JUVENILE IN VITRO FERTILIZATION-EMBRYO TRANSFER (JIVET): THE IN VITRO PRODUCTION OF VIABLE EMBRYOS FROM OOCYTES OBTAINED FROM GONADOTROPHIN-STIMULATED JUVENILE CALVES AND LAMBS

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Methods of in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) are now in widespread use for production of embryos from oocytes recovered from ovaries of slaughtered cattle and sheep. This source of oocytes provides a highly efficient method for production of embryos for research purposes and for certain commercial applications. However, application in breeding programs to increase the genetic contributions of valuable females would be greatly enhanced by efficient methods for repeated collection of oocytes from living animals.

We have previously demonstrated the feasibility of using IVM-IVF-IVC for production of embryos and offspring using oocytes collected laparoscopically or surgically from calf and lamb ovaries (Armstrong et al. 1992; Earl et al. 1994). The use of oocyte donors at early ages enables follicular oocytes to be obtained by simple procedures that require less expensive capital equipment than the ultrasonography equipment required for transvaginal aspiration from adult cattle, and the rate of genetic gain can be increased by the substantially shorter generation interval possible with the use of oocytes from prepubertal animals (Georges and Massey 1991). Our continuing studies with calves are aimed at optimizing follicle responses and yields of viable, mature oocytes for IVF and in vitro embryo production.

Results with 6-9 week-old calves pretreated with intravaginal progestagen sponges for one week indicate that their ovaries respond to stimulation with FSH enabling repeated follicular aspiration at 3-week intervals withour evident damage to ovaries (Stubbings et al. 1993) or interference with subsequent breeding capability (unpublished observations). Follicular responses to FSH plus PMSG administered as a single sub-cutaneous injection during continued progestagen treatment via intravaginal sponge pessary were similar to those of calves receiving the same FSH dose in 8 multiple injections. responses increased with repeated treatments at 3-week intervals from 3 to 9 weeks of age, with 5.1 • 1.2, 11.3 • 2.3 and 22.3 • 6.0 (mean • SEM) follicles observed in calves stimulated at 3, 6 and 9 weeks of age, respectively.

Under continued progestagen treatment, and with oocyte maturation induced by administration of an exogenous preovulatory gonadotrophin injection, maturational status of the recovered oocyte-cumulus complexes (OCC) differed with the maturation treatment employed. Combination treatment with FSH (Folltropin, Vetrepharm) plus LH (Lutropin, Vetrepharm) resulted in a significantly greater proportion of recovered oocytes with cumulus cell expansion (73%) than treatment with LH alone (22%)($p<0.05$). For oocytes recovered by aspiration of gonadotrophin-treated calves, cumulus expansion is a highly reliable indicator of nuclear maturation (Armstrong and Earl, unpublished).

Withdrawal of progestagen treatment (sponge removal) 48h after FSH + PMSG treatment resulted in significantly increased numbers of follicles available for aspiration over those observed in calves receiving

progestagen (54.5 • 10.2; range 22-80, vs 23.5 • 19.8; 2-50, respectively), and no significant effect of GnRH on follicle numbers was observed.

Following progestagen withdrawal, combined treatment with FSH + LH was more effective as an in vivo stimulus of oocyte maturation (cumulus expansion) than GnRH, and resulted in more follicles available for aspiration. Mean • SEM follicles aspirated, oocytes recovered and % oocytes with expanded cumulus cells for FSH + LH treated calves were 56.5 • 34.8, 31.9 • 23.5, 69% respectively, compared to 35.0 • 23.1, 17.1 • 10.9, 43% respectively for GnRH-treated calves.

Immature oocytes (oocytes with unexpanded cumulus cells at aspiration) were cultured for 24h in defined media containing FSH, LH and oestradiol to permit in vitro maturation before addition of spermatozoa for IVF, whereas oocytes with expanded cumulus cells (matured in vivo) were inseminated without a further period of culture for IVM. Rates of in vitro fertilization and development of blastocyst stages did not differ significantly between calf oocytes whether matured in vitro or in vivo, and results with calf oocytes were not significantly different from those of oocytes aspirated from adult cow ovaries obtained at an abattoir and subjected to IVM before IVF (Table 1)

Table 1. Embryo development from IVF of calf oocytes matured in vivo or in vitro, and cow oocytes matured in vitro.

			Embryo development in vitro		
Oocyte donor type	Maturation	Number of oocytes	Cleaved number (%)	> 4 cell (%)	Blastocyst number (%)
Calf [1]	In vivo	59	50 (85)	(78)	12 (20)
Calf [2]	In vitro	101	80 (79)	(73)	16 (16)
Cow [3]	In vitro	135	104 (77)	(59)	27 (20)

[1] Calf oocytes with expanded cumulus cells, inseminated without culture for IVM

[2] Calf oocytes with unexpanded cumulus cells, inseminated after IVM

[3] Cow oocytes from ovaries obtained at slaughter, inseminated after IVM

We are continuing investigations with juvenile lambs along lines similar to out calf studies, aimed at optimizing oocyte yields and in vitro development for subsequent embryo transfer. The ovaries of newborn lambs appear to be particularly active, with peak numbers of follicles in the range of 0.5 - 1.0 mm diameter previously reported between 4 to 10 weeks of age (Tassell et al. 1978). We have administered combined treatments of FSH + PMSG to crossbred (Merino-Border Leicester and Merino-Border Leicester-Dorset) within these age ranges and obtained very high numbers for preovulatory follicles. Lambs were administered progestagen pretreatment for one week via intravaginal sponge pessary similarly to calves, sponges removed two days after FSH-PMSG injection, and GnRH administered 24 h later to induce final follicle and oocyte maturation. Observed mean • SEM follicles aspirated, oocytes recovered and % oocytes with expanded cumulus cells were 82.8 • 40.9, 45.4 • 12.9 and 78% respectively. Observed rates for fertilization and in vitro development to blastocyst stage were 50% and 26% respectively, with no significant differences noted with sperm concentrations from 0.4 to 0.8 x 10^6/ml.

In a separate experiment in which 43 embryos were transferred laparoscopically to synchronized recipients at 8-16 cell stages, 20 (47%) were detected as viable foetuses by ultrasonography 48 days after ET. In a more recent experiment examining the effect of age and body weight on follicular response, substantially greater numbers of follicles developed in 6-7 than in 11-12 week-old lambs in response to similar

gonadotrophin treatment regimens, with high percentages of oocyte recoveries of up to 90% of follicles aspirated, yielding up to 180 mature oocytes per lamb collected, and with mean fertilization rates of 60% of mature oocytes inseminated in vitro. Based on these oocyte recovery and development rates, it should be possible to produce up to 15 offspring from a single oocyte collection per ewe lamb.

These developments in embryo technology have profound implications for the genetic improvement of sheep, for rapid multiplication of new breeds developed for cross breeding programs or through transgenesis, as well as for breeding from superior individuals selected from existing breeds. Continuing research may be expected to result in similar achievements for cattle.

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