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A novel bench top device for freeze drying sperm cells

Arav Amir & Yehudit Natan

FertileSafe Ltd., 11 Haharash st. Nes-Ziona, 7403118, Israel. Fertilesafe@gmail.com

**Introduction:**

We developed a new device for freeze drying of cells and tissue. The device can be sterilized before use by autoclave. The device is built of a stainless steel cylinder having a condenser and a vials carrier incorporated with temperature control unit and is cooled by liquid nitrogen. The cylinder is connected to vacuum pump and it can be equipped with a vials closing system.

We freeze dried human sperm cells in the device and tested the concentration and DNA integrity before and after rehydration.

**Material & Methods:**

Freezing: Ejaculated fresh sperm were analyzed for concentration and motility before being washed in order to remove the seminal plasma. Then the sperm were diluted in Lyophilized solution (LyoS) 1:1 (v/v) in α-MEM Eagle medium containing 0.25M sucrose, 0.25M trehalose and 0.6% (w/v) human serum albumin (HSA). A samples was taken for DNA integrity evaluation using the HAlosperm G2 kit (Halotech DNA, Spain). Droplets of 10ul were then directly exposed to sterile liquid air produced by a bench top device (CLAir®, FertileSafe, Israel). The frozen pellets were kept in closed 10ml glass vials (n=6). Part of the frozen drops were thawed for evaluation using Hepes medium warmed to 37°C.

Freeze-drying: The vials were opened, in sterile liquid air and placed in the lyophilization device (Darya®, FertileSafe, Israel). Prior use the Darya device was sterilized using an autoclave. Pressure was set to 10mTorr, shelf temperature was -35°C and condenser temperature was set to
-110°C. We dried the sperm for 48 hours.

Rehydration: Dried droplets (20) were directly exposed to 0.2ml of α-MEM Eagle medium pre-warmed to 37°C or to 0.2ml of warm LyoS. Sperm cells were evaluated for concentration and at least 200 sperms were evaluated in each group for DNA integrity with the Halosperm G2 Kit.

**Results:**

Fresh sperm concentration was 10•106 cells/ml and motility was more than 50%, DNA integrity was 81.06±9.2%.
Post thaw motility was 65-80% compared to the fresh (normalized) same specimen.
After drying and rehydration most of the cells concentration of the group that was rehydrated with LyoS was 8.25•106 cells/ml and DNA integrity was 81.3%±3.5% and cells concentration of the groups that was rehydrated with medium was 5.375•106 cells/ml and DNA integrity was 71.19%±7.7%.

**Conclusions:**

The Darya device allows for successful freeze drying of sperm in a sterile manner. Rehydration with LyoS was better than with medium resulting with no significant cell loss and no additional DNA damage. The dried droplets may be directly introduced into the culture medium for rehydration and used for IVF, mainly ICSI, without any need of sperm pre-washing.

