Critical Analysis of Long-Term Sperm Preservation

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Introduction

Having a sperm bank for semen preservation has always been a goal and it is important for:

1) Maintance of livestock
2) Conservation of rare or endangered species
3) Fertility male protection

Furthermore, eggs are scarce and difficult to obtain. But sperm seems that could be better to find new techniques to increase fertility.

The most widely used method for sperm preservation is cryopreservation. With this method, spermatozoa can be stored indefinitely and after thawing they maintain their motility and fertility when used in *in vitro* fertilization. However, cryopreserved sperm requires continual supplementation of liquid nitrogen and presents safety and cost problems for transportation and risk of pathogen contamination. That is why some alternative methods have been reported.

Objective and Methodology

The following review is intended to make a critical analysis of current and future following methods to preserve sperm.

Data displayed in this poster has been mainly obtained from original scientific research papers and reviews using the searching tool Pubmed.

The search was based in key words as *sperm, male fertility, long-term preservation, cryopreservation, freeze-drying* or *evaporative-drying*. All the references are chosen depending on their quality and date of publication.

Current Method for Long-Term Sperm Preservation

CRYOPRESERVATION

It consists on freezing biological material at extreme temperatures (most common -196 °C) in liquid nitrogen. At these low temperatures all biological activity stops. Cryoprotectants are needed to avoid the formation of ice crystals of intracellular water.

Pros

- Living cells obtained after thawing
- ICSI is not needed
- Indefinitely storage
- No apparently DNA damage
- High embryo development
- Healthy offspring

Cons

- High costs
- No simple storage
- Contamination risk
- Harmful cryoprotectants (DNA damage)
- High dose of cryopreserved sperm to achieve good fertility
- Not valid in all species

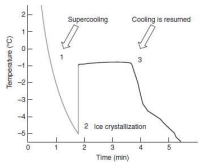

Fig. 1. Graph showing the temperature changes that occur when a sample of semen is frozen within a plastic straw. The most significant events are that the temperature first decreases to the usual freezing point (1) (super cooling), then increases again rapidly as the latent heat of fusion is released (2). Dissipation of the latent heat produces a short period when the temperature does not change, after which cooling is finally resumed (3).

Alternative Methods for Long-Term Sperm Preservation

Dried state (DESSICATION)

Desiccated spermatozoa must be considered dead because they are not motile and are unable to penetrate the egg. But the only sperm component necessary for the production of a zygote is the nucleus, so ICSI technology has lead to the possibility of storing spermatozoa in the dried state.

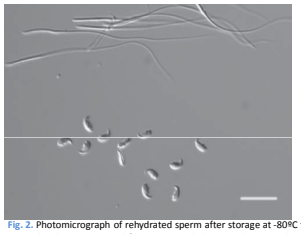

Fig. 2. Photomicrograph of rehydrated sperm after storage at -80ºC for 11 mo (mice). Sperm heads are separated from sperm tails by piezoelectricity-driven needle.

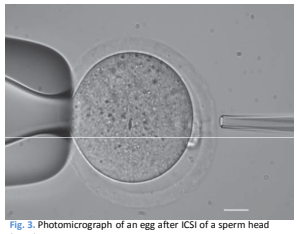

Fig. 3. Photomicrograph of an egg after ICSI of a sperm head (mice).

FREEZE-DRYING (Lyophilization)

Based on freezing spermatozoa and then reducing the surrounding pressure allowing the sublimation from solid to gas state of the inner water from the cell.

Pros

- No DNA damage (using EGTA and low temperatures)
- Simple storage
- High embryo development
- Healthy offspring

Cons

- No living cells obtained after rehydration
- ICSI is needed
- No indefinitely storage
- High costs

Fig. 4. Sperm membrane integrity assessed by the vital dye eosin in rehydrated spermatozoa (human). Eosin penetrate in all cells indicating membrane damage and lack of viability. Sperm head morphology seems to be well preserved, but tail curling is present in four cells.

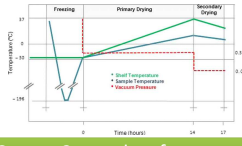

Fig. 5. Scheme of sperm freeze-drying protocol in a freeze-dryer machine: time, temperature and pressure kinetic.

Recent goal achieved: stored sperm at 24ºC up to 3 months of storage.

EVAPORATIVE-DRYING (Vacuum Drying)

Based on accumulating intracellular sugar as trehalose in their cells. Sperm is placed in a vacuum drying chamber and then dried by nitrogen gas at room temperature.

Pros

- Low costs
- Simple storage
- Healthy offspring

Cons

- No living cells obtained after rehydration
- ICSI is needed
- No indefinitely storage (improvable by decreasing trehalose concentration)
- DNA damage (improvable)
- Reduced embryo development

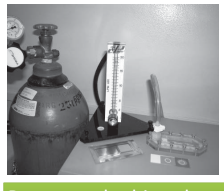

Figure 6. Evaporative drying system for sperm. Compressed ultrapure nitrogen gas is blown through a regulator and flow meter into the drying chamber, and out through a hole at the right end of the drying chamber.

Recent goal achieved: stored sperm at 24ºC up to 2 years of storage.

Future

Freeze drying seems to be the ultimate method to protect wild species from extinction and for humans too. In addition, freeze-dried human sperm surprisingly do not require EGTA to maintain their chromosome integrity. Even so, for evaporative-drying sperm, no human sperm studies have been reported yet, maybe because it is a more recent method that has got a low development potential and therefore should be further studied in animals first.

Future desiccation methods should be conducted to allow indefinitely storage at room temperature.

Conclusions

- ✓ Having a sperm bank for semen preservation is important for maintance of livestock, conservation of rare or endangered species and fertility male protection.
- ✓ The most widely used method of long-term preservation is cryopreservation. However, cryopreserved sperm requires continual supplementation of liquid nitrogen and presents safety and cost problems and risk of pathogen contamination.
- ✓ Alternative methods allow samples to be kept for a long time in a refrigerator or at ambient temperature and can also remain viable.
- ✓ In human, a high damage to cell membranes after freeze-drying is demonstrated, so it has to be applied ICSI. But DNA fragmentation is by far more prevalent with cryopreservation technique. No studies have been reported by using evaporative drying sperm in human.
- ✓ New alternative long-term methods to preserve sperm should involve optimum conditions to protect sperm from desiccation and reduce oxidative stress. Desiccation methods must be improved in order to preserve sperm permanently and to store it at room temperature.

References:

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