

## LIFE DURATION OF INBRED AND OUTBREED RABBITS, IRRADIATED WITH GAMMA RAYS

S. TANCHEV<sup>1</sup>, S. GEORGIEVA<sup>1</sup>, D. HRISTOVA<sup>1</sup>, L. SOTIROV<sup>2</sup>, Ts. KOYNARSKI<sup>2</sup> and V. PETROV<sup>2</sup>

<sup>1</sup> *Trakia University, Faculty of Agriculture, BG – 6000 Stara Zagora, Bulgaria,*

<sup>2</sup> *Trakia University, Faculty of Medicine, BG – 6000 Stara Zagora, Bulgaria*

### Abstract

TANCHEV, S., S. GEORGIEVA, D. HRISTOVA, L. SOTIROV, Ts. KOYNARSKI and V. PETROV, 2015. Life duration of inbred and outbreed rabbits, irradiated with gamma rays. *Bulg. J. Agric. Sci.*, 21: 404–408

The life expectancy of inbred and outbreed rabbits irradiated with gamma rays at a dose 4 Gy has been studied. It was found that the average life duration of inbred groups is less than that of the outbreed animals, as the differences were statistically significant. It was viewed that the animal produced from inbred strains and inbred lines are at risk from the harmful effects of environmental factors (specific gamma-radiation) compared to heterozygous hybrids and animals from populations with high heterogeneity.

**Key words:** life duration, gamma rays, inbreeding, outbreeding, homogenic animals, heterogenic animals, rabbits

### Introduction

Ionizing radiation including gamma radiation is characterized by high biological activity. When interacting with living cells, it causes ionization of different chemical compounds and bio substrates, which leads to complex reactions and processes in the cells and tissues (Auerbach, 1976).

A large amount of research in this area focus on the harmful effects of gamma radiation on the genetic structure and biochemical and physiological processes in various species and humans (Georgiev et al., 1996; Georgieva and Georgiev, 2003; Ginsberg, 2003; Carnes et al., 2003; Georgiev et al., 2005; Tanchev et al., 2004; Georgieva et al., 2005a; Georgieva et al., 2005b; Tanchev et al., 2005; Popov et al., 2007; Sasaki and Fukuda, 2008).

Other scientists have turned their attention to research opportunities for positive and stimulating effects of low doses of gamma rays on the physiological condition and life expectancy of the studied biological objects (Thomson et al., 1986; Thomson and Grahm, 1989; Furuno - Fukushi et al., 1993; Hsie et al., 1996; Calabrese and Baldwin, 2000; Par-

sons, 2002; Cameron, 2003; Tanaka et al., 2003; Cameron, 2005; Kumar et al., 2006; Mitchel, 2006; Ito et al., 2007; Le Bourg, 2007; Moskalev, 2007; Tanaka et al., 2007; Aloy et al., 2008; Moskalev, 2008).

Few studies have been devoted to the effects of gamma rays on life duration of different species - representatives of heterogeneous and homogeneous populations (Moskalev and Zainullin, 2006; Sasaki and Fukuda, 2006; Moskalev, 2008).

This study is a result of our other study, where we did comparative chromosome analysis in inbred and outbreed rabbits irradiated with gamma rays (Tanchev et al., 2006). Subsequently raised the idea to investigate the influence of the factors genotype /homozygous and heterozygous/ and inbreeding on survival of irradiated with gamma rays rabbit has become a major objective of the present study.

### Materials and Methods

Twenty four female rabbits, aged 8 to 12 months were allocated into two main groups: Group I – 12 rabbits irradiated with gamma rays and Group II – 12 rabbits – sham irradiated

\*E-mail: dhristova@uni-sz.bg

(control) group. First group was divided into two subgroups – 6 inbred rabbits ( $F_x = 0.25$ ) and 6 outbreed rabbits. Additionally, depending on the origin and the degree of inbreeding these subgroups were divided as follows:

- 3 homogeneous outbreed rabbits representatives of genetic consolidated breed California rabbit;
- 3 homogeneous inbred rabbits representatives of the same breed;
- 3 heterogeneous outbreed rabbits – hybrid forms produced by crossing the breeds Californian rabbit and chinchilla;
- 3 heterogeneous inbred rabbits with the same hybrid origin.

In the second (control) group, rabbits were allocated in the same manner as in Group I. The degree of inbreeding was considered by the classic method of Wright (Wright, 1937).

Rabbits of Group I were exposed to a single whole body irradiation with gamma rays at a dose of 4 Gy, at a dose rate 24 Gy/min with a source of radiation  $^{60}\text{Co}$ . Exposure was calculated according to the geometry parameters of the power source, its distance and capacity.

The experimental protocol was approved by the Department of Animal Care and adhered to the European Community Guiding Principles for the Care and Use of Animals. During the study, all rabbits were raised under identical conditions of feeding and rearing. The life duration after irradiation with gamma rays was calculated in days.

Data from observations of irradiated animals was processed by analysis of variance. During the processing, we used additive model with fixed effects – ANOVA. The mean values for days of life duration in rabbits and Standard Deviation were calculated by Descriptive Statistics and for evaluation of the P-values was used ANOVA Single Factor (MS Excel 7.0). Statistically significant effects of the studied factors were reported with the critical level  $P < 0.05$ .

## Results and Discussion

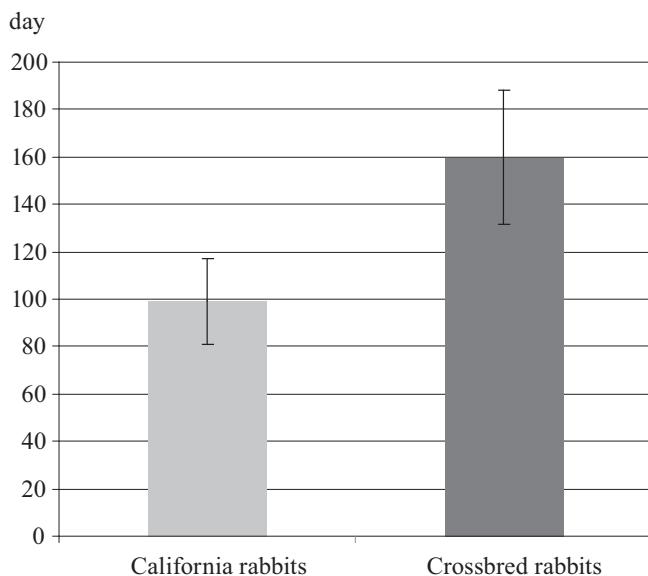
The analysis of the influence of the studied factors on survival of rabbits after irradiation with gamma rays (4Gy) are presented in Table 1. Statistically significant impact on life expectancy of female rabbits have both the studied factors separately.

**Table 1**

**Influence of origin and level of inbreeding on survival of rabbits irradiated with gamma rays at a dose of 4 Gy**

Factors of influence	df Effect	MS Effect	df Error	MS Error	F	p-level
Breed	1*	10266.75*	8*	118.4684*	86.4684*	0.000015*
Level of inbreeding	1*	6120.08*	8*	118.7500*	51.53754*	0.000094*
Both together	1	310.08	8	118.75	2.61123	0.144772

\*statistical significance

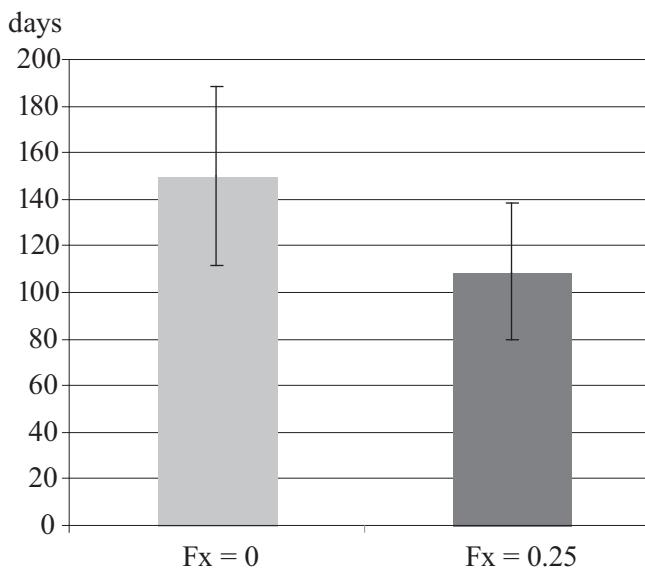


**Fig. 1. Life duration of rabbits after irradiation with gamma rays at a dose of 4 Gy, depending on the origin. Identical letters mark statistically significant differences ( $P = 0.001$ )**

Figure 1 shows the life expectancy of rabbits after irradiation, according to the origin. Heterogeneous animals statistically significantly outperform their homogeneous coevals. Most likely this is the result of retained heterosis effects in crosses in terms of resistance to gamma radiation.

Figure 2 presents the average life duration of rabbits after irradiation with a dose of 4 Gy, depending on the inbred level. Outbreed rabbits statistically significantly outperform their inbred coevals in life duration. This is confirmed by the fact that 90 days after irradiation homogeneous inbred rabbits are losing 20–25% of their live weight, while in heterogeneous inbred rabbits that loss is in the range of 5–10% (Figures 4 and 5). Increasing the level of homozygous inbreeding leads to increased sensitivity to negative effects of various environmental factors in our case – gamma rays, the most likely cause for the observed results, depending on inbred level / $F_x$ /.

Although we found no statistically significant effect of the combination of the two factors examined (Table 1), the

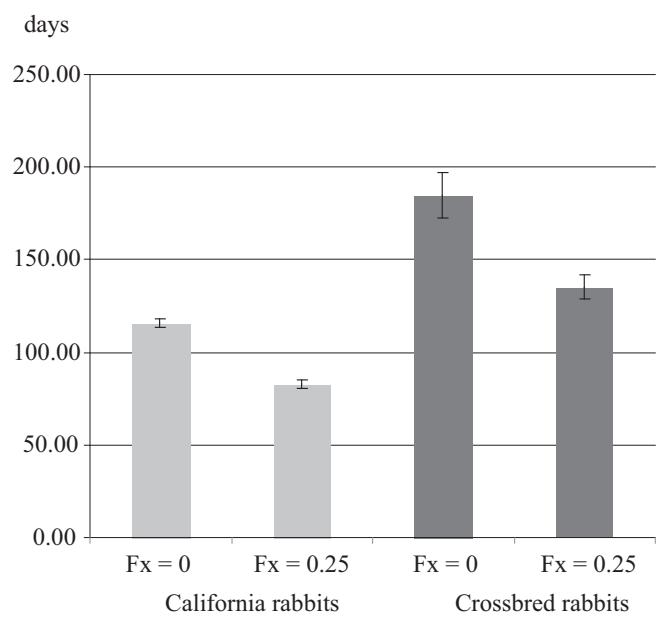


**Fig. 2. Life expectancy of rabbits after irradiation with gamma rays at a dose of 4 Gy, depending on the inbreed level. Identical letters mark statistically significant differences ( $P = 0.064$ )**

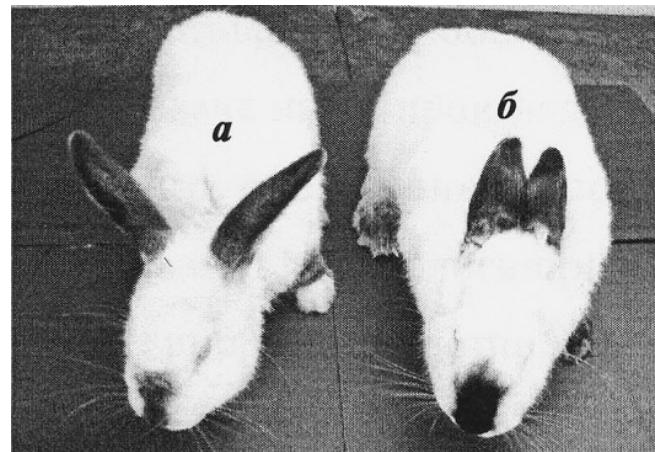
results of multiple comparisons between groups showed statistically significant superiority of outbreed compared to inbred rabbits (Figure 3). This confirms once again the above-mentioned findings that generally heterogeneous rabbits have higher adaptive capacity to ionizing radiation, which determines the longer life duration after irradiation with gamma rays compared to homogeneous group of rabbits. Body conditions of homogeneous and heterogeneous female rabbits after irradiation are presented in Figures 4 and 5.

The results of the research in this area are ambivalent. Some authors state that chronic exposure of biological objects with low doses of gamma rays increases the life duration (Calabrese and Baldwin, 2000; Parsons, 2002; Moskalev, 2008; Moskalev et al., 2009). Most researchers, however, contend that irradiation with gamma rays with both high and low doses adversely affects the life duration of irradiated organisms and cell populations (Thomson et al., 1986; Thomson et al., 1989; Furuno – Fukushi et al., 1993; Hsie et al., 1996; Tanaka et al., 2003; Kumar et al., 2006; Sasaki and Fukuda, 2006; Tanaka et al., 2007).

Our results show that the characteristics of individuals identified by the degree of inbreeding and origin are essential for the reactivity of the organism, its adaptability and consequently its survival in changing environmental conditions. Heterogeneous organisms are more adaptable and more resistant to environmental factors, while homogeneous individuals that arise through inbreeding are unstable and

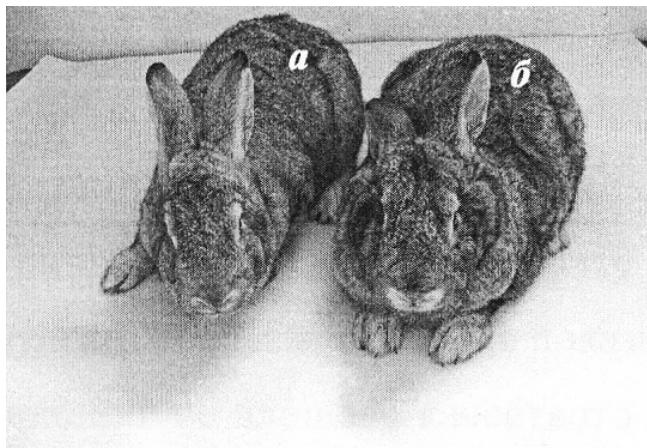


**Fig. 3. Life expectancy of rabbits after irradiation with gamma rays at a dose of 4 Gy by origin and inbred level. Identical letters mark statistically significant differences ( $P = 0.000$ )**



**Fig. 4. Body condition of homogeneous female rabbits / California/ 90 days after irradiation with gamma rays at a dose of 4 Gy: a (Fx = 0.25), b (Fx = 0)**

threatened by rapid and massive death. This is especially important for species and productive populations of laboratory animals where intensive selection in one or more directions and implementation of inbreeding in order to reinforce certain skills lead to higher homozygosity and high genetic similarity. Such inbred lines are used in livestock. Due to the high homozygosity achieved during intensive selection



**Fig. 5. Body condition of heterogeneous female rabbits /hybrid forms/ 90 days after irradiation with gamma rays at a dose of 4 Gy: a ( Fx = 0.25), b ( Fx = 0 )**

and inbreeding, individual representatives of the line will respond similarly to environmental factors. If the influence of these negatively factors arise there is danger of losing a large percentage of individuals and even the whole inbred line.

In a study of the life duration of isogenic and heterogeneous lines drosophila (wild-type) chronically exposed for 14 consecutive generations of low-dose gamma rays – 60 cGy (Moskalev and Zainullin, 2006) reported results that differ from our results. Their research showed that the life duration of the irradiated drosophila isogenic lines increased compared to non-irradiated isogenic control groups and heterogeneous line. The reason according to the authors was the increasing level of genetic heterogeneity effects induced by gamma rays in the isogenic line.

Despite the differences between the results of Moskalev and Zainullin with our results, we believe that there is a contradiction in our opinion. According to us, the main reason is the difference in the performance in the experiment and probably species-specific difference in response after irradiation with gamma rays (insects and mammals). On the other hand, the study of the Russian scientists on *Drosophila melanogaster* involves exposure to low doses of gamma rays for many generations, in which natural selection results in the preservation of those genotypes in isogenic populations providing the best adaptive qualities and best adaptability to specific environmental conditions.

This natural selection leads to strong reduction of genetic diversity and reduced variability of the quantities characterizing a sign. Opposite, the highest heterozygosity in heterogeneous populations determines large differences in the re-

action of the various genotypes to gamma rays. This defines a large range of variation of a feature in heterogeneity populations, which is observed after the first hybrid generation.

In another review article, Moskalev (2008) notes that reducing the life duration of organisms irradiated with ionizing radiation is due to stress-induced premature aging of cells and increase - the stress-induced activation of transcription factor (FOXO). According to the author such a positive activation under the influence of low doses of radiation over a number of successive generations leads to a reduction of the harmful effects of depression in inbred populations of laboratory animals.

In our study, we monitored the reaction of rabbits after a single exposure to a significantly higher dose of gamma rays (4 Gy) and made a comparative analysis of the life of the irradiated inbred and outbreed individuals. Results and statistically found significant differences are in favor of the outbreed groups, primarily heterogeneous hybrid rabbits. This allows us to conclude that the probability of homogeneous populations – product of inbreeding to be at risk from the harmful effects of environmental factors is significantly greater than heterogeneous populations.

Without exploring the influence of ionizing radiation Lacy et al. (1996); Tanchev et al. (2006); Georgieva et al. (2012), noted that along with the negative impact on reproductive performance of animals increased inbreeding and homozygosity negatively affects the vitality and adaptive abilities.

By applying advanced mathematical models for accounting the fixed effects and integrated assessment of productivity, including lifetime productivity of inbred rabbits and pigs to similar conclusions came Poujardieu and Toure (1980); Farghaly (2000).

## Conclusion

The results of this study show that after a single exposure to gamma rays (4 Gy) inbred rabbits have a shorter life duration than their outbreed coevals. This allows us to assume that animal product of inbreeding, respectively herds and highly inbred lines are at higher risk from harmful effects of environmental factors, specifically gamma rays, compared with heterozygous female hybrids and animals from populations with high heterogeneity.

## References

- Aloy, M. T., E. Hadchity, C. Bionda, C. Diaz-Latoud, L. Claude, R. Rousson, 2008. Protective role of Hsp27 protein against Gamma radiation-induced apopto-sis and radiosensitization effects of Hsp27

- gene silencing in different human tumor cells. *Int. J. Radiat. Oncol. Biol. Phys.*, **70**: 543–553.
- Auerbach, C.**, 1976. Mutation Research. Problems, Results and Perspectives. London.
- Calabrese, E. J. and L. A. Baldwin**, 2000. The effects of gamma rays on longevity. *Biogerontology*, **1** (4): 309–319.
- Cameron, J. R.**, 2003. Longevity is the most appropriate measure of health effects of radiation. *Radiology*, **229**: 14–15.
- Cameron, J. R.**, 2005. Moderate dose rate ionizing radiation increases longevity. *Br. J. Radiol.*, **78**: 11–13.
- Carnes, B. A., D. Grahn and D. Hoel**, 2003. Mortality of atomic bomb survivors predicted from laboratory animals. *Radiat. Res.*, **160**: 159–167.
- Farghaly, H. M.**, 2000. Effects of inbreeding on doe's performance traits in closed commercial rabbit populations in Egypt. In: 7<sup>th</sup> World Rabbit Congress, Valencia, pp. 39–43.
- Furuno-Fukushi, I., K. Aoki and K. Matsudaira**, 1993. Mutation induction by different dose rates of gamma rays in near-diploid mouse cells in plateau and log-phase culture. *Radiat. Res.*, **136** (1): 97–102.
- Georgiev, P. Z., S. Georgieva, G. Bonev and S. Tanchev**, 2005. Dose dependent influence of external gamma irradiation upon the pituitary-gonadal axis in female pigs. *Revue de Cytologie et Biologie vegetales-Le Botaniste*, **28**, 96–100.
- Georgieva, S. and P. Georgiev**, 2003. Dose- and gender-dependance or hormonal response following external gamma irradiation in pigs. *Bul. J. of Vet. Med.*, **6** (4): 223–232.
- Georgieva, S., P. Georgiev, E. Jeliazkov, Ts. Yablanski, S. Tanchev, D. Yarkov and M. Oblakova**, 2005b. Dose-Effect Correlation after external gamma irradiation of spermatogonia in rabbits. *Revue de Cytologie et Biologie vegetales-Le Botaniste*, **28**: 101–105.
- Georgiev, P., S. Georgieva and S. Vitanov**, 1996. Effect of ionizing radiation on the reproductive function in male pigs. *Mac. Vet. Rev.*, **25** (1–2): 79–92.
- Georgieva, S., S. Tanchev, D. Bonev, E. Tanchev and B. Popov**, 2012. The influence of inbreeding on level of sexual hormones in rabbits. *J. Anim. Sci.*, **XLIX** (2): 37–43.
- Georgieva, S., Ts. Yablanski, P. Georgiev and S. Tanchev**, 2005a. Cytogenetical changes in the progeny (F1) of rabbits following external gamma irradiation. *Revue de Cytologie et Biologie vegetales-Le Botaniste*, **28**: 92–95.
- Ginsberg, G. L.**, 2003. Assessing cancer risks from short-term exposures in children. *Risk. Anal.*, **23**: 19–34.
- Hsie, A. W., R. C. Porter, Z. Xu, Y. Yu, J. Sun, M. L. Meltz and J. L. Schwartz**, 1996. Molecular markers of ionizing radiation-induced gene mutations in mammalian cells. *Environ. Health Perspect.*, **104** (3): 675–678.
- Ito, M., Y. Shibamoto, S. Ayakawa, N. Tomita, C. Sugie and H. Ogino**, 2007. Low-dose whole-body irradiation induced radioadaptive response in C57BL/6 mice. *I. Radiat. Res.*, **48**: 455–460.
- Kumar, P. R., M. N. Mohankumar, V. Z. Hamza and R. K. Jeevanram**, 2006. Dose-rate effect on the induction of HPRT mutants in human GD lymphocytes exposed *in vitro* to gamma radiation. *Radiat. Res.*, **165** (1): 43–50.
- Lasy, R. C., G. Alaks and A. Walsh**, 1996. Hierarchical analysis of inbreeding depression. *Evolution*, **50**: 2187–2200.
- Le Bourg, E.**, 2007. Hormetic effects of repeated exposures to cold at young age on longevity, aging and resistance to heat or cold shocks in *Drosophila melanogaster*. *Biogerontology*, **8**: 431–444.
- Mitchel, R. E.**, 2006. Low doses of radiation are protective in vitro and *in vivo*: evolutionary origins. *Dose-Response*, **4**: 75–90.
- Moskalev, A.**, 2007. Radiation-induced life span alteration of *Drosophila* lines with genotype differences. *Biogerontol-Ontology*, **8**: 499–504.
- Moskalev, A.**, 2008. Genetic investigations of low dose irradiation influence on life. *Radiat. Biol. Radioecol.*, **48** (2): 139–145.
- Moskalev, A., M. Shaposhnikov and E. Tutysheva**, 2009. Life span alternation after irradiation in *Drosophila melanogaster* stains with mutations of Hsf and Hsps. *Biogerontology*, **10** (1) 3–11.
- Moskalev, A. and V. G. Zainullin**, 2006. Life-span in generation of chronic irradiated *Drosophila melanogaster* with type isogenic and heterogenic stains. *Radiat. Biol. Radioecol.*, **46** (4) 436–440.
- Parsons, P. A.**, 2002. Radiation hormesis: challenging LNT theory via ecological and evolutionary considerations. *Health. Phys.*, **82**: 513–516.
- Popov, B., V. Petkova and S. Georgieva**, 2007. Comparative analysis of chromosome damage in two groups of workers handling either radioisotopes or anti-tumour drugs in an oncology clinic of Stara Zagora, Bulgaria. *Trakia Journal of Sciences.*, **5** (2) 34–38.
- Poujardieu, B. and S. Touré**, 1980. Influence de la variation du taux de consanguinité sur les performances d'élevage de lapines utilisées en croisement de souches. In: *Proc. 2 e l World Rabbit Congress*, Barcelona, Espagne, **1**: 223–227.
- Sasaki, S. and N. Fukuda**, 2006. Dose-Response relationship for life-shortening and carcinogenesis in mice irradiated at day 7 postnatal age with dose range below 1 Gy of gamma rays. *I. Radiat. Res.*, **47** (2): 135–145.
- Sasaki, S. and N. Fukuda**, 2008. Dose-response Relationship for Induction of Ovarian Tumors in Mice Irradiated during Prenatal, Early Postnatal and Elder Periods. *J. Radiat. Res.*, **49**: 623–633.
- Sasaki, S. and N. Fukuda**, 2005. Temporal Variation of Excess Mortality Rate from Solid Tumors in Mice Irradiated at Various Ages with Gamma Rays. *J. Radiat. Res.*, **46**: 1–19.
- Tanaka, I. B., S. Tanaka, K. Ichinohe, T. Matsumoto, H. Otsu, Y. Oghiso and F. Sato**, 2007. Cause of death and neoplasia in mice continuously exposed to very low dose rates of gamma rays. *Radiat. Res.*, **167** (4): 417–437.
- Tanaka, S., I. B. Tanaka, S. Sasagawa, K. Ichinohe, T. Takabatake, S. Matsushita, T. Matsumoto, H. Otsu and F. Sato**, 2003. No lengthening of life span in mice continuously exposed to gamma rays at very low dose rates. *Radiat. Res.*, **160** (3): 376–379.
- Tanchev, S., P. Georgiev, S. Georgieva and S. Dimitrov**, 2004. Changes in semen quality and quantity and dominant lethal mutations in rabbits following external gamma irradiation. *B. J. Vet. Med.*, **7** (2): 77–84.
- Tanchev, S., S. Georgieva, E. Jeliazkov and B. Popov**, 2006. Comparative study of karyotype mutability in inbred and outbred rabbits after gamma irradiation. *Trakia Journal of Sciences*, **4** (1): 27–32.
- Tanchev, S., V. Semerdjiev, N. Sandev, L. Sotirov, E. Zhelyazkov and S. Georgieva**, 2005. Fagocytic activity of leucocytes in pigs, product of narrow inbreeding. *Trakia Journal of Sciences*, **3** (2): 39–43.
- Thomson, J. F. and D. Grahn**, 1989. Life shortening in mice exposed to fission neutrons and gamma rays. VIII Exposures to continuous gamma radiation. *Radiat. Res.*, **118** (1): 151–160.
- Thomson, J. F., F. S. Williamson and D. Grahn**, 1986. Life shortening in mice exposed to fission neutrons and gamma rays VI Studies with the white-footed mouse. *Peromyscus leucopus*. *Radiat. Res.*, **108** (2): 176–188.