# Effect of fresh seminal plasma on cryopreserved thawed sperm on motility / HOST

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**abstract**

**Some poorly motile specimens may significantly improve their motility after suspension in seminal plasma, as compared to supporting media. Freezing the seminal plasma significantly reduced its efficacy in improving motility. It is possible that some portion of the reduction of sperm motility following cryopreservation / thawing may be related not only to ice crystal formation, but possibly due to damage to some motility enhancing factor in seminal plasma. the present study presented was designed to evaluate initial sperm count / motility following therapy and then repeating these parameters after addition of either human seminal plasma (HSP) vs human tubal fluid (HTF) to equal aliquots. The mean motility of the initial thawed specimens was 15.7% and was 15.8% with HSP and 12.9% with HTF(paired t-test HSP vs HTF found $p<.05$). Motile density (MD) was significantly lower following addition of HTF ($2.3 \times 10^6$/mL) with $p<.05$. The study was performed in duplicate. The initial specimen was divided into 2 portions before freezing of both samples. Repeated tests on the second sample was 17.8% following HSP vs 14.7% with HTF($p<.05$). However, the MD was not significantly different (2.8 vs $2.5 \times 10^6$/mL). Once again the percent motility did not improve with HSP vs initial thawed specimens. Some specimens improved motility by >50% following HSP with 26%(n=29) as did 22% with HTF(n--25). Motility dropped more than 50% in 18 cases(16%) following HSP compared to 21 (18.6% with HTF). The motility scores were classified into 6 categories (0-10, 11-20, 21-30, 31-40, 41-50, and >50). 21% of the samples increased to a higher category with HSP(21%) vs 14(13%) with HTF(chi-square analysis not significant). Though there may be some advantage of HSP for preserving motility versus HTF, the HSP did not significantly improve the motility of poorly motile sperm following freeze-thawing is probably more likely related to sperm damage by ice crystal formation than to the negative effect of freezing on some motility enhancing factor in seminal plasma.**

## Introduction

There is a significant reduction in sperm motility after cryopreservation / subsequent thawing of human sperm(Crister et al., 1987;Keel et al., 1980) This damage has been attributed to ice crystal formation with damage to the sperm membrane(Wooley et al., 1978; Mahadervan et al., 1984).

The improvement in sperm motility of some asthenozoospermic specimens both from men with retrograde and antegrade ejaculates could be achieved by suspending the sperm in human seminal plasma obtained from men with very good motility(Check, JH et al., 1990; Check, DJ et al., 1991). In several instances the improvement far exceeds that by suspension in supporting media e.g. protein-supplemented Ham's F-10 media or human tubal fluid, it is possible that the seminal plasma contain some motility enhancing factor that is not present in artificial media. However, upon freezing seminal plasma, the motility enhancing effect was lost(Check et al., 1991).

When whole semen is cryopreserved / thawed, some motility enhancing factor in the seminal plasma is damaged, and possibly not all of the motility reduction is related to ice crystal damage. Alternatively, cryo-damage from some sperm may release reactive oxygen species(ROS) and these may adversely effect other sperm unless counteracted by seminal plasma scavengers. Thus it is possible that improvement of motility may occur if fresh seminal plasma is added to the thawed specimen.

The present study, the effect of human seminal plasma on both sperm motility functional integrity of the sperm membrane, was evaluated as measured by the hypo-osmotic swelling test(HOST).

## Material / Method

### 1. Experimental Design

Baseline semen analysis was performed on 112 specimens to measure sperm count, motility, motile density, and volume. the semen was divided into 2 aliquots: one half were frozen using the conventional method, the other was frozen using the rapid technique. The specimens were thawed 2 to 4 weeks later. Semen analysis(including HOST) were repeated at the time of thawing. Results were then compared(only sperm showing progressive forward motion were considered normal).

### 2. Cryopreservation / Thawing: Standard Technique

The ejaculate was placed in a 20-mL conical tube and mixed with an equal proportion of test yolk buffer(TYB) as the cryoprotectant. TYB contains sodium citrate, fructose, penicillin-G, and the active ingredient, glycerol(which comprises 12% of the whole solution). The semen and TYB were pipetted together, slowly t(to reduce bubble formation), for 4 minutes. After this time, the conical tube was capped and placed in a 500-mL water bath at $37^0$C, than placed into a freezer set at $10^0$C. The specimen in the water bath remained in the freezer for 60 minutes to reduce the specimen temperature(on average) to $12^0$C. The conical tubes were removed after the appropriate time period from the freezer and quickly placed under a sterile hood. The contents of the tubes were then pipetted into 1-mL Nunc vials. The Nunc vials were than placed into liquid nitrogen vapors(-$100^0$C, 2 in. from the actual liquid), for 90 minutes. At the end of this period, the Nunc vials were separated into canes, then plunged into the liquid nitrogen, at which time the specimen were cryopreserved.

3. **Cryopreservation / Thawing-Rapid technique**
The modifications of the standard technique were as follows:
a) The test yolk buffer was added all at once(vs one drop at a time with the standard technique).
b) Placing the specimens in a 70% isopropyl alcohol bath in a -20$^0$C freezer for only 21 minutes with the new vs a water bath in a 4$^0$C refrigerator for 90 minutes with standard.
c) A reduced time in the liquid nitrogen vapors with only 12 minutes exposure with the new method vs 1 hour with the standard technique.

4. **Preparation of human seminal plasma**
The human seminal plasma was prepared by centrifugation at high speed from volunteers' specimens which demonstrated at least 70
5 progressively motile sperm. The supernatant was decanted from the sperm pellet and checked for the absence of sperm. The seminal plasma was then added 1:1 to the frozen-thawed specimen and the sperm count, motility, and HOST were then performed.

## Results

The effects of HSP on HTF on semen parameters of cryopreserved sperm frozen by the conventional procedure is seen in Table 1. Using the paired t-test there was a statistically significant difference between HSP vs HTF($P<.05$) in % motility, but no difference compared to the post-thaw specimen. Similarly, the only statistical difference with the sperm frozen-thawed with the rapid procedure(Table 2) was the difference between % motility after HSP(17.8%) vs HTF(14.7%)($P<.05$).

HSP improved the % motility in individual cases by .50% more often than HTF, but also slightly decreased individual cases by >50% more often than HTF by 50%(Table 3). the motility was divided into 6 categories 0-10%, 21-30, 31-40, 41-51, and .50%. HSP increased the percent motility to a higher category in 24 cases(21%) compared to only 14(13%) with HTF(but chi-square analysis not significant).

## Discussion

Extensive investigations have shown that fertility rate is reduced when using cryopreserved donor sperm rather than fresh. there is a monthly fecundity rate of 20% with fresh sperm despite only an 8% pregnancy rate with frozen(Smith et al., 1981). Significant differences were also found between fresh semen(27%) and frozen semen(10%)(Richter et al.,1984; Brown et al., 1988). The results are even worse when using cryopreserved sperm from men prior to vasectomy or testicular surgery or cancer chemo or radiation therapy. There is only 1 pregnancy in 2 such couples inseminated with cryopreserved-thawed sperm(friedman et al., 1981). Even when motility is preserved, membrane damage as evidenced by reduced HOST are frequently found(Check ML et al., 1991;Check ML et al., 1991)

Though there have been many attempts to try to reduce ice crystal damage by alterations in supporting medias or change in cryoprotectant there has been little success in improving sperm quality. Based on previous data showing loss of some seminal plasma motility enhancing factor after freezing, at least part of the poor quality post-thaw motility may be related to seminal

**Table 1** Effect of human seminal plasma vs human tubal fluid on semen parameters of sperm cryopreserved using standard techniques. (n = 112)

| Parameters | Count | %Motility | Motile density |
|---|---|---|---|
| Initial pre-freeze | 53.7 | 70.0 | 38.7 |
| Baseline Post-thaw | 19.6 | 15.7 | 3.08 |
| Addition of HSP | 20.2 | 15.8 | 3.2 |
| Addition of HTF | 17.8 | 12.9 | 2.3 |

**Table 2** Effect of human seminal plasma vs human tubal fluid on semen parameters of sperm cryopreserved using rapid freezing techniques. (N = 112)

| Parameters | Count | %Motility | Motile density |
|---|---|---|---|
| Initial pre-freeze | 53.7 | 70.0 | 38.7 |
| Baseline post-thaw | 15.1 | 15.7 | 2.4 |
| Addition of HSP | 15.8 | 17.8 | 2.8 |
| Addition of HTF | 17.0 | 14.7 | 2.5 |

**Table 3** Comparison of human seminal plasma vs human tubal fluid on increasing or decreasing the baseline post-thaw specimen by 50% (N = 112)

| Parameter | HSP | HTF |
|---|---|---|
| Improved by >50% | 29(26%) | 25(22.1%) |
| Decreased by >50% | 18(16%) | 21(18.6%) |

plasma damage. Since no significant improvement was realized after fresh seminal plasma was added, intrinsic damage to the sperm, probably through ice crystal formation, accounts for most of the asthenozoospermia after thawing.

Not all seminal plasma samples have this motility enhancing effect. it would have been best to use a non-frozen asthenozooaspermic sample as a control to be sure the fresh seminal plasma could improve motility. Unfortunately, there would not have been enough seminal plasma to use this control unless a mini-method is developed.

A small but significant improved motility was seen with thawed sperm suspended in seminal plasma rather than HTF. However, pregnancy rate is improved after intrauterine insemination(IUI)(Silva et al., 1989) Fresh Donor sperm is usually placed intracervically at the time of a mature follicle but before oocyte release. This timing is based on the fact that cervical mucus may regress very quickly as one approaches ovulation, and the sperm is considered capable of fertilizing the oocyte for at least 48 h. It is recommended to be a little early than late with intracervical insemination, so one does not chance missing the oocyte. Women with cervical factor(and showing no sperm with forward progressive motion)achieved a pregnancy rate of 10% with intracervical insemination(ICI). The ICI is timed closer to intercourse rather than 36-40 h after the LH surge as IUI was timed(Check, JH et al., 1991).

Because of the presence of prostaglandins in the human semen. IUI cannot be performed if the sperm is mixed with seminal plasma. If IUI is necessary to maximize fertility rates, then the slight improvement with fresh seminal plasma is merely academic. It is possible that the benefit of IUI is more related to the timing rather than the placement in the uterine cavity.

The risk of acquiring HIV infection following intracervical insemination of sperm from an HIV infected donor is 0.3% per insemination(Chiasson et al., 1990). The American Fertility Society / the Center for Disease Control strongly recommend the use of frozen donor sperm, to allow a 6 month quarantine period. Despite all of the safeguards taken by commercial laboratories a mistake can still be made, if inadvertently and intracervical insemination is performed from an HIV infected donor the risk would be less than 3/1000 to become HIV positive. However, bypassing the cervix and placing the sperm in the uterus, and possibly exposure of blood contact, may increase the risk of HIV infection.

It is hoped that improved cryopreservation procedure may inhibit ice crystal damage. Perhaps then damage to a seminal plasma motility enhancing factor will still inhibit maximum motility and perhaps human seminal plasma may be needed. The use of fresh seminal plasma did not significantly improve motile densities of cryopreserved-thawed sperm, possibly because of an overwhelming extent of damage by ice crystal formation. Nevertheless, the data was still consistent with some damage also to a motility enhancing seminal plasma protein.

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