RAFFAELE CAVALLORO - MARIANO MUNIZ-DAZA - GIOVANNI POZZI

COMPARATIVE RADIOSENSITIVITY OF MED-FLY CELLS AND EMBRYOS

Rerum natura nusquam magis quam in minimis tota.

PLINIO

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RAFFAELE CAVALLORO (*) - MARIANO MUNIZ-DAZA (**)
GIOVANNI POZZI (*)

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INTRODUCTION

Early results obtained at the Ispra Joint Research Centre on the effects of gamma radiation on cells of *Ceratitis capitata* Wiedemann cultivated « in vitro » (CAVALLORO R. *et al.*, 1983), stimulated further investigations on young embryos at various stages of development.

Although considerable research has been carried out at the cellular level with ionizing radiation in the animal kingdom (PUNCK T.T., 1960; SINCLAIR W.K., 1968; OKOMURA Y. and UCHIYAMA Y., 1974), very few studies have been reported for invertebrates, and particularly uncommon are those on « in vitro » cells (BROOKS M.A. and KURTI T.J., 1971; KOVAL T.M., 1982) and on embryonated eggs (CAVALLORO R., 1973; WURGLER F.E. *et al.*, 1978; MIKI M. and MURAKAMI A., 1979).

The aim of this research was that of investigating the effects of gamma radiation on one single invertebrate species, both at the cellular level and on embryos in the first stage of development.

The availability of mass-rearing *Ceratitis capitata* and of cell lines of the same *Diptera Trypetidae* allowed us to carry out this research. Particular attention was placed on the induced radiobiological effects, both immediate and delayed in time.

The data obtained are considered to be very useful for a radiobiological comparison between cells and embryos, which can lead to interesting considerations.

(*) Commission of the European Communities - Joint Research Centre - Ispra, Italy
(**) Instituto Espanol de Entomologia - C.S.I.C. - Madrid, Espana.

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MATERIALS AND METHODS

The materials used were cultured « in vitro » cells of *Ceratitis capitata* Wied. (CEC CC 130 cell line), and embryonated eggs at two different stages of development.

The cells were obtained from a stabilized continuous proliferating culture of a diploid population (CAVALLORO R., 1981), and the embryos from a permanent mass-rearing culture (CAVALLORO R. and GIROLAMI V., 1969).

The « in vitro » cells were grown in 25 cm^3 Roux flasks with 4 ml of culture medium (SCHIELD G. and SANG J.H., 1977) prepared 48 h before irradiation and kept during the whole experiments at 25 ± 1 °C.

The embryos were collected at the same age, after 30 min from eggs-laid. Irradiation was carried out both on embryos stored for 24 h from the collection and immediately after the collection itself. In this way, we have treated both the embryos that had undergone 24 h of active development and 30 min old embryos. The embryos, both after the collections and after the treatments, were kept at 25 ± 1 °C on filter paper soaked in water and placed on the bottom of Petri dishes.

The irradiation was carried out with a Gammacell-220 device with a ring of 60 Co source of 2480 Ci with a dose-rate of 600 rad/min. The dosimetry for the irradiation was carefully checked with a PTW Duplex ionization chamber No. 16204. Accuracy was calculated to be ± 2.5%.

The embryos, and the « in vitro » cells were irradiated at the same time by placing them at the same position at the centre of the irradiation chamber. The doses used were 300, 600, 1200, 2400 and 4800 rad. After irradiation the cells contained in the Roux flasks were rinsed twice with fresh nutritional medium, leaving finally 4 ml of fresh culture medium per flask.

In each cell flask 20 different areas were chosen at random in order to count the cells every day, by means of a special network, placing the flask always in the same position on the microscope stage.

For the embryos, the effects of the treatments were determined by evaluating the hatching rate on batches of 100 eggs; each test was repeated at least 4 times.

RESULTS

Cells

In untreated cells (control) the normal population doubles about every 22 hours (CAVALLORO R., 1981). This occurs regularly and is evident until the population density reaches very high levels, which have an influence on the rate of proliferation of population itself, reducing proportionally the increase in development (fig. I).

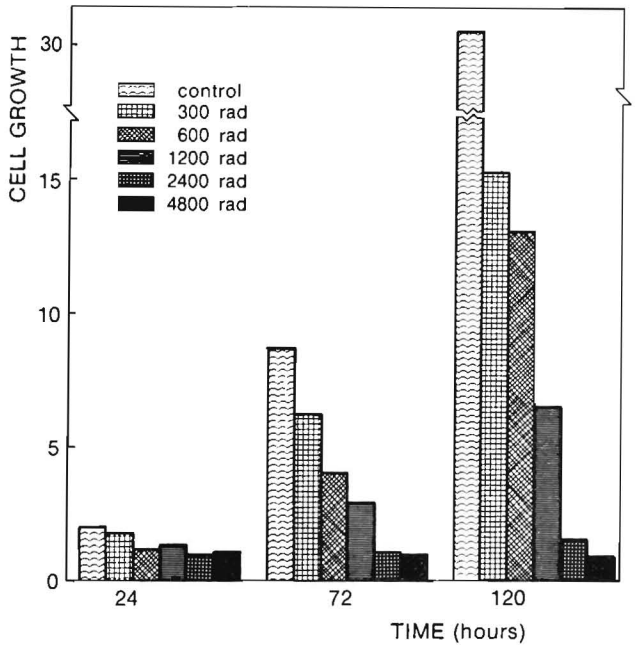

Fig. I

Ceratitis capitata Wied.: growth of irradiated and non-irradiated cells as a function of time.

It should be remembered that the cells multiply in a monolayer, adhering to the bottom of the flask and that as a rule the transfer occurs about 130 hours after the preparation of the culture.

A microscopic observation of the morphology shows the suffering state of the cells as the time for transplanting approaches. There is an irregular

superposition of the cells in masses which brings about their detachment from the bottom of the flask and cause then floating to the surface of the culture medium.

When the cell population is irradiated, the doubling time is modified, depending on the dose received.

For the intermediate values used, i.e. for those between 300 and 1200 rad, after an apparent stop in initial growth an exponential increase is observed. At higher doses, however, a block in cell growth becomes apparent, due both to cell mortality and to an arrest in cell division (CAVALLORO R. *et al.*, 1980).

Indicating cell growth with I, the development of the following expression

$$I = \frac{N(tx) - N(to)}{N(to)}$$

where N(tx) is the number of cells at observation time and N(to) is the number of cells at the beginning of the experiment, clearly shows for the various treatments that cell growth itself depends on the radiation dose received (fig. I).

Cell mortality can be very clearly determined from observation with phase contrast, which allows us to recognise the dead cells from an alteration of the cell membrane (CAVALLORO R. *et al.*, 1983).

From a careful observation of the survival curves versus time of the irradiated cell populations (fig. II) the recovery capacity shown by the cells for doses lower than 1200 rad is evident.

The survival parameter (S) is derived from the relationship between the indices of cell growth of the irradiated material (T) with respect to the non-irradiated material (C). This is expressed as a percentage, according to the following expression:

$$S(n) = \frac{N_T(tx) - N_T(to)}{N_C(tx) - N_C(to)} \cdot 100$$

where:

S (n) = cell survival at day « n »
$N_T(tx)$ = number of cells treated at observation time (tx)
$N_T(to)$ = number of cells treated at time zero (to)
$N_C(tx)$ = number of control cells at observation time (tx)
$N_C(to)$ = number of control cells at time zero (to)

Embryos

The irradiation of the eggs is expected to induce, depending on the dose used, a longer embryo development time as well as a certain embryo mortality.

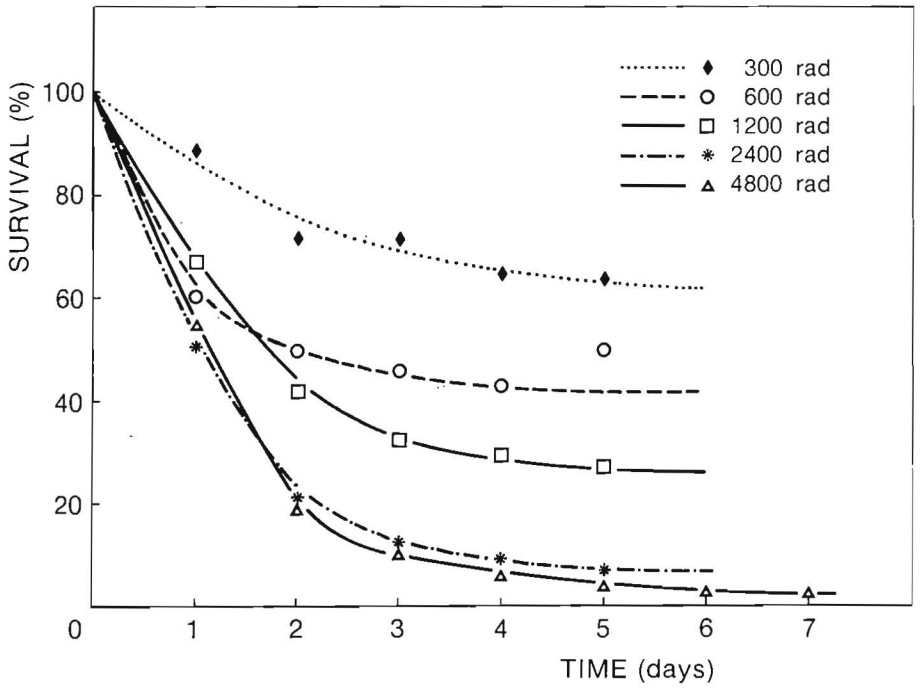

Fig. II

Ceratitis capitata Wied.: cell survival in time after various doses of γ-rays.

The phenomenon is reported in fig. III and fig. IV for eggs irradiated immediately after they were laid (30 min) and for eggs at a more advanced stage of development (24 h). One can see the different behaviour of the embryonated eggs depending on their age and thus on the stage of embryo development they have reached. The response to the same irradiation dose shows that the embryos close to the hatching have greater resistance than younger embryos which have a higher mortality rate and thus confirms the different radiosensitivities at the two stages of life of the same embryos.

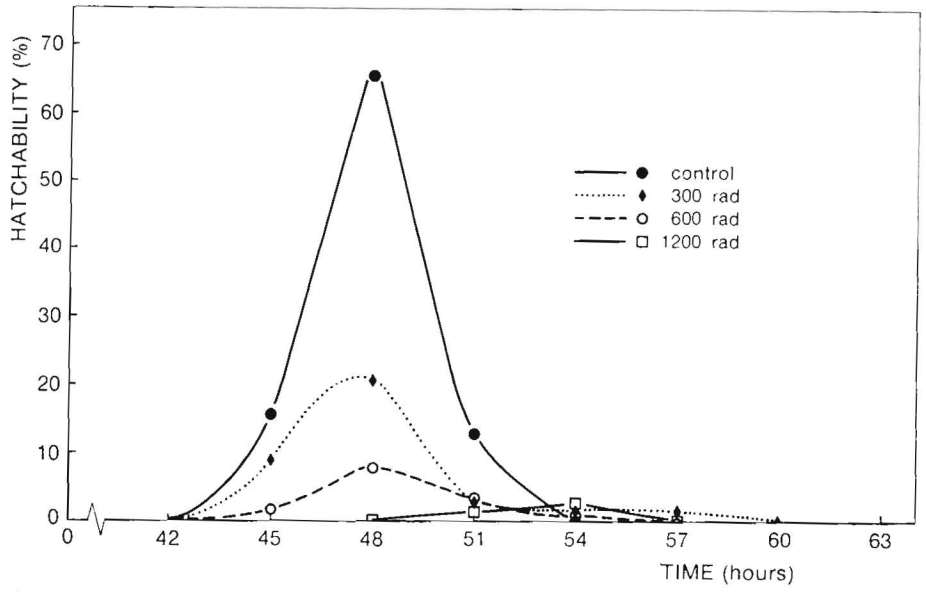

Fig. III

Ceratitis capitata Wied.: effect on hatching of irradiated eggs 30 min old (value at 26±1°C).

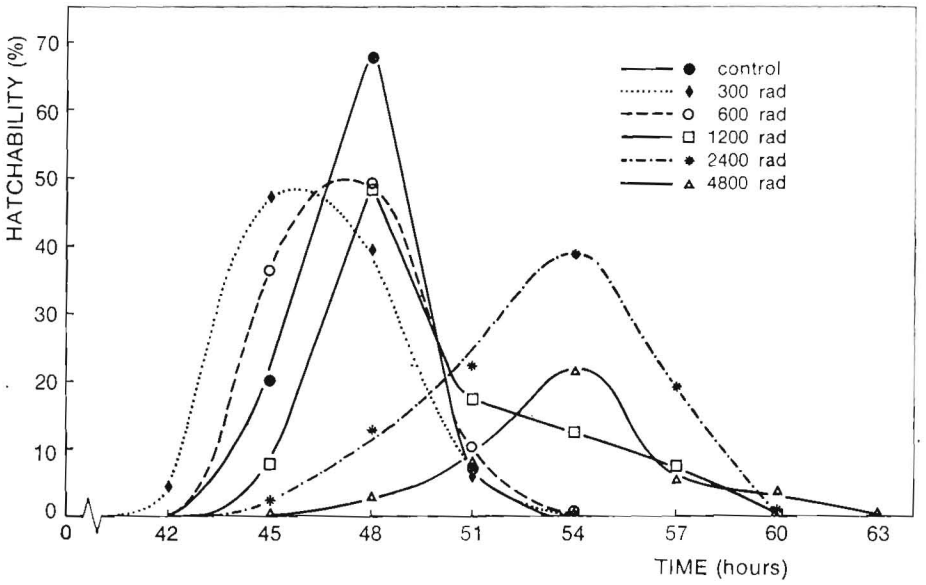

Fig. IV

Ceratitis capitata Wied.: effect on hatching of irradiated eggs 24 h old (value at 26±1°C).

The quantification of this phenomenon (fig. V) allowed to note a resistance of more than 20 times higher in the irradiated embryonated eggs in a more advanced state of development with respect to those less developed.

In fact, the LD_{50} of eggs subjected to irradiation, indicates that the same biological effect is obtained in embryonated eggs of 30 min and 24 h of age for doses of 210 and 4700 rad, respectively.

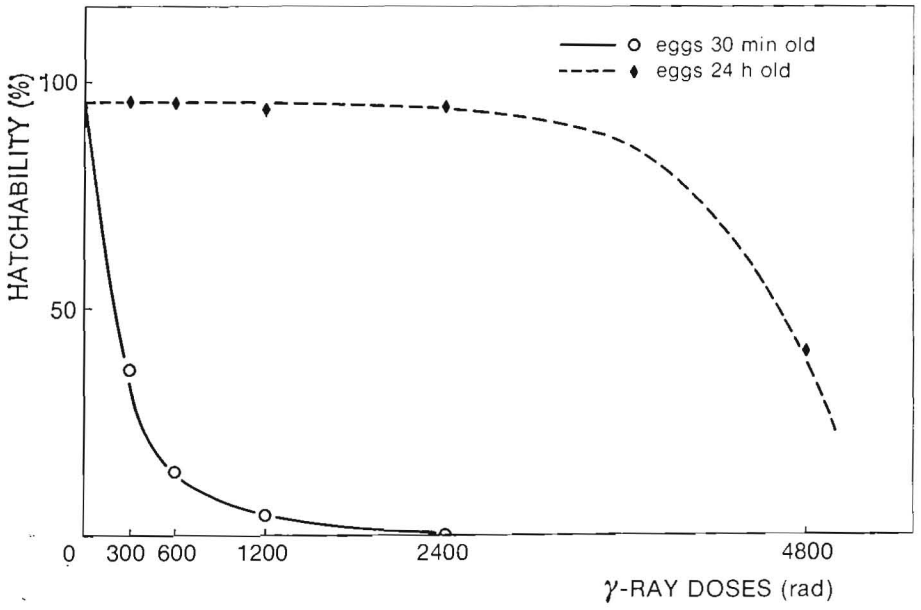

Fig. V

Radiosensitivity of Med-fly embryos at two different ages of development.

It is interesting to observe (tab. 1) how the lower doses (up to 1200 rad) cause a high rate of mortality in the young embryos while in older embryos no significant difference is noted in the hatching rate of the eggs compared to the controls.

One can also note some interesting effects not only in the stages which immediately follow the irradiation stage, but also in the next generation (F_2).

In particular, the effect of irradiation of the eggs can be found at the appearance of the adults that show a lower percentage of emergence when

dealing with younger eggs, but more harmful effects when the irradiation occurs in eggs at a more advanced stage of development.

Thus, while a few individuals, obtained e.g. after treatment with 600 rad (8.6%)are found to be fertile and with a high degree of fertility (90.0%), a higher percentage (20.7%) for the same dose received among the 24 h old eggs is not having offsprings because the adults cannot mate.

Tab. 1. — *Ceratitis capitata* Wied.: effects of eggs irradiation on subsequent development and reproduction.

Age of irradiated eggs and dose		Effects of irradiated Med-fly eggs on life-cycle development				
eggs	dose (rad)	larvae (%)	pupation (%)	emerged adults (%)	eggs and larvae from emerged adults	
					eggs laid	larvae (%)
30 min old	control	95.5	90.0	70.0	yes	95.2
	300	36.7	35.0	15.6	yes	88.9
	600	13.2	11.0	8.6	yes	90.0
	1200	4.0	2.7	1.8	no mating	—
	2400	0	—	—	—	—
	4800	0	—	—	—	—
24 hours old	control	95.2	90.1	75.7	yes	95.7
	300	96.0	89.9	54.3	yes	42.2
	600	95.7	90.0	20.7	no mating	—
	1200	93.7	5.7	0	—	—
	2400	95.0	0	—	—	—
	4800	40.5*	0	—	—	—

* Larvae reduced in size and unable to hatch.

In eggs at an advanced stage of development treated with low irradiation doses (300 rad) a delayed sterility in the first generation is found which is not noticed when younger eggs are irradiated. Thus one can observe, on irradiating 30 min eggs, an immediate effect which is greater than the delayed effect while the opposite occurs when 24 h old eggs are irradiated where no immediate effect is found but the delay is longer.

DISCUSSION AND CONCLUSIONS

For some time it has been known that, generally speaking, insects are highly resistant to ionizing radiation (O'BRIEN R.D. and WOLFE L.S., 1964). However, the effects found depending on the doses given with immediate and delayed phenomena are not yet well understood. The range of these effects (depending on decreasing dose) varies from mortality to lesions on apparatus or organs of considerable metabolic activity, to lengthening of the development times at various stages of life, to inhibition of growth and cell division, etc. (CAVALLORO R., 1975).

The radiosensitivity also varies depending on the species considered, although for the same species changes in sensitivity occur during ontogenesis. As well as other physico-chemical parameters connected with radiation, physiological factors such as mitotic activity, stage of cell cycle, etc. are also very important.

Tab. 2. — Mortality of cultured Med-fly cells and eggs irradiated at different γ-ray doses.

Dose (rad)	Cells cultured « in vitro » (%)	Eggs (*) 30 min old (%)	Eggs (*) 24 hours old (%)
300	27.3 ± 1.80	61.5 ± 5.50	0
600	57.1 ± 0.58	86.1 ± 1.38	0
1200	73.2 ± 1.29	95.8 ± 0.41	1.6 ± 1.34
2400	97.9 ± 0.33	100	0.3 ± 0.41
4800	100	—	57.5 ± 0.50

(*) The data are expressed as corrected mortality.

This leads to the conclusion that sensitivity to radiation is associated not only with the nature of the radiation itself, but also with the various physiological conditions in which the biological material treated is found.

The comparison between single cells and embryos of *Ceratitis capitata* confirms these various responses to ionizing radiation.

Table 2 shows the mortality effect for various doses in both « in vitro » cells and in embryonated eggs collected 30 min and 24 h from eggs-laid, respectively. For what concerns the radiosensitivity, a factor of greater resistance of about 3 times in « in vitro » cells is found as compared to eggs in which the embryo is undergoing differentiation (fig. VI).

The radioresistance of eggs with already developed embryos (fig. V) is higher at least 22 times as compared to embryos in the first stage of formation and about 8.5 times as compared to « in vitro » cells of the same species.

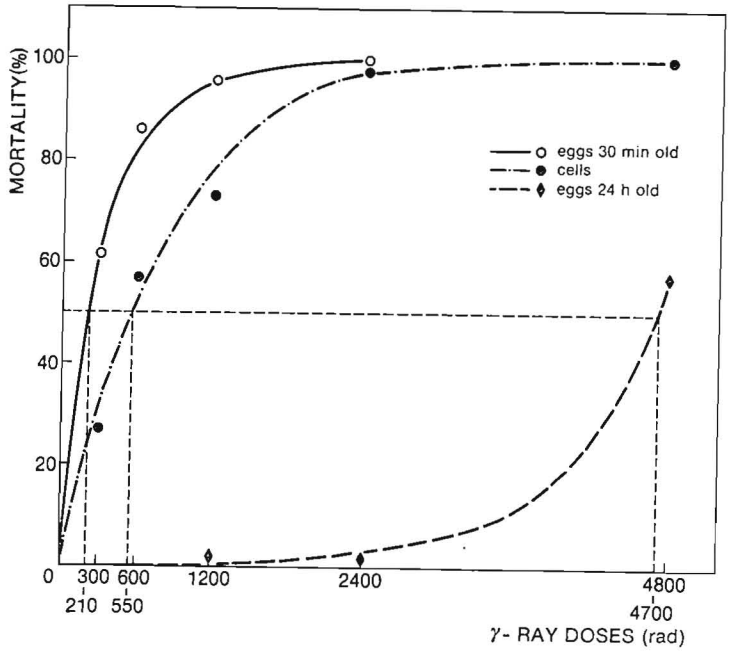

Fig. VI

Comparative radiosensitivity of Med-fly cell « in vitro » and eggs 30 min and 24 h old (the LD_{50} value are respectively 550, 210 and 4700 rad).

This may be considered a logical consequence if radiosensitivity is linked to cell differentiation. A 30 min old embryo is certainly less differentiated than the « in vitro » cell and the 24 h old embryo, which is almost completely developed.

As known, cells of the embryo at the first stage are not differentiated and thus show lower resistance. This is also due to the genetic expression of the cell itself, which needs to express a wider range of genes and thus suffers from heavy DNA lesions.

At high doses the cells definitely lose the ability to reproduce and with a careful observation (CAVALLORO R. *et al.*, « in press ») one can detect variations in the cell structure in which, as well as the classic variations in the chromatin, the cytoplasm appears to be emptied of most of its organelles, especially mytocondria and Golgi apparatus, with fewer mesh cisterns and with a dramatic reduction in the number of ribosomes.

Irradiation to a 30 min egg affects the cell at a phase of exceptional activity and thus more easily leads to mortal damage, while when it hits more mature eggs in which the DNA synthesis occurs over a longer time, the cell is less easily affected or reacts to the damages inflicted by the radiation to the DNA itself, showing a greater radiation resistance.

Examination of the irradiated « in vitro » cells also leads to the conclusion that there is a slowing down in reproduction rate at low radiation doses but they are still capable of reproduction.

SUMMARY

This research is dealing with the effect of ^{60}Co gamma radiation on cultured « in vitro » cells and on embryos at different developmental stages, of *Ceratitis capitata* Wiedemann.

The parameters analyzed for both the cells and the embryos were growth, survival and mortality rates. The immediate and late effects of irradiation were also studied at the level of egg hatching, larval life cycle, emergence of adults and their fertility.

A particular result that became evident in the comparison of the radiosensitivity was that the cells « in vitro » had a greater radioresistance than the very young embryos by a factor of 3, but the cells had less resistance by a factor of 8.5 when compared with the developed embryos.

In general, with an increase in dose there was an increase in damage; even at 1200 rad a prolonged arrest was found in the growth of the cell population, and with 2400 rad it was found in the development of 24 h old embryos. Confronting embryos of different ages, it was noted that the same quantitative effect was obtained with doses proportional to the age of the embryos: the same mortality effect in 30 min old embryos irradiated with 300 rad as in those 24 h old treated with 4800 rad was observed.

The results obtained are presented and discussed.

RIASSUNTO

La ricerca ha riguardato l'effetto delle radiazioni gamma da ^{60}Co su cellule coltivate « in vitro » e su embrioni in diverso momento di sviluppo di *Ceratitis capitata* Wiedemann.

I parametri usati sono stati la crescita, la sopravvivenza e la mortalità, sia per le cellule che per gli embrioni. Si sono evidenziati gli effetti immediati e differiti dell'irrag-

giamento sulla schiusura delle uova, lo sviluppo del ciclo larvale, lo sfarfallamento degli adulti e la loro fertilità.

È emersa in particolare una maggior radioresistenza della cellula « in vitro » rispetto a quella dell'embrione nei primi stadi di sviluppo, per un fattore di circa 3 volte, ed una resistenza inferiore di 8,5 volte quando si confronta con l'embrione in età avanzata.

In generale, con l'aumentare delle dosi si è notato un aumento del danno; un arresto prolungato nello sviluppo della popolazione cellulare si manifesta già con 1200 rad e così avviene con 2400 rad anche negli embrioni di 24 ore d'età. Nel confronto fra embrioni di differente età di sviluppo si è osservato un medesimo effetto quantitativo con dosi proporzionali all'età degli embrioni; per esempio si riscontra lo stesso grado di mortalità sia in embrioni di 30 minuti di età irradiati con 300 rad che in quelli di 24 ore trattati con 4800 rad.

I risultati della ricerca sono presentati e discussi.

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