Hereditary Radiation Effects In Offspring Of the Second and Third Generations After Irradiation Of Both Grandparents: Experimental Studies and Hereditary Radiation Effects In Offspring Of the First Generation After Irradiation Of One And Both Parents: Experimental Studies

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INTRODUCTION

Hereditary effects in mammals, due to ionising radiation, have been revealed for 90y yet several unresolved questions remain. Most studies have been on the progeny of only one irradiated parent. Recently, because of the considerable increase in the number of sources in our environment, it has been considered necessary to study hereditary effects resulting from the irradiation of both parents. There are practically no published data on this [1] to determine whether any systematic differences might be discerned with the effects of just one parent being irradiated. Such studies should also take account of the stages of gametogenesis when gonadial exposure of either parent occurred [2,3].

The parent study has investigated radiation effects in progeny of the first generation of Wistar rats after irradiation of one and both parents with doses of 0.25-4 Gy and radiation effects in progeny of the second and third generations of Wistar rats after irradiation of both grandparents with doses of 2-4 Gy. Attention was focused on the relationship between stages of gametogenesis in parents at the moment of radiation exposure and death of progeny in embryogenesis and early postnatal ontogenesis.

MATERIAL AND METHODS

Totals of 4207 mature males and females Wistar rats, 17465 offspring of the first generation (F1), 17734 offspring of the second generation (F2) and 746 offspring of the third generation (F3) were the subjects of investigation. Male and female rat parents (P) of 220-250 g were exposed to an external single irradiation by gamma rays to doses of 0,25, 0,5, 1, 2, 3 and 4 Gy at a dose rate of 0.003 $Gy.s^{-1}$ (^{60}Co source). Following irradiation they were mated with each other and also with non-irradiated intact individuals. The animals were mated at different times after irradiation so that different stages in gametogenesis were studied [4]. The stages were for males spermatozoids, spermatids, spermatocytes, and spermatogonia; and for females metaphase I to metaphase of the II^{nd} division of maturity and maturing (late dictyothena) oocytes. The beginning of the pregnancy was registered cytologically by the presence of sperm in vaginal smears. Experimental groups were formed depending on the stages of spermato- and oogenesis of P gametes at the moment of irradiation and radiation dose. Each consisted of 30-50 first-pregnant females. Embryogenesis F1 was studied after euthanasia of some females by ether on the 20th day of pregnancy. The fetuses were scored for size and mass, pathology of viscera and skeleton and the total, pre- and post-implantation death of embryos was calculated. In addition development of young rats was observed for 30 days after the birth. The numbers surviving on the 1st and 30th days after the birth were calculated, giving the death rate for this period of time. When F1 sexual maturity was achieved, F1 males of each experimental group were mated with intact females to produce F2 descendants from the father's line and convercely F1 females use mated with intact males to produce F2 descendants from the mother's line. Embryogenesis and early postnatal development F2 were studied in the same way as F1. Sexually matured males F2 were irradiated with a dose of 2 Gy, mated with the intact females and embryogenesis of the F3 was studied. All animals were kept in the standard conditions of the vivarium. The uncertanties in the resultant data was considered reliable at $p \leq 0.05$.

RESULTS

Radiation effects in embryogenesis and early postnatal ontogenesis F1 after irradiation of one or both parents (P) with doses of 0.25 - 4 Gy

The embryonic death F1 changed from 10.8 ± 1.9% to 83.1 ± 2.8% (in control 13.1 ± 2.2%) depending of radiation dose, stage of gametogenesis at radiation and whether one or two irradiated parents participated in the mating (Table 1). The embryonic death was highest levels after irradiation of germ cells of parents at stages of spermatids, spermatozoids and matured oocytes. Following irradiation of both parents with doses of 0.25, 0.5 and 1 Gy at these stages of gametogenesis and 4 Gy at the stage of spermatids and matured oocytes there was a trend of increasing radiation effects caused by the participation of two irradiated germ cells (Fig.1). After irradiation of both parents with doses of 2, 3 and 4 Gy (for spermatozoids and matured oocytes) the embryonic death F1 was essentially the same as rates for irradiated females and non-irradiated males. There was no dependence of F1 embryonic death, with respect to parental doses, on germ cells exposed as spermatogonia,

spermatocytes or maturing oocytes. This is shown in Fig.2 where it is also interesting to note that for irradiated mature oocytes universal death rate in F1 embryos followed irradiation of the female that was unaffected by whether or not the male parent was also exposed. However following irradiation of the maturing oocytes the F1 death rate from both irradiated parents is very similar to that observed when only males were irradiated (except for the care of irradiated spermatocytes + maturing oocytes). Therefore one may postulate that the F1 embryonic consequences were determined by the female parent if the irradiation occurs to the mature oocytes, but by the male if the oocyte was still maturing.

Radiation Dose for P (Gy)	**Embrionic death of F1 (%)**		
	Irradiated Females + Irradiated Males	Irradiated Females + Non-irradiated males	Non-irradiated Females + Irradiated Males
0,25	26,9±3,7*	15,7±2,1	11,0±1,9
0,5	28,9±3,6*	17,6±2,4	10,8±1,9
1	44,0±5,2*	30,7±3,1*	22,9±2,6*
2	61,5±2,8*	62,4±4,2*	29,1±3,4*
3	68,9±4,2*	72,0±3,5*	35,5±3,3*
4	83,1±2,8*	75,5±3,1*	48,8±3,2*
Control		13,1±2,2	

Table 1: Numerical values of F1 embryonic death after irradiation of germ cells of parents (P) with doses of 0.25-4 Gy at the stages of spermatozoids and matured oocytes

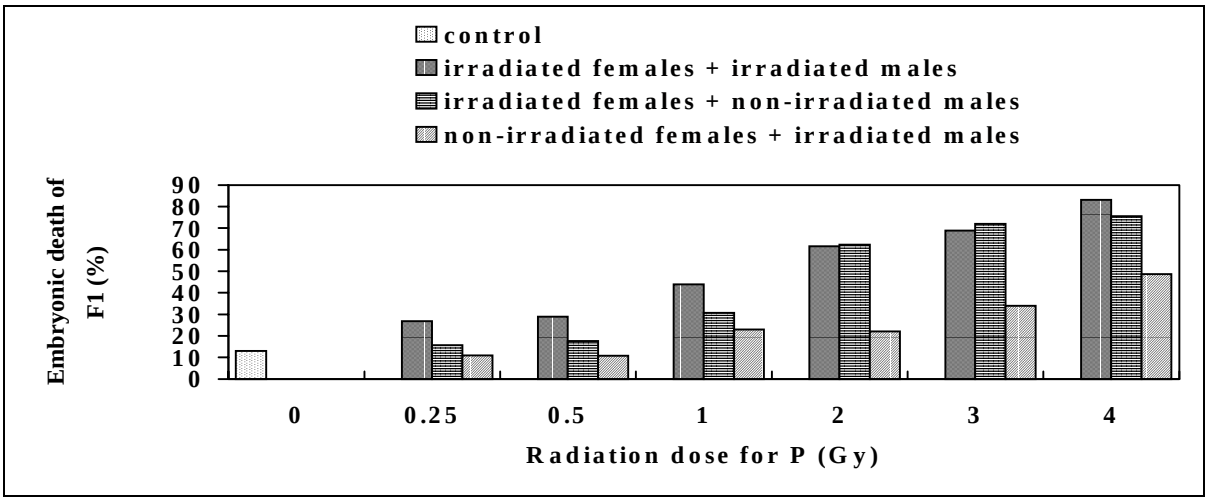

Figure 1: Embryonic death of the first generation (F1) after irradiation of germ cells of parents (P) with doses of 0.25-4 Gy at the stages of spermatozoids and matured oocytes.

The F1 death rate in early postnatal development exceeded the control (9.9 ± 3.1%) only after irradiation with doses of 2, 3 and 4 Gy. It changed from 9.6 ± 3.5% to 33.5 ± 3.5% and depended to a greater degree on development stages of parent's germ cells at the time of irradiation (Fig.3) than on radiation doses. F1 death reached maximum values usually after irradiation of germ cells of both parents at the stage of spermatids, spermatozoids and matured oocytes. Following irradiation of germ cells with a dose of 2 Gy at the stage of spermatozoids and the matured or maturing oocytes an increase in radation effects was noted when two irradiated rats were mated (Fig.3). In some cases deviations occurred in late development. But cases of F1 death in the period 1-3 months post natal when the animals were reaching maturity were rare.

Overall the results lead to a conclusion that the production of radiation effects in F1 depends on both radiation dose and stage of the development of germ cells at the moment of irradiation and occurs mainly in embryogenesis. In the particular situation of both parents having been irradiated, hereditary effects may be dominated by dose to one or other of the parents depending on the particular stage of germ cell maturation when the exposure occurred. From these results we may draw up a list of gamete irradiations in the following order of decreasing effect: matured oocytes + spermatids /spermatozoids/ spermatocytes/ spermatogonia and maturing

oocytes + spermatids/ spermatozoids/ spermatocytes/ spermatogonia.

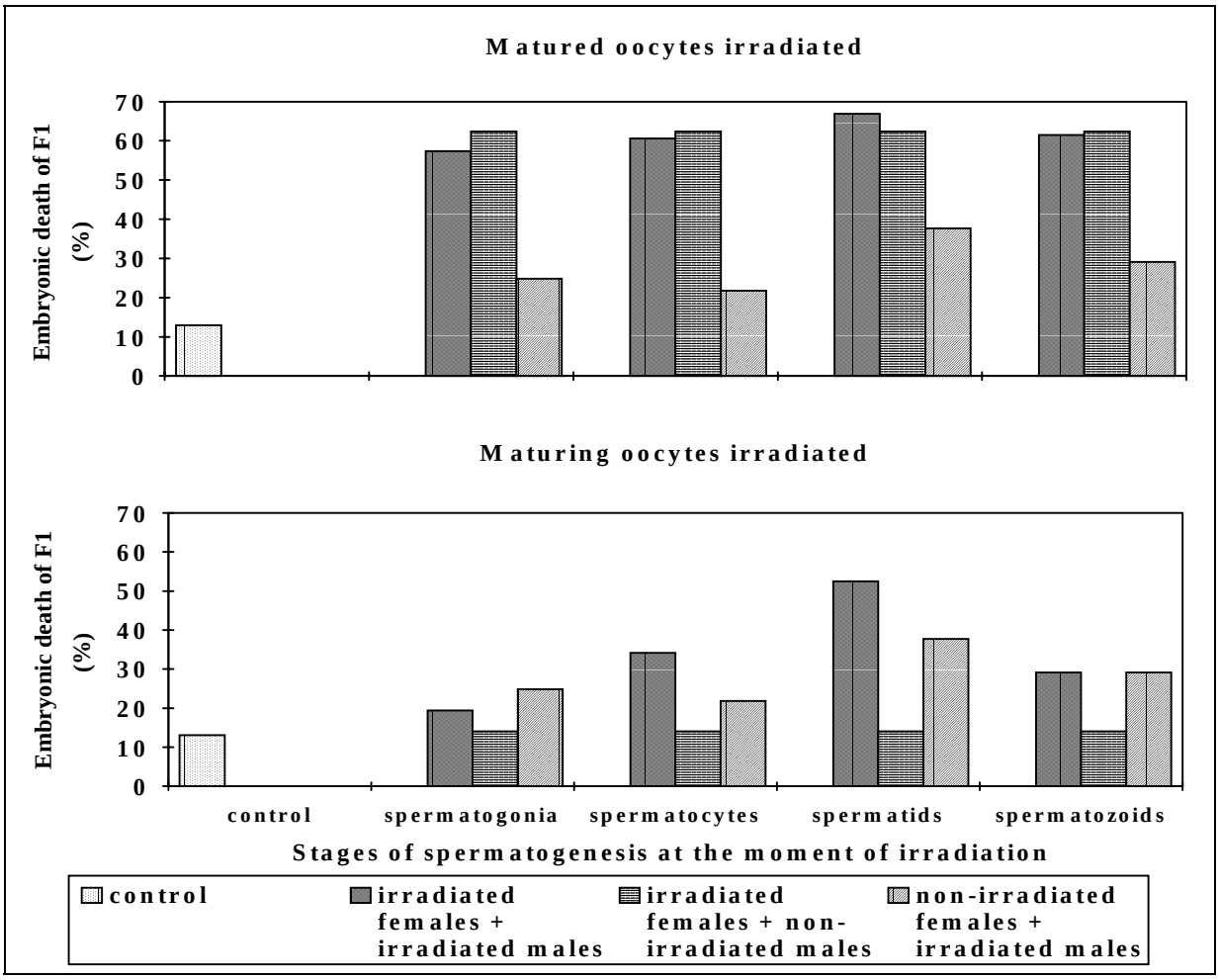

Figure 2. Embryonic death of the first generation (F1) after irradiation of germ cells of parents (P) with dose of 2 Gy at the different stages of gametogenesis.

Conclusions:

1) radiation effects depended on a dose and stage of gametogenesis of both P at the time of radiation exposure;
2) F1 death occurred mainly in embryogenesis and achieved maximum after irradiation of spermatozoids, spermatids and matured oocytes;
3) after irradiation of both P in doses of 0.25, 0.5, 1 and 4 Gy aggravation of radiation effects takes place in F1 embryogenesis compared to irradiation of only one of P;
4) after irradiation of both P with doses of 2 and 3 Gy radiation effects for F1 are determined only by exposed female P or only exposed male P. It depends on the stage of gametogenesis in both P at the time of radiation exposure;
5) a high rate of F1 death after birth was observed only in case of irradiation of both P with a dose of 4 Gy.

Radiation effects in embryogenesis and early postnatal ontogenesis F2 after irradiation of both grandparents (P*) with doses of 2-4 Gy.

F2 death in embryogenesis did not exceed the control (12.9 ± 1.9%) if both parents were irradiated with doses of 2 and 3 Gy, but it rose up to 47.8 ± 4.0% following irradiation of P* with a dose of 4 Gy. The frequency of visceral abnormalities in 20-day F2 embryos did not differ from the control, but disorders in the rate of skeleton ossification occurred in all experimental groups. In this case no dependence on radiation doses and stages of gametogenesis at the time of parental irradiation was found. In early postnatal development the F2 death from both irradiated grandparents had practically no dependence on exposure over the range of doses.

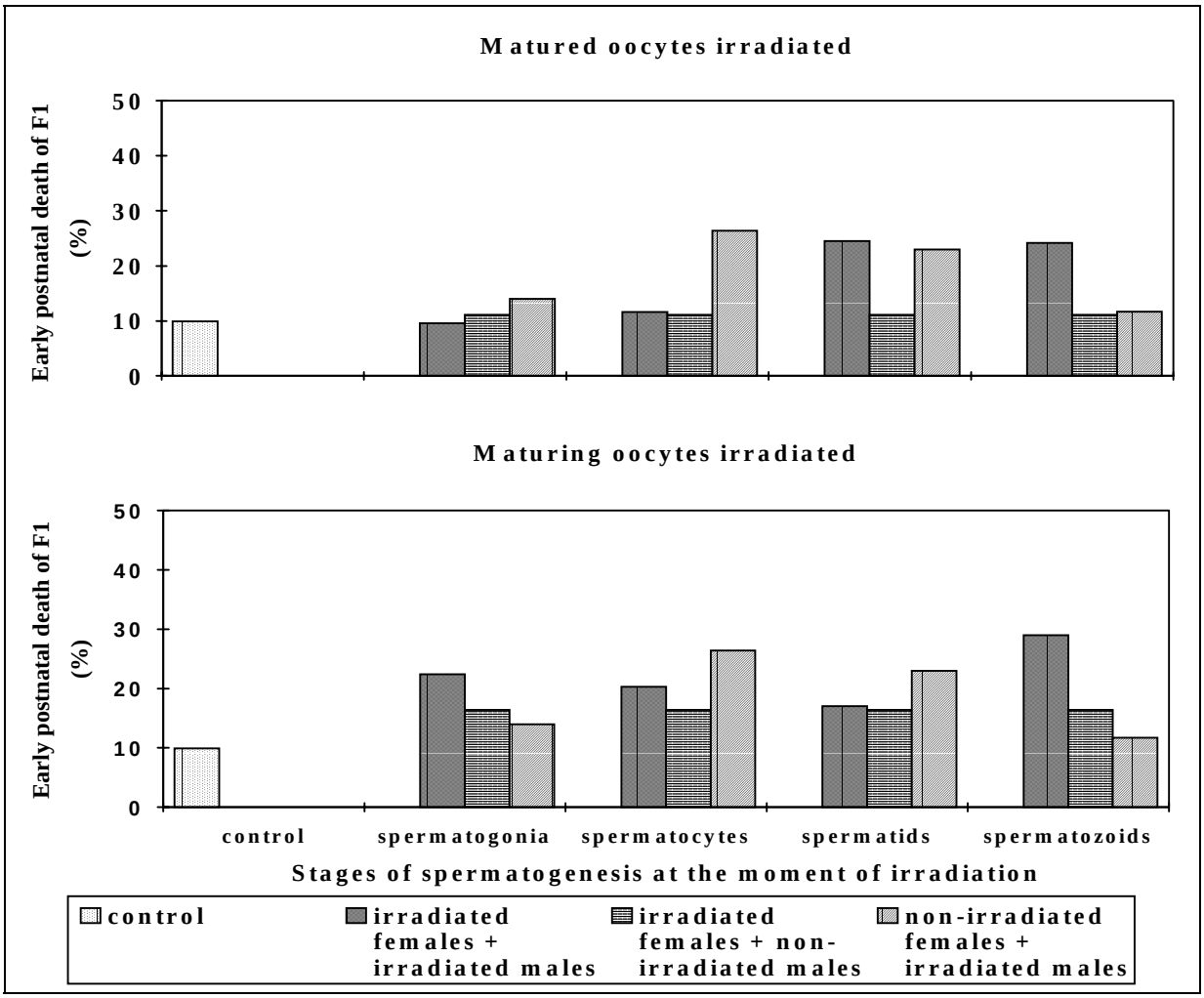

Figure 3. Early postnatal death of the first generation (F1) after irradiation of germ cells of parents (P) with dose of 2 Gy at the different stages of gametogenesis.

Studied death of F2 by father's line was higher than by mother's line and reached maximum values after the irradiation of germ cells of male-parents at the stages of spermatogonia and spermatocytes (Table 2, Fig.4). Irradiation of the parents'mature oocytes resulted either in a higher F2 death rate, or a rate equal to that which was observed following irradiation of the maturing oocytes. F2 death in early postnatal development depended more on stage of parents at the time of irradiation than on radiation dose (Fig. 5). The total F2 death (without subdivision into specific experimental groups) after irradiation of both grandparents by a dose of 2 Gy was 26.7±0.6% (1 517 of 5 679 subjects died) and in control 9.2 ± 1.8%.

Stage of Spermatogenesis at the Moment of Irradiation	**Father's Line**		**Mother's Line**	
	Irradiated Matured Oocytes of P*	Irradiated Maturing Oocytes of P*	Irradiated Matured Oocytes of P*	Irradiated Maturing Oocytes of P*
Spermatogonia	62,6±4,4*	43,6±3,8*	7,1±3,4	6,7±2,5
Spermatocytes	63,5±3,5*	60,7±5,2*	30,8±4,8*	14,4±2,8
Spermatids	40,8±4,4*	40,5±3,7*	22,2±4,4*	6,1±1,7
Spermatozoids	8,5±2,4	12,2±2,9	20,0±2,9	25,9±4,2*
Control	9,2±1,8			

Table 2: Numerical values of F2 early postnatal death after irradiation of germ cells of both grandparents (P*) with dose 2 Gy at the different stages of gametogenesis

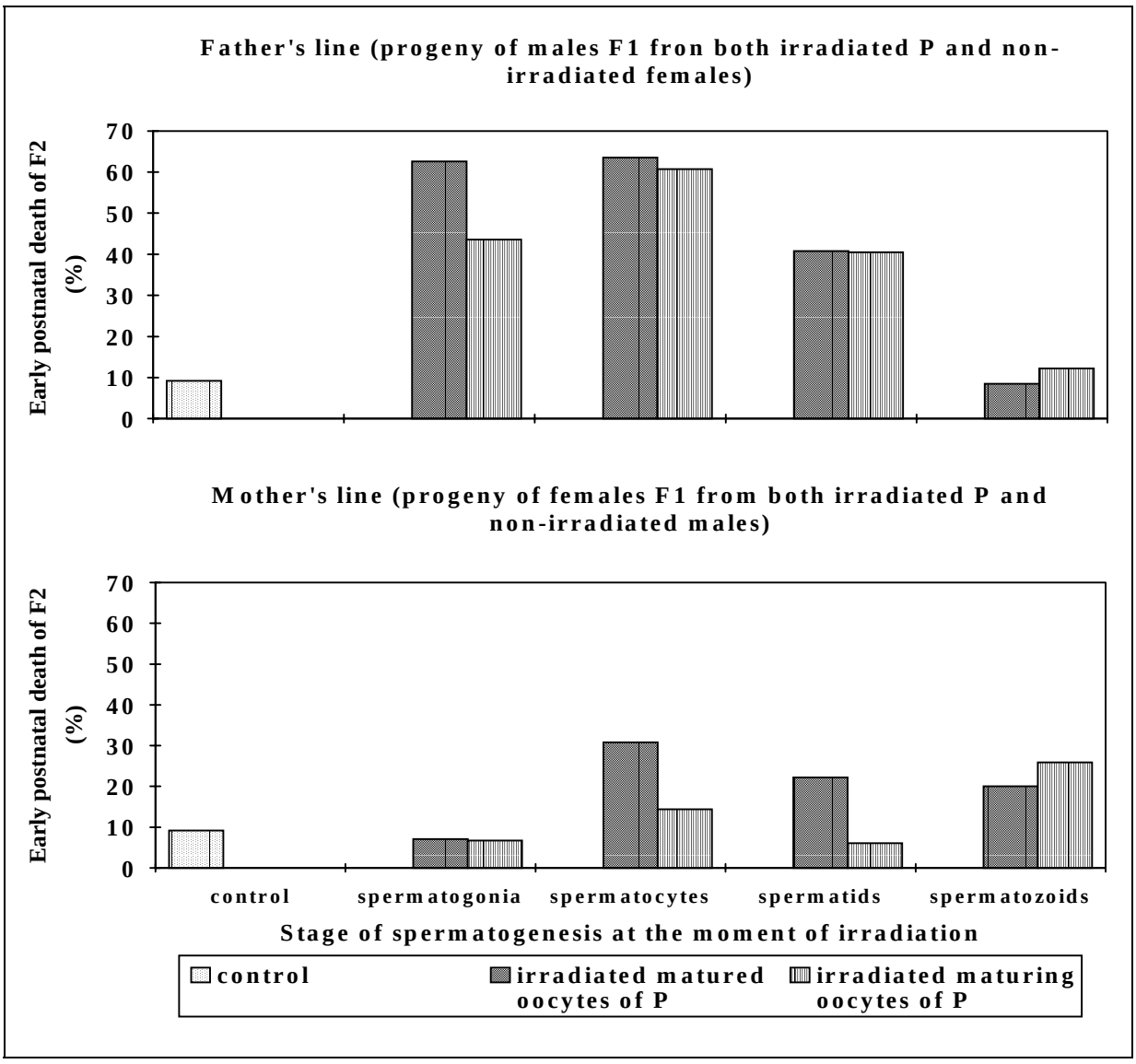

Figure 4. Early postnatal death of the second generation (F2) after irradiation of germ cells of both grandparents (P*) with dose 2 Gy at the different stages of gametogenesis.

In some experimental groups puberscent F2 males born to P* which had both been irradiated with a dose of 4 Gy were given to "testing" radiation dose of 2 Gy and mated with non-irradiated females to study F3 embryogenesis. It was found that gametes of such males had a higher radiosensitivity of a latent character (Fig.6). In general, these results lead to a conclusion that radiation effects in F2 depend, to a greater degree, on the stages of gametogenesis of both P* at the time of irradiation than on radiation dose. Radiation effects in F2 mainly occur in the early post natal period and they were more expressed through the father's line than the mother's. Gametes of puberscent F2 males of both irradiated grandparents have a higher radiosensitivity compared to F2 of non-irradiated parents. Based on these observed radiation effects in F2 one may draw up a list of parental germ cells, irradiated at different stages of maturation, in the following decreasing order of effect: matured/maturing oocytes + spermatocytes/ spermatogonium/ spermatids/ spermatozoids.

Conclusions:

1) radiation effects depend upon a dose and stage of gametogenesis of both P* at the time of radiation exposure;
2) F2 death in embryogenesis occurred only after irradiation of P* with a dose of 4 Gy;
3) ossification disorder of the skeleton in F2 embryogenesis was found in all experimental groups;
4) high F2 death is observed after birth which depends much more on the stage of gametogenesis P* than radiation dose;
5) maximum death F2 occurs after irradiation of spermatogonia and spermatocytes P*,

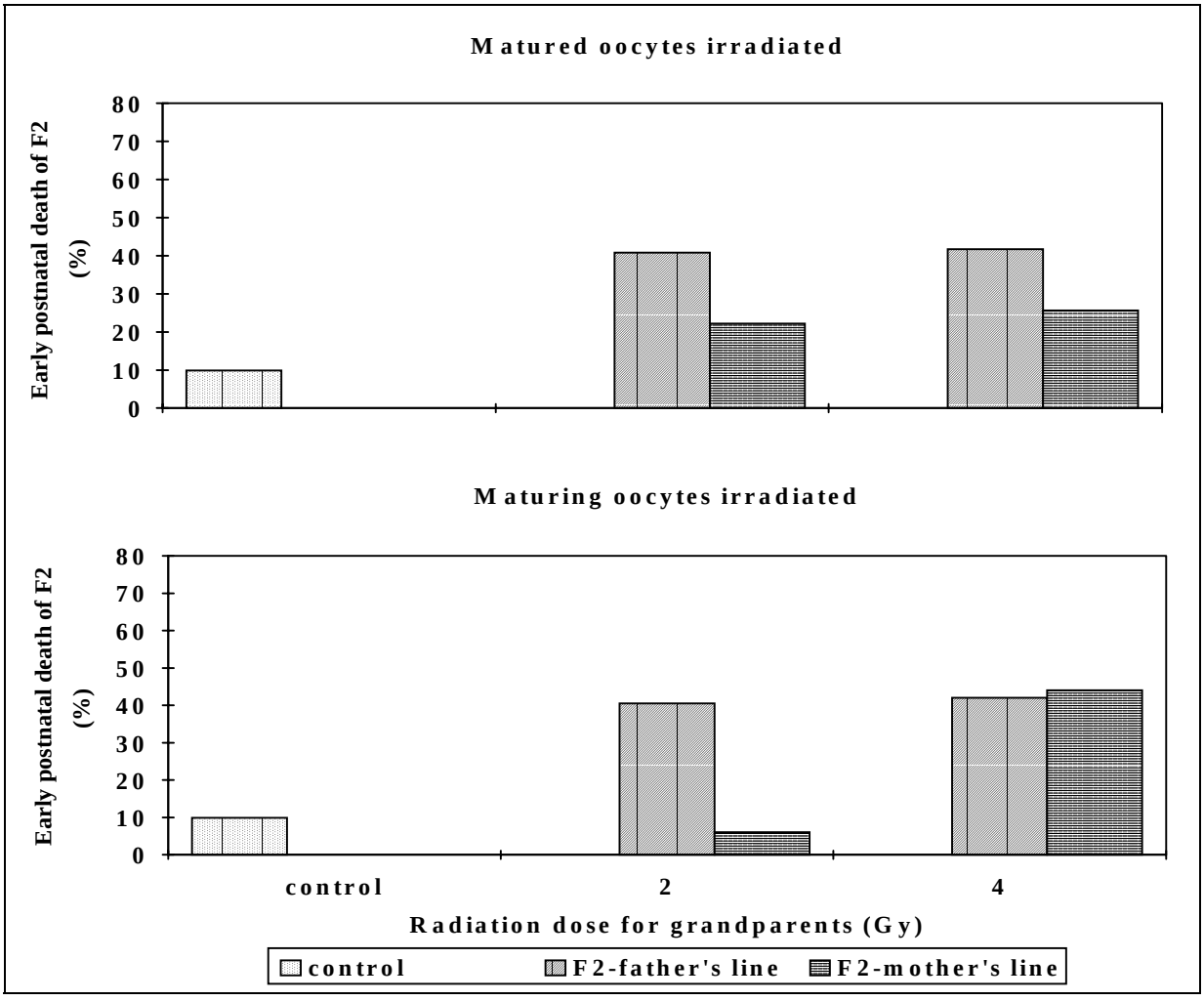

Figure 5. Early postnatal death of the second generation (F2) after irradiation of germ cells of both grandparents (P*) at the stages of spermatids and matured/maturing oocytes.

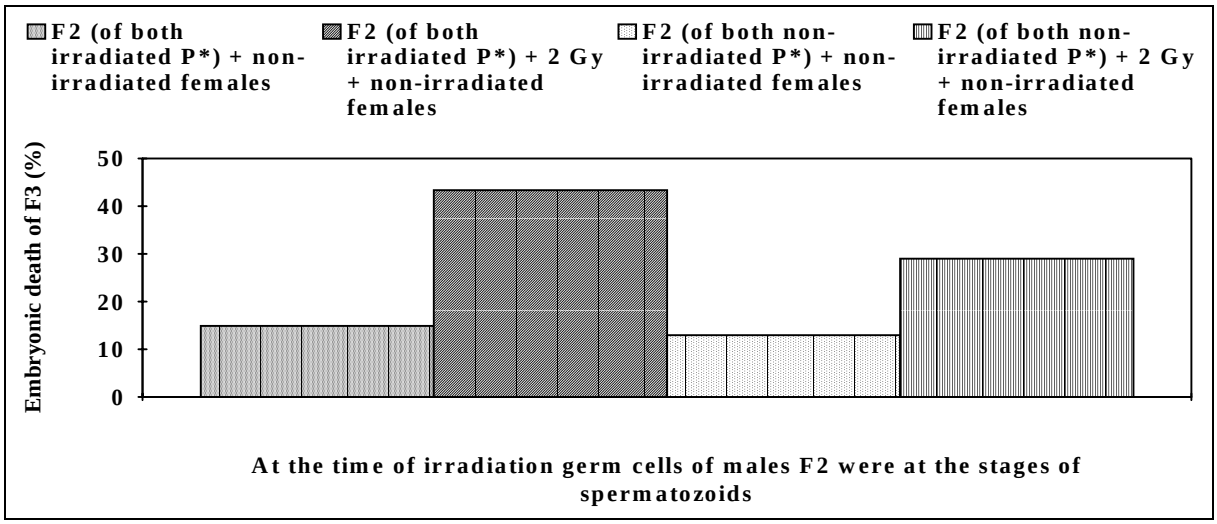

Figure 6. Embryonic death of the third generation (F3) after "testing" irradiation of puberscent males of the second generation (F2) with dose of 2 Gy (of both irradiated or non-irradiated grandparents (P*)) and mating them with non-irradiated females.

6) death F2 from males F1 and non-irradiated females is higher than from females F1 and non-irradiated males;
7) radiation exposure to males F2 (from both irradiated P) and their pairing with non-irradiated females results in more significant radiation effects in F3 than the same radiation exposure to F2 from both non-irradiated parents (control). This fact indicates higher sensitivity of sex cells F2 to irradiation.

DISCUSSION N

The studies of the casual relationship between the stages of development of parents'germ cells at the time of irradiation and effects in development over 2 following generations showed certain trends which have led to suggestions of possible mechanisms. The increase in radiation effects in the F1 from the mating of two irradiated parents appears to be associated with a mechanism demonstrating additivity or synergism.

The outcome in the F1 of mating between one or both irradiated P is influenced by variations in genetic radiosensitivity of both male and female germ cells at different stages in their maturation. The mature oocytes are more radiosensitive than the maturing oocytes or male gametes at any stage of spermatogenesis. The high death rate of F1 during embryonic development is likely to be associated with gross chromosomal and genomic abnormalities, whiest the death of F2 after the birth is connected with non-repaired point DNA mutations induced by radiation in gametes P*. The point DNA mutations in germ cells may lead to the allowance of DNA instability of the somatic cells in organisms developed from irradiated gametes [5]. This process may results in the reduction of the specific effects, or altered sensitivity of individuals to various pathogens. It may not manifest phenotypically but rather appears as a latent characteristic "physiological inferiority" [6] against some specific pathogen or environmental pressure.

Transmission of some radiation effects from parents to future generations may be connected with the mechanism of inactivation and reactivation of the X-chromosome in the gametogenesis of parents and hence "conservation" and "deconservation" of radiation disorders in the germinal line of their progeny.

Taking into account the universal nature of the biological effect of ionizing radiation on mammals, it is reasonable to assume that the same mechanisms as shown here in rats would also operate in humans. It is, however, not possible to predict how the actual numerical results presented here would apply human species.

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