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Animal Reproduction Science 105 (2008) 332–343

ANIMAL REPRODUCTION SCIENCE

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The temporal relationship between oocyte maturation and early fertilisation events in relation to the pre-ovulatory LH peak and preimplantation embryo development in red deer (*Cervus elaphus*)

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Received 1 September 2006; received in revised form 4 March 2007; accepted 23 March 2007
Available online 27 March 2007

Abstract

The temporal relationships among oocyte maturation, gamete transport and fertilisation following the pre-ovulatory luteinsing hormone surge in red deer were established; and secondly, early preimplantation development to the blastocyst stage in relation to the onset of oestrus was determined for red deer. In the first series of observations, oestrus was synchronised in April ($N = 22$), for the fixed time recovery of gametes from 0 to 36 h after the estimated pre-ovulatory LH peak. Matings were observed and the time of the LH peak was determined from the retrospective analysis of blood plasma collected at 3 h intervals. Gametes were recovered surgically and the meiotic status of follicular and ovulated oocytes assessed. Spermatozoa were recovered from the oviduct and their motility analysed by videomicroscopy. Nineteen of 22 hinds exhibited a pre-ovulatory LH surge and were observed to mate. Oocyte metaphase I occurred between 11 and 18 h, and metaphase II was completed within the follicle between 20 and 25 h following the pre-ovulatory LH peak. Fertilised ova were recovered from 30 to 36 h in both the ampulla and isthmic portions of the oviduct. Motile spermatozoa were first recovered from the isthmus and the ampulla at 13 and 21 h, respectively, after the LH peak. Hyperactive spermatozoa were observed in both the isthmus and the ampulla flushings but only from the eight hinds that had ovulated.

In the second series of observations, 16 mature hinds were synchronised and allocated to groups for embryo collection on days 3, 5 and 7 after oestrus. Eight embryos were recovered; an 8-cell at 90 h, 3 morulae at 137, 138 and 186 h, and 4 blastocysts at 180, 182 and 190 h post-mating. Blastocysts were only recovered from the

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0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2007.03.013

uterine horns and the mean ± S.E.M. number of nuclei per blastocyst was 93.5 ± 10.0 with a range of 66–114 cells. The results of this study will improve the application of assisted reproductive technologies to red deer as they indicate that oocyte maturation, fertilisation and early embryonic development of the red deer is similar to other domestic ruminants with the exception that the red deer embryo enters the uterus at the blastocyst stage.

Keywords: In vivo oocyte maturation; Sperm motility; In vivo fertilisation; Embryo development; Embryo quality; In vivo; LH surge; Oviduct; Red deer; *Cervus elaphus*

1. Introduction

From its beginnings in 1971, the New Zealand red deer industry has matured and is beginning to select breeding animals based upon genetic breeding values. During this time, considerable research and development has been invested in various reproductive technologies to increase the rates of genetic gain. Advances in out-of-season breeding, artificial insemination (AI) and embryo transfer have been well documented (Asher et al., 1993; Fennessy et al., 1994).

Much of the development of multiple ovulation-embryo transfer (MOET) proceeded on an ad hoc basis using adaptations of sheep and cattle protocols (Fennessy et al., 1994) and the same is true for red deer in vitro embryo production. Cattle in vitro embryo production systems have not transferred well to red deer; reported fertilisation rates are low and no embryo development occurred beyond the 16-cell stage (Fukui et al., 1991; Bainbridge et al., 1999; Berg et al., 2002a). Fertilisation levels significantly improved (to approximately 70%) with the addition of sheep serum (20%) to either cattle or sheep fertilisation media (Comizzoli et al., 2001; Berg et al., 2002b; Locatelli et al., 2005). Berg et al. (2002b) demonstrated that 30% of fertilised zygotes resulted in live calves upon transfer into the oviducts of synchronised red deer recipients.

A variety of non-invasive morphological and biochemical markers have been used to assess in vitro blastocyst quality in domestic ruminants. These include gross embryo morphology, the timing of development, cell numbers and metabolic tests, all of which are then compared to the ideal, the in vivo embryo (Van Soom et al., 2001). Cell numbers of red deer in vitro produced morulae and blastocysts have been reported with a range of 11–25 and 40–80 cells, respectively (Berg et al., 1995; Comizzoli et al., 2001). These cell numbers are low when compared with in vitro sheep and cattle embryos (Knijn et al., 2003; Gardner et al., 1994; Leoni et al., 2006; Pomar et al., 2005), and both studies suggest that the in vitro culture system was inadequate. However, in vivo red deer embryo cell numbers as well as embryo morphology and the timing of development have not been reported and these lower cell numbers observed may also reflect a significant species difference.

This paper describes the temporal relationships, among the onset of oestrus, oocyte maturation, gamete transport and fertilisation, following the pre-ovulatory LH peak in red deer. Secondly, it describes the in vivo early preimplantation embryonic development to the blastocyst stage.

2. Materials and methods

2.1. Experiment 1: Gamete transport and maturation

2.1.1. Animals, management, synchronisation treatment and oestrus detection

Twenty-two mixed-aged (4–8 years) red deer hinds at Ruakura Research Centre were separated from the general herd and were run as a single group with a vasectomised stag until the start of