

DEVELOPMENT OF TECHNIQUES FOR THE PRODUCTION OF VIABLE EMBRYOS FROM SIX TO SEVEN WEEK OLD LAMBS

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Recently there have been reports of offspring from oocytes collected from 6-9 week old calves. This study investigates whether these techniques could be transferred to similar aged lambs. A preliminary experiment was conducted to establish if there was any difference between transferring embryos, developed in *vitro* from immature oocytes obtained from abattoir ovaries, at day 2.5 post in *vitro* fertilisation (IVF) (S-12 cell stage) or at day 6 post IVF (blastocyst stage). *In vitro* maturation (IVM), IVF and embryo culture was as per Walker *et al.* (1994). Embryos were transferred to synchronised recipients via a laparoscopic method to the utero-tubal junction (UTJ) for the S-12 cell embryos or to the uterus for the blastocysts. Fetal numbers were determined by ultrasound on day 48 (Table 1). In the second study, six 6-7 week old crossbred lambs underwent ovarian stimulation via the following protocol. Progestagen intravaginal sponges (Repromap; Upjohn, Australia) were inserted on day 0. On day 7, 8.3 mg FSH (Embryo-S; Jurox, Australia) was injected subcutaneously together with 400 IU PMSG intramuscularly (im) (Folligon, Intervet, Holland). Forty eight hours later (day 9) sponges were removed and 24 hours after this (day 10) 40 µg GnRH (Fertagyl; Intervet, Holland) was injected im. Nineteen hours after the GnRH injection (day 11) ovaries were exposed via mid-ventral laparotomy and the follicles were aspirated with a 20 gauge needle and a 10 mL syringe. Those oocytes that had expanded cumulus cells at collection were classified as mature and put into IVF within 3-4 hours, while oocytes with tight cumulus cells underwent 24 hours of IVM prior to IVF. All S-12 cell stage embryos on day 2.5 post IVF were transferred to the UTJ of synchronised recipients.

Table 1. *In vitro* development and transfer results of oocytes collected from adult abattoir sourced ovaries, transferred as 8-12 cell embryos (day 2.5) or blastocysts (day 6) (all % are of oocytes)

Transfer day	No. oocytes	No. cleaved (%)	≥ 4 cell at 52 hours post IVF (%)	No. embryos transferred (%)	No. fetuses on day 48 of pregnancy (%)
2.5	55	26 (47)	25 (45)	19 (34)	11 (20)
6	57	28 (49)	28 (49)	15 (26)	10 (18)

Table 2. *In vitro* development and transfer data of mature and immature oocytes collected from lambs (all % are of oocytes)

Oocyte type at collection	No. oocytes	No. cleaved (%)	≥ 4 cell at 52 hours post IVF (%)	No. 8-12 cell embryos transferred (%)	No. fetuses on day 48 of pregnancy (%)
Mature	30	21 (70)	21 (70)	18 (60)	8 (27)
Immature	16	9 (56)	5 (31)	5 (31)	0 (0)

Mean ±s.d. of follicles aspirated and oocytes recovered per lamb were 14.8 ± 16.6 (range: 0-40) and 11.2±11.5 (range: 0-29) respectively. Mean oocyte recovery rate and the percentage of recovered oocytes that were mature were 88.8% (range: 63-100%) and 83.2% (range: 40-100%) respectively. There were no differences in oocyte survival to day 48 fetuses, regardless of whether the transfers were as S-12 cell embryos or as blastocysts (Table 2).

WALKER, S.K., HILL, J.L., BEE, CA. and WARNES, D.M. (1994). *Theriogenology*.**41**:330.