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Polyploid Abnormalities in Day 3 and Day 5 Merino Sheep Embryos

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Abstract

The chromosome complement was assessed in Merino sheep embryos collected at 3 and 5 days after the onset of oestrus. Donor ewe treatments were: untreated, or immunized against androstenedione (day 3); and untreated, or treated with follicle-stimulating hormone (FSH), or treated with FSH plus immunization against androstenedione (day 5). No significant differences in the frequency of chromosomally abnormal embryos between treatment groups within each age group were observed, so the data have been combined. Euploid abnormalities were observed in 10·8% of the day-3 embryos (4/37), with the abnormalities being one $1n$, one $3n$ and two $5n$. Embryos with euploidy (10%) were also observed at day 5, with three $1n/2n$ mosaics and a $3n$ embryo present in a sample of 40. These data suggest that chromosomally aberrant embryos are not lost before day 5 of development.

Introduction

The presence of cytogenetic abnormalities in cleavage-stage embryos has been correlated with early embryonic mortality in mammals. Considerable data now exist for man (Chandley 1981) and laboratory animals (see review table in Binkert and Schmid 1977), but the number of studies on chromosome abnormalities in embryos of livestock species is quite small.

Analysis must be carried out on cleavage stage embryos to obtain an accurate assessment of the frequency of chromosomally abnormal embryos, as unbalanced embryos are usually lost before reaching the blastocyst-elongation or egg-cylinder stage (Murray *et al.* 1986*a*). However, the time of loss of aberrant embryos between day 3 and day 13 is not known. There are no data available on the frequency of chromosome abnormalities in cleavage-stage embryos from pigs, and the data available for cattle are confounded by the use of exogenous hormones to produce superovulation (King *et al.* 1981; Gayerie de Abreu *et al.* 1984; Murray *et al.* 1985*b*). The possible effects of superovulation on the incidence of cytogenetically unbalanced embryos is not clear, as conflicting evidence has been published from both livestock and laboratory species (see Murray *et al.* 1986*b*).

The incidence of chromosome abnormalities in cleavage-stage sheep embryos has been estimated to be 6% (Long and Williams 1980) and 11% (Murray *et al.* 1985*a*), with the frequency dropping to 1·9% in day 13–14 blastocysts (Murray *et al.* 1986*a*). Long and Williams (1980) principally observed aneuploid abnormalities (5/6), whereas in the study by Murray *et al.* (1985*a*) 7 of the 8 abnormalities in early embryos were of the euploid type involving entire chromosome sets.

The present paper reports results of the chromosomal analysis of 37 Merino embryos collected on day 3 following oestrus in untreated and androstenedione-immune ewes and from 40 day-5 embryos collected from ewes treated with follicle-stimulating hormone (FSH) with or without immunization against androstenedione. Embryos enter the tip of the uterine horn between day 4 and day 5; the day-5 embryos were therefore analysed to assess whether the time of loss of chromosomally abnormal embryos coincided with the movement of the embryos into the uterus.

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Materials and Methods

Embryos for this study were collected from ewes being used in two unrelated experiments, the first studying embryo mortality and the second studying methods for increasing ovulation rate. In both experiments, ewes were syncronized in the previous oestrous cycle using progestagen-impregnated sponges (Repromap, Upjohn Pty Ltd, Rydalmere, N. S. W.) and left untreated or immunized against androstenedione-7-human serum albumin (Fecundin, Glaxo Animal Health Pty Ltd, Boronia, Vic.). Two injections of Fecundin were given, with the booster injection timed such that it was given 21 days after the primary injection and 14 days before expected mating. Ewes were exposed to two relays of harnessed Merino rams (1 ram per 15 ewes), which were alternated at the morning and evening checks for oestrus. All day-3 embryos were collected from ewes either left untreated or given these treatments and two day-5 embryos were collected from untreated ewes.

In the second experiment, the protocol was altered by the addition of a superovulatory treatment. Starting 24 h before sponge removal, ewes were given four injections at 12-h intervals of 6, 5, 3 and 2 mg equivalents (Armour units), respectively, of FSH (gift from Y. Combarnous, Nouzilly, France) to give a total dose of 15 mg Armour equivalents of FSH. The donors of most of the day-5 embryos were ewes which had been treated with FSH, or with FHS plus immunization against androstenedione.

Embryos were collected at either 48–60 h (day 3; 1–4 cell) or 108–120 h (day 5; 16–64 cell) after mating by surgically flushing the top of the uterine horn and oviducts with 3–4 ml phosphate-buffered saline (PBS; 0·1M, pH 7·8) containing 5% heat-inactivated sheep serum. Embryos were cultured in PBS containing 15% sheep serum, 0·8 μg colchicine ml^{-1}, 100 i.u. penicillin G ml^{-1} and 100 μg streptomycin sulfate ml^{-1} for 10 h at 37°C with a 5% CO_2 in air atmosphere before preparation of chromosomes by the method of Long and Williams (1978). Embryos were individually transferred to clean slides and the excess hypotonic solution removed. One drop of methanol:acetic acid fixative (1:1, v/v) was dropped onto the embryo from a height of 27 cm. Chromosome preparations were stained in 5% Giemsa in 0·004 M phosphate buffer for 3·5 min, air-dried and mounted in DPX.

Results

Chromosome counts were accurately obtained from 37 of the 106 day-3 embryos processed (Table 1). There was no significant difference with respect to the incidence of chromosome

Table 1. Chromosomal composition of day-3 and day-5 Merino embryos

			Chromosome complement					
Day	Group	No.	$2n = 54$	$\simeq 54$	$1n$	$1n/2n$	$3n$	$5n$
3	Control	48	11	1	1			
	Immunized	58	19	2			1	2
	Total	106	30	3	1		1	2
5	Control	2				1	1	
	FSH	34	10	7		1		
	FSH + immunized	36	12	7		1		
	Total	72	22	14		3	1	

abnormalities between the untreated or immunized groups and therefore the data have been pooled. Three embryos had $\simeq 54$ chromosomes, but a complete count was not possible because of overlapping chromosomes. The frequency of day-3 embryos with euploid abnormalities is 4 out of 37 or 10·8% (Table 1). The two $5n$ embryos were from the same ewe. The $5n$ embryos were males as both contained Y chromosomes. A $5n$ cell is illustrated in the composite photograph in Fig. 1.

There was no significant difference between the FSH only and the FSH-plus-immunization groups with respect to the frequency of day-5 embryos with abnormal chromosome complements, so the data have been combined. The two embryos from untreated ewes have also been included in the total even though they were both abnormal. The $3n$ embryo was a male, while the $1n/2n$ mosaics lacked a Y chromosome in both the $1n$ and the $2n$ cells. The incidence of day-5 embryos with euploid abnormalities is thus 15·4% (4/26), or, if near-diploids are included, 10% (4/40).

Discussion

The overall frequency of day-3 embryos observed in this study with euploid chromosome abnormalities was 10·8%. This result is not significantly different from the 11% abnormalities

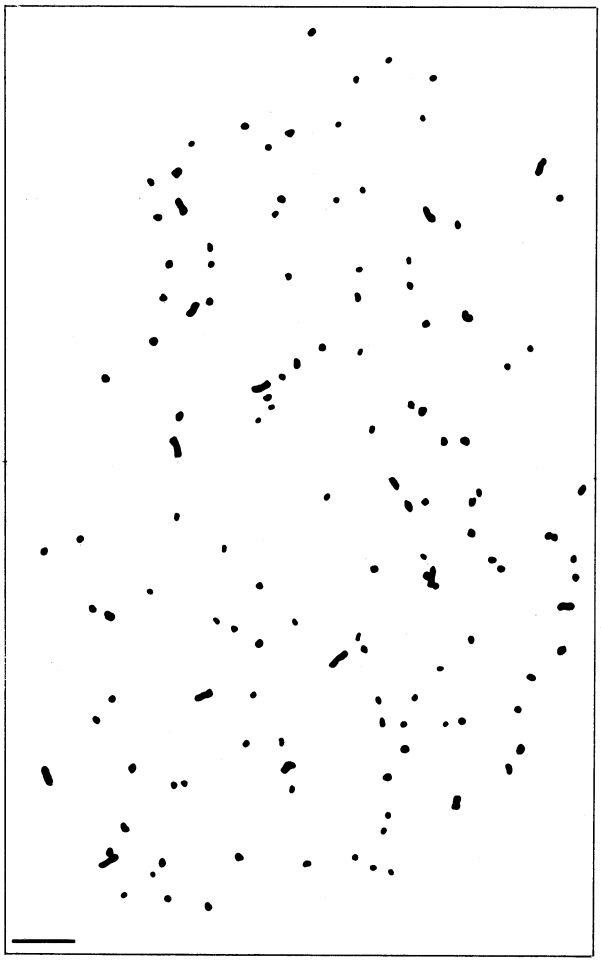

Fig. 1. A composite picture of a 5*n* cell from a 3-cell embryo. (scale bar, 20 μm).

observed in an earlier study (Murray *et al.* 1985*a*). By combining the two data sets, the estimated frequency of chromosomally abnormal day-3 embryos in Merino sheep becomes 11·2% (12/107). The estimated frequency of chromosomally aberrant embryos in Merino sheep does not differ from the 6% incidence of abnormal embryos reported by Long and Williams (1980) in British breeds.

The frequency of abnormal embryos at day 5 was 15·4%, all observed abnormalities being of the euploid type. When near-diploid embryos were included, the frequency of abnormal embryos dropped to 10·0%. The incidence of euploid aberrant embryos observed here does not differ from the 6·25% (3/48) abnormal rate observed in embryos collected from Merino ewes superovulated with pregnant mare serum gonadotrophin (PMSG) (Murray *et al.* 1986*a*).

The frequency of chromosomally aberrant embryos did not significantly drop between the day-3 and day-5 groups in this study (11·8% and 15·4%, respectively); this indicates that the unbalanced chromosomal state of the abnormal embryos is not lethal until some time after the embryos have entered the uterine horn. Most of the abnormal embryos are lost by day 13 of development in the Merino, as Murray *et al.* (1986*b*) observed chromosome abnormalities in only 1·9% of the elongated blastocysts examined. This is also consistent with other work

where the incidence of chromosome abnormalities was very low in blastocysts of sheep (Long 1977) and cattle (Hare *et al.* 1980).

The majority (18/19) of chromosome abnormalities observed in early Merino embryos in this and our previous studies (Murray *et al.* 1985*a*, 1986*b*) were of the euploid or fertilization-error type. Embryos with aneuploid abnormalities, arising from meiotic non-disjunction, may be confused with hypo- or hyperdiploid chromosome spreads produced by the fixation technique and may thus not be detected. A single hyperdiploid embryo ($2n=55$) was identified in our previous study (Murray *et al.* 1985*a*). Thus, assuming hypo- and hyperdiploidy occur at the same frequency, approximately 10% of the chromosomally abnormal cleavage-stage Merino embryos are of the aneuploid type.

The 18 Merino embryos thus far identified in this and in our previous studies as having euploid abnormalities have been four $1n$, eight $1n/2n$, three $3n$, one $4n$ and two $5n$ (Murray *et al.* 1985*a*, 1986*b*). Murray *et al.* (1985*a*) presented evidence that the single $4n$ embryo and three of the $1n/2n$ mosaic embryos originated by polyspermy, as, in the latter case, the $1n$ cells contained Y chromosomes. In the present study, one of the $3n$ embryos and both of the $5n$ embryos had two Y chromosomes. Polyspermy is therefore the major, if not the only, cause of haploid/diploid mosaicism and polyploidy in cleavage-stage Merino embryos. The origin of the four haploid embryos is still unclear, as none contained a Y chromosome and a 27-X complement is consistent with either a parthenogenetic or androgenetic origin.

Haploid and polyploid embryos are lost by day 13 of development in the Merino (Murray *et al.* 1986*a*). The fate of the $1n/2n$ mosaic embryos is, however, unclear. In recent work in mice, androgenetic and normal diploid embryos were combined and normal young were produced with no detectable androgenetic cells (Surani *et al.* 1986). This indicates that the $1n$ cells derived by polyspermy may be lost from the $1n/2n$ embryos without affecting the normal development of the diploid cells.

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