

# Comparison of the survival of fertilized eggs from adult ewes in the uteri of adult ewes and ewe lambs

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**Summary.** Fertilized eggs, obtained from mature donor ewes, were transferred into the uteri of adult ewes and ewe lambs (1 or 2 eggs per recipient). The survival rate to term of the transferred eggs was similar in the two classes of recipients. The percentage of adult ewe and ewe lamb recipients which gave birth to twins was 45·3 and 41·1 respectively. Gestation length was shorter ( $P < 0\cdot01$ ) and lamb birth weight lower ( $P < 0\cdot01$ ) for ewe lamb recipients. The results indicate that the generally lower lambing rate of ewe lambs compared to adults is unlikely to be due to unfavourable uterine conditions.

## Introduction

The lower reproductive performance of mated ewe lambs compared with adult ewes is well recognized (Dyrmundsson, 1973, 1981; Quirke, 1981). The magnitude of this problem at farm level, in Britain for example, can be judged from the flock records collected by the Meat and Livestock Commission (1977) which show that 37% of young female sheep mated in their first year fail to produce a lamb. The reasons for this high rate of reproductive failure are not fully understood although factors such as the phenomenon of oestrus unaccompanied by ovulation (Edey, Chu, Kilgour, Smith & Tervit, 1977; Quirke, 1979a), inadequacies in the behavioural responses of ewe lambs to rams at oestrus (Edey, Kilgour & Bremner, 1978) and insemination failure (Killeen & Quirke, 1979) may be implicated in particular breeds and circumstances. There is also evidence (Hamra & Bryant, 1979; Quirke, 1979a) that embryonic mortality is particularly high in ewe lambs and may be a major contributory factor to their reduced lambing rate. Quirke (1979a) estimated that 63% of fertilized eggs in Galway ewe lambs were not represented by viable embryos at 26 days after mating. This is more than twice the value suggested by Edey (1969) as being normal for mature ewes (25%). Embryonic development in young ewes may be limited because of unfavourable environmental conditions in or limited capacity of the uterus and it was to investigate these possibilities that the present work was undertaken. A preliminary report has been published (Quirke, 1979b).

## Materials and Methods

### *Experiment 1*

Finnish Landrace adult ewes, aged 3–6 years, were induced to superovulate and mated with Finnish Landrace rams; 179 fertilized eggs were obtained from the 32 ewes and transferred. Sheep blood serum was the medium used for recovery, storage and transfer of the embryos. The recipients

consisted of 89 adult ewes (65 Galway and 24 crossbreds), aged 3–6 years, and 84 Galway ewe lambs which were about 8 months old at the beginning of the experiment in late October 1976. One egg was transferred to each recipient and placed in the uterine horn ipsilateral to the ovary containing the greater number of corpora lutea.

Blood samples, for progesterone assay, were taken from each recipient on Days 12 and 18 after the onset of oestrus (day of oestrus = Day 0). The samples were taken by jugular venepuncture into evacuated tubes containing EDTA and centrifuged. The plasma was stored at  $-20^{\circ}\text{C}$  until assayed. Duplicate plasma samples were used for progesterone determination by a conventional radioimmunoassay procedure which has been described elsewhere (Quirke, Hanrahan & Gosling, 1979). The sensitivity of the assay, as calculated from the errors in the blanks ( $2 \times \text{s.d.}$ ) and the slopes of the standard curves, was  $0.18 \pm 0.02 \text{ ng/ml}$  ( $\pm \text{s.e.m.}$ ,  $n = 20$ ). The intra- and inter-assay coefficients of variation for plasma samples containing 2.23 ( $n = 21$ ), 3.14 ( $n = 13$ ) and 5.80 ( $n = 8$ ) ng progesterone/ml were 7.8, 10.2 and 4.9%, and 16.8, 13.5 and 7.1% respectively.

### Experiment 2

The 73 donor ewes which provided fertilized eggs for transfer in this experiment were 3–6 years old and consisted of 46 Finnish Landrace, 16 Galways and 11 crossbreds (mainly Fingalway). After superovulation 40 of the Finn ewes and all of the Galways were mated with rams of their own breed and the crossbreds and remaining Finn ewes were mated with Suffolk rams. The medium used for collection, storage and transfer of embryos was a modification of Dulbecco's medium (Whittingham, 1971; Trounson, Willadsen & Rowson, 1976) which was obtained commercially (Ovum Culture Medium: Flow Laboratories Ltd, Irvine, U.K.). A total of 273 fertilized eggs were transferred of which 171 and 26 were provided by intra-breed mating of Finn and Galway donor ewes respectively. The Finn and crossbred ewes mated with Suffolk rams provided 36 and 11 fertilized eggs, respectively. The recipient ewes consisted of 108 adult ewes, aged 3–6 years (46 Galways, 53 Fingalways, 4 Finnish Landrace and 5 Greyfaces), and 77 spring-born Galway ewe lambs which were about 8 months old at the start of the experiment in October 1977. One or 2 eggs were transferred to each recipient; if there were 2 eggs one egg was placed in each uterine horn; the procedure for one-egg recipients was as described for Exp. 1.

### General procedures

The experiments were conducted during the breeding seasons of 1976 and 1977 and in both years practically all of the surgical work was completed during the second half of October and November. Oestrous cycles were synchronized in all donor animals by treatment, for 12–14 days, with intravaginal sponges containing 30 mg cronolone (Laboratories Searle, Montrouge, France). Superovulation was induced at the second oestrus after sponge removal by injecting 1200–1500 i.u. PMSG intramuscularly on the 15th day after sponge removal (i.e. on Day 12 or 13 of the oestrous cycle; day of oestrus = Day 0). Eggs were recovered at surgery 3–6 days after the onset of oestrus, usually on Days 4 or 5, by flushing the uterus and oviducts by essentially the same procedures as those outlined by Hunter, Adams & Rowson (1955).

Oestrus was synchronized in the recipient animals by the use of cronolone-impregnated sponges and all the recipients were used at the first oestrus after sponge removal. Ewe lamb recipients, in both experiments, were given 500 i.u. PMSG intramuscularly at sponge removal to ensure induction of oestrus. The adult recipient ewes did not receive PMSG. All of the adult recipient ewes had experienced at least one previous pregnancy.

Eggs were transferred into the uterine horns of recipient ewes and only those that were at the 8-cell stage or greater were used. Recipients were weighed a few days before surgery and were managed uniformly until lambing. Gestation length was calculated as the period between the time of onset of oestrus in the donor and lambing in the recipient.

### Statistical analysis

Categorical data were analysed using the  $\chi^2$  statistic while the *t* test was employed in comparisons of group means for birth weight and gestation length. Breed differences (of recipient and embryo) were ignored since previous work had shown that the breeds used here had no effect on pregnancy rate or embryo survival (Hanrahan, 1979).

## Results

### Experiment 1

Ovulation rate, plasma progesterone concentration on Day 12 and egg survival to term were similar for the ewe lambs and both groups of adult ewes (Table 1). The pregnancy rate on Day 18, as evaluated by progesterone assay, was substantially higher than at lambing time in both age classes of recipients. Gestation length was shorter ( $P < 0.05$ ) and lamb birth weight lower ( $P < 0.01$ ) for the ewe lamb recipients.

**Table 1.** Survival of one fertilized egg from mature donors in the uteri of ewe lambs and adult ewe recipients (Exp. 1)

	Recipient		
	Galway ewe lambs	Galway adult	Cross-bred adult
No. of recipients	84	65	24
Body wt at time of egg transfer (kg)	48.9 $\pm$ 0.54	70.2 $\pm$ 0.93	51.8 $\pm$ 1.10
Ovulation rate	1.64 $\pm$ 0.10	1.49 $\pm$ 0.07	1.67 $\pm$ 0.13
Plasma progesterone conc. on Day 12 (ng/ml)	3.0 $\pm$ 0.17	2.7 $\pm$ 0.11	3.0 $\pm$ 0.21
No. pregnant at Day 18* (%)	47 (55.9)	39 (60.0)	17 (70.8)
No. lambed (%)	40 (47.6)	23 (35.4)	11 (45.8)
Gestation length (days)	141.4 $\pm$ 0.30	144.7 $\pm$ 0.40	142.9 $\pm$ 1.17
Lamb birth weight (kg)	2.9 $\pm$ 0.09	4.0 $\pm$ 0.15	3.7 $\pm$ 0.17

Values are mean  $\pm$  s.e.m.

\* Estimated after assay of plasma progesterone 18 days after the induced oestrus (ewe pregnant if concentration  $\geq 1.0$  ng/ml).

### Experiment 2

For one- and two-egg transfers the lambing rate and egg survival rate was similar in ewe lamb and adult ewe recipients (Table 2). The values for lamb birth weight and gestation length given in Table 2 refer only to pure-bred Finnish Landrace lambs and to dams which gave birth to pure-bred Finn lambs respectively. This restriction was imposed because Finn transfers were the most numerous class and gestation length is known to be influenced by the breed of the lamb *in utero* (Bradford, Hart, Quirke & Land, 1972). Gestation length was similar in singleton- and twin-bearing recipients within both age classes but was significantly shorter for ewe lamb recipients ( $P < 0.05$ ). Lamb birth weight was lower (singletons and twins) for the Finn progeny of ewe lamb recipients ( $P < 0.01$ ) than for the corresponding progeny of adults.

There was significant variation ( $P < 0.01$ ) in the survival rate of fertilized eggs from individual donor ewes in both experiments. This did not, however, affect the interpretation of the results because of the large number of donors used and the eggs of individual donor ewes were distributed among the two age classes of recipients as far as possible.

**Table 2.** Survival of 1 and 2 fertilized eggs transferred from mature ewes into the uteri of ewe lamb and adult ewe recipients (Exp. 2)

	Recipient	
	Ewe lamb	Adult ewe
Body wt of recipients at time of egg transfer (kg)	46.5 ± 0.55	72.5 ± 1.02
<i>Single egg transfers</i>		
No. of recipient ewes	43	54
No. lambled (%)	26 (60.5)	31 (54.4)
<i>Two egg transfers</i>		
No. of recipient ewes	34	54
No. lambled (%)	23 (67.6)	40 (74.1)
No. of single births (%)	9 (39.1)	11 (27.5)
No. of twin births (%)	14 (60.9)	29 (72.5)
Gestation length (days)*	143.8 ± 0.39 (23)†	145.5 ± 0.24 (53)†
<i>Birth wt of purebred Finnish Landrace progeny (kg)</i>		
Singles	3.1 ± 0.22 (18)†	4.0 ± 0.10 (36)†
Twins	2.1 ± 0.28 (10)†	3.7 ± 0.08 (34)†

Values are mean ± s.e.m.

\* Only for recipients that gave birth to purebred Finnish Landrace lambs.

† No. of records.

## Discussion

The results of these experiments show that the uteri of adult ewes and of ewe lambs are similar in terms of their ability to facilitate the survival of 1 or 2 fertilized eggs transferred into them at about Day 4 of the cycle. Progesterone is essential for the establishment and maintenance of pregnancy in the sheep and the similarity in the plasma levels of this hormone in lambs and adults in the present study and in others (Smith, Drost, Fairclough, Peterson & Tervit, 1977; Quirke *et al.*, 1981) is a further indication of the equality of opportunity for egg survival in the uteri of ewes of these two age groups. It is unlikely, therefore, that the high levels of embryonic mortality which have been observed in ewe lambs (Hamra & Bryant, 1979; Quirke, 1979a) can be attributed to unfavourable developmental conditions in the uterus.

Evidence has been provided in previous studies (Wright, Anderson, Cupps, Drost & Bradford, 1976; Quirke & Hanrahan, 1977) of a difference in egg quality between ewe lambs and mature ewes. In the study by Quirke & Hanrahan (1977), for example, the survival rate to term of 8–16-cell eggs from ewe lambs of the Galway breed after transfer to the uteri of adult ewes was less than half that of similar eggs from mature ewes (33 compared with 73%). Because of this and of the evidence of a different pattern of oestrogen secretion by lamb follicles *in vitro* and *in vivo* (Trounson, Willadsen & Moor, 1977; Quirke *et al.*, 1981) it has been suggested (Quirke *et al.*, 1981) that conditions in the developing follicle or in the reproductive tract between ovulation and the 8–16-cell stage may be related to the reduced fertility of very young ewes. A high frequency of errors of oogenesis or of fertilization, because of maternal age, could also be implicated and a karyotyping study, similar to that by Long & Williams (1980) with mature ewes, could provide useful clues in this direction. The early ovine embryo is capable of secreting a variety of compounds (Martal, Lacroix, Loudes, Saunier & Wintenberger-Torrès, 1979; Gadsby, Heap & Burton, 1980) and it is possible that some embryos from young ewes may lack the ability to secrete substances required for maternal recognition of pregnancy or for its continuation up to and including implantation.

The birth weight of lambs born to ewe lambs is generally lower than for those born to mature ewes (Dyrmondsson, 1973, 1981). This was apparent in the present study also and the data were used to consider whether this was merely a reflection of differences in maternal liveweight. The

formulae used by Hinkelman, Bradford, Pollak, Anderson & Cupps (1979), which predict lamb birth weight in inter-breed pregnancies, were used to predict the weight of purebred Finn lambs born to ewe lambs and mature ewes. The predicted mean birth weight of the progeny of mature ewes was 4.2, 4.2 and 3.5 kg for single-born lambs in Exp. 1 and for single- and twin-born lambs in Exp. 2, respectively, and these values are in close agreement with the mean birth weights which were actually observed (Tables 1 & 2). The progeny of the ewe lamb dams, however, weighed considerably less than predicted. The predicted mean birth weight of singletons in Exps 1 and 2 and of twins in Exp. 2 were 3.9, 3.9 and 2.9 kg respectively, which are 26–38% greater than those actually observed (Tables 1 & 2). These calculations suggest that ewe lambs cannot be considered merely as 'small adults' in terms of their ability to support fetal growth. This departure from expectation may be due to differences in the pattern of nutrient utilization by pregnant ewe lambs compared with mature ewes, as suggested by the results of Robinson, Fraser, Corse & Gill (1971) and Quirke, Sheehan & Lawlor (1978). It may also be a reflection of the inability of the ewe lamb uterus to interact adequately with the developing conceptus so that the placenta can accommodate fetal growth potential.

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