Frequency of chromosomal abnormalities in early embryos of the domestic sheep (*Ovis aries*)

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Summary. Embryos or unfertilized eggs were collected 2 or 3 days *post coitum* from mature sheep of various breeds and crosses. The karyotypes of 89 of the 376 collected were established. There were 44 embryos with $2n = 54XX$, 30 with $2n = 54XY$; 1 was a $2n/1n$ mosaic; 4 had $2n + 1$ chromosomes giving an incidence of trisomy of 4.7%; and 1 unfertilized egg had $n = 28$. The incidence of chromosomal abnormalities was 6%, and 1.3% of the eggs or embryos had a cracked zona pellucida.

Introduction

Early embryonic death in domestic animals represents a considerable economic loss. In sheep the wastage has been estimated to be 20–30% of all fertilized ova (Edey, 1969). Similar levels of embryonic loss have been reported in the pig (Hanly, 1961) and cow (Boyd, Bacsich, Young & McCracken, 1969). Such losses are exclusive of those caused by infectious conditions and have been attributed to a number of factors (for reviews, see Boyd, 1965; Edey, 1969). Bishop (1964) suggested that a proportion of the loss might be due to an abnormal genetic load rendering the embryo non-viable. A high proportion of spontaneous human abortuses have a chromosomal abnormality and it has been assumed that some of the embryonic loss in domestic animals has a similar aetiology. Chromosomally abnormal embryos have been reported in the pig (McFeely, 1967; Smith & Marlow, 1971; Åkesson & Henricson, 1972; Moon, Rashad & Mi, 1975) and the cow (McFeely & Rajakoski, 1968), but examination of preimplantation blastocysts in sheep failed to detect any with such abnormalities (Long, 1977). Of the embryos studied by Long (1977), 25% were undiagnosed and it was suggested that abnormal embryos may have been degenerating by the late preimplantation stage and that more accurate information would be obtained from an examination of earlier embryos. A preliminary investigation revealed the presence of some 2-day *post coitum* sheep embryos with chromosomal abnormalities (Long & Williams, 1978). The present paper presents further information on the incidence of chromosomally abnormal embryos from 54XX ewes mated to 54XY rams.

Materials and Methods

The work was carried out during the breeding seasons of 1976–1978. The 162 mature ewes of various breeds (Table 1) were naturally mated to a Suffolk or Clun ram. The chromosome complement of all the ewes and rams was established by routine lymphocyte culture.

Embryos and unfertilized eggs were collected 2 or 3 days *post coitum* at laparotomy and *post mortem*. At 2 days *post coitum* the embryos were at the 2–4-cell stage, whereas by Day 3 they had reached the 8-cell stage. Collection at laparotomy was by retrograde flushing of the oviducts (Long & Williams, 1978). After surgery the ewes were returned to the flock and were mated again at the next oestrus. The ewes were slaughtered 2–3 days after this second service and the embryos and eggs were collected *post mortem*.

Table 1. Breeds of sheep used for egg collection

Breed	No. of ewes
Scottish halfbred	40
Clun cross	30
Suffolk cross	26
Dorset horn × Scottish halfbred	25
Dorset down × Finnish Landrace	9
Mashams	19
Welsh Mountain	13

The embryos, fertilized and unfertilized eggs were cultured for 24 h in medium containing colchicine to accumulate cells at metaphase and chromosome preparations were made as previously described (Long & Williams, 1978). The slides were stained with a 1:10 Giemsa (Gurr R66): phosphate- buffered saline solution, pH 6.8, for 5–15 min.

Results

The chromosome complements were $2n = 54XX$ for all the ewes and $54XY$ for all the rams. A total of 376 eggs or embryos was collected: 5 (1.3%) had a cracked zona pellucida (3 of these were unfertilized eggs (Pl. 1, Fig. 1) and 2 were 8-cell embryos (Pl. 1, Fig. 2)) and 209 (56.3%) of the remaining 371 had mitotic metaphase chromosomes. Only 35% of the unfertilized eggs and 41% of the 8-cell embryos went into cell division, whereas 87% of the 2–6-cell embryos began to divide in culture: 89 of those dividing (42.6%) produced metaphase spreads suitable for karyotype analysis (Table 2). The remainder were discarded because of overlapping or excess spreading. There were 44 embryos with $2n = 54XX$ and 30 with $2n = 54XY$. This was not a statistically significant difference from a 1:1 ratio ($\chi^2 = 2.64$; $0.1 < P < 0.2$).

Table 2. Chromosome complements of eggs and embryos from $54XX$ ewes mated to $54XY$ rams

Chromosome no.	27X	54XX	54XY	Abnormal	Total
No. of eggs or embryos	2	44	30	13	89

Thirteen of the 89 (14.6%) were considered to deviate from the normal; 1 egg (28X) showed evidence of non-disjunction at meiotic anaphase I (Pl. 2, Fig. 3); 1 embryo appeared to be a $2n/1n$ mosaic; 4 embryos (2 with $55XY$, 2 with $55XX$) had an extra chromosome (Pl. 2, Fig. 4); 1 single-cell zygote ($54XX$) had a chromatid break in the long arm of chromosome number 1; and the remaining abnormalities (2 with $26X$, 1 with $53XX$, 2 with $53Y$ and 1 with $53X$) were deficiencies of whole chromosomes.

Discussion

The present work confirms the earlier finding (Long & Williams, 1978) that chromosomally abnormal sheep embryos are present at 2 days *post coitum*. However, the results need to be interpreted with caution. The nature of the technique is such that individual chromosomes are easily lost during the spreading process. Therefore, since none of the hypomodal eggs or embryos was diagnosed on spreads from more than one blastomere, the possibility that these

PLATE 1

Fig. 1. An unfertilized sheep egg with a cracked zona pellucida.

Fig. 2. An 8-cell sheep embryo with a cracked zona pellucida.

(Facing p. 198)

Fig. 3. Karyotype from an unfertilized sheep egg with 28 chromosomes.

Fig. 4. Karyotype from an 8-cell sheep embryo with 55 chromosomes. The arrow indicates a chromatid break.

were technical artefacts cannot be excluded. Similarly, it is well recognized that such defects as chromatid breaks may be due to viral contamination of the culture medium (Fechheimer, 1971, 1972). Even relatively minor changes in specific cultural conditions can give rise to enhanced secondary constrictions (Palmer & Funderburk, 1965; Bruère & McLaren, 1967). These considerations make it impossible to draw firm conclusions from the present data on the incidence of such abnormalities as autosomal or X monosomy.

The presumptive $2n/1n$ mosaic embryo is extremely interesting. The preparation showed two clear diploid spreads and one haploid (27X) spread. The haploid spread could not have been from a normal polar body since, in our experience, the chromosomes from these are much more elongated and spidery. One possibility is that this was a dispermic egg and the haploid spread was from the extra male pronucleus. A true $2n/1n$ mosaic has been seen in a mouse morula (E. P. Evans, personal communication). Again, the haploid spreads were female so that their origin remained obscure.

The finding of four $2n + 1$ embryos gave an incidence of trisomy in the diagnosed material of 4.7%. In each case the extra chromosome was an acrocentric. It was tentatively identified as an autosome but definite identification was not possible. C-banding was attempted with some preparations using the method described by Sumner (1972) but was unsuccessful.

Such trisomic individuals may arise as a result of non-disjunction at the first or second meiotic division in the formation of an ovum or spermatozoon. In man, for example, the extra chromosome 21 in Down's syndrome has been shown to be of maternal or paternal origin, although with differing frequencies. It can also arise at metaphase I or metaphase II (Jacobs & Morton, 1977; Hansson & Mikkelsen, 1978). No information is available on the source of the extra chromosomes in the sheep embryos.

The 1-cell egg with 28 chromosomes in the present work had an extra acrocentric autosome since the X and three metacentric chromosomes were clearly identifiable (Pl. Fig. 3). Direct calculations of non-disjunction at metaphase I in the ewe have not been made since only a few cells at meiotic metaphase II have been successfully analysed (Logue, 1977). However, estimates from the present data suggest that the incidence of ova with the extra chromosome lies in the range of 1–5%. Calculations of autosomal non-disjunction in the ram from reports in the literature (Table 3) give a mean value of 2.8% using the formula: no. of (hyperhaploid cells $\times 2 \times 100$)/total no. of cells counted. The level of trisomy found in the present study is therefore compatible with current estimates for non-disjunction in the gametes.

Table 3. Distribution of chromosome number at meiotic second metaphase in 54XY rams

Authors	No. of animals	No. of chromosomes					Total no. of cells
		<25	25	26	27	28	
Chapman & Bruère (1975)	10	6	1	3	52	0	62
Logue (1977)	2	0	3	8	42	2	55
Long (1978)	1	0	2	10	86	2*	100
Total	13	6	6	21	180	3 (4)	217

* One cell of 28X and one of 28XY.

If only hypermodal eggs and embryos are considered, since these were unlikely to have been technical artefacts, the incidence of chromosomal abnormalities was 6% (5/84). This compares with the values of 11 and 8% reported in preimplantation blastocysts of pigs (McFeely, 1967) and cattle (McFeely & Rajakoski, 1968) respectively. Results from work with laboratory animals have been reviewed by Ford (1975) and show a similar low incidence.

The abnormalities found in sheep embryos differ from those reported in the pig in which polyploidy, predominantly triploidy and tetraploidy, has been the most common finding (McFeely, 1967; Moon *et al.*, 1975). In addition, the one abnormal bovine blastocyst has been described as a diploid/tetraploid mosaic (McFeely & Rajakoski, 1968). Polyploid cells have also been found in pig blastocysts, 10 days *post coitum*, by N. S. Fechheimer (personal communication) who suggested that these may have originated from trophoblast cells rather than from the inner cell mass. Giant cells and binucleate cells are present in the pig trophoblast at this stage (E. C. Polge, personal communication). By contrast, binucleate cells do not appear in the sheep trophoblast until Day 16 (Boshier, 1969) and have not been reported in the cow trophoblast until Day 17 of gestation (Greenstein, Murray & Foley, 1958).

It is, however, possible that the polyploid embryos in the pig may have arisen due to ageing of the ova after ovulation. Ageing of gametes is known to increase the incidence of triploidy (Shaver & Carr, 1967, 1969), with ageing of ova being the more important (Fechheimer & Beatty, 1974). In addition, ageing of spermatozoa *in utero* increases the incidence of mixaploid blastocysts in the rabbit (Martin & Shaver, 1972).

Differences in the duration of oestrus and times of ovulation in domestic animals may be important in that they will influence the likelihood of abnormal embryos arising. Animals such as ewes or cows, which have short oestrous periods and ovulate after the female ceases to accept coitus, are less likely to incur ovum ageing and hence the production of triploid embryos than, for example, sows or mares in which oestrus continues for several days and the female will accept coitus even after ovulation has occurred. The incidence of trisomy, however, depends on the incidence of non-disjunction in the gametes and is not dependent on the time of fertilization.

The incidence of chromosomally abnormal embryos in domestic animals is probably lower than that in man. Estimates from data from surveys of chromosomal abnormalities in newborn infants (Sergovich, Valentine, Chen, Kinch & Smout, 1969; Lubs & Ruddle, 1970; Hamerton, Canning, Ray & Smith, 1975) and from spontaneous abortuses (Boué, Boué & Lazar, 1975; Alberman & Creasy, 1977) suggest that 7–8% of recognized pregnancies have a chromosomal abnormality. However, this is almost certainly an underestimate of the incidence at conception, which has been placed as high as 50% by some workers (Boué *et al.*, 1975). In the sheep, not all the early embryonic loss can be attributed to gross chromosomal abnormalities. However, as techniques of chromosome identification improve they may reveal the existence of more minor chromosomal re-arrangements which are incompatible with life.

One final result of interest is the 1.3% of eggs or embryos found with a cracked zona pellucida. It is difficult to know whether these were present *in vivo*. They could not have represented the normal degeneration of unfertilized eggs since many such eggs were collected with an intact zona. Trounson & Moore (1974) have shown that mechanical rupture of the zona of 2- and 3-day embryos seriously impairs their potential for development. It may be that such abnormalities of the zona are a further cause of early embryonic loss in the sheep.

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