Frequency of chromosomal abnormalities in embryos from superovulated Merino ewes

J. D. Murray, M. P. Boland† and C. Moran*

CSIRO, Division of Animal Production, P.O. Box 239, Blacktown, New South Wales 2148, and

*Department of Animal Husbandry, University of Sydney, Sydney, New South Wales 2006, Australia

Summary. Chromosomal analysis was carried out on 48 Day 2–7 embryos collected from superovulated Merino ewes. Three embryos had abnormal chromosome complements ($1 \times 1N$, $1 \times 1N/2N$, $1 \times 3N$), yielding an incidence of 6.25% abnormal embryos. It is concluded that superovulation does not cause an increase in the incidence of chromosomal abnormalities in embryos of Merino sheep.

Introduction

Abnormal chromosome complements are recognized as one factor which can lead to early embryonic mortality in domestic animals (King, 1985). Long & Williams (1980) reported an incidence of 6% chromosomally abnormal Day-2 and -3 sheep embryos in a sample from untreated British breeds, while Murray *et al.* (1985a) observed a higher frequency of abnormal embryos (11%) at the same developmental stage from untreated or androstenedione-immune Merino ewes. Abnormal embryos are largely lost before Day 13 of gestation (Long, 1977; Murray *et al.*, 1986).

There is now some evidence to suggest that the incidence of abnormal embryos may increase when superovulatory treatments are used (King, 1985). The data for sheep supporting this case came from a study of Williams & Long (1980) in which they observed that 20.9% of the Day-2 or -3 embryos flushed from Clun and Herdwick ewes treated with pregnant mares' serum gonadotrophin (PMSG) were chromosomally unbalanced.

In this report, we present the results of the chromosomal analysis of 48 embryos obtained from PMSG superovulated Merino ewes.

Materials and Methods

The embryos used in this study were collected during the establishment of an embryo production regimen for use in gene transfer experiments (Nancarrow, Murray, Boland, Sutton & Hazelton, 1984; Boland *et al.*, 1985). Ewes were synchronized with progestagen-impregnated sponges (Repromap: Upjohn Pty. Ltd, Rydalmere, New South Wales, Australia) and given 750 or 1500 i.u. PMSG (Folligon: Intervet Aust. Pty. Ltd, Artarmon, New South Wales, Australia) either alone ($N = 38$) or in conjunction with active immunization against oestradiol-17 β ($N = 14$). Ewes were killed between 2 and 7 days after natural or artificial insemination and the embryos were collected by reverse flushing the top of the uterine horn through the oviduct with 3–5 ml phosphate-buffered saline (PBS: 0.1 M, pH 7.8) containing 5% heat-inactivated sheep serum.

†Present address: Department of Agriculture, University College, Dublin, Lyons, Newcastle P.O., Ireland.

Embryos were cultured in PBS containing 15% sheep serum, 0.75–1.5 µg colchicine/ml, 100 i.u. penicillin G/ml and 100 µg streptomycin sulphate/ml for 4–10 h at 37°C before preparation for chromosome spreads by the method of Long & Williams (1978). Chromosome preparations were stained in 5% Giemsa in 0.004 M-phosphate buffer for 3.5 min, air dried and mounted in DePeX.

Results

Spreads suitable for mitotic analysis were obtained from 48 of the 290 (20%) embryos processed. Significantly more of the 2- and 4-cell cleavage stages and morulae successfully yielded chromosome preparations than did 8- and 16-cell embryos (Table 1; $\chi^2_1 = 9.378$, $P < 0.01$).

Table 1. Sheep embryos yielding analysable chromosomes from various cleavage stages

Stage	No. of embryos		
	Yielding chromosomes	Processed	%
1-cell	0	1	0
2-cell	12	63	19
4-cell	19	63	30
8-cell	5	58	8.6
16-cell	3	25	12
Morula	8	22	36
Blastocyst	1	6	17

Table 2. Chromosomal composition of 48 Merino sheep embryos collected from PMSG superovulated ewes

	Chromosome composition	No. of embryos			
		PMSG dose		Pooled PMSG	Total
		750 i.u.	1500 i.u.		
Normal	54XX	5	12	5	22
	54XY	4	9	8	21
Abnormal*	53		2		2
	1N		1		1
	1N/2N	1			1
	3NXXY		1		1

*All were 4-cell embryos.

Abnormal chromosome complements were observed in 5 of the 48 embryos (Table 2). Eleven embryos were collected from ewes immunized against oestradiol-17β, with only one of these being abnormal (1N/2N mosaic). The remaining abnormal embryos were amongst the 37 embryos collected from ewes treated with PMSG only. The sex ratio of the embryos was not affected by the treatments, there being 23 male and 22 female embryos scored, with the breakdown between PMSG only and PMSG + immunization being 19♂♂:17♀♀ and 4♂♂:5♀♀ respectively.

Two embryos were monosomic, having 1 scorable cell each with 53 chromosomes. One embryo had two haploid cells, while another 4-cell embryo yielded 3 mitotic figures, 2 haploid and 1 diploid. The final abnormal embryo was a triploid with all 3 mitotic cells having a 3NXXY chromosome composition (Fig. 1). The hypodiploid embryos ($2N = 53$) may be artefacts, as chromosomes can be lost from the metaphase plate during slide preparation. Therefore, the incidence of chromosomally abnormal embryos observed in the study is between 6.25% (3/48) and 10.4% (5/48).

Fig. 1. A 3N cell from the triploid 4 cell embryo. Arrow indicates the Y chromosome. $\times 1420$.

There was no effect of the dose of PMSG administered to the ewes on the incidence of chromosomally abnormal embryos collected (Table 2). The remaining 13 embryos came from groups of embryos from the immunization experiments which were pooled with respect to PMSG dose at the time of collection.

Discussion

The frequency of chromosomally abnormal embryos observed in this study ranged from 6.25 to 10.5%, but the 6.25% figure is more acceptable as the 2 embryos with hypodiploid chromosome counts could have been artefacts. A conclusive monosomic embryo based on 2 or more cell counts has yet to be observed in over 100 Day-2 and -3 Merino embryos so far analysed (Murray *et al.*, 1985a; unpublished observations). The frequency of chromosomally aberrant embryos found in this study is not different from the 6–11% incidence reported in untreated (Long & Williams, 1980) and control or androstenedione-immunized ewes (Murray *et al.*, 1985a).

The level of abnormalities observed in preimplantation sheep embryos in this study (6.25%) is significantly less than the 21% observed in Day 2–3 sheep embryos collected from superovulated donors by Williams & Long (1980) ($\chi^2_1 = 5.38$, $P < 0.05$). If the hypodiploid embryos are included in both data sets the difference is no longer significant (10.5% vs 23.1%; $\chi^2_1 = 3.34$, $P > 0.05$).

The chromosomal abnormalities observed in early embryos can have 3 origins: (1) meiosis, i.e. non-disjunction at either meiotic division resulting in hypodiploid or hyperdiploid gametes; (2) fertilization errors resulting in 1N, 1N/2N, 3N etc, and (3) non-disjunction in the cleavage-stage mitosis resulting in $2N/2N \pm$ mosaicism. Only one of the abnormal embryos observed by Long & Williams (1980) from untreated ewes of various British breeds was of the fertilization type while 15 out of the 20 abnormal embryos from PMSG-treated Clun and Herdwick ewes were of this type (Williams & Long, 1980). However, in both the present study and the previous data set concerned with abnormalities in embryos from untreated and immunized Merino ewes (Murray *et al.*, 1985a) the abnormalities have largely been of the fertilization type (3/3 and 7/8 respectively). It is therefore

inconclusive as to whether superovulation with PMSG leads to increased errors of fertilization in sheep.

Considerable caution needs to be exercised in concluding that superovulation treatments will lead to an increase in the frequency of chromosomally abnormal embryos in livestock animals. To be relevant, the chromosome analysis must be made on early stage embryos, as chromosomally aberrant embryos are lost before Days 12–15 of gestation in sheep (Long, 1977; Murray *et al.*, 1986) and cattle (Hare *et al.*, 1980). In addition care must be taken when analysing blastocysts to avoid assessment of the normally occurring polyploid trophoblast cells (Long & Williams, 1982; Murray *et al.*, 1986) as abnormal embryos.

In pigs the only data are from Day-10 embryos for which 9/87 embryos from naturally ovulating sows were classified as abnormal (McFeely, 1967) while all of the 169 embryos collected from PMSG-treated gilts were chromosomally normal (Dolch & Chrisman, 1981). However, Dolch & Chrisman (1981) were using a level of PMSG stimulation which did not produce a superovulatory response in the treated gilts and McFeely (1967) was not aware of the normal occurrence of polyploidy in the trophoblast.

There are no data available for early stage embryos from untreated cows, but the overall incidence of cytogenetically abnormal embryos from PMSG-treated cows is around 9% (combined data from King, Linares & Gustavsson, 1981; Gayerie de Abreu, Lamming & Shaw, 1984; Murray, Moran, Boland, Doff & Nancarrow, 1985b), which is in the range of abnormalities observed in embryos from untreated sheep (Long & Williams, 1980; Murray *et al.*, 1985a). Data cited by King (1985) concerning embryos collected from FSH-superovulated cows suggest that this treatment may increase the incidence of chromosome abnormalities, but most of the data were confounded by the selection of poor quality embryos for analysis or by the use of in-vitro fertilization techniques.

The data which are available from rabbits concerning the effects of superovulation on chromosomal abnormalities in blastocysts are also conflicting. Fechheimer & Beatty (1974) concluded that superovulation induced with FSH had no demonstrable effect on the incidence of chromosomal abnormalities in Day 5.5 rabbit blastocysts. However, Fujimoto, Pahlavan & Dukelow (1974) reported that PMSG treatment led to an increased incidence of chromosomal abnormalities in Day 5–7 rabbit blastocysts.

There has been much work on the incidence of chromosome abnormalities in mice, with most studies using superovulation protocols (see Table 4 of Binkert & Schmid, 1977). The incidence of chromosomally abnormal embryos was variable, ranging from 2.9 to 29.2%. The data of Gosden (1973) do not indicate any increase in the incidence of abnormalities when 3 i.u. PMSG were used to superovulate immature females, while Takagi & Sasaki (1976) reported a significant increase in chromosomally abnormal embryos in mice induced to superovulate with 10 i.u. PMSG. Maudlin & Fraser (1977) concluded that the incidence of polyploidy increased in a linear fashion with increasing doses of PMSG coupled with in-vitro fertilization. Aneuploidy did not increase with increased doses of PMSG. In contrast, we note that the data of Maudlin & Fraser (1977) show a decrease in polyploidy with increasing PMSG dose associated with in-vivo fertilization ($\chi^2_1 = 4.6$, $P < 0.05$). The data from laboratory animals is thus as conflicting as the more limited data from livestock species as to the effects of superovulation on the incidence of chromosomal abnormalities in early embryos. Further work to increase the sample sizes for all species is needed to understand the effect that superovulatory treatments may have on the incidence of cytogenetic abnormalities in early embryos.

We thank Mr Ian Hazelton and the animal house staff at Prospect for animal handling; Dr R. M. Hoskinson and Dr C. D. Nancarrow for help with the immunization and embryo collections, respectively; and Mrs A. Dafter for general technical assistance. M.P.B. was the recipient of a Reserve Bank of Australia fellowship while on leave from University College, Dublin.

References

- Binkert, F. & Schmid, W. (1977) Pre-implantation embryos of Chinese hamster. I. Incidence of karyotype anomalies in 226 control embryos. *Mut. Res.* **46**, 63–76.
- Boland, M.P., Murray, J.D., Hoskinson, R.M., Hazelton, I.G., Sutton, R. & Nancarrow, C.D. (1985) Ovarian response to PMSG treatment in ewes immunized against oestradiol-17 β . *Aust. J. biol. Sci.* **38**, 339–345.
- Dolch, K.M. & Chrisman, C.L. (1981) Cytogenetic analysis of preimplantation blastocysts from prepuberal gilts treated with gonadotrophins. *Am. J. vet. Res.* **42**, 344–346.
- Fechheimer, N.S. & Beatty, R.A. (1974) Chromosomal abnormalities and sex ratio in rabbit blastocysts. *J. Reprod. Fert.* **37**, 331–341.
- Fujimoto, S., Pahlavan, N. & Dukelow, W.R. (1974) Chromosome abnormalities in rabbit preimplantation blastocysts induced by superovulation. *J. Reprod. Fert.* **40**, 177–181.
- Gayerie de Abreu, F., Lamming, G.E. & Shaw, R.C. (1984) A cytogenetic investigation of early stage bovine embryos—relation with embryo mortality. *Proc. 10th Int. Congr. Anim. Reprod. & A.I., Urbana*; Brief commun. 2, 82, Abstr.
- Gosden, R.G. (1973) Chromosome anomalies of pre-implantation mouse embryos in relation to maternal age. *J. Reprod. Fert.* **35**, 351–354.
- Hare, W.C.D., Singh, E.L., Betteridge, K.J., Eaglesome, M.D., Randall, G.C.B., Mitchell, D., Bilton, R.J. & Trounson, A.O. (1980) Chromosomal analysis of 159 bovine embryos collected 12 to 18 days after estrus. *Can. J. Genet. Cytol.* **22**, 615–626.
- King, W.A. (1985) Intrinsic embryonic factors that may affect survival after transfer. *Theriogenology* **23**, 161–174.
- King, W.A., Linares, T. & Gustavsson, I. (1981) Cytogenetics of preimplantation embryos sired by bulls heterozygous for the 1/29 translocation. *Hereditas* **94**, 219–224.
- Long, S.E. (1977) Cytogenetic examination of pre-implantation blastocysts of ewes mated to rams heterozygous for the Massey I (t₁) translocation. *Cytogenet. Cell Genet.* **18**, 82–89.
- Long, S.E. & Williams, C. (1978) Chromosomal abnormalities in ova and zygotes from normal ewes mated to normal rams. *Vet. Rec.* **102**, 153.
- Long, S.E. & Williams, C.V. (1980) Frequency of chromosomal abnormalities in early embryos of the domestic sheep (*Ovis aries*). *J. Reprod. Fert.* **58**, 197–201.
- Maudlin, I. & Fraser, L.R. (1977) The effect of PMSG dose on the incidence of chromosomal anomalies in mouse embryos fertilized *in vitro*. *J. Reprod. Fert.* **50**, 275–280.
- McFeely, R.A. (1967) Chromosome abnormalities in early embryos of the pig. *J. Reprod. Fert.* **13**, 579–581.
- Murray, J.D., Boland, M.P., Moran, C., Sutton, R., Nancarrow, C.D., Scaramuzzi, R.J. & Hoskinson, R.M. (1985a) Occurrence of haploid and haploid/diploid mosaic embryos in untreated and androstenedione-immune Australian Merino sheep. *J. Reprod. Fert.* **74**, 551–555.
- Murray, J.D., Moran, C., Boland, M.P., Doff, A.M. & Nancarrow, C.D. (1985b) Cytogenetic analysis of 34 early stage bovine embryos from superovulated Hereford donors. *Can. J. Genet. Cytol.* **27**, 483–486.
- Murray, J.D., Moran, C., Boland, M.P., Nancarrow, C.D., Sutton, R., Hoskinson, R.M. & Scaramuzzi, R.J. (1986) Polyploid cells in blastocysts and early fetuses from Australian Merino sheep. *J. Reprod. Fert.* **78**, 439–446.
- Nancarrow, C.D., Murray, J.D., Boland, M.P., Sutton, R. & Hazelton, I.G. (1984) Effect of gonadotrophin releasing hormone in the production of single-cell embryos for pronuclear injection of foreign genes. In *Reproduction in Sheep*, pp. 286–288. Eds D. R. Lindsay & D. T. Pearce. Australian Academy of Science, Canberra.
- Takagi, N. & Sasaki, M. (1976) Digynic triploidy after superovulation in mice. *Nature, Lond.* **264**, 278–281.
- Williams, C.V. & Long, S.E. (1980) The effect of superovulation on the chromosome complement of early sheep embryos. *Proc. 4th Eur. Collog. Cytogenet. Domest. Anim., Uppsala*, pp. 168–171.

Received 4 February 1986