Cryopreservation or low temperature(-196°C) storage of spermatozoa is believed to result in considerable impairment of post that sperm quality in terms of motility and viability when compared to fresh ejaculates, the condition led mainly by detrimental effect of freezing on the acrosomal structure and acrosin activity. Since optimum sperm motility correlates with improved fertilisation capacity and subsequent pregnancy rate, the role of cryopreservation in the respect is highly controversial. Numerous investigations are being carried out worldwide towards improving the existing condition in alleviating the afore-mentioned shortcoming of sperm cryopreservation in general. But no rational approach has till date been adopted to find out the merits and demerits of the technique with respect to various categories of semen samples eg, oligozoospermic, asthenozoospermic and normozoospermic sample produced. Keeping this objective in mind a study was conducted to explore the factors of cryopreservation that will maximise the rates of conception emphasising mainly in the class specific response, if any, of the samples treated.

The advent of assisted reproductive technique (ART) demands a continued role for the use of donor sperm in the management of fertility secondary to uncorrectable seminopathies on the basis of cost effectiveness and availability of the technology and the recognised risk of infectious disease transmission associated with therapeutic donor insemination, especially human immunodeficiency virus (HIV) and hepatitis B virus have made the use of quarantined semen mandatory in all facets of ART. A recent case study also reports that a donor sample transmitting HIV-1 infection when fresh after being frozen for 1-4 months did not infect the recipients due probably to a decline in the viral titre by cryopreservation. In case of homogenous insemination i.e., insemination with husband’s sperm also cryopreserved turns out to be very useful for absentee husbands, those facing problems in producing sample when they are enrolled in an ART programme, those having premature ejaculation or when the husband is expected to undergo chemotherapy or radiation treatment. Anonymity of donor is also better managed with frozen sperm. Thus the primary requisite of an ideal fertility clinic appears to be establishing and maintaining proper sperm bank which was the ultimate aim of the present study.

MATERIAL AND METHOD
Ejaculates of 90 samples were collected from screened donors as well as husbands (undergoing treatment for male factor subfertility) by masturbation in sterile graduated containers after a fixed period of 3 days' abstinence. The samples after liquefaction (complete in most cases by 30 minutes) were mixed thoroughly and routine semen analysis was performed in each with a drop of well mixed semen according to common practice following World Health Organisation (WHO) guidelines.5

Depending on seminal parameters the samples were classified into different (viz, 3) categories. Next each sample was divided into two portions and part, depending on its sperm density, was diluted 1:1 or 2:1 with an appropriate amount of 13.5% glycerol-egg-yolk-citrate buffer cryopreservation medium (CPM) at pH7.4. The mixture was next stored in 1.8 ml sterile autoclaved screw top cryovials labeled properly before storage in liquid nitrogen (-196°C) by the vapour-freeze technique6 ie, by holding it in liquid nitrogen vapour for 30 minutes and then plunging into liquid nitrogen. The other half of each sample was rendered free of seminal plasma by washing thoroughly with ham F-10 (HF-10) medium and centrifuging at 1000rpm twice and the pellet formed was resuspended in a small volume of the same medium and stored at 37°C in the incubator. After one hour postwash sperm recovery was examined under light microscope magnifying 400 times.

For thawing purpose the frozen samples were taken out of liquid nitrogen after a period of 6 months and put into water bath at 37°C for 10 minutes. They were mixed and washed free of CPM by double centrifugation at 1000 rpm with HF-107. Postwash sperm recovery was determined in these frozen thawed samples suspended in HF-10 medium and incubated at 37°C for one hour in an exactly similar way as in case of fresh samples. A total number of 90 samples (30 in each group) were analysed during the course of this study and the same procedure was strictly followed in all the cases.

OBSERVATIONS AND DISCUSSION

Depending on sperm density, motility and morphology the semen samples were classified into three main groups eg, oligozoospermic, normozoopermic and asthenozoospermic categories. Post –thaw survival rate, percent motility and viability of cryosamples were assessed with respect to the values obtained from fresh semen. It has been observed that survival of spermatozoa is greatly influenced by the conditions they are exposed to during thawing eg, temperature, time and also the quality of the cryocontainer used, etc; the rate being optimum when thawing was done at 37°C for 10 minutes. A significant reduction in the rate was observed in cases where cryovials used contained rubber.

Regarding motility cryosamples belonging to oligozoospermic group did not produce any noticeable change as compared to the fresh ones, the negligible increase and/or decrease being in the range 4-6.7%. Asthenozoospermic samples, on the other hand, exhibited marked increase in motility in all the cases irrespective of the count, the change being over a wide range of 15-50% with an average of 27.5%. Normozoospermic samples, however, showed two distinct types of reaction depending on their total count. In samples with less than 275x10^6 spermatozoa post-thaw motility reduced on an average by 25% over a range of 18-30% whereas in those with more than 275x10^6 cells motility enhanced by 13-32%, average being 19%. In case of viability also normozoospermic samples exhibited exactly similar count oriented change pattern.
In any intra-uterine insemination (IUI) cycle other factors like selection of patients, follicular maturation monitoring, identification of ovulation window, etc, remaining favourable, the rate of pregnancy depends to a great extent on the number of total motile sperm inseminated. The above results therefore clearly indicate that cryopreservation can be strongly recommended for asthenozoospermic samples in general and for normozoospermic samples with total count more than \(275 \times 10^6\) provided freezing thawing conditions are optimal. In addition cryopreservation in spite of having some deleterious effect on spermatozoa can be effectively utilized for manipulation in certain cases such as high volume oligozoospermia. For this purpose the semen can be concentrated before freezing by centrifugation followed by discarding most of the supernatant seminal plasma leaving behind a small volume to resuspend the pellet. Moreover in case of any indication that the seminal plasma is detrimental to the sperm cells the pellet can be frozen in donor seminal plasma.

So it may be concluded that semen cryopreservation possesses definite beneficial role in the management of infertility provided the cases to be treated are selected properly keeping in mind the potentials as well as the limitations of the technique. All the patients with true positive peritoneal lavage had intra-abdominal injuries seen at laparotomy. The complication of diagnostic peritoneal lavage (DPL) was nil.

**DISCUSSION**

Blunt abdominal trauma is increasing day by day in our life. In this study maximum patients involved were in the 2\(^{nd}\) and 3\(^{rd}\) decades. Diagnostic error may lead to unnecessary laparotomy and prolonged hospital stay. Abdominal paracentesis was used as a diagnostic aid, but validity and limitation of the technique has been well established. Only 78% of the taps were positive in presence of 500ml intraperitoneal blood\(^2\). No positive tap could be obtained if less than 200ml were present\(^2\), but it is easy to perform, safe and rapid. So it should be carried out in all patients of blunt abdominal trauma. In this study 13 out of 30 taps were positive, 12 were true positive and was false positive. Morton and Hinshaw\(^3\) reported 57-75% positive taps. Byrne\(^4\) reported 64% positive taps. Peritoneal lavage is an accurate technique for diagnosis of abdominal visceral injury. In this study 37 patients in which lavage was done 22 showed true positive results. Caffee and Benfield\(^5\) and That and Shives\(^6\), reported the accuracy of test from 90.2% to 98.3%. All cases with true positive lavage had intra-abdominal injuries. False positive observations by Root et al\(^7\) varied from 0-6%, and false negative results varied from 0-5%.

It is concluded from this study that four-quadrant abdominal paracentesis should be carried out in all patients with blunt abdominal trauma because it is safe, easy and quick method of detecting haemoperitoneum, although diagnostic accuracy of this procedure was less; so DPL should be carried out in all stable patients with abdominal trauma with negative taps and patients who had associated head injury, alcoholic intoxication, and in children where it is difficult to assess the extent of intra-abdominal injuries. The diagnostic accuracy of DPL was very high.

So it recommended that four-quadrant abdominal paracentesis and DPL should be carried out as a routine investigative procedure in all stable cases of blunt abdominal trauma where physical
examination is equivocal due to any reason, as it saves time, avoids unnecessary laparotomy and hospital stay.

REFERENCES