

## **OVARIAN TRANSFER FOLLOWING IRRADIATION AS AN ALTERNATIVE TO RESTORE REPRODUCTIVE FUNCTIONS.**

**Andréia Ruis Salgado<sup>1</sup>, Luiz Augusto Corrêa Passos<sup>1</sup>, Patrick Jack Spencer<sup>2</sup>, Ana Paula Ginemes<sup>1</sup>, Viviane Liotti Dias<sup>1</sup> and Nanci do Nascimento<sup>2</sup>**

1-Centro Multidisciplinar para a Investigação Biológica da UNICAMP (CEMIB/UNICAMP)  
Universidade Estadual de Campinas, Rua 5 de Junho nº 230, Caixa Postal 6095

2-Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP  
Av. Professor Lineu Prestes, 2242 – Cidade Universitária  
CEP: 05508-000 – São Paulo – SP – Brasil  
<sup>1</sup>e-mail: [viviliotti@cemib.unicamp.br](mailto:viviliotti@cemib.unicamp.br)

### **ABSTRACT**

Assisted reproduction technologies are essential for restoring reproductive functions, especially in ovary cancer cases, which besides impairing fertility; present the highest lethality amongst gynecological diseases. Classical treatment involves surgery, radiotherapy and chemotherapy, and in young women, the recommended treatment is the removal of the affected ovary, leading to physical, psychological and emotional problems. An alternative would be the ovarian transfer after the treatment. However, there are no data in the literature about experiments specifically designed to investigate the interference of this procedure on reproductive functions. In the present work, we used the C57BL/6Unib and B6CF1 hybrid mice strains to evaluate the viability of ovarian transfer before and after irradiation, as well as possible differences in radiosensitivity between the strains. To do so, female mice from both strains were irradiated with 4 Gy and submitted to either partial or total ovarian transfer from healthy donors. After the surgery, the animals were mated and the results obtained so far for mating 7 days after the procedure indicate that irradiation promoted a significant decrease in fertility ( $p=0.0127$ ). Also, our data show that the recovery of fertility is proportional to the amount of grafted ovarian tissue. Furthermore, there seems to be differences in radiosensitivity, from genetic origin, between the two mice strain, since, after irradiation, the hybrid mice had bigger litters than the donor strain.

### **1. INTRODUCTION**

The advances in reproductive medicine have enabled the development of extremely useful methods and techniques for the treatment of women with reproductive difficulties.

Nevertheless, a challenge remains: the incapacity to preserve oocytes in patients submitted to chemo and radiotherapy. In such cases, the graft of fresh or cryopreserved ovarian tissue could be important by preserving the patient's fertility and as an alternative to hormonal reposition.

Despite of the advances in life quality and medicine, diseases like cancer have significantly increased.

The treatment of cancer patients has become increasingly successful. However, the treatment that these patients receive may affect germ cell survival. Ovarian damage results in both sterilization and loss of hormone production.

The results of those treatments like radiotherapy cause family problems and justify the researches in this subject. This study was undertaken to evaluate fertility in

the irradiated female mice and the efficacy of ovarian transfer technique to recover the fertility.

Generally, three major effects are observed following ovarian cancer treatment: pos-chemotherapy, pos-radiotherapy and pos surgery ovarian failure.

In the case of pos radiotherapy ovarian failure, that is the focus of this study, different authors showed that the irradiation process induces gonad damage due the direct and indirect cytotoxic effects in DNA.

It is estimated that the radiation dose requested to destruct 50% of oocytes is less than 2 Gy [8]. On the other hand, while a total of 20 Gy can induce the ovarian failure in women around 40 years old, a dose like 6 Gy, that represents a small dose, is enough to induces a ovarian failure in women older than 40.

Similarly to chemotherapy treatment, the use of radiation to treat other diseases can affect the fertility. Technically, a fractionated total dose is normally used, to get a lower toxicity [8], nevertheless the uterine exposition to doses as low as 10 Gy can induce an elevation on infertility and abortion[9,10].

In the present work we employed hybrid and isogenic mice in order to evaluate the viability of ovarian transplant before and after irradiation effects, as an alternative to restore the reproductive functions.

## 2. MATERIAL AND METHODS

### 2.1. Animals

The mice strains employed were C57BL/6-Unib (ovary donor) and CB6F1 (ovary receptor) that were accommodated in flexible isolated units and ventilated racks (ALESCO), until the use, receiving water and food *ad libitum*

### 2.2 Ovaries

Both ovaries from the C57BL/6-Unib strain were surgically removed, transfered to an endorph with 1,0ml of Whitten medium (fresh prepared) and kept in an incubator (5% of CO<sub>2</sub> and 37°C) during the preparation of receptor female.

### 2.3 Animals Irradiation

The animals were whole-body irradiated with gamma rays (<sup>60</sup>Co, dose rate: 0.5 Gy/min., Panoramic Irradiator, Atomic Energy of Canada Ltd.) in doses of 4.0 Gy and 8.0 Gy, doses rate of 28,4 Gy/h. The irradiation process occurred at Radiation Technology Center (CTR) of Ipen-Cnen/SP. After irradiation, the animals were divided in groups consisting of 4 animals each. The animals were observed for 48 hours after irradiation and their mortality was recorded.

### 2.4 Ovarian transplant

Ovaries from C57BL/6-Unib mice were collected and kept at 37°C with 5% of CO<sub>2</sub>, inside an incubator as described before (2.2).

The ovarian transplant were performed in hybrid receptor females, obtained by mating females from the C57BL/6-Unib inbred strain, with males from the BALB/c inbred strain.

For the surgical procedures, the animals were anesthiated with 0,2 ml of Avertin@ (2,2,2,tribromoethanol), injected intraperitoneally.

While under anesthetic effects, the animals were opened and the ovarian bursa was carefully cut in order to remove the original ovaries. After that, the donor ovaries were inspected with a microscope and transfered to the recipient female.

The animals were observed for the post surgicals side effects, during 15 days, and after that they could be mated.

### 2.5. Reagents

All the reagents employed during the experiments were of pro-analysis quality and the water was purified in a Milli-Q system.

### 2.6 Experimental groups

Each experimental group had 4 female mice and we had a total of nine experimental groups:

G1: Unilateral ovariectomy (left side) + Transplant to the right side (2/3 of donor ovary + 1/3 of the receptor female);

G2: Unilateral ovariectomy (left side) + Transplant of total right ovary;

G3: Bilateral Transplant of total ovary;

G4: Irradiated B6C-F1 Female (control);

G5: Irradiated B6 Female (control);

G6: B6 Female with 2/3 of the ovary (1/3 one third was transferred to G1);

G7: Removal and reintroduction of the ovaries (surgery control);

G8: Normal mating - B6 Female (control);

G9: Normal mating - B6C-F1 Female (control).

The animals from the control group, as well as the irradiated ones, were mated with C57BL/6-Unib males one week or 30 days after irradiation.

### 2.7 Statistical analysis

The GraphPad InStat statistical software was used to analyses the results.

## 3. RESULTS

The results with the animals of the groups that were mated 7 days after the ovarian transfer are shown below.

The females of the non irradiated control groups had an average litter size as indicated in table 1.

Table 1: Litter size (average) observed in the non-irradiated controls.

GROUP	STRAIN	TREATMENT	LITTER
G7	B6CF1	Surgery control (*)	2,8 ± 2,58
G8	C57BL/6Unib	Non-irradiated control	6,8 ± 1,64 (**)
G9	B6CF1	Non-irradiated control	11,0 ± 3,24 (***)

(\*) Surgery control = removal and re-insertion of the ovaries.

(\*\*) Values obtained with the unirradiated donor strain.

(\*\*\*) Values obtained with the unirradiated receiver strain.

The groups irradiated with 4 Gy presented an litter size as indicated in table 2.

Table 2: Litter size (average) observed in the irradiated controls.

GROUP	STRAIN	TREATMENT	LITTER
G4	B6CF1	Irradiated (control)	8,8 ± 1,09 (*)
G5	C57BL/6Unib	Irradiated (control)	3,8 ± 1,30 (**)

(\*)Values obtained with the irradiated receiver strain without ovarian transfer.

(\*\*)Values obtained with the irradiated donor strain without ovarian transfer.

Table 3 shows the 4 Gy irradiated experimental groups were mated after 7 days of ovary transplant and their numeric litter.

Table 3. Mean numeric offspring in ovary transplanted mice

GROUP	MICE STRAIN	TREATMENT	LITTER
G1	B6CF1	Unilateral left oophorectomy with right ovary transplant (2/3 donor)	1,2 ± 1,64
G2	B6CF1	Unilateral right oophorectomy with complete left ovary transplant	1,8 ± 2,49
G3	B6CF1	Oophorectomy and ovary transplant in both sides	3,4 ± 1,81
G6	C57BL/6Unib	♀ C57BL/6Unib with 2/3 ovary (1/3 transferred to G1)	2,4 ± 1,50

The comparison of litter size in all groups, with respective variation, could be seen in figure 1.

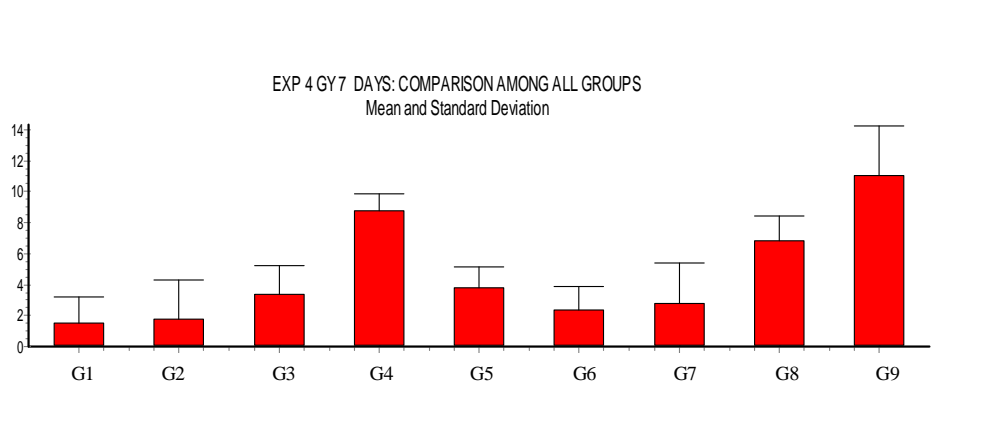


Figure 1: Litter size comparison per female in 4Gy irradiated mated 7 days after ovary transplant.

Animals from different groups that have been mated 30 days after ovary transplant were evaluated.

Females from control group that were not submitted to a 4 Gy radiation dose presented a number of births by litter as described at Table 4:

Tabela 4: N° of births by litter (average) presented by control group

GROUP	STRAIN	TREATMENT	LITTER
G7	B6CF1	Transplant control (*)	5,0 ± 1,41
G8	C57BL/6Unib	Non irradiated control	7,6 ± 3,50 (**)
G9	B6CF1	Non irradiated control	12,4 ± 2,19 (***)

(\*) Technique control = removal and reinsertion of the own ovary

(\*\*) Values from ovary donor strain, mated without radiation treatment

(\*\*\*) Values from ovary receptor strain, mated without radiation treatment

The number of births by litter from 4 Gy irradiated control group are presented at table 5.

Table 5: N° of births by litter from irradiated controls

GROUP	STRAIN	TREATMENT	LITTER
G4	B6CF1	Irradiated control	0 (*)
G5	C57BL/6Unib	Irradiated control	0 (**)

(\*)Values from irradiated ovary receptor strain without ovary transplant

(\*\*)Values from irradiated ovary donor strain without ovary transplant

The table 6 shows the number of births by litter presented by 4 Gy irradiated experimental group that have mated after 7 days ovary transplant.

Table 6: N° births by litter from irradiated and ovary transplanted animals.

GROUP	STRAIN	TREATMENT	LITTER
G1	B6CF1	Unilateral oophorectomy +R transplant (2/3 donor; 1/3 receiver)	2,6 ± 0,54
G2	B6CF1	Unilateral oophorectomy L + total R ovary transplant	2,2 ± 0,83
G3	B6CF1	Bilateral transplant: total ovary	3,0 ± 0,70
G6	C57BL/6Unib	♀ C57BL/6Unib with 2/3 ovary (1/3 transferred to G1)	1,6 ± 1,14

A comparison of births among all groups could be observed at figure 2.

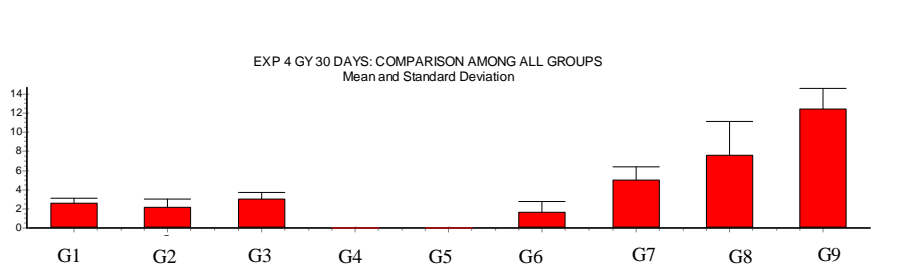


Figura 2: evaluation of the numbers of offspring by birth from 4 Gy irradiated female and mate seven days after ovary transplant.

#### 4. DISCUSSION AND CONCLUSIONS

Our data from control non-irradiated mice were similar to reported number in hybrid or inbred strains. As reported elsewhere, hybrid strains presented reproductive indexes higher than their parental isogenic strains [14]. Our data confirmed those findings as the litter size of group 8 (parental C57BL6/Unib) was significantly lower ( $p < 0.05$ ) than the hybrid group 9 (hybrid CB6F1).

Our data have also shown that irradiation, at least at early stages after irradiation, do not block their pregnancy ability, as both strain, donor or receptor, presented some offspring, suggesting that this dose of irradiation do not acutely block the ability to fertilization, implantation or uterine growth of offspring of irradiated female.

However, the comparison between irradiated and non irradiated C57BL6/Unib strain presents a significant  $p = 0,0127$  while hybrid strain, employed as female receptor presented a  $p = 0,1883$  between irradiated and non irradiated female, that represents a non significant value, suggesting that this lineage is less radiosensitive.

In 30 days mating groups, hybrid female also confirms the difference in reproductive indexes of this strain, being more reproductive both in donor or receptor strains ( $p < 0.05$ ).

The irradiation with mating after 30 days resulted in no offspring in donor strains, suggesting a late effect of irradiation in the reproductive status of those females. However, receptor females presented offspring, suggesting that the main effect could be on reproductive cells.

Those data suggest that the ovary transplant could be an alternative in the management of irradiated strains, allowing the development of studies detailing the effects of ionizing radiation on reproductive patterns.

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