BIRTH OF LAMBS AFTER CULTURE OF SHEEP OVA *IN VITRO* FOR UP TO 6 DAYS

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(Received 29th August 1973)

Early cleavage stage (one-cell to eight-cell) sheep ova cultured by Tervit, Whittingham & Rowson (1972) in a synthetic oviduct fluid medium (SOF) with an atmosphere of 5% CO₂/5% O₂/90% N₂ cleaved readily during culture and twelve ova, which had been cultured for 3 to 6 days, developed to viable Day-13 or Day-14 conceptuses in recipient animals. Similar cleavage-stage ova had previously been reported to undergo only limited development during culture (Wintenberger, Dazier & Thibault, 1953; Hancock, 1963; Kraemer, 1966; Tervit & McDonald, 1969; Moore, 1970; Moor & Cragle, 1971; Moore & Spry, 1972) and to be viable after culture for 1 or 2 days (Hancock, 1963; Tervit & McDonald, 1969; Moore, 1970; Schwartz & Ulberg, 1972; Schwartz, Thompson, Goode & Ulberg, 1972). This communication describes the birth of lambs after the culture of early cleavage-stage ova for up to 6 days.

Welsh Mountain ewes were induced to superovulate and were mated at the ensuing oestrus. They were subjected to laparotomy 48 to 72 hr after the onset of oestrus and the ova were flushed from the reproductive tract in a modified (Tervit *et al.*, 1972) Dulbecco's phosphate-buffered salt solution (PBS). The ova were held at 37°C in this medium until they were to be placed in culture, when they were removed from the PBS and washed in 2 ml SOF which had been previously gassed with a mixture of 5% CO₂/5% O₂/90% N₂. The ova were then cultured in this atmosphere for up to 6 days in SOF, using the test-tube technique described previously (Tervit *et al.*, 1972). After culture, the tubes were gently agitated, uncorked and the ova and medium were removed with a Pasteur pipette. The ova were isolated, examined for cleavage stage and were then placed into dishes containing PBS at 37°C and stored until transfer. At transfer, recipients were chosen whose stage of the oestrous cycle was approximately synchronous, i.e. from 12 hr more advanced to 48 hr less advanced, with the stage of development of the ova. Most recipients received two ova, one being transferred to each uterine horn.

The development and viability of the various cleavage-stage ova cultured in SOF are presented in Table 1. The ova recovered from the culture tubes were usually retarded compared to ova developing *in vivo* for the same length of time (Rowson & Moor, 1966). Except for the eight-cell ova, increased culture length tended to be associated with the recovery of embryos which were increasingly retarded compared with ova developing *in vivo*. These effects are similar to those reported in the mouse (Bowman & McLaren, 1970). Of the eighteen

Table 1. The development and viability of sheep ova cultured in a synthetic oviduct fluid medium

No. of ova cultured	Developmental stage at start of culture	Length of culture (days)	Developmental stage reached at end of culture (no. of ova/stage)					No. of lambs born to recipient animals	
			12-cell	16-cell	Early morula (approx. 20 cells)	Morula (approx. 32 cells)	Late morula (approx. 64 cells)		Early blasto-cyst
6	1-cell	3	5	1	—	—	—	—	1
17	1-cell	6	—	1	7	8	1	—	0
6	2- to 4-cell	3	—	2	—	4	—	—	1
27	2- to 4-cell	5	—	—	11	14	2	—	10
4	2- to 4-cell	6	—	—	—	—	—	4	1
18	8-cell	3	—	—	4	10	4	—	10
4	8-cell	4	—	—	—	—	1	3	2

eight-cell ova transferred after culture for 3 days, ten (56%) survived to term. Moor & Cragle (1971) are the only other workers who have cultured eight-cell ova for 3 days and then transferred the ova to recipients. They reported that none of fifteen ova cultured in enriched TCM-199 survived after transfer. The four eight-cell ova cultured for 4 days in SOF produced proportionately fewer lambs than the ova cultured for 3 days. The two- to four-cell ova cultured for 5 or 6 days cleaved readily during culture and 37% (10/27) and 25% (1/4), respectively, of the ova survived to term. The one-cell ova cultured for 6 days also cleaved readily but none (0/17) survived to term. The lower viability of the two- to four-cell ova and lack of viability of the one-cell ova, relative to eight-cell ova cultured for 3 days, may have been due to the increased length of culture and not to the cleavage stage at commencement of culture. It was noted, however, that the six one-cell and six two- to four-cell ova cultured for 3 days also showed poor viability in the recipient animals.

Of the twenty-five lambs born, twenty-two (88%) were alive after birth and three (12%) died during difficult births. The three dead lambs were found to be anatomically normal and the twenty-two live lambs are healthy and are now between 3 and 7 months of age.

The results show that early cleavage-stage sheep ova are viable after culture for up to 6 days in a synthetic oviduct fluid medium and a gas phase containing 5% oxygen. This successful culture system can now be used to study aspects of embryo development and manipulation and for the transport of ova from country to country.

The assistance of Dr D. G. Whittingham in preparing a batch of medium is acknowledged. One of us (H.R.T.) is the recipient of a New Zealand Department of Scientific and Industrial Research Fellowship.

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