In vitro maturation and fertilization of red deer (*Cervus elaphus*) oocytes.

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The increased development of deer farming has stimulated interest in applying artificial breeding techniques to deer. Information concerned with red deer (*Cervus elaphus*) *in vitro* maturation/*in vitro* fertilization (IVM/IVF) has been limited to one report by Fukui *et al.* (1991). Maturation rates were acceptable (70%) but fertilization rates were low (20%), and may have been attributable to inappropriate timing of IVF with respect to oocyte maturation or inadequate *in vitro* capacitation of sperm. This study investigated the time course of meiotic maturation during IVM of red deer oocytes and fertilization procedures of the resulting matured oocytes.

Oocytes were aspirated from 2-6mm follicles derived from abattoir ovaries. Ten cumulus-enclosed oocytes (COC) were maturated in 50µl of maturation medium (TCM 199 supplemented with 10 µg/ml FSH and LH, 1 µg/ml E_2 and 10% fetal calf serum) under oil, at 39°C under 5% CO_2 in air. Oocytes were fixed with acetic acid/alcohol at 0, 6, 9, 12, 15, 18, 24 and 27 hours (h), fixed and lacmoid-stained to access nuclear maturation.

Frozen-thawed red deer sperm, pooled from 2 stags, was separated onto a Percoll gradient, washed once in HEPES Synthetic Oviduct Fluid (SOF) and divided into 3 aliquots for fertilization treatments: A) SOF + 20% Sheep Serum (SS); B) SOF + heparin (10 µg/ml); C) SOF + BSA. Sperm were added to 10 COC (matured for 24-25 hrs) at a final concentration of 1 million/ml and co-incubated for 24 hours at 39°C under 7% O_2, 5% CO_2, 88% N_2. Subsequent IVF used SOF + 20% SS at various sperm concentrations (1, 0.5, 0.3 million sperm/ml). At the cessation of culture all oocytes were lacmoid-stained to assess fertilization. An oocyte was considered fertilised when at least one sperm tail and a swollen sperm head with associate female chromatin was present in the cytoplasm. Polyspermic fertilization occurred when more than two pronuclei and sperm tail(s) were present.

The timing of *in vitro* oocyte maturation was similar to other domestic ruminants. Timing of germinal vesicle breakdown (s.e.) occurred at 7 (0.6)h, metaphase I, 12 (0.4)h; anaphase or telephase I, 19 (0.4)h; and metaphase II, 25 (0.9)h.

The addition of SS to fertilization medium significantly increased the fertilization rate (s.e.): A) 100% (0.2); B) 2% (0.1); C) 0% ($P<0.001$). However, a high percentage of the oocytes, (37%) were polyspermic. Decreasing the sperm concentration to 1,0.5 and 0.3 million sperm/ml decreased the percentage (s.e.) of polyspermic fertilization to 46 (13)%; 39 (6)%; 4 (3)% respectively $P<0.01$), without decreasing the overall fertilization rate of 79 (3)%. These results demonstrate that red deer oocytes complete meiotic maturation by 25 hours of culture. Such oocytes are readily fertilised *in vitro* when sheep serum is added to the fertilization medium.

REFERENCES

Fukui, Y., McGowan, L.T., James, R.W., Asher, G.W. & Tervit, H.R. 1991. Effects of culture duration and time of gonadotropin addition on *in vitro* maturation and fertilization of red deer (*Cervus elaphus*) oocytes. *Theriogenology* **35**:499-512.