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Original Contribution

RESPONSE OF THE PITUITARY-SEXUAL GLAND CHAIN IN FEMALE PIGS FOLLOWING EXTERNAL GAMMA IRRADIATION

S.Georgieva[1], P.Georgiev[2]*

[1]Department of Genetics, Breeding and Reproduction, Faculty of Agriculture;
[2]Department of Radiobiology and Radioecology; Faculty of Veterinary Medicine,
Trakia University, Stara Zagora, Bulgaria

ABSTRACT

The reaction of the pituitary gland - sexual glands chain is established by quantitative changes in LH, FSH and estradiol concentrations together with morphological studies of ovaries.
Sexually immature female pigs, aged 2 months, were irradiated with single doses of 1.5 and 2.5 Gy. The controlled hormonal parameters were monitored up to the onset of sexual maturity.
Early post-irradiation studies, following 1.5 Gy, showed a significant decrease in LH levels, compared with the control values. Three months post irradiation, its level was 0.035±0.003 IU/L *vs* control concentrations of 0.186±.0.07.IU/L. Subsequently, the level increased significantly to 0,210±0.003 IU/L at the end of the experimental period.
2.5 Gy irradiation triggered a peak in LH secretion 3 months post irradiation and, subsequently, by sustained suppression.
The irradiation at 1.5 Gy resulted in early elevation in FSH levels which lasted to 3 months post irradiation and then remained at a value close to those in controls. The dose of 2.5 Gy provoked a lasting suppression in FSH secretion during the entire period of study.
The irradiation at both doses inhibited, to different extents, 17β estradiol increase; this result was confirmed by histological examinations of ovaries.

Key words: Pituitary gland , sexual glands, irradiation, pigs

INTRODUCTION

The earliest responses occurring immediately following irradiation are mediated by functional disorders in nervous and endocrine systems. It is demonstrated beyond any doubt that prior to the onset of organic disorders in the different cells, tissues and systems, irradiation triggers functional changes at first

The functional status of the pituitary gland, assessed via the content of tropic hormones, is highly dependent upon the type and the phase of the radiation damage. In the first post irradiation days, the concentrations of gonadotropins in pituitary gland usually increase and thereafter decrease proportionately to the irradiation dose. The LH and FSH levels do not change in the early hours following irradiation but increase significantly 30, 60 and 90 days after that(1,2).

All those facts are evidences for the effect of ionized radiation on gonadotropic hormonal production whose alteration after irradiation is probably realized jointly with the other neuro-endocrine functions.

Sexual glands are the most radiosensitive of all endocrine organs and are as sensitive as bone marrow, lymphoid tissue and the small intestine.

Compared to testes, ovaries are very sensitive to radiation. The radiosensitivity of the functional ovarian structures ranges in the following ascending order: yellow body, stroma, primordial follicles, ovum, developing follicles, mature follicles, granulosa cells (3,4,5).

When the functional activity of ovaries is stimulated, that is, with gonadotropic hormones, their sensitivity to radiation is considerably increased. Due to the positive and negative correlation between gonadotropic hormones-hypothalamus-pituitary gland, the levels of the different hormones are dynamically changed. Several

*Correspondence to: P.Georgiev, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria ; sgeorg@af.uni-sz.bg

authors report that the irradiation of mammals results in pathological changes in endocrine gland function and primarily in the gonads (6,7,8).

The changes in the function of gonads occur in two directions, with both germinal cells and sexual hormone-producing cells being involved in the pathological processes, depending on the power and the dose of irradiation and the systemic functional status (9,10).

The sensitivity of germinal ovarian cells to radiation is different. Furthermore, ovary-produced hormones are in a complex relationship with both hypothalamus and pituitary gland on one part and with glands of internal secretion on the other.

The state of the central nervous system also influences the synthesis of sexual hormones and that is why the blood plasma levels of gonadotropins (LH, FSH and sexual steroids) reflect the functional state, not only of gonads but of pituitary gland as well.

Due to the correlation between hypothalamus-pituitary gland and target glands of internal secretion on one part and the relations among the glands themselves on the other and the negative feedback effect, the occurring hormonal changes are very complex and require a detailed analysis.

While the reaction of the pituitary-gonads chain has been established, there are no data about the quantitative changes in LH, FSH and estradiol levels within the irradiation dose range of 1.5-2.5 Gy in sexually immature pigs and the consequential hormonal profile and structural changes during the three stages of sexual maturation of pre-puberty, puberty and sexual maturity.

The present study aims to provide a satisfactory information for qualitative (morphological ovarian) and quantitative changes in gonadotropic (LH, FSH) and sexual (17-β-estradiol) hormones occurring in sexually immature pigs irradiated within the dose range 1.5-2.5 Gy up to the onset of sexual maturity.

MATERIALS AND METHODS

Material

The experiments were performed in sexually immature female pigs from the Danubian White Breed of same age, body weight and housing conditions. A 2-week period of adaptation was allowed prior to the experiments. Each group comprised 6 pigs in order to allow statistical processing.

Irradiation and conditions of irradiation

The total single irradiation of pigs was performed at the age of 2 months (i.e. prior to the onset of sexual maturity) using a ^{60}Co gamma source "Rokus" at a dose rate of 1,5 and 2,5 Gy and a density of 0,48 rad/s. The time of irradiation was calculated according to the distance and the intensity of the source (11).

Experimental design

The influence of ionized radiation upon the reproductive performance of female pigs was determined in the following experiments:

I. Determination of the influence of gamma irradiation within the dose range 1,5 Gy – 2,5 Gy upon the hormonal status of female pigs – luteinizing hormone (LH), follicle stimulating hormone (FSH) and 17-β estradiol.

II. Determination of the influence of gamma irradiation within the dose range 1,5 Gy – 2,5 Gy upon the morphological structure of ovaries.

The animals were divided into the following groups:

- Group I – female pigs – non-irradiated (controls)
- Group II– female pigs – irradiated at D = 1,5 Gy;
- Group III – female pigs – irradiated at D = 2,5 Gy

Samples of heparinized blood was obtained from orbital sinuses for determination of hormonal parameters at the following periods:

- One month post irradiation (age of 3 months)
- Two months post irradiation (age of 4 months)
- Three months post irradiation (age of 5 months)
- Four months post irradiation (age of 6 months)
- Five months post irradiation (age of 7 months)
- Six months post irradiation (age of 8 months)

The studies upon the ovarian structure were done similarly via a unilateral ovariectomy.

Methods

The morphological studies on ovaries were done by routine histological methods.

The quantitative determination of those hormonal parameters was done using radioimmuno assays (RIA) (12,13).

Statistical analysis

The results were tabulated in arithmetic means ± SD. The significance of the differences was calculated using the Student's t-test.

RESULTS AND DISCUSSION

The results for LH levels in female pigs irradiated at 1,5 and 2,5 Gy are presented on **Fig.1**.

In the early periods, the irradiation at 1,5 Gy provoked a decrease in LH and its levels were very low during the first 1-3 months after irradiation, that is, at the age of 3-5 months: 0,043 ± 0,0024 IU/L, 0,032 ± 0,009 IU/L and 0,035 ± 0,003 IU/L without a clear tendency towards increase.

During the 4th and the 5th post irradiation months (age of 6-7 months) there was a significant increase in LH concentrations (#p<0.05, p<.001). By post irradiations months 5 and 6 (age of 7-8 months), they were higher than those in Controls (*p<0.05). This fact could be explained by the sustained increase in LH after an irradiation at 1,5 Gy and by the compensatory pituitary response.

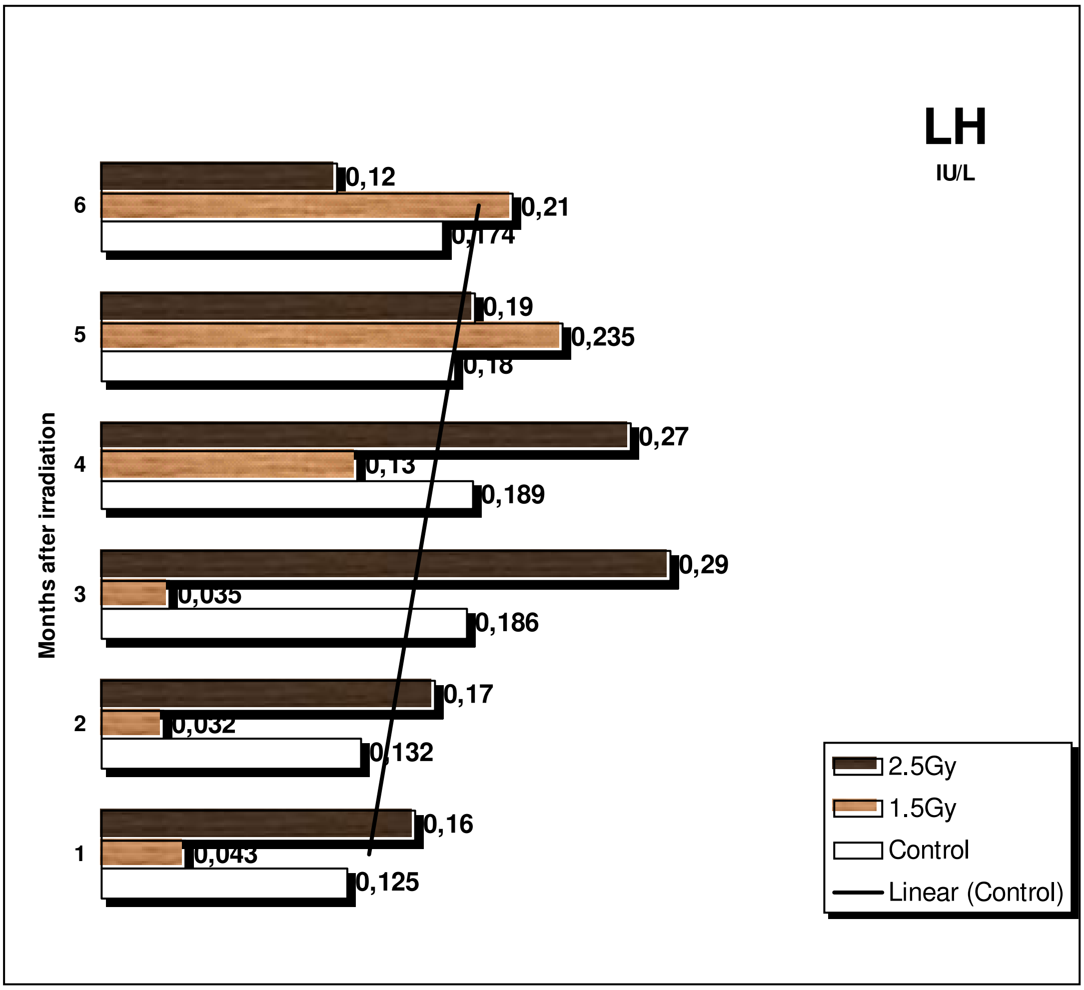

Fig. 1. Serum LH levels in female pigs after irradiation at doses 1.5 and 2.5 Gy; *p< 0.05; **p>0.01; ***p<0.001versus control, #p<0.05;### p<0.001 versus 3rd month

The quantitative analysis of LH in female pigs, irradiated at 2,5 Gy, showed that this dose triggered permanent and completely different changes in the gonadotropic hormone. In the early post irradiation periods, serum LH levels were 0,16 ± 0,08 and 0,17 ± 0,07 IU/L (age of 3–4months). Although statistically insignificant, they were higher than the respective values in the Controls (0,125 ± 0,05 and 0,123 ± 0,04 IU/L). By post irradiation months 3 and 4 (age of 5-6 months), LH levels reached their peak values – 0,029 ± 0,08 and 0,27 ± 0,09 IU/L. Subsequently, there was decrease in LH concentrations.

To summarize, the dynamics of LH

after irradiation within the dose range of 1,5-2,5 Gy showed that with the onset of sexual maturity, the compensatory physiological mechanisms prevailed over the influence of the ionization factor. Following irradiation at 2,5 Gy, the effect of radiation remained, however, hardly visible.

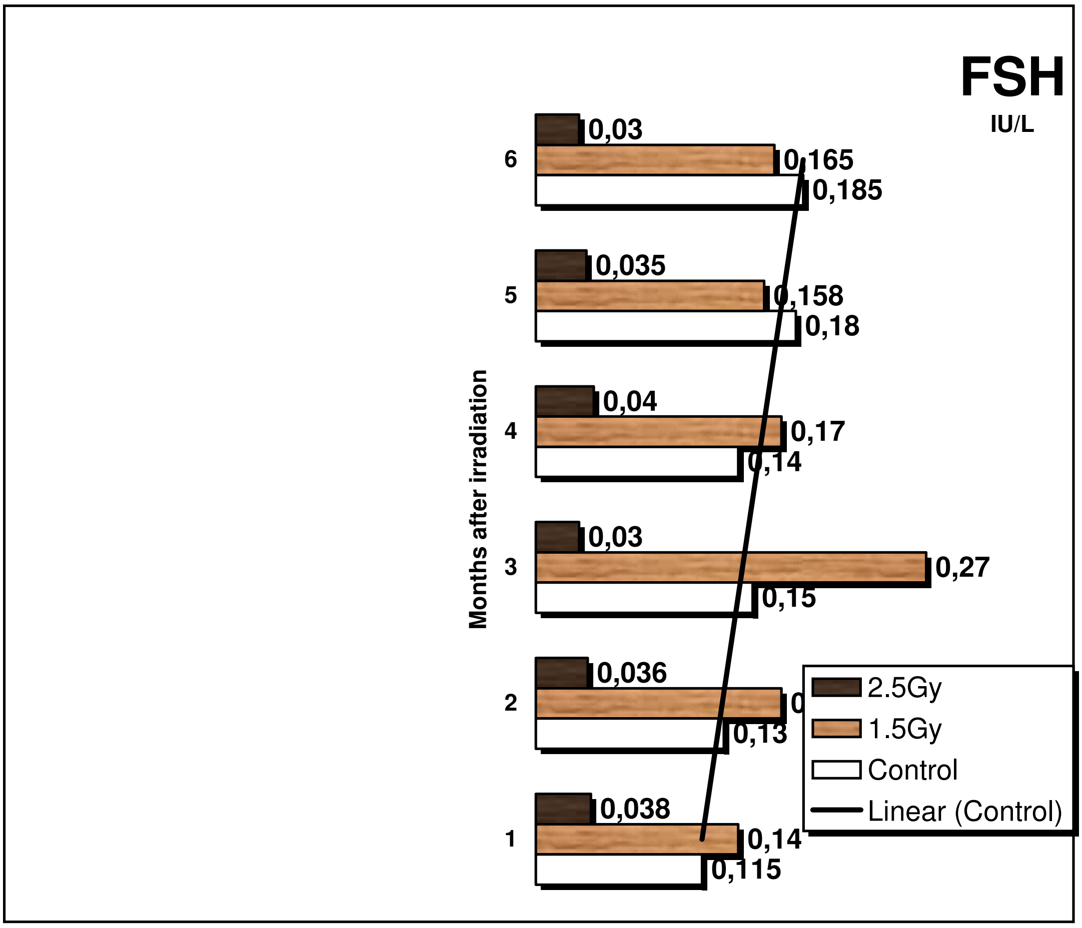

Fig. 2. Serum FSH in female pigs after irradiation at dose 1.5 and 2.5 Gy, *p<0.05; **p<0.01; ***p< 0.001versus Control

FSH determinations in female pigs irradiated at 1,5 and 2,5 Gy are presented on **Fig. 2**.

During the first and the second post irradiation months (age of 3-4 months) FSH levels (0,14 ± 0,021, 0,17 ± 0,006 IU/L) were not significantly higher (p<0.05) compared to Controls – 0,115 ± 0,02, 0,13 ± 0,005 IU/L.

By post irradiation month 3 (age of 5 months), serum FSH concentrations in the 1,5 Gy group reached a peak of 0,27 ± 0,04 IU/L and subsequently decreased.

The results in pigs irradiated at 2,5 Gy showed that as early as the first post irradiation month (age of 3 months), hormone levels were significantly lower that those in Controls (0,038 ± 0,009 IU/L) (p<0.001). The hormone levels at the next periods showed irrelevant fluctuations and the levels measured by post irradiation months 2, 3, 4, 5 and 6 varied within 0,03 ± 0,006 – 0,04 ± 0,008 IU/L and were significantly lower, compared to the control values at the same periods (p<0.001).

The dynamics of FSH in female pigs irradiated at 1,5 and 2,5 Gy were dissimilar – the dose of 1,5 Gy resulted in a biphasic model of change whereas the dose of 2,5 Gy caused a sustained decrease in FSH levels.
The quantitative determinations of 17-β estradiol in 1,5 Gy and 2,5 Gy irradiated female pigs are shown on **Fig. 3**.

One month after the irradiation (age of 3 months) 17-β estradiol concentrations were not considerably different from those in Controls (110 ± 5 pmol/L and 109 ± 15 pmol/l, respectively). By post irradiation month 2 however, 17-β estradiol levels in the 1,5 Gy group were 104 ± 6 pmol/L, significantly lower than Controls (126 ± 6 pmol/l) (p<0.05). In later periods (age of 5-6 months) 17-β estradiol concentrations decreased significantly. At the end of the experiment (6^{th} post irradiation month or age of 8 months), 17-β estradiol levels were by 84% higher compared to the 5^{th} month (age of 7 months) and close to the respective Control levels.

After the irradiation with 2,5 Gy (age of 3–4 months) there was not a distinct change in 17-β estradiol levels and they ranged between 113 ± 16 and 112 ± 5,1 pmol/L, being insignificantly lower compared to control hormonal values at the same intervals. After the 3rd post irradiation months however, 17-β estradiol concentrations were 43% higher, compared to the previous month and reached 161 ± 6,1 pmol/L.

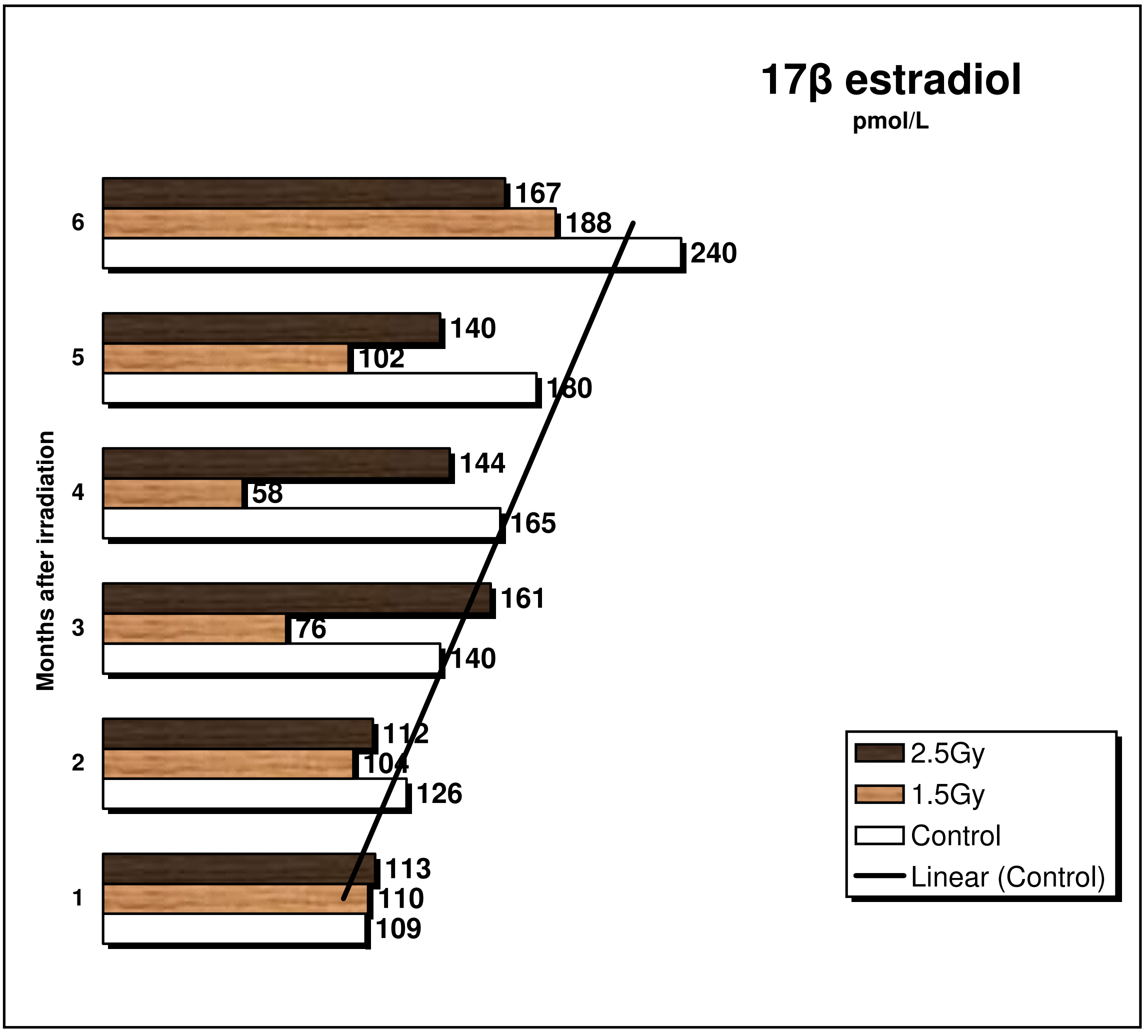

Fig.3. Serum 17β estradiol in female pigs after irradiation at doses 1.5 and 2.5 Gy.
*p<0.05; **p<0.01; ***p<0.001; versus Control
#p<0.05; ##p<0.01; versus 4th month (for 1.5 Gy)
#p<0.05; ##p<0.01; versus 2nd month (for 2.5 Gy)

At the end of the period, there was a tendency towards increase in 17-β estradiol. For the interval between post irradiation months 5 and 6 (age of 7-8 months) there was 19% increase, compared to a corresponding increase by 33% in Controls. Nevertheless, this elevation did not attain the Control concentrations.

Notwithstanding the tendency towards compensation of low 17-β estradiol concentrations, measured in the early periods in the 1,5 Gy group, 17-β estradiol levels decreased in the 2,5 Gy group after the 3rd month (age of 5 months) and by post irradiation months 4 and 5 was lower than in Controls. At the end of the study, i.e. the 6th month, 17-β estradiol concentrations increased, compared to the previous month but were still significantly lower than Controls (p<0.05).

The changes in the studied hormonal parameters were influenced by the process in ovogenesis. During the first post irradiation month, there was no significant difference in ovarian structure of pigs from both irradiated groups. In comparison with non-irradiated pigs, an increased number of primary and secondary follicles with atresia was observed. The primary oocysts within them showed clear dystrophic changes; the follicular epithelium was disorganized and was undergoing a connective tissue organization (**Fig. 4**).

By post irradiation month 2, the dystrophic changes in secondary follicles prevailed. The number of tertiary follicles was elevated in both irradiated groups compared to Controls. A part of them was with dystrophic changes affecting the oocyte and the follicular epithelium (**Fig. 5**).

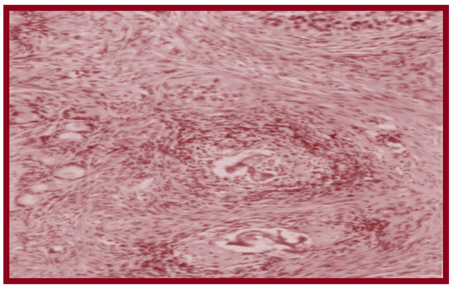

Fig. 4. Ovary of the pig at age of 3 months dose 2.5 Gy. dose 1.5 Gy. Atretic primordial and growing follicles

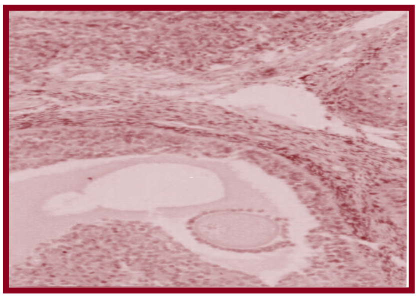

Fig. 5. Ovary of pig at age of 4 months Atretic vesicular follicles

By month 3, in 1.5 Gy pigs there was a considerable number of activated follicles, whereas in 2.5 Gy pigs the part of secondary and tertiary follicles with atresia was big. A luteinization of non-ovulated tertiary follicles was observed (**Fig.6**).

In the period that followed (months 4 and 5) the tendency towards a superfollicular effect was preserved in the 1.5 Gy group while in ovaries of 2.5 Gy pigs the dystrophic changes affected a major part of secondary and tertiary follicles, some of them being with a marked hyperplasia of the granulose zone and others - with signs of cystic degeneration (**Fig. 7**).

At the end of the experimental period, the ovarian histostructure was normalized. The number of follicles with atresia remained elevated in the 2.5 Gy group (**Fig 8**).

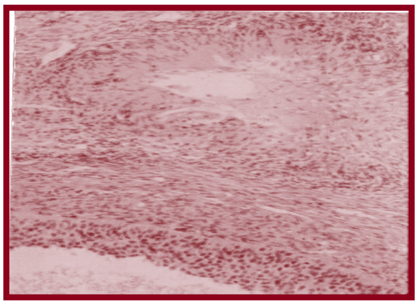

Fig.6. Ovary of pig at 5 months of age– dose 2.5 Gy. Luteinization of nonovulated vesicular follicle

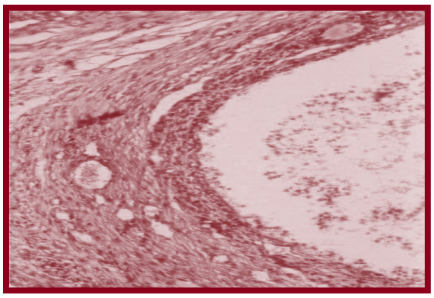

Fig. 7. Ovary of pig at age of 6 months – dose 2.5 Gy. Cystic changed vesicular follicle

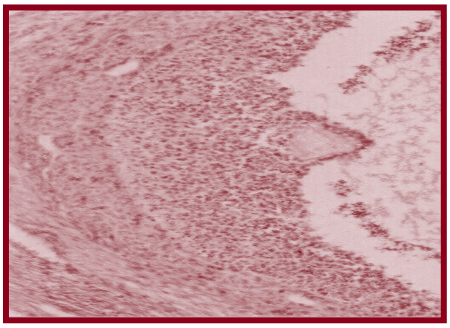

Fig. 8. Ovary of pig at age 8 months – dose 2.5 Gy. Atretic vesicular follicle with hyperplasia of zona granulosa and dystrophic changed oocyte

The recapitulation of data suggested that the irradiation within the dose range of 1,5 – 2,5 Gy prior to the onset of the sexual maturity resulted in significant alterations in the dynamics of pituitary and peripheral sexual hormones that were dose-dependent.

The hormonal changes in female pigs correlated positively with the changes observed in female gonads (3).

The dynamics of LH, FSH and 17-β estradiol confirmed the fact, as reported by others (1,14), that gonadal reaction could be regarded as indirect or secondary. This phenomenon could be explained by degenerative structural changes in ovaries resulting from the biological effect of radiation (15, 17).

The analysis of FSH and 17-β estradiol determinations showed that both hormones followed a biphasic pattern of change, manifested to a larger extent following a 2,5 Gy irradiation in 17-β estradiol. The biphasic model of FSH was present in the 1,5 Gy group while the bigger dose of 2,5 Gy led to a permanent suppression of hormonal increase.

It must be emphasized that at the end of the experimental period, 17-β estradiol concentrations reached levels indicative of the presence of transforming (ripening) follicle in ovaries.

The 17-β estradiol dynamics were determined at a significant extent by those of LH and FSH (16). From the other side, the changes in FSH after a 2,5 Gy irradiation could not be explained by 17-β estradiol fluctuations. In our opinion this was due to changes in ovarian structure following irradiation (development of damaged primary, secondary and tertiary follicles) (15).

Regardless of the evidences for quantitative changes in serum LH and FSH concentrations, the mechanisms of action of hypothalamo-pituitary system under the effect of radiation remains unclear (9). Neither the known data, nor our studies could explain whether the irradiation influenced the hypothalamus or the pituitary secretory mechanism. Our data, however, revealed indisputably the inhibiting effect of ionizing radiation upon both pituitary function (LH, FSH) and the function of peripheral sexual glands in female (17-β estradiol) individuals.

CONCLUSION

The results of our experiments have shown the dose-dependent pattern of LH, FSH and 17β estradiol changes; and that could also be explained by the known subordination of hypothalamus-pituitary gland-sexual glands interrelationships.

The quantitative changes in LH and FSH after irradiation at different doses were also confirmed by 17β estradiol changes that are known to be FSH-dependent.

Despite the suppressing effect of irradiation within this dose range upon LH, FSH and 17β estradiol increase, histological examinations of ovaries evidenced that the irradiation of female pigs at 1.5 and 2.5 Gy did not result in degeneration in all follicles and the reproductive potential of ovaries, although reduced, was preserved.

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