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Sperm viability in human semen specimens cryostored at 5°C using the Bio-Tranz™ container system for semen transport

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ABSTRACT

Objective: To asses a protocol designed for transport of unprocessed human semen specimens from the production site to distant laboratories.

Design: The viability of semen specimens stored from the time of collection to the time at which the specimens were to be processed and used (24 hr after collection) was evaluated using the Bio-Tranz™ technology. Specimens were assessed for percentage and grade of motility, and for the sperm membrane functional integrity as measured by the hypoosmotic swelling (HOS) test. The semen specimen was split into two aliquots (Aliquot 1 and 2) and transferred to 15.0 ml conical centrifuge tubes. Aliquot 1 was used without further processing and Aliquot 2 was mixed 1:1 (v/v) with TYB media. Aliquot 1 was maintained at 21°C. Aliquot 2 slowly cooled to 5°C by placing the tube into the middle compartment of the Bio-Tranz™ container.

Setting: Andrology Institute of Lexington, Lexington, Kentucky.

Patients: Semen specimens (n=30) were collected by each participant at intercourse via the use of the MFP ™ and delivered to the Andrology Institute of Lexington for processing.

Main Outcome Measure(s): Viable cryostorage of semen specimens during transport at 5°C for 24 hr for andrological evaluation or use in assisted reproductive technologies.

Result(s): Significant differences ($P<0.05$) in all sperm parameters assessed were noticed between the unprocessed and TYB-prepared specimens after storage for 24 hr. Sperm characteristics were improved when preparing the specimens using TYB (Time 0; $P<0.05$). Sperm characteristics between the unprocessed (Time 0) and TYB-prepared specimens (24 hr) were not different ($P>0.05$).

Conclusion(s): Collection and preparation of human semen for transport at 5°C is possible and that the Bio-Tranz ™ container maintains adequate sperm viability after 24 hours of cryostorage. The use of the Bio-Tranz™ container is convenient for patients that request semen processing services or for other clinical purposes at distant locations.

Key words: semen, spermatozoa, cryostorage, viability, transport

Transport of unprocessed human semen specimens from the production site to distant laboratories for andrological evaluation and other clinical uses requires the development of proper protocols and devices for the maintenance of sperm viability and fertilizability during and after transport (1). Spermatozoa recovered following storage at 5°C in TEST-Yolk buffer (TYB), show enhanced survivability and fertilizing capacity than spermatozoa frozen at subzero (-196°C) temperatures following short-term incubation between 24 and 96 hr (1-7). It has also been documented that, the treatment of human spermatozoa with TYB can enhance their ability to

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penetrate zona-free hamster oocytes (ZFHO) and bind to the zona pellucida as measured by the sperm penetration assay (SPA) or hemizona (HZA) assays (1, 5, 8-11). Preincubation of spermatozoa in TYB have been shown to increase the percentage of human oocytes fertilized via in vitro fertilization (IVF) procedures (1, 5, 11, 12). It seems that a higher proportion of the sperm population undergo capacitation during TYB incubation, which results in synchronization of the acrosome reaction following sperm washing and preparation for use in the various assisted reproductive technologies (5, 6, 11). Subsequently, the increased percentage of acrosome-reacted spermatozoa that is seen after incubation in TYB may indicate that a larger percentage of the sperm population acquired the ability to penetrate the ovum and account for the higher rates of fertilization observed (1, 5, 6, 11).

The Bio-Tranz™ container, which was designed to cool and maintain semen specimens at 5°C during transport, consists of a properly insulated Polystyrene container, TYB, a nonspermicidal condom-shaped semen collection device and test tubes. The viability of semen specimens stored from the time of collection to the time at which the specimens were to be processed and used (24 hr after collection) was assessed using the Bio-Tranz™ technology.

MATERIALS AND METHODS

Semen Collection and Assessment

Thirty normozoospermic (World Health Organization standards) (13); donors participated in this study. Each donor produced a semen specimen after 3 to 4 days of abstinence via intercourse using a semen collection device provided with the Bio-Tranz™ (14, 15). The Bio-Tranz™ technology (ZDL, Inc., Lexington, KY, USA) consists of a Polystyrene (Styrofoam) container which contains a cold pak, TYB, the Hygiene™ seminal collection kit, and a Styrofoam separator that divides the Bio-Tranz™ container into two compartments (Fig. 1). The Hygiene™ semen collection kit consists of a nonspermicidal condom-shaped collection device (Male Factor Pak™), funnel and two 15.0 ml conical centrifuge tubes. Semen specimens were assessed for percentage and grade of motility, and for the sperm membrane functional status as measured by the hypoosmotic swelling (HOS) test (16). Grade of motility was measured using a scale of 0 to 4 to describe the progressive motility as described by Zavos et al. (17).

Experimental Design

Semen specimens were split into two aliquots (Aliquot 1 and 2) and transferred to 15.0 ml conical centrifuge tubes following initial assessment. Aliquot 1 was used as control without further processing and Aliquot 2 was mixed 1:1 (v/v) with TYB media. Aliquot 1 was maintained at 21°C (ambient room temperature). Aliquot 2 was slowly cooled to 5°C (approximately 0.1°C/min) by placing the tube containing the diluted seminal specimen into the middle compartment of the Bio-Tranz™ container. A separator cover (thin piece of Styrofoam with two holes) was placed over the test tube. The specimen preparation procedure was completed by placing a frozen cold pak on top of the separator and then closing the Bio-Tranz™ container prior to transport. The semen-TYB mixture was cooled via conduction as it was placed closed to the frozen cold pack (-20 °C). The low temperature of the frozen cold pack coupled with distance of the semen-TYB mixture in reference to frozen cold pack allowed the rate of cooling of the semen-TYB mixture (approximately 0.1°C/min). Semen specimens (Aliquot 1 and 2) were assessed after 24 hr of storage as previously described.

Semen Rewarming

The semen-TYB mixture was removed from the Bio-Tranz™ container and placed in water bath (37° C) for 15-20 minutes as previously described (2, 3). The semen-TYB mixture was gently agitated

Table 1. Sperm viability of semen specimens diluted and cryostored in TEST-Yolk buffer (TYB) at 5°C during 24 hours of cryostorage (means± S.D.)

Semen treatments	Sperm characteristics assessed		
	Motility (%)	Grade (0 to 4)	HOS* (%)
Fresh, 0 hours†	55.0±7.5[a]	3.4±0.3[a,b]	64.0±11.0[a,b]
Fresh, 24 hours†	17.7±6.1[b]	1.6±0.5[c]	23.0±11.6[c]
TYB, 0 hours‡	61.8±7.6[c]	3.5±0.2[a]	68.7±10.8[a]
TYB, 24 hours‡	52.8±9.0[a]	3.3±0.2[a]	58.7±11.4[b]

*HOS= hypoosmotic swelling test (% swollen spermatozoa).
†Fresh specimens were maintained at 21°C and assessed at 0 or 24 hr following collection.
‡Semen specimens were cryostored at 5°C in TYB and assessed at 0 or 24 hr following collection.
[a,b,c]Values with different superscripts within columns are significantly different (P<0.05).

periodically during the incubation period in order to keep the rewarmed spermatozoa in suspension.

Statistics

The results were reported as means±SD. The data was analyzed by ANOVA procedures using the SAS Statistical Package (18). A level of P<0.05 was considered statistically significant. The statistical model included the effects of patients, temperature and sperm characteristics assessed.

RESULTS

The results obtained are summarized in Table 1. Significant differences (P<0.05) in all sperm parameters assessed were noticed between the unprocessed and TYB-prepared specimens after storage for 24 hr. Sperm characteristics were also improved when preparing the specimens using TYB after collection (Time 0; P<0.05). Sperm characteristics between the unprocessed (Time 0) and TYB-prepared specimens (24 hr) were not significantly different (P>0.05). The percentage and grade of motility, as well as, the integrity of the sperm membrane declined by 68, 53 and 65% after 24 hr in specimens stored at 21°C, respectively. Similarly, in specimens stored at 5°C using TYB, those sperm characteristics declined by 15, 6 and 15% after 24 hr of cryostorage.

DISCUSSION

The use of cryopreserved semen for artificial insemination using donor semen (AID) is steadily increasing, although the cryopreservation (-196°C) techniques presently employed reduce significantly the fertilization potential of human spermatozoa (19). Spermatozoa cryostored at 5°C in TYB show improved survivability than spermatozoa frozen at subzero temperatures (2, 15). The use of cryopreserved spermatozoa provides the advantage of arranging patients and performance of assisted reproductive techniques (ART) such as intrauterine insemination (IUI), IVF, intra cytoplasmic sperm injection (ICSI) and others (3, 20, 21). A newly established technique has been introduced to optimize fertility rates and to decrease the difficulty in aligning patients or making semen available from patients that either travel on the day that the procedure is to be performed or they happen to be residing at another distant location from where the procedures are performed. This technique consists of collecting, diluting and cryostoring (5°C) human spermatozoa diluted with TYB for approximately 24 to 48 hr and up to 96 hr (3, 6, 7). The fertilization potential of spermatozoa incubated and cryostored in TYB can

also be enhanced by selection of spermatozoa via various sperm preparation techniques employed in ART after the cryostorage period (3, 17, 22-24). It has also been documented that the treatment of human spermatozoa with TYB can enhance their ability to penetrate ZFHO (1). Preincubation of spermatozoa in TYB have been shown to increase the percentage of human oocytes fertilized via IVF procedures (12). It seems that following sperm incubation in TYB at 5°C, more spermatozoa undergo capacitation and synchronization of the acrosome reaction (5, 6). Subsequently, the increased proportion of capacitated spermatozoa that is noted after TYB incubation may indicate that a higher percentage of spermatozoa achieve the acrosome reaction stage and consequently the ability to penetrate the ovum, which could account for the higher fertilization rates observed (1, 5, 6, 8-11). Most recently, it was also shown that incubation of spermatozoa in TYB followed by filtration using the Sperm Prep™ Sephadex filtration method can improve the recovery of antisperm antibody (ASA)-free spermatozoa by selectively masking and entrapping spermatozoa with ASA bound to its surface (25).

The development of methods for the transport of semen specimens processed and cryostored in TYB combines the advantages of maintaining sperm viability, and possibly the sperm fertilizing ability, while in transit. Following delivery to the andrological or ART laboratory, the specimens are then processed for clinical evaluation or used for various forms of ART including IUI. The employment of this technology also allows the performance of various inseminations obtained from the cryopreserved specimen, without the patient having to produce multiple specimens within a short period of time (24 to 96 hr). The results obtained in the current study show that the collection and preparation of human semen for transport at 5°C is possible and that the Bio-Tranz™ container maintains adequate sperm viability after 24 hr of cryostorage. The use of the Bio-Tranz™container is convenient for patients from distant locations that request semen processing services or for other clinical purposes such as semen cryopreservation, evaluation and processing for use in the various forms of ART and also IUI procedures. The Bio-Tranz™ is a rather unique product and will revolutionize the way liquid (nonfrozen) semen is shipped and utilized for patients that require various clinical and laboratory procedures performed on their semen and they happen to be located at distant locations from the site where those procedures are performed.

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