13. A Preliminary Note on Chromosome Abnormalities of Intratubal Rabbit Embryos

By Seiichiro FUJIMOTO,*¹⁾ Thomas J. PASSANTINO,*²⁾
and Ilona KOENCZOEL*³⁾

The Population Council, the Rockefeller University,
New York, N.Y. 10021, U. S. A.

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The air-drying technique for the chromosome study of mouse eggs (Tarkowski 1966) could be applied with some modifications to other mammals in early embryonic stages, and has been contributed to cytogenetics of the mouse, rat, golden hamster and Mongolian gerbil at various phases from egg meiosis to pre-implantation stages. There are cytogenetic studies on pre-implantation blastocysts of the rabbit (Shaver and Carr 1967; Austin 1967; Shaver 1970; Hofsaess and Meacham 1971; Widmeyer and Shaver 1972; Martin and Shaver 1972; Fechheimer and Beatty 1974; Fujimoto *et al.* 1974a), but little has been known on the chromosomes of very early intratubal embryos.

This paper describes preliminarily some chromosomal aspects of the intratubal rabbit embryos with an improved double fixation method (Fujimoto *et al.* 1974b), with attention to the effect of superovulation on the chromosomes of rabbit intratubal embryos at the 8- to 16-cell stage.

Four mature virgin white rabbits from six to eight months of age (3.67 ± 0.2 kg, $M \pm SE$) were mated to fertile males 4 days after treatment with 100 i.u. PMS i.m. (Calbiochem). The does received 100 i.u. HCG i.v. (A.P.L. Ayerst Laboratories) immediately after mating. Seven females of comparable age (3.49 ± 0.1 kg) were injected with saline for control. All animals were sacrificed on day-2 (mating=day-0) with an overdose of sodium pentobarbitone.

Oviducts were flushed with warm culture media consisting of 30% fetal bovine serum, 70% minimum essential medium, and 0.08 γ /ml of colcemid. The embryos thus obtained were cultured for 4 to 5 hours at 37°C in a moist atmosphere of 5% CO₂ in air. The eggs were pretreated with hypotonic solution and fixed twice with methylalcohol, and glacial acetic acid and deionized water (4:1:3, v/v), and methylalcohol and glacial acetic acid (3:1, v/v). They were air-dried on slides and stained with ammoniacal Giemsa or basic Fuchsin

Address for reprint request: Dr. Seiichiro Fujimoto, Department of Obstetrics & Gynecology, School of Medicine, Hokkaido University, Sapporo, Japan.

Fig. 1. Whole preparation from a day-2 embryo. Fig. 2. Two metaphase plates enlarged from Fig. 1. Figs. 3-4 Metaphase plate and karyotype of a haploid ovum. Fig. 5. Karyotype of a trisomic embryo.

after hydrolysis with 1N HCl. Technical procedures were detailed in another paper (Fujimoto 1975).

Results. Figures 1-5 show the quality of preparations made by this method. Figure 1 is a representative whole preparation from a day-2 embryos, showing 13 nuclei and 3 metaphase plates with a karyotype of 44, XX in each. Figure 2 depicts 2 enlarged plates from Figure 1. Figures 3-4 demonstrate a haploid chromosome set in each, and Figure 5 is a trisomic karyotype.

The results of cell counts and karyological analyses, together with other findings, are summarized in Tables I and II. It was shown that the mean number of cells per embryo was relatively high ($p < 0.05$) in the control group as compared to the superovulated one, though the difference appeared to have been influenced by the presence of 5 haploid ova, 4 of which were in 1-cell stage. These haploid plates seemed to represent the first cleavage division or second meiotic metaphases of ova which retained intact during very early intratubal stages after ovulation. The remaining haploid ovum consisted of 6 blastomeres including 2 haploid metaphase plates: it may be a product of gynogenesis.

Usually, the number of metaphase cells was very limited in these early embryos, and an exact karyotype analysis was possible only in one or two plates in each sample (Table II). Hence, a mosaic case as indicated by 43/45, XY (no. 6) in Table II is dubious. For the same reason, some embryos in which only one metaphase was analysed may not always be reliable.

Table I. Summary of observations in rabbit early embryos recovered on day-2

	No. of anim.	No. of ovulat.	No. of embryos		Blastomere no. per embryo	Mitotic cell no. per embryo	Chromosome analysis		
			obs.	prep.			Embryo no.	Diploid	Abnormality
Super-ovulat.	4	112 (M=28.0)	104 (M=26.0)	91 (M=22.8)	1052 (11.6±0.5)*	221 (2.3±0.2)*	56	44,XY(19) 44,XX(15) 44(18)	Haploid (5) Triploid (2) Tetraploid (1) Trisomy (4) Double trisomy (1) Mosaic (1)
Control	7	73 (M=10.4)	70 (M=10.0)	67 (M=9.6)	877 (18.1±0.4)	161 (2.2±0.2)	42	44,XY(13) 44,XX(18) 44(6)	Triploid (1) Hypodiploid (3) Trisomy (1)

* Mean ±S.E.

Table II. Cytological features of chromosomally abnormal embryos in superovulation and control series

Embryo	Blasto- mere no.	Mitosis no.	Chromosome numbers distribution										Karyo- typed cell no.	Abnormality
			Diploid and near diploid											
			41	42	43	44	45	46	~2N	~1N	~3N	~4N		
RI-1-3	8	3					3					(1)*	Trisomy 43/45,XY (no. 6)**	
RI-1-5	10	5					2					2	(1)	Triploid
RI-1-6	13	2											1	22,X
RD-8-3	1	1								1			1	22,X
RD-8-4	1	1								1			1	22,X
RD-8-5	1	1								1			1	22,X
RD-8-6	1	1											1	22,X
RD-8-7	12	1											(1)	Tetraploid 45,XX (no. 12)
RD-11-6	6	2					2						2	Triploid 46,XX (no. 11)
RD-11-7	8	5						4					(1)	46,XX (no. 20,21)
RD-11-11	4	4											1	45,XY (no. 12)
RD-11-20	12	3											2	22,X
RD-11-25	16	4					4						1	41,XY (no. 3,18,18)
RD-11-54	6	2										2	(1)	Triploid 42,XX (no. 22,22)
RG-8-1	16	4	2										1	43,XX (no. 18)
RG-8-10	11	3												45,XY (no. 12)
RG-8-11	16	1		1	3								3	
RN-3-6	16	3											2	
RN-3-9	8	3					3							

* Precise karyotyping and sexing were difficult. ** Missing or extra chromosome (possibly) is indicated.

Nevertheless, it may be interesting that the present data indicate a higher incidence of polyploidy and aneuploidy in the superovulation group than in the control series.

Remarks. Technical difficulties in obtaining an ideal preparation suitable for karyological analysis of rabbit intratubal eggs seem to lie in the remnant or undissolved mucin and zona pellucida, loss of embryos by swinging or floating on the slide glass, and breaking or overscattering of blastomeres. These shortcomings are overcome by the present double fixation technique. The mucin and zona pellucida could be perfectly dissolved by the enzyme and hypotonic treatments. Pre-fixation of embryos in the first fixative solution could decrease the dissociation or overscattering of blastomeres. The remnants of cytoplasm, particularly rich in this species, are satisfactorily negligible after hydrolysis with 1N HCl.

Recovery rates of embryos obtained in this study (92.9% in superovulation and 95.9% in control) are in accordance with those reported by Adams and Chang (1962) and Adams (1970).

The effect of exogenous gonadotropins on the chromosome abnormalities of rabbit embryos has been a matter of repeated discussion (Shaver and Carr 1969; Hansen-Melander and Melander 1970; Shaver 1970; Fujimoto *et al.* 1974a; Fechheimer and Beatty 1974). The reported incidence of chromosomal anomalies in rabbit blastocysts to which gonadotropins were not administered ranged from 0 to 1.7% (Shaver and Carr 1969; Widmeyer and Shaver 1972; Fujimoto *et al.* 1974a). In contrast, considerably increased incidence ranging from 1.0 to 12.0% occurred after administration of various doses of gonadotropins (Shaver and Carr 1969; Shaver 1970; Hofsaess and Meacham 1971; Martin and Shaver 1972; Fechheimer and Beatty 1974; Fujimoto *et al.* 1974a). The same tendency was shown in the present intratubal embryos, although the number of embryos analysed was not large enough.

Further data are needed to estimate chromosome abnormalities in relation to pre-implantation wastage of embryos, because of the importance of cytogenetic data of early intratubal embryos.

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