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Non-surgical deep intra-uterine transfer of in vitro produced porcine embryos derived from sex-sorted frozen–thawed boar sperm

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Abstract

Embryos and offspring of a pre-determined sex have been produced in pigs using AI and IVF with unfrozen sperm, and after surgical insemination with sex-sorted frozen–thawed sperm. The aims of this study were to demonstrate that sex-sorted frozen–thawed boar sperm could be incorporated into pig IVF for the production of embryos of a pre-determined sex and that these embryos could be successfully non-surgically transferred. Oocytes were matured in vitro, fertilised with either unsorted or sex-sorted frozen–thawed sperm and cultured until the eight-cell stage. These embryos were then transferred to recipients ($n = 7$) non-surgically ($n = 70$ embryos per sow). Oocyte cleavage was similar between sex-sorted (1538/5044; 30.5%) and unsorted (216/756; 28.6%) frozen–thawed sperm, and PCR sex-determination of the embryos confirmed that they were of the predicted sex ($n = 16$). Delayed return to oestrus (>23 days) was observed in five recipient sows (71.4%). Fetal sacs were observed by transcutaneous ultrasound on Day 18 in one of these sows. Pre-sexed porcine IVP embryos can be successfully produced using sex-sorted frozen–thawed boar sperm, and these embryos are capable of initiating pregnancies when transferred to recipients. However, further refinement of porcine ET protocols are required to enable development to term.

Keywords: Sex preselection; Sperm sexing; Cryopreservation; In vitro fertilisation; Non-surgical embryo transfer

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1. Introduction

Offspring from a number of species have been produced from artificial insemination (AI) and in vitro fertilisation (IVF) with embryo transfer (ET) using sperm that has been sorted by flow cytometry into X- and Y-chromosome bearing populations (Johnson, 2000). Due to the time constraints of the sex-sorting procedure, commercialisation requires cryopreservation of the sex-sorted sperm. Combination of sex-sorting and cryopreservation of sperm prior to AI has been successfully implemented in cattle (Seidel et al., 1999a,b), horses (Lindsey et al., 2002), pigs (Johnson et al., 2000) and sheep (Hollinshead et al., 2002). Despite this, the technology has thus far become commercially available only in cattle, horses and humans (Seidel and Garner, 2002).

One limitation of the sex-sorting procedure that is particularly relevant to pigs is the vast quantity of sperm required to achieve acceptable fertility levels from AI of sows and gilts (a double insemination of 6×10^9 frozen–thawed sperm per dose compared to a single insemination of 20×10^6 for cattle). Recent technology has allowed the number of sperm required for an insemination to be reduced by depositing the sperm deep into the uterine horn (Martinez et al., 2001a). This has allowed for the dose to be reduced to 250×10^6 frozen–thawed sperm without significant loss of fertility compared to conventional cervical insemination with chilled semen (Bathgate et al., 2003). However, this is still prohibitively high in terms of time taken to sort each dose.

The use of sex-sorted, frozen–thawed sperm in combination with IVF and ET offers a more efficient use of sorted sperm, as vastly lower numbers are required to fertilise each oocyte. The same technology developed by Martinez et al. (2001b) to deposit semen into the uterine horn has been used for non-surgical ET (Martinez et al., 2001b). This heightens the potential of incorporating the use of sex-sorted, frozen–thawed sperm into commercial production of pigs.

The aims of this study were to incorporate sex-sorted frozen–thawed boar sperm into an in vitro embryo production system for the production of pre-sexed embryos, and demonstrate that in vitro derived porcine pre-sexed embryos are capable of developing to term.

2. Materials and methods

2.1. Animals and experimental design

Procedures described herein were approved by The University of Sydney's Animal Ethics Committee. Cumulus oocyte complexes (COCs) were collected from sows slaughtered at a local abattoir. In vitro matured oocytes ($n = 5800$; three replicates) were incubated with either unsorted or Y-chromosome bearing frozen–thawed sperm and cultured until the eight-cell stage. Embryos at the eight-cell stage or greater (Day 4; $n = 490$) were then transferred to synchronised recipients to observe the in vivo development.

2.2. Collection and preparation of sperm for sorting

The sperm rich fraction of semen was collected from a mature boar, diluted with Androhep (1:3 semen:Androhep; Minitube, Germany), and transported to the laboratory at ambient temperature (approximately 1 h at 20–25 °C). Upon reaching the laboratory the semen was further diluted with Androhep to a concentration of 100×10^6 sperm/ml. Extended semen (990 μl) was aliquoted into 1.5 ml eppendorf tubes (Livingstone, Rosebury, NSW, Australia) to which 10 μl of 5 mg/ml Hoechst 33342 (bis-benzimide, Sigma–Aldrich, St. Louis, MO, USA) was added. The tubes were