Developing an effective diluent for transport of rabbit semen

Dr Roslyn Bathgate and Danielle Johinke

Issue

The farming of rabbits for meat in Australia is an industry with great potential for growth as it provides a useful source of diversification in regional and rural areas where farm incomes are low. Commercial rabbit breeding is becoming more dependent upon successful artificial insemination (AI) programs. Unfortunately, rabbit farms in Australia are still limited to the use of freshly diluted sperm collected from bucks on-site due to the low fertility and prolificacy rates achieved using stored rabbit semen.

Considerable research has been undertaken into the preservation of rabbit sperm. The low levels of fertility and prolificacy achieved with frozen semen do not allow its use at a commercial level, thus the improvement of liquid storage protocols is imperative. Chilled storage protocols are commonly used in the European rabbit industry to preserve the fertilising capacity of rabbit spermatozoa for up to 48 hours post-semen collection. In the Australian industry, this is not sufficient time to allow for transport between rabbit breeders due to the combination of greater distances between farms and poorer transport infrastructure. Indeed, a minimum preservation time of 72 hours between semen collection and insemination is required to facilitate the transfer of superior buck genetics between Australian farms. At present, the available semen diluents do not support storage for this length of time and as such, the development of a diluent that meets the logistical needs of the Australian industry is required.

Project Objectives

This project aimed to formulate a semen diluent that would enable the storage of rabbit semen for a minimum of 72 hours and facilitate the transportation of rabbit semen across the Australian rabbit industry.

To this end, the project aimed to:

- Compare semen extenders and storage temperatures currently employed in the chilled storage of rabbit semen in terms of \textit{in vitro} sperm quality and \textit{in vivo} fertility
- Provide a comprehensive assessment of \textit{in vitro} sperm quality during prolonged chilled storage of rabbit semen by flow cytometric analyses
- Identify protective additives to reduce the deleterious effects associated with chilled storage and prolong sperm longevity
- Optimise \textit{in vitro} dilution and storage protocols for rabbit semen
Results

Comparison of different semen extenders and storage conditions revealed that the Tris-citric acid-glucose (TCG)-based extender described by Boiti et al. (2005) and a storage temperature of 15°C best preserved in vitro rabbit sperm quality for up to 96 hours.

During chilled storage, damage to sperm quality due to intracellular reactive oxygen species (ROS) production, lipid peroxidation and associated oxidative stress was reduced by supplementation of the sperm medium with 100µM quercetin.

Chilled storage at a concentration of 30x10⁶ spermatozoa/mL was optimal for rabbit semen as excessive dilution to a low concentration of 15x10⁶ spermatozoa/mL exerts a detrimental effect on sperm motility, while preservation at a high concentration results in significantly higher production of intracellular ROS. The excessive generation of ROS during chilled storage can be further limited by storage of rabbit spermatozoa in sealed straws, rather than tubes.

Implications

Commercial rabbit breeding in Australia can now take advantage of the described chilled storage techniques to preserve rabbit semen of high quality or superior genetic value for AI up to 72 hours post-semen collection. In doing so, the Australian rabbit industry will benefit from a faster growing, more prolific population on farm and will be able to improve production rates so as to meet growing consumer demand. Furthermore, the deleterious effects of long-term preservation on rabbit sperm quality are now better understood and can be alleviated by controlling the conditions to which spermatozoa are exposed during storage.

One of the major impediments in the successful preservation of sperm fertilising capacity over time is the production of intracellular ROS by spermatozoa and associated oxidative damage. We have been able to effectively reduce the level of ROS produced by rabbit spermatozoa chilled for up to 96 hours through supplementation of the diluent with 100µM quercetin prior to semen dilution.

This finding provides future researchers with a starting point for the improvement of chilled storage protocols in rabbits. While the rates of pregnancy achieved with chilled rabbit semen for more than 72 hours may not yet be sufficient for commercial rabbit meat production, this research has demonstrated the ability of quercetin supplementation in the semen extender to improve both the in vivo quality and in vivo fertility of rabbit semen during chilled storage up to 96 hours.
Reference


This project has resulted in the following journal articles and conference papers


D. Johinke, S.P. de Graaf and R. Bathgate. Flow cytometric analyses of rabbit spermatozoa incubated with Merocyanine-540, Yo-Pro-1 or FITC-PNA revealed a minimal effect of staining time on the proportion of cells with high fluorescence. Reproduction in Domestic Animals (2015); Submitted.


D. Johinke, S.P. de Graaf and R. Bathgate. The effects of various diluents on sperm motility of rabbit semen stored at 5 degrees C for up to 96 h. Reproduction in Domestic Animals (2012); 585–6.


For more information

Dr Roslyn Bathgate
Faculty of Veterinary Science
The University of Sydney
roslyn.bathgate@sydney.edu.au

Danielle Johinke
Faculty of Veterinary Science
The University of Sydney
danielle.johinke@sydney.edu.au

Contact RIRDC

Phone: 02 6271 4100  Email: rirdc@rirdc.gov.au  Web: www.rirdc.gov.au

Pub. No. 15/015