# Utilization of high-security straws for embryo freezing in an in vitro fertilization program: a prospective, randomized study

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**Objective:** To compare the outcome of frozen-thawed ET cycles where embryos had been stored in conventional versus ionomeric resin-based, high-security straws (HSSs).
**Design:** Prospective, randomized study.
**Setting:** Private assisted-reproduction unit.
**Patient(s):** Three hundred and six freeze cycles, and 197 thaw cycles.
**Intervention(s):** Day 3 embryos (n = 1,268) were frozen, and 517 were thawed using HSSs. Alternately, day 3 embryos (n = 1,228) were frozen, and 505 were thawed using conventional straws.
**Main Outcome Measure(s):** Cryosurvival, cleavage and morulae formation rates, and clinical pregnancy, implantation, and multiple pregnancy rates.
**Result(s):** Although cycle characteristics did not show any differences, the cryosurvival rate was higher in the HSS group (94.7%) than in the conventional straw group (86%), as was the morulae formation rate (58.7% versus 42.7%). Despite a similar number of embryos being transferred, the clinical pregnancy rate (PR) was higher in the HSS group, but the difference lacked statistical significance (42.5% versus 31.2). Implantation rates (19.4% versus 11.4%) and multiple PRs (41.8% versus 16.6%) were significantly higher in the HSS group than in the conventional straw group.
**Conclusion(s):** High-security straws are high effective in human embryo cryopreservation, because they provide higher cryosurvival and implantation rates, as well as a lower risk of cross-contamination compared to conventional straws. (Fertil Steril® 2007;87:691–6. ©2007 by American Society for Reproductive Medicine.)

**Key Words:** Human embryo cryopreservation, freezing, thawing, high-security straw

Assisted reproduction generally results in surplus embryos that can be cryopreserved for later use. A cryopreservation program will undoubtedly increase the cumulative conception rates attained in IVF and intracytoplasmic sperm injection (ICSI). A cryopreservation program has been performed in our center since its legalization in Turkey in 1998. Since then, cleavage-stage embryos have been frozen and stored in conventional straws within liquid nitrogen tanks.

For cryopreservation purposes, samples from numerous couples are preserved in the same tank for a long period of time. This situation raises the concern of cross-contamination of cells or tissues with viral pathogens. By 2000, a different type of straw (high-security) was introduced into the market to prevent any risk of cross-contamination of frozen embryos or spermatozoa in the tank (1). High-security straws (HSSs) are composed of a different material than conventional straws. Many reports demonstrated the benefits of these new straws against hepatitis C virus (HCV) or HIV contamination (1–3).

In addition to their different composition, there are differences in size, and in plugging and sealing properties, between HSSs and conventional straws. Although many studies were published showing the efficacy of HSSs against viral contamination, there are limited data showing the effect of HSSs on the clinical outcome of frozen-thawed ET cycles. This prospective, randomized study was designed to compare the outcome of frozen-thawed ET cycles where embryos had been stored in conventional straws versus HSSs.

## MATERIALS AND METHODS

### Study Design

The study group consisted of 396 freeze cycles and 197 thaw cycles which had been performed between March 2004–May 2005. During 14 months of the study period, 1,448 fresh cycles were performed, and the cryopreservation rate was 27.3%. Testicular sperm extraction and percutaneous epididymal aspiration cases were excluded from the study group. Good-quality supernumerary embryos were frozen on day 3 of cleavage. These 396 freeze cycles were randomly

Received January 17, 2006; revised and accepted July 20, 2006.
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divided into two groups, according to a computer-generated randomization list: 198 freeze cycles were assigned to the HSS group, and 198 freeze cycles to the conventional straws group. The randomization sequence was concealed from researchers and patients until freezing had been assigned. Approval was obtained from the Institutional Review Board of the American Hospital, Istanbul, Turkey.

In total, 1,268 day 3 embryos were cryopreserved in HSSs, and 1,228 in conventional straws. Thawing was performed for 101 cycles (51%) in the HSS group, and for 96 cycles (48.4%) in the conventional straw group. Five hundred and seventeen embryos were thawed from HSSs, and 505 embryos were thawed from conventional straws. Laboratory environment, culture media, stimulation protocols, and ET policy were unchanged during the study period.

The outcomes of fresh cycles performed in the HSS group were as follows: 92 cycles (91.0%) failed to achieve pregnancy, whereas out of nine cycles with positive pregnancy results, two ended with biochemical abortions, and seven with clinical abortions. Out of 96 thawing cycles in the conventional straw group, 86 (89.5%) fresh ET cycles failed, whereas three had biochemical abortions, and seven had clinical abortions. Hence, there were no ongoing pregnancies following fresh ET cycles in both study groups.

### Embryo-Freezing With Conventional Straws

A method modified from that of Testart et al. (4) was used for early embryo freezing, whereby 1,2 propanediol (PrOH) was used as a permeating cryoprotectant, and sucrose was used as a nonpermeating cryoprotectant. A phosphate-buffered solution was also used, so that all steps could be performed outside the incubator at ambient temperature. Freeze Kit-1 (refrence no. 10012; Vitrolife, Gothenburg, Sweden) was used throughout the freezing procedure.

Only good-quality embryos with equal, homogeneous blastomeres and <20% fragmentation (grades 1–2), which reached at least cell stage 5 on day 3, were cryopreserved (5). All solutions used for the procedure should be equilibrated to ambient temperature before usage. Embryos were first rinsed for approximately 2 minutes in Cryo-PBS (Vitrolife), a phosphate buffer solution with 25 mg/mL human serum albumin (HSA). They were then gently replaced into Freezing Solution-1 (FS1) (1.5 M PrOH in Cryo-PBS) for 10 minutes. The cells of the embryo were shrunken and then reequilibrated in this solution. The embryos were moved across to FS2 (1.5 M PrOH + 0.1 M sucrose in Cryo-PBS) and loaded into straws (reference no. 014103; Cryo Bio System Groupe, I.M.V. Technologies, Normandie, France) by attaching each straw to a 1-mL syringe, which was connected to the straw by 1-cm silastic tubing. Straws were rinsed with the storage medium before loading, to remove any traces of fiber or powder constituents of the plug. Straws were then attached to a sterile plug (reference no. 007442; Cryo Bio System Groupe, I.M.V. Technologies) to avoid leaking inside the straw and into the liquid nitrogen ($LN_2$) tank during storage. A maximum of two embryos was placed in each straw. Straws were then placed into the freezing chamber at ambient temperature, and the program began. Planer Kryo 10 Series III (Planer Products Ltd., Sunbury on Thames, United Kingdom) was used for cryopreservation. The freezing program used was as follows:

Starting temperature: 18–25°C.
Step 1: −2.0°C/min to −7.0°C.
Step 2: Hold at −7.0°C for 10 minutes. Seed after 2 minutes. Straws were manually seeded at −7.0°C with $LN_2$-cooled forceps close to the cotton plug.
Step 3: −0.3 C°/min to −30.0°C.
Step 4: −30.0°C to below −80.0°C (at least 10°C/min). Straws were then removed and plunged into the $LN_2$ storage tank immediately.

### Embryo-Freezing With High-Security Straws

All procedures were the same as for freezing with conventional straws, until embryos were loaded into CBS HSSs (reference no. 010286; Cryo Bio System Groupe, I.M.V. Technologies). The straw was attached to a 1-mL syringe which was connected to the straw by 1-cm silastic tubing. Loading of the straw was as follows: a medium column of 0.02 mL was aspirated first, then an air bubble of 0.03 mL (approximately 7 mm), medium of 0.05 mL (10 mm) with the embryos (embryos aspirated at the last point), air bubble of 0.03 mL, medium of 0.02 mL, and finally an air bubble of approximately 0.02 mL were aspirated. The seeding procedure was performed on the medium column where the embryos were placed, but on the opposite side from where the embryos were aspirated. After the embryos were loaded, straws were heat-sealed on both ends with the SYMS unit especially designed by the Cryo Bio System Group (CBS) for this purpose. A maximum of two embryos was placed in each straw. Straws were then placed into the freezing chamber at ambient temperature, and the program began. Planer Kryo 10 Series III (Planer Products Ltd.) was used for cryopreservation. The freezing program and tank storage were the same as for conventional straws.

### Embryo-Thawing in Conventional and High-Security Straws

Straws were thawed one at a time, and all steps were performed at ambient temperature. Thaw-kit 1 (reference no. 10013; Vitrolife) was used for the thawing procedure. All solutions were preequilibrated to ambient temperature before use. The manual for recommended use of Vitrolife Fertility Systems was followed throughout the procedure.

Each straw was removed from the $LN_2$ tank and air-thawed for 30 seconds. During this time, the straws were handled carefully and examined for air bubbles, cracks on