Sperm Cryopreservation in Australian Farmed Greenlip (*Haliotis laevigata*) and Blacklip (*H. rubra*) Abalone

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DECLARATION

I certify that this work contains no material which has been accepted for any other degree or diploma in any university or institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

In addition, I certify that no part of this work will be submitted for any other degree or diploma in any university or institution without the prior approval of the University of Flinders.

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ABSTRACT

Greenlip *Haliotis laevigata* and blacklip *H. rubra* abalone as well as their hybrid are the major abalone farmed in Australia. To ensure the long-term sustainable and competitive development of the industry, genetic improvement programs have been applied. However, the efficiency of these programs has been compromised due to: (1) asynchronous spawning in males and females within and between species; (2) the short spawning window period in a breeding season; and (3) risks associated with keeping superior broodstock alive and healthy. Sperm cryopreservation is a proven technique to overcome similar issues and sperm collected from wild greenlip abalone has been investigated and 90% post-thaw sperm fertilization rates have been achieved. Nevertheless, when the developed protocol was applied to sperm from farmed stocks, low and highly variable results were observed, hindering its application. The Australian abalone aquaculture industry now relies entirely on domesticated farmed abalone as broodtsock. Therefore, understanding factors causing this discrepancy, and development of sperm cryopreservation techniques suitable for Australian farmed abalone species, are the focus of this PhD project.

Factors affecting sperm cryopreservation and strategies to improve the sperm quality were evaluated using both programmable and/or non-programmable freezing techniques in farmed greenlip and blacklip abalone. In greenlip abalone, broodstock physiological conditions, such as broodstock age and sperm collection time over a natural spawning period, were also evaluated.

Among the single cryoprotectant agent [dimethyl sulfoxide (DMSO), propylene glycol, ethylene glycol, and glycerol] evaluated, DMSO produced the best post-thaw sperm motility and/or fertilization rates using both programmable and non-

programmable freezing techniques in farmed greenlip abalone. However, to achieve the highest post-thaw sperm fertilization rates of 60 to 70%, a higher DMSO concentration and a sperm to egg ratio were required in the former technique (10% and 40,000:1) than in the latter one (6% and 10,000:1). The addition of sugar (glucose, sucrose or trehalose) in DMSO to further improve the post-thaw sperm quality showed that the post-thaw sperm motility was significantly improved by the addition of 4% sucrose using programmable freezing technique, whereas the post-thaw sperm fertilization rates were not. In contrast, when a non-programmable freezing technique was used, the addition of 1% glucose in 6% DMSO significantly improved both post-thaw sperm motility and fertilization rates, with the latter reaching 80%. Fluorescent stain analyses revealed that the addition of glucose significantly improved the post-thaw sperm plasma membrane integrity (PMI) and mitochondrial membrane potential (MMP).

Data from the addition of amino acids (taurine and glycine) and vitamin (L-ascorbic acid) revealed that the addition of 0.6% glycine in 10% DMSO or 6% DMSO + 1% glucose significantly improved the post-thaw sperm fertilization rates to over 90% when using the programmable and non-programmable freezing techniques, respectively. The addition of glycine improved the post-thaw sperm MMP with application of the former technique, whereas PMI and acrosome integrity (AI) with application of the latter one. After the addition of 0.6% glycine, the optimal sperm to egg ratio remained the same in programmable freezing technique whereas this ratio reduced from 10,000:1 to 2,000:1 in the non-programmable freezing technique. Flow cytometry analyses showed higher post-thaw sperm PMI, MMP and AI values were achieved when using non-programmable verses programmable freezing techniques.

Evaluation of the effects of glucose, fructose and galactose using the non-

programmable freezing technique indicated that in farmed greenlip abalone, galactose and fructose had a similar ability to protect sperm from cryoinjury as glucose. The replacement of glucose with either of these monosaccharides resulted in a similar level of post-thaw sperm fertilization rate, PMI, MMP and AI.

Results from evaluation of broodstock physiological conditions in farmed greenlip abalone showed that sperm collected at the middle of a natural spawning period had a better ability to tolerate the cryopreservation processes than those collected at the beginning or at the end of a natural spawning period, resulting in significantly higher post-thaw sperm motility, fertilization rate, PMI, MMP and AI. In contrast, little difference in these parameters was found between 2 and 3 years old animals.

Among the single cryoprotectant agents assessed in farmed blacklip abalone using the non-programmable freezing technique, 6% DMSO achieved the highest post-thaw sperm motility, which was the same as found for farmed greenlip abalone. Further addition of sugar (glucose, sucrose or trehalose) in 6% DMSO showed that the addition of 2% glucose significantly improved the post-thaw sperm quality, producing the highest post-thaw sperm fertilization rate of 70% at a sperm to egg ratio of 10,000:1, although the rate was significant lower than the control (83%).

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