Available online at www.sciencedirect.com
SCIENCE DIRECT
Theriogenology
ELSEVIER
Theriogenology 64 (2005) 363–377
www.journals.elsevierhealth.com/periodicals/the

The effect of gamete co-incubation time during in vitro fertilization with frozen-thawed unsorted and sex-sorted ram spermatozoa on the development of in vitro matured adult and prepubertal ewe oocytes

Katherine M. Morton[a,*], Sally L. Catt[b,1], Fiona K. Hollinshead[a], W.M. Chis Maxwell[a], Gareth Evans[a]

[a]*Centre for Advanced Technologies in Animal Genetics and Reproduction (ReproGen), Faculty of Veterinary Science, The University of Sydney, NSW 2006, Australia*
[b]*Sydney IVF, 4 O'Connell St. Sydney, NSW 2000, Australia*

Received 29 September 2004

Abstract

In vitro matured adult (Experiment 1) and prepubertal (Experiment 2) ewe oocytes were co-incubated with unsorted or sex-sorted frozen-thawed spermatozoa for 2 to 3 h (short) or 18 to 20 h (long) to determine the effects of reducing the gamete co-incubation time during IVF on subsequent embryonic development in vitro. For oocytes derived from adult ewes, there were no differences in oocyte fertilization and cleavage at 24 h post insemination (hpi) between types of spermatozoa or co-incubation times ($P > 0.05$). By 48 hpi, oocyte cleavage was higher after a short (390/602, 64.8%) compared with a long (381/617, 61.7%) co-incubation ($P < 0.05$), and was not significantly different for unsorted (266/372, 71.5%) and sex-sorted (505/849, 59.9%) spermatozoa. Blastocyst formation from cleaved oocytes was similar for unsorted (150/266, 56.4%) and sex-sorted (295/505, 58.4%) spermatozoa, but was higher after a short (240/390, 61.5%) than long (205/381, 53.8%) co-incubation ($P < 0.05$). Oocyte development to the blastocyst stage was not different for unsorted (150/372; 40.3%) and sex-sorted (295/847; 34.8%) spermatozoa but was significantly increased by a short (240/

* Corresponding author. Tel.: +61 2 9351 2463; fax: +61 2 9351 3957.
E-mail address: kmorton@vetsci.usyd.edu.au (K.M. Morton).
[1] Present address: Academic Unit of Paediatrics Obstetrics and Gynaecology, School of Medicine, University of Leeds, LS2 9NS, UK.

0093-691X/$ – see front matter
doi:10.1016/j.theriogenology.2004.12.004

602, 39.9%) compared with a long (205/617, 33.2%) co-incubation. Fertilization of oocytes from prepubertal ewes was similar for types of spermatozoa and for duration of co-incubation. Oocyte cleavage (48 hpi) was similar for a short (241/377, 63.9%) and long (226/349, 64.8%) co-incubation with unsorted spermatozoa, but was increased ($P < 0.05$) by a long co-incubation (286/500, 57.2% versus 163/517, 31.5%) with sex-sorted spermatozoa. Blastocyst formation from cleaved oocytes was similar for unsorted (230/467, 49.3%) and sex-sorted (186/449, 41.4%) spermatozoa, and a short (200/404, 49.5%) or long (216/512, 42.1%) co-incubation. However, oocyte development to the blastocyst stage was higher ($P < 0.05$) after IVF with unsorted (230/726, 37.1%) than sex-sorted (186/1017, 18.3%) spermatozoa. Reducing the duration of gamete co-incubation did not deleteriously affect the in vitro development of adult and prepubertal ewe derived oocytes after IVF with unsorted and sex-sorted spermatozoa. In general, sex-sorting had no substantial influence on fertilization and embryo development rates.

Keywords: Sperm sexing; Prepubertal; In vitro fertilization; Embryo; Sex pre-selection

1. Introduction

Offspring of a pre-determined sex have been produced using fresh and frozen-thawed sex-sorted spermatozoa after both AI and IVF [1]. Sex-sorted spermatozoa have been successfully incorporated into IVF systems in cattle (fresh [2]; frozen-thawed [3]), pigs (fresh [4]; frozen-thawed [5]) and sheep (fresh [6]; frozen-thawed [7,8]), although reductions to the in vivo [9–14] and in vitro [2,3,8,15] fertility of sex-sorted spermatozoa have been reported. In vitro assessment of flow cytometrically sorted spermatozoa has demonstrated that, compared with unsorted spermatozoa, sorted spermatozoa have altered patterns of motility [16–20], contain a higher proportion of capacitated spermatozoa [14,21,22], and have a reduced lifespan [14].

Prolonged gamete co-incubation during IVF results in the exposure of oocytes and embryos to high levels of reactive oxygen species (ROS) produced by spermatozoa [23], which may have detrimental effects on embryonic development [24,25]. Reducing the duration of gamete co-incubation during IVF has been reported to increase oocyte fertilization [25,26], blastocyst formation [27–29], and the rate of embryonic development [25]. Given the reduced in vitro lifespan of sorted frozen-thawed spermatozoa, reducing the gamete co-incubation length during IVF with sex-sorted frozen-thawed spermatozoa may enhance subsequent embryonic development in vitro.

The objectives of the present study were to investigate the effects of reducing the duration of gamete co-incubation during IVF with unsorted and sex-sorted frozen-thawed spermatozoa from 18 to 20 h (long) to 2 to 3 h (short), on the subsequent embryonic development of in vitro matured adult and prepubertal ewe-derived oocytes.

2. Materials and methods

2.1. Animals and experimental design

Procedures described herein were approved by The University of Sydney's Animal Ethics Committee. Two experiments were performed. Cumulus oocyte complexes (COCs)