

POOR HYPO-OSMOTIC SWELLING TEST RESULTS FROM CRYOPRESERVED SPERM DESPITE PRESERVATION OF SPERM MOTILITY

MATTHEW L. CHECK and JEROME H. CHECK

Cryopreservation/thawing of sperm leads to decreased motile density. Most donor programs select for cryopreservation a male with a high initial motile density and if the post-thaw semen has a motile density over $10 \times 10^6/\text{ml}$, the specimen is thought to represent a fertile specimen. Some recent data suggest that males with normal motile densities but subnormal hypo-osmotic swelling (HOS) tests may be infertile. A study was thus performed to see if males with an adequate motile density after cryopreservation may still demonstrate membrane damage as evidenced by decreased viability and a poor HOS test. The semen species from seven men with motile densities $\geq 10 \times 10^6/\text{ml}$ after freeze-thawing were evaluated for HOS changes and viability. Despite preservation of normal motile density, all 7 men had HOS scores below 50% following cryopreservation (though all were significantly above this level pre-freeze). The mean viability and HOS scores prefreezing were 70 ± 9.7 and 68.5 ± 9.5 , and post thaw they dropped to 33.7 ± 6.9 and 32.8 ± 6.2 . These data suggest a mechanism for impaired fertility even with adequate motile density of a thawed specimen that had been cryopreserved.

Key Words: Hypo-osmotic swelling test; Cryopreservation.

INTRODUCTION

Cryopreservation results in reduced motility and fertility of human semen [2, 13]. Freezing/thawing results in damage to the plasma membrane and acrosome of human spermatozoa as evidenced by significant ultrastructural changes demonstrated by electron microscopy [10, 15]. The most common method of assessing the presumed fertilizing capacity of frozen-thawed sperm is to evaluate motile density. Some recent data suggest that men with motile densities below the norms established by the World Health Organization (10×10^6 motile sperm/ml) do not demonstrate significant reduction in fertilizing capacity as long as the hypo-osmotic swelling (HOS) test is above 50% [4]. In fact, men with perfectly normal semen parameters but low HOS scores failed to fertilize [4, 5]. The dichotomy between normal parameters and low HOS scores was not common, occurring in only 6% of the cases studied.

Received 7/18/90; accepted 7/30/90.

From the University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department OB/GYN, Division of Reproductive Endocrinology and Infertility, Camden, NJ (J.H.C.).

Address reprint requests to: Jerome H. Check, M.D., 7447 Old York Road, Melrose Park, PA 19126.

Matthew L. Check was the 1990 Andrology Young Investigator Award Recipient (Camden, New Jersey, USA).

This study was designed to evaluate whether cryopreservation and subsequent thawing causes significant membrane damage as evidenced by low HOS scores and reduced viability even when the thawed specimen maintained a motile density above $10 \times 10^6/\text{ml}$. Furthermore, the study would determine if freeze-thawing causes a difference in the type of damage to sperm membrane; perhaps structural injury might not occur as evidenced by normal viabilities but problems with the functional integrity of the sperm membrane might be manifested by HOS scores under 50% [8].

MATERIALS AND METHODS

Seven males were selected from seven couples registering for infertility investigation. Only men with at least a motile density of 30×10^6 sperm per ml with progressive forward motion were chosen. HOS tests were performed as previously described [8]. Viability testing was performed using an eosin stain [1]. The cryopreservation procedure involved mixing the sperm with test yolk buffer in a 1 : 1 ratio. The buffer mixture was brought down gradually in temperature over a 5-h time period. The specimen was then thrust into liquid nitrogen. After 1-2 weeks the frozen sperm was allowed to thaw at room temperature. Motile densities, viabilities, and the HOS test were now repeated on the thawed specimens.

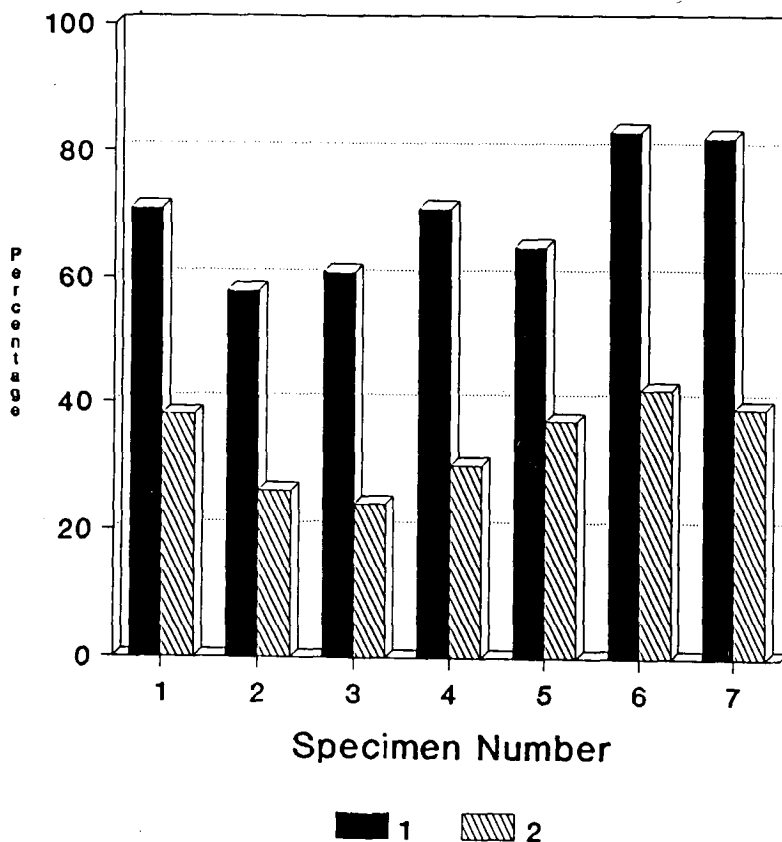
RESULTS

Each thawed specimen had a motile density above $10 \times 10^6/\text{ml}$ sperm with PFM. The results of pre- and post-thawed specimens for the viability and HOS test scores are seen in Figures 1 and 2, respectively. Though all started with viability and HOS tests above 50%, not one of the 7 specimens attained this level post thaw (Table 1). In fact, the highest post-thaw viability and HOS tests of 42% and 40% respectively were seen in patient number 6 whose prefreezing baseline percentages were 83% and 82% respectively.

The precryopreservation viability was 70 ± 9.7 and the mean HOS scores were 68.5 ± 9.5 . The mean viability after freeze/thaw was 34 ± 6.9 and the mean HOS scores were 33 ± 6.2 . Statistical analysis was performed using the two-tailed Student's *t*-test comparing pre- and post-freeze/thaw specimens for viability and HOS, with $p < .002$ and $p < .006$ respectively.

DISCUSSION

The results demonstrated that despite preserving reasonable motility post cryopreservation and thawing, the viability and HOS changes may drop significantly below fertilization potential. Whether the reduced viability and HOS scores portend poor pregnancy rates for the frozen-thawed specimens as in natural circumstances remains to be seen. More clinical trials are needed comparing therapeutic donor insemination (TDI) using frozen-thawed sperm manifesting poor viability, low HOS test scores, and fair to good motility with sperm manifesting comparable motility but with good viability and HOS scores. Though many groups have found reduced fertility rates following insemination of cryopreserved sperm [2, 9, 13], some reports have found TDI with cryopreserved sperm to be as effective as fresh sperm [7, 11]. The sperm used for testing in this study were from infertile patients as opposed to that used for TDI, which originate from known fertile donors. Perhaps even those demonstrating normal viability and HOS scores have subtle membrane damage impairing their longevity. This suggests that improved pregnancy rates might possibly be achieved by more precise intrauterine placement of the cryopreserved thawed specimen using precise timing by measuring LH levels. A report



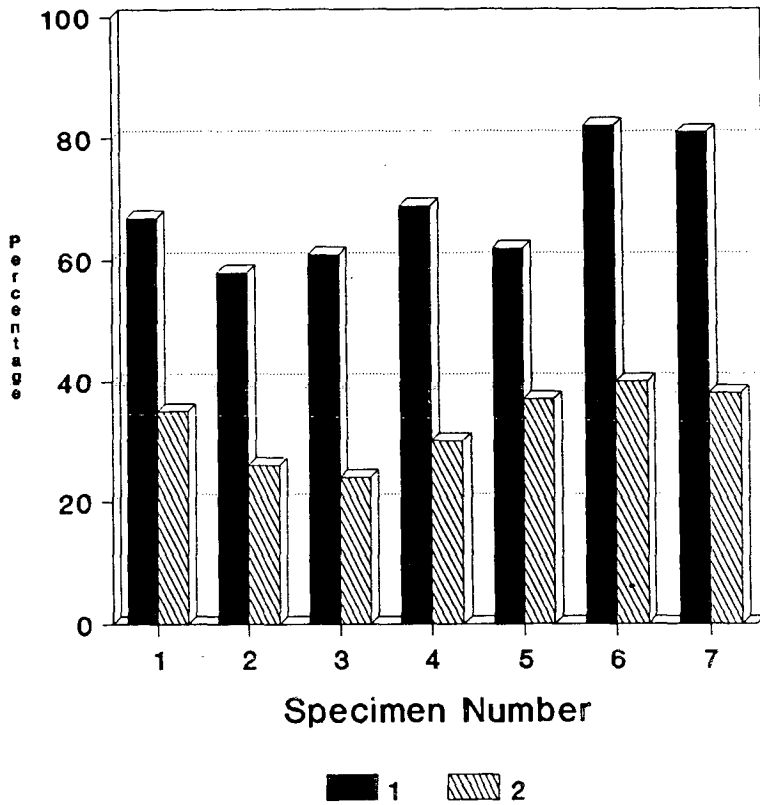
1 - Before freezing; 2 - After freezing

FIGURE 1 Comparison of viability testing of sperm both before and after cryopreservation and thawing.

by Silva et al. has suggested improved fertility rates after cryopreserved TDI using intrauterine insemination [14].

Since the extent of how low the viability and HOS scores drop appears to be a reflection of how high the level was to start, the data would suggest that in a donor program potential candidates be screened by these two tests as well as baseline motility density and morphology. In addition, these tests should be performed on the cryopreserved-thawed specimens to determine whether a potential donor candidate should be included in the donor program.

The importance of cryopreservation of sperm extends beyond its use to ensure less risk of infection during TDI by allowing a quarantine period. Cryopreservation of sperm is needed for young men prior to potentially sterilizing testicular surgery or chemotherapy or radiation therapy. Furthermore, a man undergoing vasectomy for contraception might want cryopreserved sperm in case he and his partner change their minds about having more children or he



1 = Before freezing; 2 = After freezing

FIGURE 2 Comparison of hypo-osmotic swelling test on semen both before and after cryopreservation and freezing.

TABLE 1 Results of Viability and HOS Scores Before and After Freezing

Donor Number	Viability		HOS Test	
	Before Freezing	After Freezing	Before Freezing	After Freezing
1	71%	38%	67%	35%
2	58%	26%	58%	26%
3	61%	24%	61%	24%
4	71%	30%	69%	30%
5	65%	37%	62%	37%
6	83%	42%	82%	40%
7	82%	39%	81%	38%

remarries; surgical vasovasostomy is not always successful. Unfortunately, 40×10^6 sperm/ml with 60% motility is considered a minimum level for successful cryopreservation (our data with poor viability and HOS tests does not make this level so secure) and yet there is data suggesting that in testicular cancer patients only 4% met these minimum requirements [3]. Indeed only 23% of men with testicular cancer or lymphoma post therapy had specimens considered potentially fertile compared with 60% of age-matched healthy men [12]. Finally, another study has suggested that only 1 of 21 couples conceived following insemination of previously cryopreserved specimens from a group of 475 men depositing semen either before elective vasectomy or therapeutic insemination [6]. These findings underscore the need to develop more successful methods of cryopreservation of semen rather than just looking for the superior specimen that can be frozen.

The data presented herein did not reveal any subtle evidence of functional impairment to the sperm membrane (as evidenced by subnormal HOS test scores) without causing structural damage to the membrane (as evidenced by poor viability). It is possible that with better specimens the viability may be normal but the HOS test may still be subnormal. For now it is still probably advisable to perform both tests.

REFERENCES

1. Adams JE, Compton MG (1985): Clinical laboratory diagnosis and treatment of infertility. Proc ASCP National Meeting
2. Beck NW Jr (1978): Artificial insemination and preservation of semen. Urol Clinics North Am 5:593-605
3. Bracken RB, Smith RD (1980): Is semen cryopreservation helpful in testicular cancer? Urology 15:581-583
4. Check JH, Epstein R, Nowroozi K, Shanis BS, Wu CH, Bollendorf A (1989): The hypo-osmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential. Fertil Steril 52:159-161
5. Check JH, Nowroozi K, Wu CH, Bollendorf A (1988): Correlation of semen analysis and hypo-osmotic swelling test with subsequent pregnancies. Arch Androl 20:257-260
6. Friedman S, Broder S (1981): Homologous artificial insemination after long-term semen cryopreservation. Fertil Steril 35:321-324
7. Gillett WR, Cameron MC, Maekay-Duff M, Seddon RJ (1986): Pregnancy rates with artificial insemination by donor: The influence of the cryopreservation method and coexistent infertility factors. New Zealand Med J 891-893
8. Jeyendran RS, Van der Ven HH, Perez-Palaez M, Carbo BG, Zaneveld LJD (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fertil 70:219-227
9. Lecton J, Selwood T, Trouson A, Wood C (1980): Artificial donor insemination: Frozen versus fresh semen. Aust NZJ Obstet Gynaecol 20:205-207
10. Mahadervan MM, Trouson AO (1984): Relationship of fine structure of sperm head to fertility of frozen human semen. Fertil Steril 41:257-293
11. Richter MA, Haning RV Jr, Shapiro SS (1984): Artificial donor insemination: Fresh versus frozen semen; the patient as her own control. Fertil Steril 41:277-280
12. Sanger WG, Armitage JO, Schmidt MA (1980): Feasibility of semen cryopreservation in patients with malignant disease. JAMA 244:789-790
13. Sherman JK (1973): Synopsis of the use of frozen human semen since 1964: State of the art of human semen banking. Fertil Steril 24:397-412
14. Silva PD, Meisch J, Schauburger CW (1989): Intrauterine insemination of cryopreserved donor semen. Fertil Steril 52:243-245
15. Woolley DM, Richardson DW (1978): Ultrastructural injury to human spermatozoa after freezing and thawing. J Reprod Fert 53:389-394