in cancer patients. In the present study 94 samples were taken and evaluated with two different cryopreservation protocol. One method was to mix the pellet with the media and the other was the pellet mixed with the seminal plasma. From the above two adopted protocols the pellet mixed with the seminal plasma showed 55- 40% of sperm retrieval after thawing which was higher than the percentage obtained by the other method.
Keywords: Cryobiology, Cryopreservation, Cryoprotectant

INTRODUCTION

Cooling to low subzero temperatures (cryopreservation), is used to store human oocytes, embryos, sperm and gonadal tissues. Several well-established cryopreservation protocols for human sperm and embryos are available. Most of them were established >15 years ago. They are classified as `slow cooling' or equilibrium cooling procedures [1, 2]. The history of human sperm cryobiology was introduced in late 1940s. The discovery that glycerol protected spermatozoa against damage from freezing led to the use of human spermatozoa stored on dry ice at –79°C [3, 4]. Subsequently, liquid nitrogen was used. After that semen cryopreservation developed rapidly with the establishment of commercial sperm banks or coordinated national services in many countries [5]. Several freezing and sperm bank management protocols are available [6, 7]. Several cryoprotectants are available commercially. Detail of a commonly used cryoprotectant, glycerol-egg-yolk-citrate (GEYC).

Progress in assisted reproductive techniques (ART) has enhanced the use of frozen-thawed semen. In fact, radiotherapy, chemotherapy, some malignant diseases, or even invasive surgery can induce testicular failure or ejaculatory dysfunction. Semen cryostorage is the only proven method that may offer couples a chance to have children in the future after such condition [8]. Sperm cryostorage is also indicated in ART programs in case of donor insemination procedure or for men with severe oligozoospermia likely to deteriorate Liquefied semen samples were cryopreserved by a standard protocol using Sperm Freeze, a commercial cryoprotectant consisting of 15% glycerol in HEPES buffer (Fertipro, Beernem, Belgium). The samples were diluted (1:1 with freezing medium in a drop wise manner. After 10 minutes of equilibration at room temperature, the mixture was frozen in liquid nitrogen vapour for 15 minutes and plunged into liquid nitrogen (-196°C) in cryovials (Nunc International, Roskilde, Denmark) for storage. Samples were cryopreserved in liquid nitrogen until required. During thawing procedure it was bring at room temperature until completely thawed and then checked for the presence, number and motility of the spermatozoa [9]. Novel cryopreservation approaches are needed to improve recovery and post thaw parameters in the highly compromised sperm specimens.

The current evidence on the feasibility and efficacy of various methods for cryopreservation of surgically retrieved sperm for usein conjunction with assisted reproductive technology including techniques specifically designed for cryopreservation of small quantities/numbers of spermatozoa. The studies used the frozen–thawed spermatozoa for ICSI and ET of the

resultant embryos [10-12]. Two studies used the empty zonapellucida as the sperm carrier [10, 11], whereas Serini *et al*. [12] froze the spermatozoa in microdroplets on a plastic dish. In five patients using their frozen–thawed spermatozoa,Walmesly et al. reported an average fertilization and cleavage rates of 65 and 90%, respectively.

MATERIALS AND METHODS

Sample collection

The present study was carried out in NCARE IVF center, Parappanangadi, Malappuram (DT), Kerala. A total of 94 subjects selected for the studies were Olgoasthenoteratozoosperma. The sample was collected in a private room near the laboratory, in order to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis .The sample was collected after a minimum of 1days and a maximum of 2 days of sexual abstinence.

The instructions concerning the collection of the semen sample was emphasize that the sample need to be complete and that the man should report any loss of any fraction of the sample. The following information was record, the man's name, age and personal code number, the period of abstinence, the date and time of collection, the completeness of the sample.

Freezing Procedure

Transfer the liquefied semen sample into a sterile 14ml tube and dilute the semen sample with equal volume of sperm wash medium and gently mix then Centrifuge at 1600 rpm for 7 minute for moderate sample, if above moderate sample then the rpm can reduce to 1500 for 5 mins , for below moderate and viscous sample 2000 rpm for 10 mins..Discard the supernatant leaving the pellet mix it with 0.5 ml seminal plasma or sperm wash medium. Take 0.8 ml or 0.7ml of freezing medium and bring it to room temperature after that add drop wise of sperm freezing media to the sample pellet and gently mix. Transfer it to a cryovial. Place cryovial in clips on metal canes and immediately expose to liquid nitrogen vapour 2-8°C for 5mins after that quicky transfer the vial to -80◦C (vapour phase) for a minimum of 30mins to a maximum of 1 hour and finally plunge the vial to liquid nitrogen. For severe oligo, occasionally motile or cryptozoospermia patients samples are advised to collect at least 6 times and freeze. All procedures should be carried out at room temperature in a non-heating stage.

Thawing Procedure

For thawing the frozen sample taken from the cryo-can .The frozen sample directly showed under running tap water. When the sample liquefies quickly process the sample based on the sperm count and motility. The sample is below moderate, if 2-3 samples frozen then pooled the sample and undergo simple sperm wash method for cryptozoospermia and below moderate sample single density gradient method was use.

Table 1: Modified cryopreservation protocol and sperm retrieval rate

No. of samples	Sperm retrieval rate for pellet mixed with media (%)	Sperm retrieval rate for pellet mixed with seminal plasma (%)
10	30	43
15	28	40
12	35	44
10	37	50
15	39	55
22	33	43
10	32	47

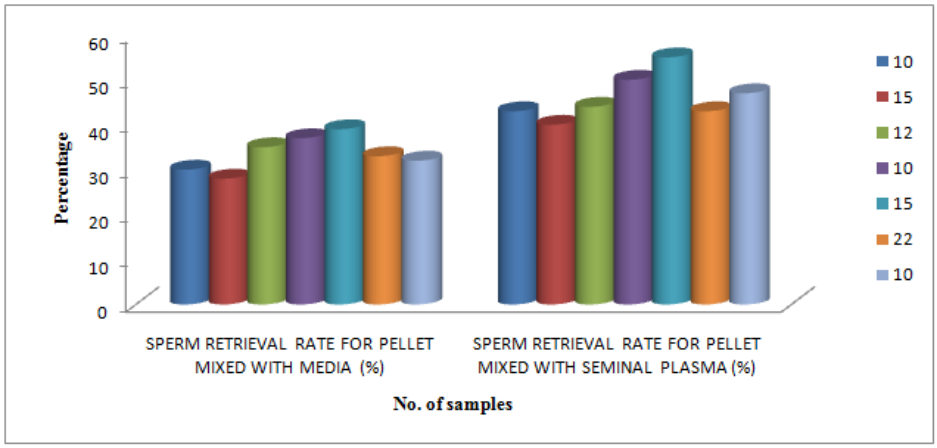

Fig. 1: Modified cryopreservation protocol and sperm retrieval rate

RESULTS AND DISCUSSION

In the present study 94 samples were taken and evaluated with two different cryopreservation protocol. One method was to mix the pellet with the media and the other was the pellet mixed with the seminal plasma. From the above two adopted protocols the pellet mixed with the seminal plasma showed 55- 40% (table1, Fig.1) of sperm retrieval after thawing which was higher than the percentage obtained by the other method [13-15]. The cryopservation of sperm pellet mix with seminal plasma method assess the feasibility and efficiency of various low sperm count freezing methodologies so this method economical cheaper because it needed only minimum quantity of cryoprotectant.

CONCLUSION

Cryopreservation of spermatozoa is a valuable component in effective treatment and management of male infertility. Biological and non-biological carriers have been tried for cryopreservation of low numbers of spermatozoa. Thawed spermatozoa were used for subsequent ICSI. It is indeed to improve on the quality of care provided to that subset of patients a novel cryopreservation technology specifically designed to handle small numbers and quantities of sperm needs to be further explored. The current method of evidence is sufficient to support the use of one technology over the other.

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