

IMPROVED RESULTS OF THAWED SPERM CRYOPRESERVED WITH SLOW STAGE COOLING WITH A CELLEVATOR

M. L. CHECK
D. J. CHECK
D. KATSOFF
J. H. CHECK

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

There is a need to develop a sperm cryopreservation technique that will allow good pregnancy rates following intrauterine insemination of thawed semen specimens that have been frozen prior to sperm-destructive procedures, such as surgery, chemotherapy, or radiation therapy. A slower cooling rate using a commercial semiprogrammable freezer may provide improved post-thaw motility and hypoosmotic swelling (HOS) test scores. However, the cost of this apparatus precludes it from being used in most andrology centers. This study compares the efficacy of slow stage cooling using an inexpensive cellevator (a device used to freeze lymphocytes) to liquid nitrogen vapor freezing. The semen from 27 males was equally divided and one aliquot was cryopreserved with the cellevator stage cooling and the other with the liquid nitrogen vapor technique. The percent motility and percent grade A sperm post-thaw were significantly higher when cryopreserved with the cellevator than with vapor freezing, as was the mean percentage of sperm showing HOS changes.

Keywords cellevator, cryopreservation, motility, semen, sperm

By properly selecting appropriate sperm donors whose specimens have good survival upon freeze/thawing, many commercial sperm bank centers can now provide cryopreserved sperm, which results in normal pregnancy rates (PRs) following intrauterine insemination (IUI). PRs have not been nearly so successful following IUI of cryopreserved-thawed sperm from males whose specimens were frozen prior to undergoing sperm destructive procedures, e.g., radiation therapy, chemotherapy or surgery. But the advent of assisted reproductive technology, especially intracytoplasmic sperm injection (ICSI), has resulted in good PRs even with thawed specimens of patients undergoing ablative therapy. Unfortunately, ICSI is an expensive procedure and requires the female partners to undergo potentially risky ovarian hyperstimulation. Therefore, there is still a great need to develop better sperm cryopreservation techniques to

Address correspondence to Jerome H. Check, MD, 7447 Old York Road, Melrose Park, PA 19027, USA.

allow patients to approach the good PRs following IUI as seen following therapeutic donor insemination.

The study presented herein evaluated semen parameters after thawing of sperm cryopreserved using a new technique that employed a stage cooling device known as a cellevator (a device originally used to freeze lymphocytes) and the results were compared to the post-thaw values after freezing with the standard nitrogen vapor technique.

MATERIALS AND METHODS

The study group consisted of 27 males ranging in age from 20 to 42 years. Specimens were obtained from patient seeking routine semen analysis as part of their infertility investigation. Semen parameters evaluated were viability, volume, count, motility, motile density (MD) (count/mL \times % motility) and hypoosmotic swelling (HOS) test. The semen was mixed by pipetting with an equal amount (1:1) of 7% Glycerol Test Yolk Buffer (TYB). After approximately 5 min of slow mixing, the semen/TYB mixture was placed in 1-mL Nunc cryopreservation vials. The vials were then equally split into control and experimental categories.

Control Vials. The experimental control vials were frozen utilizing a vapor-cryopreservation technique. The Nunc vials were suspended approximately 2–2.5 in. above the level of liquid nitrogen for 15 min. After this time, the vials were plunged into the liquid nitrogen and stored in metal canes.

Experimental Vials. The experimental vials were placed within the lower "wheel" portion of the cellevator device. All freezing of the cellevator specimens was performed in a fully charged dry-shipper container. Only liquid nitrogen vapors were present but the specimen was not plunged into liquid nitrogen. The main housing of the cellevator (a cylinder above the attached wheel) is placed within the mouth of the dry shipper, with the Nunc vials underneath secured in the neck of the dry shipper. Over a period of 3.5 h, the specimen is lowered at 1-cm increments into the lower main body of the dry shipper. After the cycle was completed, the specimens were plunged into the liquid nitrogen specimen storage tank. Thawing was performed in a similar manner for all specimens. Semen/TYB mixture was thawed at room temperature for 5–10 min. Light pipetting was performed to ensure proper mixture. The average freezing time for all specimens was 3 weeks.

Specimens were then reevaluated for the same semen parameters as on the baseline precryopreservation specimens. The HOS test was performed by combining 0.1 mL of ejaculate with 1.0 mL hypoosmotic solution (fructose/sodium citrate) following precisely the technique described by Jeyendran et al. [7]. After incubation of the mixture for at least 30 min at 37°C, 100 spermatozoa were observed with a phase-contrast microscope for tail changes typical of a reaction in the HOS test. The HOS tests were performed on unprepared specimens during standard semen analysis.

The analysis consisted of sperm concentration and percentage motility using the MicroCell counting chamber (Conception Technologies, La Jolla, CA, USA). Sperm motility was subjectively classified into grades using the World Health Organization [6, 10] criteria: "A" having both rapid and linear forward progression (linear velocity $\geq 22 \mu\text{m/s}$); "B" either slow or nonlinear forward progression (linear velocity ≥ 5 but $< 22 \mu\text{m/s}$); "C" having nonprogressive motility (velocity $< 5 \mu\text{m/s}$); and "D" as nonmotile [5]. All motility analyses were performed at 37°C.

RESULTS

The mean baseline sperm count was $74.3 \times 10^6/\text{mL}$ with a range of 32.0 to $125.0 \times 10^6/\text{mL}$. The mean HOS score was 75.2% and the range was 52.0 to 87.0%. The mean percent progressive motility was 71.6 with a range from 44.4 to 90.0.

Mean percent motility post-thaw was significantly higher in specimens frozen with the cellevator ($49.3 \pm 14.3\%$) than with vapor freezing ($33.4 \pm 12.9\%$, $p < .01$). The percentage of sperm with grade A quality was 17.8 ± 13 vs. $9.2 \pm 8.2\%$ ($p < .01$).

The mean HOS score following thawing of sperm cryopreserved with the cellevator was 57.5 ± 9.7 vs. 45.8 ± 10.9 with vapor freezing ($p < .01$). The number of specimens at or above the critical level of 50% was 2 of 11 (18%) following freezing with nitrogen vapors vs. 6 of 11 (55%) with the cellevator, but there were inadequate numbers to show significance (Fisher's exact test, $p > .05$).

DISCUSSION

McLaughlin et al., using sperm from the donor pool, found improved percent motility after thawing the unwashed specimens following freezing in a commercial semiprogrammable freezer vs. vapor freezing and also found better preservation of HOS scores in the frozen-thawed, postwashed specimen [8]. A study by Ragni et al. evaluated specimens from patients prior to therapy for testicular tumors or Hodgkin's disease and found improvement using a slow-staged freezing with a biological freezer versus vapor freezing in both percentage of progressive motility ($24.0 \pm 12.4\%$ vs. $15.0 \pm 11.2\%$) and in HOS scores (33.3 ± 11 vs. 27.6 ± 12.8) [9].

The data presented herein also demonstrated superiority of cryopreservation with a slower rate cooled technique than vapor freezing. The main advantage of the cellevator vs. commercial freezers is that it is an inexpensive device and thus it would be available for use by many more andrology centers.

The HOS test was originally described by Jeyendren et al., and a level of $<60\%$ was considered abnormal [7]. However, Check et al. stated that a level $<50\%$ better defined the subnormal population [2, 3]. Cryopreservation has been shown to have a detrimental effect on the HOS score [4]. Attempting to slow the vapor freezing process by an intermediate hold did not improve the HOS percentage [1]. However, the slow cooling process with the cellevator not only allowed the mean post-thaw level to remain above the critical 50% level, but also led to superior levels compared to the commercial freezer used by Ragni et al., even in those who started out with apparently normal semen.

Future studies will need to determine if sperm cryopreserved by the cellevator method will lead to improved PRs following IUI of the thawed specimens.

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