Effect on Pituitary in Rats by Differences in Timing of γ-ray Irradiation

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We have no COI regarding this presentation.

Objective

Radiation is likely to affect **male and female reproductive organs**, and fetuses and children are more susceptible. **Azoospermia and Delayed puberty** in humans are currently a problem, there are many causes, and fetal and childhood influences may be one of them.

We have revealed at past annual meetings (50th and 51st) that **differences in the timing of irradiation result in clear differences in the subsequent development of the testis and ovary**, and the degree of their changes depends on the irradiation dose.

Since gonadotropin secretion from the anterior pituitary is important for the development of the reproductive organs, this study investigated how irradiation at fetal, neonatal, weaning, and early sexual maturation periods affect the pituitary development until adulthood.

Materials and Methods

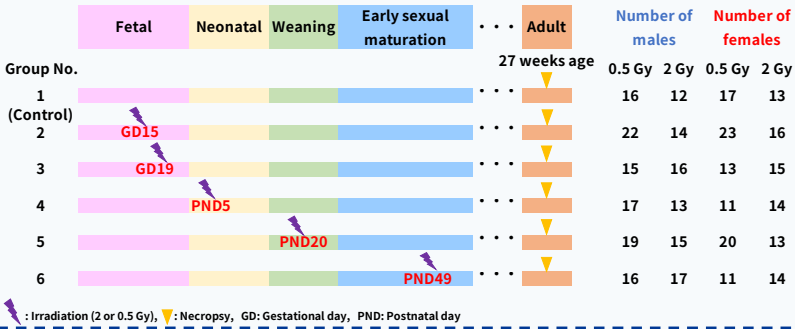
All experimental protocols involving rats were reviewed and approved by the IACUC of the National Institute of Radiological Sciences (NIRS) and were performed in strict accordance with the NIRS Guide for Care and Use of Laboratory Animals.

Animals: F1 hybrids of male Eker rats and female Fischer-344 (F344) rats (CLEA Japan)

Mating: Pro-estrous or estrous females were placed with an individually housed male overnight. Pregnancies were dated from gestational day 0 (GD0), being the day after mating.

Radiation exposure: Pregnant F344 rats, at **GD15** and **GD19**, and F1 rats, on **postnatal day 5 (PND5)**, **PND20**, and **PND49**, were whole-body irradiated with **0.5 or 2 Gy** of ^{137}Cs gamma irradiation at the dose rate of 0.7 Gy/min. Exposure was conducted using a Gammacell 40 (Atomic Energy of Canada). The F1 rats not irradiated with γ-rays, were set up as control animals.

Experimental protocol is as follows.

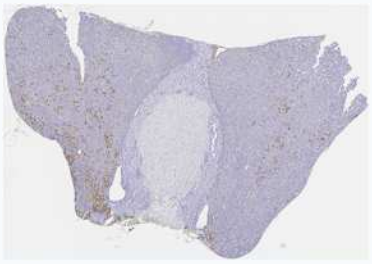


Histopathological examination: The **pituitary** was removed and prepared the histopathological specimens in accordance with the prescribed method.

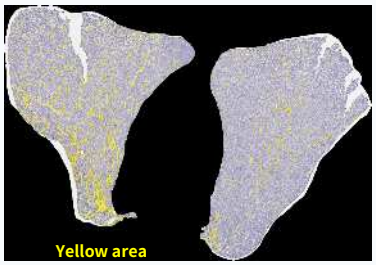
Immunostaining for FSH was performed to label gonadotropic cells.

The first antibody used was as follow: anti-FSH rabbit polyclonal antibody (1:500, Millipore Corp., USA)

Image analysis was performed using Area Quantification v2.2.4 module in HALO® (Indica Labs Ind.) to calculate the FSH-positive area/anterior pituitary area (μm²).



Immunostaining for FSH



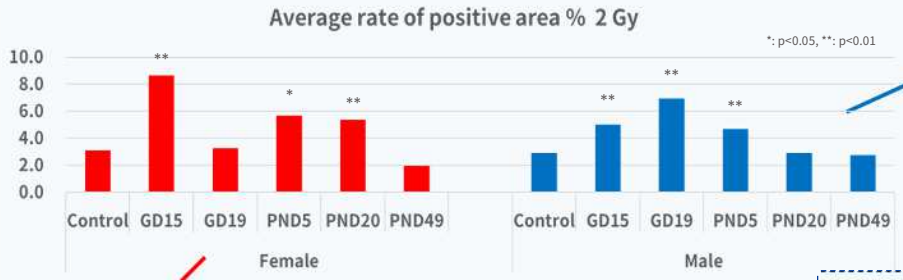
Identifying FSH-positive area using HALO

<Histopathological examination>

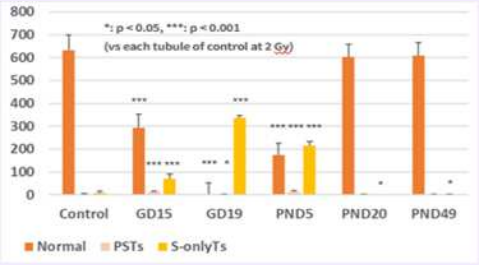
At 2 Gy, **minimal hypertrophy of basophils in the pituitary** was observed in males of the GD15, GD19, and PND5 groups and females of the GD15 and PND20 groups.

Results and Discussion

<Immunostaining for FSH and image analysis using HALO® in the pituitary>

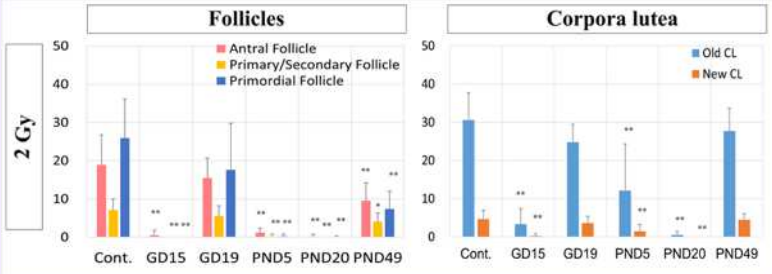


Effects on the testis (51st annual meeting)

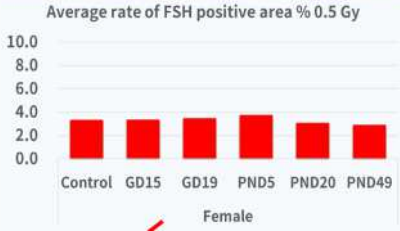


Severe decreases in the tubules in males of GD15, GD19, and PND5

Effects on the ovary (50th annual meeting)

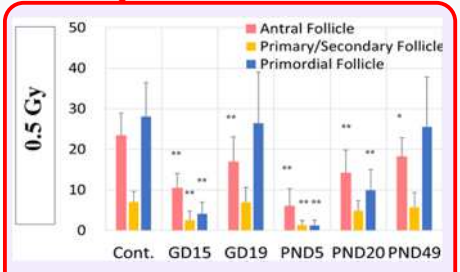


Severe decreases in the follicle and corpus lutea in females of GD15, PND5, and PND20



At 0.5 Gy, there were no changes in average rate of FSH positive area in both sexes of any group.

In the testis at 0.5 Gy, a slight decrease in tubules of GD15 and GD19 was noted; however, there were no severe changes like that seen at 2 Gy. (No data are shown in males.)



- Decreases in the follicle
- The degree is less than 2Gy.
- No change in corpus lutea (Normal: No data are shown.)

The decrease in follicle number showed a similar tendency to that observed in the 2 Gy irradiation group; however, the FSH-positive area did not increase as in the 2 Gy irradiation group, suggesting that the **secretion ratio of estrogen and progesterone was not affected.**

At 2 Gy, in the irradiation groups, in which there was reduction or depletion of follicles and loss of seminiferous tubules, the FSH positive area was higher .

The changes in the number of FSH-positive cells in the pituitary reflected the condition of the ovaries and testes, and the feedback function was maintained, suggesting that **irradiation exposure had no direct effect on the pituitary**.

Acknowledgement

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