Iodixanol is an effective cryoprotectant for mouse spermatozoa

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Introduction

- Cryopreservation of sperm, eggs, and embryos provides a valuable means of maintaining transgenic mouse strains used in biomedical research[1,2]
- Sperm is easy and inexpensive to collect, store, and transport between research institutes[3]
- Freezing sperm minimizes the potential for genetic drift or spontaneous loss of phenotype[1]
- The ability of thawed sperm from inbred mice to successfully fertilize an egg (sperm viability) is routinely low[4]
- Freezing protocols need improvement to maximize survival and viability of frozen-thawed mouse sperm
- Sperm quantity and quality between mouse strains is also highly variable[4] and freezing protocols need to be standardized to maximize post freezing viability between lines
- Previous research indicates that iodixanol (OptiPrep™) has cryoprotectant properties for sperm from cattle[5] and rats [6]

Hypothesis

The addition of iodixanol (OptiPrep™) to a standard freezing solution will improve survival and viability of mouse sperm following freezing and thawing.

If true, we predict that more sperm will:

- Survive freezing and thawing
- Show rapid, progressive motility
- Have an intact acrosomal membrane
- Have high mitochondrial membrane potential

Results

Iodixanol improves sperm survival after freezing

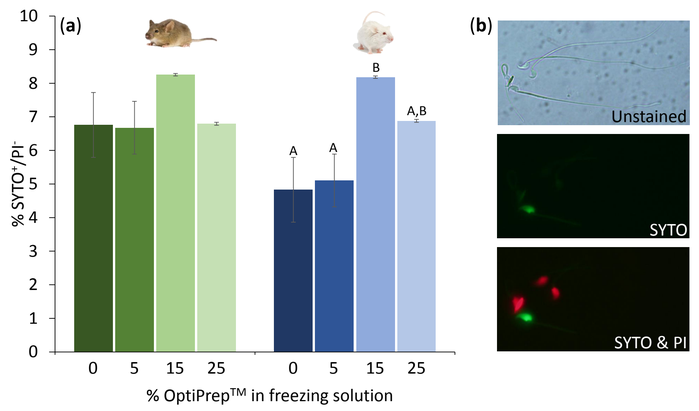

Figure 1: (**a**) The effect of OptiPrep™ on plasma membrane integrity of thawed sperm from 129/SV (green) and FVB/NJ (blue) inbred mouse lines. Four freezing solutions with 0, 5, 15 or 25% OptiPrep™ were compared. Survival was significantly higher for sperm from FVB/NJ mice frozen with solution containing 15% OptiPrep™ compared to the other freezing solutions (difference indicated with letters above bars). Sperm from 129/SV mice showed a similar trend. (**b**) Viable cells stain with SYTO (green) but not PI (red).

Iodixanol improves motility of thawed sperm

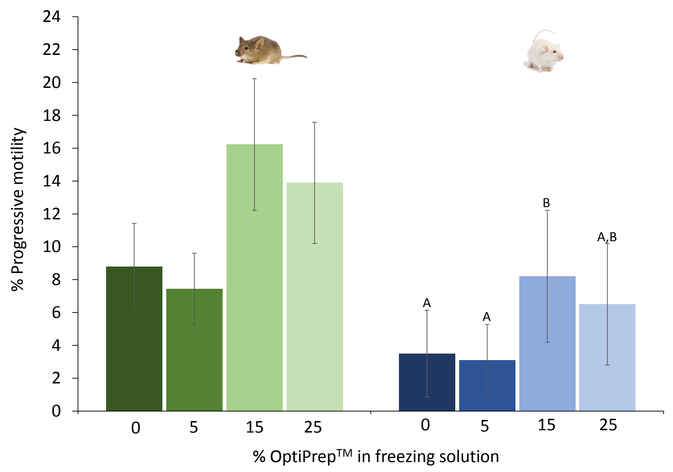

Figure 2: The effect of OptiPrep™ on progressive motility of thawed sperm from 129/SV (green) and FVB/NJ (blue) inbred mouse lines. Four freezing solutions with 0, 5, 15 or 25% OptiPrep™ were compared. More sperm from FVB/NJ mice frozen with solution containing 15% OptiPrep™ showed progressive motility compared to the other freezing solutions (difference indicated with letters above bars) Sperm from 129/SV mice showed a similar trend.

Iodixanol protects acrosomal membrane from freezing damage

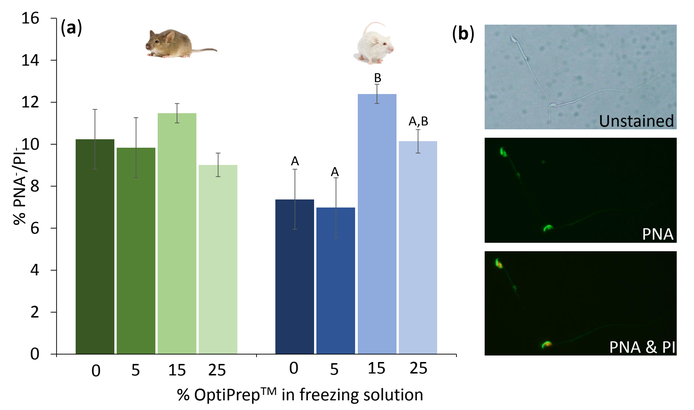

Figure 3: (**a**) The effect of OptiPrep™ on acrosomal membrane integrity of thawed sperm from 129/SV (green) and FVB/NJ (blue) inbred mouse lines. Four freezing solutions with 0, 5, 15, or 25% OptiPrep™ were compared. More sperm from FVB/NJ mice frozen with solution containing 15% OptiPrep™ had an intact acrosomal membrane compared to the other freezing solutions (difference indicated with letters above bars). Sperm from 129/SV mice showed a similar trend. (**b**) Viable cells with an intact acrosomal membrane do not stain with either dye. Dead cells with a reacted or damaged acrosomal membrane stain with both SYTO (green) and PI (red).

Iodixanol doesn't affect mitochondrial membrane potential of thawed sperm

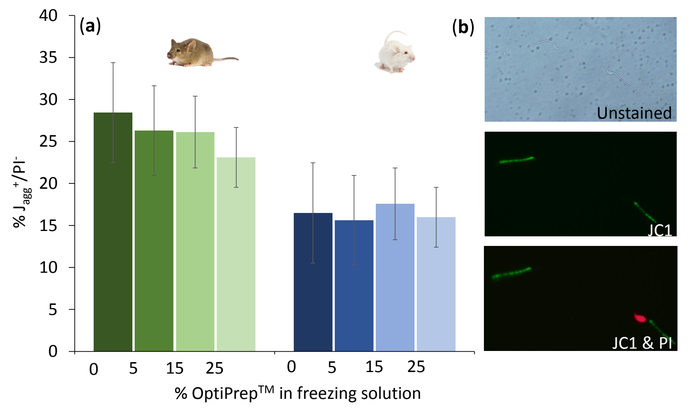

Figure 4: (**a**) The effect of OptiPrep™ on mitochondrial membrane potential of thawed sperm from 129/SV (green) and FVB/NJ (blue) inbred mouse lines. Four freezing solutions with 0, 5, 15 or 25% OptiPrep™ were compared. (**b**) Viable cells with a high mitochondrial membrane potential stain in the mid-piece with SYTO (green) but the head does not stain with PI (red).

Summary & Conclusions

- Addition of iodixanol to a standard freezing solution improves survival of sperm from two inbred mouse strains
- Surviving sperm also show improved viability, measured as progressive motility and acrosomal membrane integrity

Future Directions

- Test the cryoprotectant potential of iodixanol for sperm from other common mouse strains
- Use artificial insemination to determine if sperm frozen with iodixanol has improved fertilizing ability

Methods

Animals: 10-12 week old 129/SV and FVB/NJ mice

129/SV
- Widely used for gene target studies
- Moderate fresh sperm quality
- Very poor sperm viability after freezing

FVB/NJ
- Widely used for DNA microinjection
- Highly fecund, excellent sperm quality
- Good sperm viability after freezing

Freezing solutions: Iodixanol (OptiPrep™) was added to a standard raffinose/skim milk freezing solution. The concentration of raffinose was altered to maintain solution osmolarity at less than 500mOsM. Four solutions were compared:

% Raffinose	% OptiPrep™	Osmolarity (mOsM)
18	0	496
18	5	503
15.5	15	489
14	25	476

Sperm collection, freezing & thawing: Sperm was collected from the cauda epididymis. Sperm suspension was mixed with freezing solution, frozen in liquid nitrogen (LN_2) vapour for 10min, and then plunged into LN_2. Sperm samples were thawed in a 40°C water bath for 5-10 seconds and diluted in TL-HEPES with bovine serum albumin (BSA) for function evaluation.

Evaluation of sperm function:

Sperm viability measure	Analysis technique	Fluorescent dye
Motility	Computer-assisted sperm analysis (CASA)	
Plasma membrane integrity	Flow cytometry (FACSCalibur, Becton Dickinson)	SYTO 10/PI
Acrosome membrane integrity		PNA-Alexa Fluor 488 /PI
Mitochondrial membrane potential		JC-1/PI

Analysis: Generalized linear models (GLM) were run in SAS 7.3 for Windows (SAS Institute Inc., Cary, NC) to compare the effects of OptiPrep™ concentration on sperm viability measures. P-values less than or equal to 0.05 were considered significant.

References: [1]Agca 2012 Theriogenology 78; [2]Ostermeier *et al* 2008 PLoS One 3; [3]Critser *et al* 2000 ILAR Journal 41; [4]Landel 2005 Lab Animal 34; [5]Saragusty *et al* 2009 Theriogenology 71; [6] Kim *et al* 2016 Reprod Biol Endocrinol 14.

Acknowledgments

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