Cryoprotective Effect of Glycerol Against Sperm DNA Damage in Frozen-Thawed Boar Spermatozoa

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Introduction

The use of frozen-thawed boar sperm still lags behind chilled boar semen in artificial insemination (AI) operations despite its advantages [1]. Freezing however, may damage the structure and function of sperm, leading to reduced fertilization success. Moreover, glycerol, which is a critical cryoprotectant in most sperm freezing protocols, can be toxic to cells in high concentrations [2]; affecting sperm motility and acrosomal integrity. This study was conducted to determine the cryoprotective effect of glycerol on sperm DNA integrity and motility in frozen-thawed boar sperm.

Materials and Methods

Semen Collection and Cryopreservation

Semen was collected from six mature Large White boars and frozen in liquid nitrogen in 0.5 mL CBS straws (IMV Corporation, USA) using the IceCube programmable freezer (Minitube, Germany) following a standard freezing protocol [3] in either 3, 6 or 8% glycerol. After three months storage, samples were thawed in a 38 °C water bath for 30 s, diluted in BTS and maintained at 38 °C for analysis.

Determination of Sperm Motility and DNA Integrity

Sperm concentration was determined by haemocytometer and motility of 20 x 10^6 sperm/ml at 38°C was analyzed using CASA (Hamilton Thorne). Sperm DNA damage in 20,000 Percoll-purified sperm per boar per treatment was evaluated using TUNEL (Roche) & flow cytometry (Dako Cytomation; Fig. 1) [4].

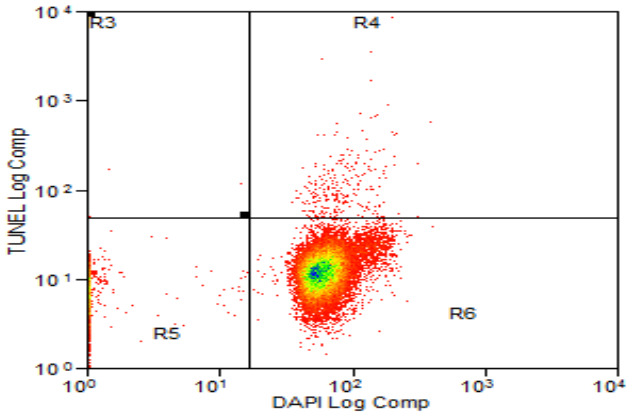

Figure 1. Scatter plot of boar sperm co-labelled by FITC and DAPI. DNA-damaged sperm in R4.

Results

There was no significant difference in DNA damage between fresh or frozen-thawed sperm at each concentration of glycerol, although it tended to be lower in fresh sperm (Table 1; P > 0.05). Both total and progressive motility were higher in fresh than frozen-thawed sperm (P ≤ 0.05), however no difference between frozen-thawed samples cryopreserved in either 3, 6 or 8% glycerol was observed (P > 0.05).

Table 1: Mean (± SEM) percentage of DNA damage and total and progressive motility in fresh and frozen-thawed boar sperm cryopreserved using different concentrations of glycerol.

Parameter	Fresh	Post-thaw		
		3% Glycerol	6% Glycerol	8% Glycerol
DNA damage	1.9 ± 0.4	3.51 ± 0.8	2.8 ± 0.5	3.0 ± 0.8
Total	72.1 ± 2.4[a]	35.3 ± 4.1[b]	26.8 ± 2.5[b]	28.6 ± 3.0[b]
Progressive	39.5 ± 2.2[a]	23.84 ± 3.2[b]	19.5 ± 2.7[b]	18.1 ± 2.2[b]

Values with different letters differ significantly between treatments for each parameter ($P \leq 0.05$); n= 6 boars per treatment.

Discussion

Using standard protocols for boar sperm freezing, our results demonstrated that glycerol at 6% appears to be the most suitable concentration to protect boar sperm against DNA damage, while maintaining the 2nd highest progressive sperm motility. Being able to cryopreserve boar sperm without inducing additional DNA fragmentation, provides confidence that frozen-thawed sperm could contribute to IVF and AI operations without causing increased rates of DNA damage-related early embryonic loss.

References

1. Bailey, J.L., Lessard, C., Jacques, J., Breque, C., Dobrinski, I., W. Zeng, W., Galantino-Homer, H.L., Cryopreservation of boar semen and its future importance to the industry, Theriogenology, 2008, 70: 1251-1259.
2. Buhr, M.M., Fiser, P., Bailey, J.L., Curtis, E.F., Cryopreservation in different concentrations of glycerol alters boar sperm and their membranes, Journal of Andrology, 2001, 22: 961-969.

3. Pursel, V.G., Johnson, L.A., Freezing of boar spermatozoa: fertilizing capacity with concentrated semen and a new thawing procedure, Journal of Animal Science, 1975, 40: 99-102.
4. Peña, S.T.J., Stone, F., Gummow, B., Parker, A.J., Paris, D.B.B.P., Tropical summer induces DNA fragmentation in boar spermatozoa: implications for evaluating seasonal infertility, Reproduction Fertility and Development, 2018, DOI: 10.1071/RD18159.

GENERAL PROGRAM

22 FEBRUARY 2019 (FRIDAY) - Attire: Business /Smart Casual					
09:00am-12:00pm	**Casting of Ballots / Election of New PVMA Officers and Council Members (COMELEC Room)**				
	TECHNICAL BREAK OUT SESSIONS – 1				**POSTER PRESENTATION**
	FUNCTION ROOM 5,6, 7	FUNCTION ROOM 1,2,3,4	HALL D	HALL A,B,C	
	LABORATORY / RESEARCH *Moderator* **DR. HERCULES P. BALDOS**	**POULTRY** *Moderator* **DR. LINA S. POLICARPIO**	**SWINE** *Moderator* **DR. NENETTE ALVIS**	**COMPANION ANIMAL** *Moderator* **DR. MAYSIE T. BATINGA**	
08:00am-08:30am	*Accomplishing Animal Care and Use Statement for IACUC Review* DR. DARIA L. MANALO	*Black Soldier Fly Larvae (Hermetia Illucens) Production, a Potential Supplementary Source of Protein for Free-Range Chickens* DR. ERWIN JOSEPH S. CRUZ	*Blood Iron, Hematocrit and Hemoglobin Values of Piglets Injected with Iron Dextran: Use of Iron Amino Acid Chelate (Iron Glycine Chelate) in Preventing Day 3 to 10 Piglet Anemia* DR. JOEL F. MANGALINDAN	*Diaphragmatic Hernia Repair with the Assistance of Anesthesia Rebreathing Bag* DR. IVY A. ALVAREZ	*Newcastle Disease Prevention and Control Related Practices of Gamecock Raisers in Western Leyte* DR. AGNES M. TAVEROS
08:30am-09:00am	*Detection of Anchoring, Communicating and Occluding Junction Proteins in Rat Tendon Cells* DR. ERROL JAY Y. BALAGAN	*Chlamydia gallinacean as an emerging pathogen in Chickens: Research from 2014-present* DR. ANA MARQUIZA M. QUILICOT	*Cryoloop and Minimum Drop Size Methods in the Vitrification and In-Vitro Maturation of Immature Philippine Native Pig Oocytes* DR. MARVIN BRYAN S. SALINAS	*Entropion: Doing it right the first time* DR. CUCU KARTINI	*Risk Perception of Newcastle Disease on Backyard Chicken Raisers in Baybay City, Leyte Philippines* DR. MELVIN A. BAGOT
09:00am-09:30am	*Health Monitoring of Laboratory Mice and Rats* Dr. Luida C. Cruz	*Results of Epidemic Simulation Modelling to Evaluate Strategies to Control an Outbreak of Avian Influenza (H5N6) among Commercial Poultry Farms in Central Luzon, Philippines* DR. RODERICK T. SALVADOR	*Cryoprotective Effect of Glycerol Against Sperm DNA Damage in Frozen-Thawed Spermatozoa* DR. SANTIAGO T. PEÑA JR.	*Gastric Dilation and Volvulus (GDV): Improving our Diagnosis, Treatment Plan Based on Prognosis* DR. IVY A. ALVAREZ	*New Castle Disease: Evaluation of the Attitude of Gamefowl Raisers in Western Leyte* DR. JANE P. DAUTIL
09:30am-10:00am	*Optimizing Laboratory Diagnostic Tests for Case Problem Solving and Disease Diagnosis* DR. VERONICA A. MATAWARAN	*The Avian Influenza Status of Layer Farms in San Jose, Batangas after the First Avian Influenza Outbreak in the Philippines* DR. ANTONIO AUGUSTUS C. LARANAS	*Swine Veterinary Practice Trends* DR. ROSELLE F. CUDAL	*Genetic Characterization and Phylogenetic Analysis of Canine Parvovirus from Domestic Dogs (Canis familiaris) in Quezon City, Philippines* DR. DENNIS V. UMALI	*Knowledge of Gamefowl Raisers on Newcastle Disease in Western Leyte, Philippines* DR. CARL LEONARD M. PRADERA
10:00am-10:30am	**COFFEE BREAK and BOOTH VISIT**				
10:30am-11:00am	*Physiological Responses, Cortisol Level and Analgesia of Goats Subjected to Exploratory Laparatomy Under Acupuncture and Regional Analgesia* DR. JESSIE A. ACORDA	*Prevalence and Antimicrobial Susceptibility Profile of Escherichia coli O157:H7 in the Feces of Native Chickens (Gallus gallus L.) from Selected Backyard Farms in Upland Cavite, Philippines* DR. MA. CYNTHIA R. DELA CRUZ	*Upgrading Swine Value Chain through Public Initiated Food Safety Standards* DR. ZEAM VOLTAIRE E. AMPER	*Using L-Asparaginase to Improve Treatment of Canine Transmissible Venereal Tumor Resistant to Vincristine* DR. ROUCHELLE B. ALONTE	*Pet Owners' Awareness on RA 9482 (Anti-Rabies Act of 2007) in Magalang, Pampanga* DR. REMEDIOS D. SAN JOSE