

IN VITRO CULTURE OF EARLY CLEAVAGE STAGE EMBRYOS RECOVERED FROM
SUPEROVULATED RED DEER (*Cervus Elaphus*).

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An increase in deer farming and captive propagation of endangered deer species have stimulated an interest in applying artificial breeding techniques to deer. There have been few published studies investigating cervine embryo collection and in vitro culture. One report has described the culture of in vitro derived red deer embryos and found that development did not proceed past the 8-cell stage (Fukui, Y. *et al.*, 1991, *Theriogenology* 35: 499).

This study reported here compared the efficacy of: two superovulation protocols for producing early cleavage stage red deer embryos and three culture systems for their ability to support further embryo development.

Twenty hinds (2-7 years old) were synchronized with intravaginal CIDR® devices inserted for 12 days. All hinds received a total of 0.4 units of ovine follicle stimulating hormone (FSH; Ovagen, ICP, Auckland, NZ) administered as eight equal doses, each twelve hours apart, beginning 72 hours before removal of CIDR® devices. In addition, hinds received 200 iu pregnant mares serum gonadotrophin (PMSG), either with the first, (Treatment 1, n=10), or last FSH injection (Treatment 2, n=10). Hinds were placed with a fertile stag at CIDR® removal and prepared for surgery 63 hours post CIDR® withdrawal. The number of corpora lutea and large follicles were recorded following mid-ventral laparotomy and embryos were recovered by retrograde flushings of oviducts. The recovered embryos (1- to 4-cells) were washed and randomly allocated into 1 of 3 culture treatments; ligated sheep oviducts; cervine oviduct monolayer + TCM 199 supplemented with 10% deer serum under 5% CO₂ in air (CC); synthetic oviduct fluid plus 20% human serum under 88% N₂, 7% O₂, 5% CO₂ atmosphere (SOF + HS). Embryos were cultured 4 to 7 days at 39°C.

The hinds in Treatment 2 had a significantly greater mean (\pm sem) ovulation rate (11.2 ± 2.4 vs 5.3 ± 2.4 , $p < 0.05$), with more hinds in Treatment 2 responding to the gonadotrophin treatment than in Treatment 1 (10/10 vs 4/10). Embryo recovery did not differ between treatments (range 73-87%). Embryo development to the morula (range 50-58%) or blastocyst stage (range 22-26%) was not affected by culture system or superovulation treatment. Four embryos (2 late morulae from CC and 1 morula and 1 blastocyst from SOF + HS) were transferred into recipient hinds (1 embryo/hind), using a non-surgical laproscopic transfer technique. Two live calves were born with normal birth weights and gestation lengths.

Superovulation with PMSG, given with the last FSH injection, increased ovulation rate and increased the number of hinds responding to gonadotrophin stimulation. *In vitro* culture of early stage red deer embryos resulted in a lower proportion developing to blastocysts when compared to studies on other ruminants. However, some of the *in vitro* cultured embryos were viable upon transfer to recipient hinds.