

FAILURE OF THE ADDITION OF FRESH SEMINAL PLASMA TO CRYOPRESERVED-THAWED SPERM TO IMPROVE SEMEN PARAMETERS

D. J. CHECK, M. L. CHECK, A. BOLLENDORF, and J. H. CHECK

Previous data has shown that subnormal motility in some semen specimens can be improved by the addition of fresh human seminal plasma (HSP). However, if the HSP was first frozen the motility-enhancing factor was lost. We hypothesized that some of the reduction in sperm motility of cryopreserved-thawed sperm may be related to damage of the "motility-enhancing factor" of HSP. This study evaluated whether the addition of fresh HSP could improve the motility of frozen-thawed sperm. Each frozen-thawed specimen was evaluated for motile density and hypoosmotic swelling and then divided into two aliquots. Equal volumes of HSP, human tubal fluid (HTF), and control media were added and the semen parameters were reevaluated. The mean scores for motile density and percent motility did not change compared with baseline thawed volumes with either HSP or HTF additives. There were some isolated cases that did improve with either HSP (21%) or HTF (14%). Future studies are needed to determine whether this improvement is coincidental or consistent, and to determine whether at least some individuals can benefit from the addition of fresh HSP to frozen-thawed sperm.

Key Words: Semen; Seminal plasma; In vitro fertilization; Cryopreservation.

INTRODUCTION

There is a significant reduction in motility after thawing of cryopreserved human sperm [8, 10]. This has been attributed to ice crystal formation damaging the sperm membrane [11, 15]. Improvement in sperm motility of some asthenozoospermic specimens from men with both retrograde and antegrade ejaculates has been reported by suspending the sperm in human seminal plasma (HSP) obtained from men with very good motility [2, 3]. In several instances the improvement far exceeds that by suspension in a supporting media, *eg.* protein-supplemented Ham's F-10 media or human tubal fluid (HTF) [2]. It is possible that the HSP contains some motility-enhancing factor that is not present in artificial media. However, upon freezing HSP the motility-enhancing effect was lost [2].

This study evaluates the effect of adding fresh HSP to frozen-thawed sperm to determine whether any improvement in motility can be noted.

From the University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, New Jersey, USA.

Address correspondence to Jerome H. Check, 8002 E. Greentree Commons, Marlton, NJ 08053, USA.

MATERIALS AND METHODS

Experimental Design. Baseline semen analyses were performed on 112 specimens to measure sperm count, motility, motile density, and volume. The semen was divided into two aliquots: one was frozen using the conventional methods, the other using the rapid technique. The specimens were thawed 2 to 4 weeks later, and the semen analyses were repeated. Results were then compared (only sperm showing progressive forward motion were considered normal).

Cryopreservation and 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. The semen and TYB were pipetted together slowly (to reduce bubble formation) for 4 min. The conical tube was then capped and placed in a 500-mL water bath at 37°C, and placed into a freezer set at 10°C. The specimen remained in the freezer for 60 min to reduce the specimen temperature (on average) to 12°C. After the appropriate time period, the conical tubes were removed 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 then placed into liquid nitrogen vapors (-100°C, 2 in from the actual liquid) for 90 min. At the end of this period, the vials were separated into canes, then plunged into the liquid nitrogen, at which time the specimens were cryopreserved.

Cryopreservation and Thawing: Rapid Technique. The modifications of the standard technique were as follows:

- 1) The TYB was added all at once (vs one drop at a time);
- 2) The specimens were placed in a 70% isopropyl alcohol bath in a freezer at -20°C for only 21 min (vs a water bath in a 4°C refrigerator for 90 min); and
- 3) A reduced time in the liquid nitrogen vapors with only 12-min exposure (vs 1 h).

Preparation of Human Seminal Plasma. The HSP from volunteers' specimens demonstrating at least 70% progressive forward motility was prepared by centrifugation at high speed. The supernatant was decanted from the sperm pellet and checked for the absence of sperm. The seminal plasma was then added at a ratio of 1:1 to the frozen-thawed specimen.

RESULTS

The effects of HSP versus HTF on the semen parameters of cryopreserved sperm frozen by the standard procedure is shown in Table 1. In the paired *t*-test there was a statistically significant difference between HSP and HTF ($p < 0.05$) in percent motility, but no difference compared with the baseline postthawed specimen (paired *t*-test). Similarly, the only statistical difference with the

TABLE 1 Effect of Human Seminal Plasma (HSP) vs Human Tubal Fluid (HTF) on Semen Parameters of Sperm Cryopreserved Using Standard Techniques

Parameter	Count (n)	Percent Motility	Motile Density
Initial prefreeze	54	70	39
Baseline postthaw	19.6	15.7	3.08
Addition of HSP	20.2	15.8	3.2
Addition of HTF	17.8	12.9	2.3

Note. $n = 112$.

TABLE 2 Effect of Human Seminal Plasma (HSP) vs Human Tubal Fluid (HTF) on Semen Parameters of Sperm Cryopreserved Using Rapid Freezing Techniques

Parameter	Count (n)	Percent Motility	Motile Density
Initial prefreeze	54	70	39
Baseline postthaw	15.1	15.7	2.4
Addition of HSP	15.8	17.8	2.8
Addition of HTF	17.0	14.7	2.5

Note. $n = 112$.

sperm frozen-thawed with the rapid procedure (Table 2) was the difference between percent motility after HSP (17.8%) vs that after HTF (14.7%) ($p < 0.05$).

Human seminal plasma improved the percent motility in individual cases by 50% more often than did HTF, but also slightly decreased in individual cases by more than 50% more often than HTF (Table 3). Motility was divided into six categories: 0%–10%, 11%–20%, 21%–30%, 31%–40%, 41%–50%, and greater than 50%. HSP increased the percent motility to a higher category in 24 cases (21%) compared with only 14 cases (13%) with HTF (chi-square analysis was not significant).

DISCUSSION

Extensive investigations have shown that the fertility rate is reduced when using cryopreserved donor sperm rather than fresh specimens. There was a monthly fecundity rate of 20% with fresh sperm despite only an 8% pregnancy rate with frozen sperm in one report [14]. Significant differences were also found between fresh (27%) and frozen semen (10%) in other studies [1, 12]. The results are even worse when using cryopreserved sperm from men prior to vasectomy or testicular surgery, or chemotherapy or radiation therapy for cancer: only one pregnancy was recorded in two such couples inseminated with cryopreserved-thawed sperm without the use of assisted reproductive technology [9]. Even when motility is preserved, membrane damage as evidenced by reduced hypoosmotic swelling test scores is frequently found [5, 6].

Although there have been many attempts to try to reduce ice crystal damage by alterations in supporting media or cryoprotectant, there has been little success in improving sperm quality. Based on previous data showing loss of some HSP motility-enhancing factor after freezing, at least part of the poor-quality postthaw motility may be related to HSP damage. Since no significant improvement was realized after fresh HSP was added, intrinsic damage to the sperm, probably through ice crystal formation, accounts for most of the asthenozoospermia after thawing.

TABLE 3 Comparison of Human Seminal Plasma (HSP) vs Human Tubal Fluid (HTF) on Increasing or Decreasing the Baseline Postthaw Specimen by 50%

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

Note. $n = 112$.

Not all HSP samples have this motility-enhancing effect; it would have been best to use a nonfrozen asthenozoospermic 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 minimethod were developed.

Because of the presence of prostaglandins in human semen, intrauterine insemination (IUI) cannot be performed if the sperm is mixed with HSP. If IUI is necessary to maximize fertility rates, as is suggested by Silva et al. [13], then the slight improvement with fresh HSP is merely academic. However, it is possible that the benefit of IUI is more related to the timing rather than the placement in the uterine cavity [4].

The risk of acquiring human immunodeficiency virus (HIV) infection following intracervical insemination of sperm from an HIV-infected donor is 0.3% per insemination [7]. The American Fertility Society and the Centers for Disease Control strongly recommend a 6-month quarantine period when using frozen donor sperm. Despite all of the safeguards taken by commercial laboratories, a mistake can still be made. If this is the case, and intracervical insemination is performed from an HIV-infected donor, the risk would be less than three in 1000 to become HIV positive. However, bypassing the cervix (and possibly the exposure of blood contact) by placing the sperm in the uterus, may increase the risk of HIV infection.

It is hoped that improved cryopreservation procedures may inhibit ice crystal damage. Perhaps then damage to the HSP motility-enhancing factor will still inhibit maximum motility and additional HSP may be needed. It is possible that freeze-thawing releases harmful reactive oxygen species and there may be natural antioxidants in seminal plasma that may neutralize the adverse effect of oxidants that are not present in artificial supporting media.

REFERENCES

1. Brown CA, Boone WR, Shapiro SS (1988): Improved cryopreserved semen fecundability in an altering fresh-frozen artificial insemination program. *Fertil Steril* 50:825-827
2. Check DJ, Check JH, Bollendorf A (1991): Fresh versus frozen seminal plasma for enhancing sperm motility in asthenozoospermic males. *Arch Androl* 25:79-81
3. Check JH, Bollendorf A (1990): Improved motility of retrograde ejaculates by adding donor seminal plasma. *Arch Androl* 25:141-143
4. Check JH, Chase JS, Spirito P (1991): Efficacy of intrauterine insemination versus sexual relations versus intracervical insemination for treatment of cervical factor infertility. *Am J Obstet Gynecol* 5:11-17
5. Check ML, Check JH, Long R (1991): Detrimental effects of cryopreservation on the structural and functional integrity of the sperm membrane. *Arch Androl* 27:155-160
6. Check ML, Check JH (1991): Poor hypo-osmotic swelling test results from cryopreserved sperm despite preservation of sperm motility. *Arch Androl* 26:37-41
7. Chiasson MA, Stoneburner RL, Joseph SC (1990): Human immunodeficiency virus transmission through artificial insemination. *J Acquir Immune Defic Syndr* 3:69-72
8. Crister JK, Huse-Benda AR, Aaker DV, Arneson BW, Ball GD (1987): Cryopreservation of human spermatozoa: I. Effects of holding procedure and seeding on motility, fertility, and acrosome reaction. *Fertil Steril* 47:656-663
9. Friedman S, Broder S (1981): Analogous artificial insemination after long-term semen cryopreservation. *Fertil Steril* 35:321-324
10. Keel BA, Black JB (1980): Reduced motility longevity in thawed human spermatozoa. *Arch Androl* 4:213-215
11. Mahadervan MM, Trounson AO (1984): relationship of fine structure of sperm head to fertility of frozen human semen. *Fertil Steril* 41:257-293
12. Richter MA, Haning RV Jr., Shapiro SS (1984): Artificial donor insemination: fresh vs frozen semen, the patient as her own control. *Fertil Steril* 41:277-280

13. Silva PD, Meisch J, Schauburger C (1989): Intrauterine insemination of cryopreserved donor semen. *Fertil Steril* 52:243-245
14. Smith KD, Rodriguez-Rigau LJ, Steinberger E (1981): The influence of ovulatory dysfunction and timing of insemination on the success of artificial insemination donor with fresh or cryopreserved semen. *Fertil Steril* 36:496-499
15. Woolley DM, Richardson DW (1978): Ultrastructural injury to human spermatozoa after freezing and thawing. *J Reprod Fertil* 53:389-394