CHROMOSOME ABNORMALITIES IN RABBIT PREIMPLANTATION BLASTOCYSTS INDUCED BY SUPEROVULATION*

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While there are undoubtedly multiple causes of spontaneous abortion and congenital birth defects, high incidences of chromosomal abnormalities in human abortuses (Carr, 1965; Geneva Conference, 1966; Makino & co-authors, 1967; Ikeuchi, Sasaki, Kohno, Hayata & Fujimoto, 1972), and in early embryos of the pig (McFeely, 1967) suggest that chromosomal imbalance may be one of the primary causes of early embryonic death.

Excessive administration of HCG has been reported to affect sperm capacitation in rabbits (Soupart, 1967; Wettemann & Hafs, 1970), and the development of the zona pellucida in baboons (Katzberg & Hendrickx, 1966). Recently, a high incidence of triploid embryos has been associated with PMSG–HCG-induced superovulation in mice (N. Takagi, personal communication).

The present investigation was conducted to study the effect of superovulation on the karyological constitution of preimplantation rabbit blastocysts.

Nine mature nulliparous female rabbits from 6 to 8 months of age and of mixed breeding were mated to fertile males 4 days after an intramuscular injection of 100 i.u. PMSG (Lilly Laboratories). Immediately after mating, the females received an intravenous injection of 100 i.u. HCG (A.P.L. Ayerst Laboratories). Ovulation and fertilization are assumed to have occurred 10 hr later (Harper, 1963). Six additional females received saline injections as controls. All does were caged separately for at least 4 weeks before mating. Leucocytes from fifteen females and three males were cultured for chromosomal analysis.

The uterine horns were flushed with warm saline for the recovery of intact preimplantation blastocysts at 5 to 7 days of pregnancy. After size determination, by a filar micrometer eyepiece, the blastocysts were separately placed in fresh culture medium (TC-199) to which colchicine (0.5 µg/ml) had been added. Following incubation for 1½ hr at 37°C in 5% CO₂ in air, chromosome preparations were made by a modification of the method of Shaver & Carr (1967). The total numbers of blastocyst cells and the mitotic indices were determined for each blastocyst as we have previously reported (Fujimoto,

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Pahlavan & Dukelow, 1973). Karyotypes were prepared according to the rabbit idiogram of Nichols, Levan, Hansen-Melander & Melander (1965). The numbering of the autosomal pairs followed that of Shaver & Carr (1967).

The peripheral blood leucocyte cultures from the three males and fifteen females yielded no cells with abnormal karyotypes.

A total of 187 blastocysts was observed from the fifteen does, which included the control group (Table 1). Of the ova ovulated, 73.9% (147/199) in the superovulation group and 86.9% (40/46) in the control group were observed as blastocysts (Table 1).

Seventy-two and thirty-six intact blastocysts were used for karyological examinations from the experimental and control group, respectively. As shown in Table 1, seven chromosomally abnormal blastocysts (9.7%) were found in the experimental group. In the control group, no chromosomal abnormalities were observed. The abnormal cytological features are summarized in Table 2. They included three triploids, a mosaic, a monosomy, a trisomy, and a sex chromosome chimaera.

Table 1. Numbers of corpora lutea and blastocysts, and their chromosome analysis, following collection from does subjected to superovulation or control does

Treatment	Days of pregnancy	No. of rabbits	No. of CL	No. of blastocysts					
				Observed	Examined	Normal 44 XY	Normal 44 XX	Abnormal	Abnormality
Superovulation	5	5	106	70	32	16	15	1	43/44 XX 45 XY, 43 XY, Three 60 XXY 44 XX/44 XY
	6	3	79	69	35	13	17	5	
	7	1	14	8	5	3	1	1	
	Total	9	199	147	72	32	33	7	
Control	5	3	28	25	24	14	10	0	None
	6	3	18	15	12	5	7	0	None
	Total	6	46	40	36	19	17	0	None

Three triploid blastocysts had an XXY sex chromosome constitution. Plate 1, Fig. 1 shows the metaphase plate and karyotype of a triploid blastocyst.

In the mosaic blastocysts (43/44XX), the diploid cell population had a female sex chromosome complex, and in karyotypes prepared from metaphases with 43 chromosomes, the missing chromosome was found to be one of the small subtelocentric chromosomes (pair 18). Karyotypes of a trisomic blastocyst showed an extra large acrocentric chromosome (pair 20). A monosomic blastocyst lacked one large submetacentric chromosome (possibly pair 9). A chimaera blastocyst had eight female and nine male sex chromosome complexes out of seventeen diploid cell karyotypes (Pl. 1, Fig. 2).

The five abnormal blastocysts, except for the two cases of 43/44XX and 45XY, had fewer blastomere numbers and lower mitotic indices than the normal diploid litter mates (Fujimoto *et al.*, 1973).

Table 2. Cytological features of chromosomally abnormal rabbit blastocysts

Rabbit no.	Blastocysts			Chromosome no. distribution										No. of cells karyotyped	Abnormality
	Outer diameter (mm)	No. of blastomeres	Mitotic index (%)	No. of cells counted	Diploid and near diploid						Exact or near 3n	Exact or near 4n			
					<43	43	44	45	46	>46					
M6-3	4.2 × 4.2	44,020	3.2	23	3	8	12	—	—	—	—	—	8	43/44 XX	
B5-5	4.5 × 4.0	105,440	3.0	24	4	1	3	13	1	—	—	2	8	45 XY	
A1-1	3.0 × 2.5	33,140	2.2	24	—	—	—	—	—	6	18	—	17	66 XXY	
A1-19	3.0 × 2.5	62,400	0.6	18	—	—	—	—	—	—	17	—	7	66 XXY	
A1-20	2.5 × 2.5	53,640	1.0	26	—	—	—	—	—	4	22	—	4	66 XXY	
A1-23	2.0 × 2.0	56,460	0.8	28	5	23	—	—	—	—	—	—	2	43 XY	
C3-4	—	39,500	2.9	22	4	1	17	—	—	—	—	—	22	44 XX/44 XY	

The outer diameters of the one monosomic and three triploid blastocysts were also reduced compared to those from normal diploid litter mates.

Kennelly & Foote (1965) reported no differences in the fertilization rate of does which had or had not been subjected to superovulation and had been inseminated immediately after HCG administration. Tesh (1966) suggested, however, that superovulated eggs were in some way deficient, and reported a decreased fertilization rate in such does with spermatozoa aged *in vivo*.

In mice, superovulation may give rise to fewer litters and to abnormal pregnancies (Fowler & Edwards, 1957). Recently, high incidences of triploid conceptuses have been reported in pregnant mice following superovulation (N. Takagi, personal communication).

In rabbits, Shaver & Carr (1969) reported no significant difference in the incidence of chromosomal abnormalities of blastocysts collected following natural mating with and without HCG injection (25 i.u.). Shaver (1970) also gave no indication that excess HCG (75 to 300 i.u.) adversely affected the chromosomal constitution of developing ova, reporting only two abnormalities (43/44 XY and 44 XY with deletion of the short arm of a submetacentric chromosome) among the seventy-five blastocysts examined. The ovulation rates in those reports, however, were 10.5 (287/27) and 10.0 (110/10), respectively; while that of our present data was 22.1 (199/9), which was assumed to be superovulatory. The percentage recovery (number of observed blastocysts) in the superovulation group (73.9%) was less than in the control group (86.9%) and also less than the levels reported by Martin & Shaver (1972) and Widmeyer & Shaver (1972) and might be due to the effect of the superovulation (Table 1).

The effects of delayed fertilization have been studied in various species. In rabbits, when mating was delayed between 6 and 9 hr after injection of HCG (25 i.u.), 13% of the blastocysts were found to be triploid, but no triploids were observed with delay intervals of 0 to 4 hr (Shaver & Carr, 1969). Our previous laparoscopic study concerning hormonal influence on the time of ovulation in rabbits (Fujimoto, Rawson & Dukelow, 1974) should, however, eliminate the possibility of delayed fertilization.

The incidences of chromosomally abnormal blastocysts of rabbits mated naturally without any gonadotrophins were reported as 2.0% (1/58) (Shaver & Carr, 1969), and 1.4% (1/72) (Widmeyer & Shaver, 1972).

We agree with the suggestion of Hansen-Melander & Melander (1970) that blastocysts obtained following superovulation are not suitable as test subjects for many types of reproductive studies.

Induction of superovulation by PMSG and HCG has been widely adopted for obtaining a large number of eggs or conceptuses from laboratory animals. The results of the present study indicate the need to consider the possible

EXPLANATION OF PLATE 1

FIG. 1. Metaphase plate and karyotype of a triploid blastocyst (Rabbit A1-19, 66XXY) collected from a doe following induction of superovulation with PMSG and HCG.

FIGS 2 and 3. Karyotypes of a chimaera blastocyst collected from Rabbit G3-4 following induction of superovulation with PMSG and HCG.

FIG. 2. Karyotype with 44XX chromosomes.

FIG. 3. Karyotype with 44XY chromosomes.

(Facing p. 180)

genetic effects of superovulation on ovum maturation, fertilization and early growth of embryos.

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