PREGNANCIES FOLLOWING THE TRANSFER OF *IN VITRO* MATURED AND FERTILIZED RED DEER (*Cervus elaphus*) OOCYTES

D. K. Berg, G. W. Asher, P.A. Pugh., H.R. Tervit and J.G. Thompson
AgResearch, Ruakura Agricultural Research Centre, Hamilton, New Zealand

Compared to heparin, the addition of Day 1.5 (Day 0 = estrus) sheep serum to IVF medium greatly enhances the penetration rate of IVM red deer oocytes. This study examined the effects of serum source; estrous deer serum (EDS), Day 1.5 deer serum (DS), or Day 1.5 sheep serum (SS), on the fertilization rates of IVM oocytes. The viability following transfer of IVM/IVF oocytes was also assessed.

Cumulus enclosed oocytes (COC) were aspirated from 2 to 6 mm follicles on abattoir-derived ovaries and matured for 22 h under standard conditions. Frozen-thawed red deer sperm, pooled from 2 stags, were Percoll separated, washed once in Hepes synthetic oviduct fluid (HSOF) and resuspended in the appropriate IVF medium. The IVF medium used was a modified SOF (1mM pyruvate, 10mM lactate, no glucose) containing 20% heat-inactivated EDS, DS, or SS. Sperm were co-incubated with 10 COC in a 100 µl drop at a final concentration of 3 x10^5 sperm/ml for 24 h at 39°C under 7% O_2, 5% CO_2 and 88% N_2. All oocytes were fixed and stained with 1% lacmoid. An oocyte was considered normally fertilized when a sperm tail plus 2 pronuclei were present in the cytoplasm.

Table 1. *In vitro* fertilization of IVM red deer oocytes in SOF supplemented with different sera.

Treatment	Oocyte no.	Fertilized (%)	Abnormal Fertilization (%)	Unfertilized (%)
EDS	70	8 (11)	9 (13)	53 (76)
DS	84	22 (24)	3 (4)	59 (70)
SS	82	60 (73)	9 (11)	13 (16)

High fertilization rates were observed when SS but not EDS or DS was added to the IVF medium (Table 1; $p<0.001$, $\chi^2=86.5$). Polyspermic/abnormal fertilization rates were similar among the groups.

In a second experiment, oocytes were matured and fertilized in SOF+SS with semen from a single sire. Fifteen resulting embryos (2- and 4-cell) were transferred to 15 synchronized hinds after IVF. Fourteen embryos (8- to 10-cell) were transferred to 13 hinds following 48 h *in vitro* culture in either SOF+BSA (n=10) or co-culture with red deer oviduct epithelial cells in TCM199 + 10% DS (n=4). An additional 12 control hinds were synchronized and naturally mated with the IVF sire. Pregnancies were confirmed ultrasonographically 45 d after transfer.

Five of 15 (33%) hinds were pregnant following the transfer of 2-cell (3 hinds) or 4-cell (2 hinds) embryos immediately after IVF while only 1 pregnancy of 14 (7%) was established from the transfer of an 8-cell embryo that had been cultured in SOF+BSA. Six of the 12 control hinds were pregnant at scanning.

The results show that SS is more effective for induction of the capacitation of red deer sperm than DS and EDS. In addition, IVM/IVF oocytes are capable of establishing pregnancies when transferred to recipient hinds. However, their viability decreases after 48 h of *in vitro* culture, possibly due to an inadequate culture system. We report the first pregnancies from IVM/IVF red deer oocytes which may serve as a model for endangered cervid species.