IN VITRO MATURATION AND FERTILISATION OF RED DEER (*Cervus elaphus*) OOCYTES

Y. Fukui, L.T. McGowan, R.W. James, G.W. Asher and H.R. Tervit

MAF Technology, Ruakura Agricultural Centre, P.B., Hamilton, New Zealand.

Successful IVM/IVF techniques can be used to produce large numbers of embryos cheaply for transfer and other manipulations. The technology has not previously been reported for deer. The objectives of this study were to determine culture duration and timing of gonadotrophin (FSH and LH) addition for successful IVM of Red deer oocytes and to examine the fertilisability of the oocytes produced.

Immature oocytes were aspirated from 1-4 mm diameter follicles on slaughterhouse-sourced ovaries and then cultured in TCM199 supplemented with 10% FCS, $1x10^6$ granulosa cells/ml and 1 μg/ml oestradiol at 39 C under 5% CO_2 in air for 16, 20, 24 or 28 h. Gonadotrophins (10 μg/ml FSH and LH) were added to the culture medium at the start of culture (0 h) or after 6 h. The trial was replicated 8 times and used a total of 1208 oocytes. In each replicate, approximately one-third of the oocytes were examined at the end of culture for maturation (M-II and polar body) and the remainder were IVF with frozen-thawed semen collected from a stag by electroejaculation. The thawed sperm were swum up and heparin treated (100 μg/ml for 15 m), and oocytes and sperm ($2.5x10^6$ sperm/ml) were incubated for 18-20 h at 39 C under 5% CO_2 in air. After incubation, oocytes were examined for evidence of fertilisation. IVF oocytes from 4 of the IVM treatments (n=309, 5 replicates/treatment) were cultured for 7 d in SOF supplemented with 10% FCS at 39 C under 5% CO_2, 5% O_2, 90% N_2 and examined for cleavage (2- to 8-cells) on Day 2 and further development on Day 7. The results are as follows:

Time of FSH/LH addition {h}	Culture duration {h}	Mean percent oocytes Matured	Fertilised	Cleaved
0 (6)	16	2.2 (7.2)	8.4 (5.8)	- (-)
	20	64.9 (61.1)	13.0 (23.6)	6.3 (18.2)
	24	76.6 (63.7)	20.6 (20.3)	5.1 (6.8)
	28	74.0 (72.8)	5.3 (10.2)	- (-)
	Total	54.4 (51.2)	11.8 (15.0)	5.7 (12.5)

A 16 h culture duration resulted in significantly ($P<0.001$) lower IVM rates than the longer durations. IVF rates were low in all treatments, but oocytes IVM for 20 and 24 h had significantly higher ($P<0.001$) IVF rates than those IVM for 16 and 28 h. The timing of gonadotrophin addition did not affect IVM rate, but tended to increase IVF rate and significantly ($P<0.05$) increased the percent of oocytes cleaving. Oocytes IVM for 20 h and with delayed addition of gonadotrophins cleaved most readily to 2- to 8-cells. No embryos continued development between Day 2 and 7 of culture. Further studies are needed to improve the success of IVF and development during culture.