IMPACT OF CRYOPRESERVATION ON SEMEN PARAMETERS OF HEALTHY DONORS IN AN ASSISTED REPRODUCTION PROGRAM

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SUMMARY

Semen parameters of 100 donor semens were analysed before and after cryopeservation. Cryopreservation was done with glycerol egg yolk cryopreservation medium using cryovials. A 49% reduction in count and 44% reduction in motility was noted. Post-thaw samples were then sujected to sperm wash by swim up technique and reanalysed. A 35% increase in motility with a post thaw recovery rate of 77% was noted. The pregnancy rate per cycle with post thaw donor insemination was 16% in our assisted reproduction program. Hence this study indicates that initial count and motility should be good for cryopreservation. Although there is a reduction in count and motility, it can be compensated by sperm wash technique.

INTRODUCTION

The increasing demand for artificial insemination by donor and the heightened concerns regarding sexually transmitted diseases related to this procedure, primarily the transmission of the acquired immunodeficiency syndrome virus (AIDS) has dramatically increased the interest in cryopreservation of sperm over the past few years.

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AIM

(1) To evaluate the effect of cryopreservation and thawing on semen parameters

(2) To note the quality of cryopreserved spermatozoa after swim up technique.

MATERIALS & METHODS

This prospective study was carried out at Manipal Assisted Reproduction Centre between January and June 1995. Hundred semen samples from healthy donors were analysed. Sperms were evaluated at three different time points - (1) Before cryopreservation, (2) After Cryopreservation and thawing and (3) 30 minutes after final preparation of sperm just before insemination. Donor samples with total count > 40 million / ml and motility > 40% were taken into the study.

Crypreservation was done with glycerol egg yolk cryopreservation medium using cryovials. These cryovials containing the semen sample and cryoprotectant in equal amounts were refrigerated at 4 degree C for 2 hours and then exposed to liquid nitrogen vapours for 5 minutes and finally immersed in liquid nitrogen (-196 degree C). When required, these samples were thawed at room temperature, 1/2 hour before insemination and semen parameters were analysed. These semen samples were then subjected to sperm wash by swim up technique. About 1 - 1.5 ml of semen is added into 2 tubes containing 3 ml of sperm wash medium. This is centrifuged for 10 minutes at 351 g and the supernatant fluid is discarded. Now the pellet is resuspended in 2 ml sperm wash medium and centrifuged again for 10 minutes. The supernatant is discarded and 0.5 to 1 ml of sperm wash medium is carefully layered over the pellet and incubated for an hour in loosely capped tubes. The supernatant is collected and pooled from both tubes and the analysis repeated before insemination.

Pregnancy outcome was used as the measure of success for patients in this study. Pregnancies were determined by at least one postitive BhCG determination and was confirmed by the presence of fetal motion by endovaginal ultrasound detection.

OBSERVATION AND RESULTS

Table I shows the semen parameters before and after cryopreservation. There was a 49% reduction in count and 44% reduction in motility after freezing and thawing process. The motility yield (post-thaw motility / prethaw motility) reached an average of 56%. The average duration of cryopreservation was 58 days (range 11 to 124 days). There was no statistical difference between the duration of pres-

Table I
SEMEN PARAMETERS AND CRYOPRESERVATION OUTCOME

Semen parameters	Count (mill./ml)		Motility %	
	Average	Range	Average	Range
Before Cryopreservation	66	40-95	60	44-71
After Cryopreservation	34	10-58	34	7-57

ervations and semen parameters (correlation coefficient - 0011). After sperm washing, there was a 35% increase in motility. The postwash recovery rate was 77%. The pregnancy rate per cycle with post-thaw donor insemination was 16%.

DISCUSSION

In this prospective study, we have examined the influence of freezing-thawing and processing on semen parameters and the subsequent fecundity of cryopreserved sperm cells. We have demonstrated a substantial reduction in sperm motility after freezing-thawing. Semen cryopreservation without a protective media results in severe damage to sperm, especially to the acrosome. Cryoprotectant are added to protect cells from drying and intracellular ice crystal formation and proteins from thermal destruction.

The most critical time for forming ice crystals is from 0 to 10 degree C. Check et al 1994 got better post thaw semen parameters by reducing the exposure time of 0 to - 10 degree C temperature range. Henry et al 1993 found maximum sperm survival at a cooling rate of 10 degree C/mt. The general consensus is that rapid freezing at room temperature is the best method. Bryd et al 1994 showed that quick thawing in 5 minutes at 37 degree C increased motility by 31% compared to slow thawing at 4 degree C over 30 minutes. Davis R O et al 1995 have suggested that freezing with a computerized staged system in the refrigerant stage and then storage in the vapours of liquid nitrogen might be advantageous in terms of improved sperm penetration assay with thawed specimen, but to date increased pregnancy rates have not been demonstrated.

Pregnancy rates with fresh and frozen sperm appears to be quite similar, perhaps becuase of improvements in ovulation detection and induction as well as use of the minimum number of active sperm for each insemination (40 millions/ml and 40% motility). The fecundability is 20% in women with no signs of impaired fertility in the 20-25 years age group. But in women with a diagnosis of impaired fertility, eg. endometriosis, tubal factors, pelvic adhesions, or significant ovulatory dysfunction, the fecundability drops to 5%. In our series, we had a pregnancy rate per cycle of 16% and this compares very favourably with our previous experience with fresh semen.

Although further studies are needed to improve pregnancy rate after therapeutic donor insemination, we conclude that intrauterine insemination with sperm prepared by sperm wash technique is a simple, cost effective means to prepare sperm. The success and outcome of this process can be influenced by a number of different variables that remained to be examined.

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