A NOVEL CLOSED SYSTEM VIAL WITH SENTINEL TEST SEGMENT FOR SPERM CRYOPRESERVATION

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OBJECTIVE

This study investigated a novel closed system vial (CellSeal®, Indianapolis, IN, USA) for cryopreservation of human spermatozoa with an integrated test-segment for motility assessment (FIGURE 1).

DESIGN

This study was designed to determine if the system could maintain its container closure integrity (CCI) under liquid nitrogen (LN2) submersion and still maintain post-thaw motility comparable to controls. An attached segment integral to the vial was also evaluated as a sentinel for representative testing, again evaluating post-thaw motility as compared to the control as well as the vial body.

MATERIALS AND METHODS

First, vials frozen/thawed with saline or bacterial support medium were subjected to container closure (CCI) testing by dye or bacterial immersion, respectively, under pressure or vacuum. Dye ingress was measured using spectrophotometry and bacterial ingress was measured via CFU assay.

Next, a total of 11 semen samples from 4 different donors were processed for long term storage using either a standard 2ml Corning cryovial (control), a 2ml CellSeal® cryovial and the CellSeal® vial integral test segment. CellSeal® vials were filled and sealed as shown in FIGURE 2. Each sample was then frozen for at least 24 hours at -196°C under liquid nitrogen (LN2). After freezing, samples were visually evaluated for LN2 ingress and then evaluated for post-thaw motility using standard methods. Means (normalized to non-frozen control) and standard deviation of the mean were calculated for the sperm from the CellSeal® vial, the vial segment and control (standard screw-cap vials) and compared statistically via student's t-test.

RESULTS

All vials passed the CCI testing with no dye or bacterial ingress. Post-thaw samples were comparable among the control and experimental groups and no difference in motility as per t-test comparing post-thaw control to the CellSeal® vials or the segments (FIGURE 3).

DISCUSSION

Storage under LN2 is optimal for stability, however Examples of clinical cross contamination through liquid nitrogen have been reported. In validation studies our lab has demonstrated that screw-cap vials with inner threads and an o-ring (Corning). closed tightly and consistently with 5 in-Lb of torque and submerged for 2 months resulted in 93% failure. Tests conducted by an independent Andrology Department published previously in Human Reproduction (Clarke, 1999) indicated 45% of Cryovials without an O-ring gasket (Nunc, Nalge International) and 85% of vials with an O-ring (Iwaki Cryovials; Iwaki, Japan) absorbed liquid nitrogen during 3 hours immersion in liquid nitrogen.

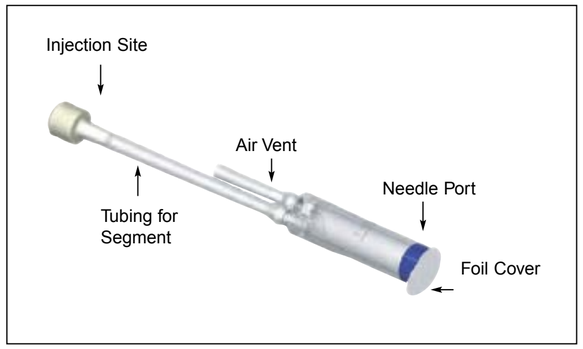


Figure 1: Concept and schematic of the CellSeal® cryogenic vial. The vial allows for a closed system with an integrated test-segment for motility testing or future disease screening.

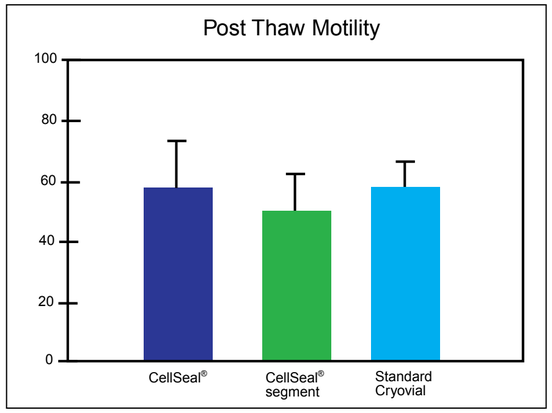
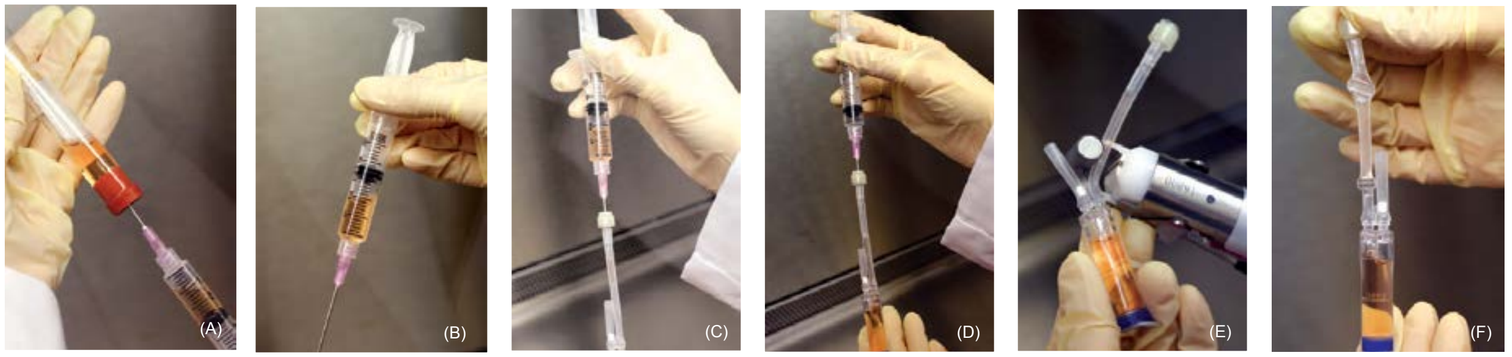


Figure 3: Comparison of post thaw motility (normalized to prefreeze) among the CellSeal® vial, vial segment and standard screw cap cryovials (inner thread with o-ring). Data expressed as means ± Standard Deviation of an average of 11 samples over 4 donors. There was no significant difference (student's t-test) between CellSeal® vial main chamber and standard vials (p=0.18); CellSeal® vial main chamber and segment (p=0.23); or CellSeal® segment and standard vials (p= 0.57).



1. Remove vial from packaging. Hold vial upright with tubing and injection site at the top, optional foil cover at the bottom

2. Working inside an appropriate biosafety hood, draw up sample to be stored with an appropriate sized syringe and needle. It is recommended that for the 5mL vial, use a 10 mL syringe and for the2mL vial, use a 5mL syringe; both syringes using an 18g needle. It is recommended to draw a small volume of air into the syringe first and then draw the sample (diagram B). The extra air will be used to level the column of liquid in the tubing or to push the entire sample into the vial.

3. Puncture the injection site with syringe and inject sample into vial as indicated in diagram C. Push the air into the tubing to place the liquid column at the desired level in the tubing if a segment is desired, or push the entire sample into the vial.

4. If a segment is desired, pull back on the syringe to create a clear space in the tubing column above and below the liquid for the segment (diagram D).

5. Seal the tubing below and above the liquid in the tubing for a segment. Seal an additional time below the first bottom seal for optimal folding over of segment for storage (diagram E). Seal close to the vial body alone if no segment is desired. (diagram F) Detach the injection site using the sealer or clean scissors. If a segment is used do not detach the segment.

6. Seal the air vent tubing above the microbial filter making this a closed system. Vial is now ready for freezing.

7. Segment can be folded over for easy storage. Place vial and integral segment into box or cane for storage.

8. When segment is needed, remove vial from storage and break off immediately after removal or cut quickly with clean scissors.

9. When sample is needed, remove vial from cold storage. Cut open the vent tube with clean scissors and thaw per specific sample thawing instructions. If using a water bath, do not submerge the open vent tube.

10. Remove the foil on the bottom of the needle port. NEEDLE PORT IS STERILE; DO NOT SIT VIAL DOWN ONCE FOIL IS REMOVED.

12. Using an appropriate sized syringe and needle puncture the needle port and extract sample. It is recommended to use an 18g needle for this port.

13. If used with infectious, hazardous, or bodily fluids dispose of vial as appropriate.

11. Thoroughly swab the top of the port with a sterile alcohol wipe. Allow to air dry up to 1 minute before accessing.

FIGURE 2. Handling and use of the CellSeal® vials.

CONCLUSION

The novel closed system vials present a means of assuring sample integrity even under liquid nitrogen and allows measurement of post-thaw motility or further testing of a specimen without thawing the primary container.

REFERENCES
Clarke G.N. 1999. Sperm cryopreservation: is there a significant risk of cross-contamination? Human Reproduction 14:2941-2943.