Influence of Deuterium Depleted Water on Rat Physiology: Reproductive Function, Forming and Posterity Development

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Abstract: Reproductive function and postnatal progeny development of rats in four generations treated with deuterium depleted water (40 ppm) were investigated. The targeted generations were parent (F0), first (F1), second (F2) and third (F3). Replacement of tap water to deuterium depleted water did not influence on fertility index as well as on survival and postnatal offspring development. Reproductive function, physical parameters and reflexes development in rats and pups consumed DDW was similar or more intensive in comparison with control group. Therefore, DDW consumption did not possess any toxic effects and may enhance general postnatal development.

Keywords: Fertility, deuterium depleted water, rats, generation, adaptation.

Biological objects are sensitive to water isotopic composition. Decrease of deuterium content in water as well as its increase can lead both to activation and to inhibition of biological functions. Isotope effects on biological objects depend on deuterium content in water. Thus, deuterium depleted water consumption can influence on biological processes more remarkable than deuterium enriched water [1].

Heavy water isotopes accumulate in organism primary with food consumption. Products processing with the implementation of deuterium depleted water are the opportunity to correct heavy isotopes content *in vivo* due to diet modification [2]. Moreover, modern technologies allow producing food substances with a known ratio of light and heavy isotopes [3].

Oxygen and hydrogen isotopes concentration in animal tissues correlates with the concentration of these isotopes in natural habitat. Additionally, isotopes variations in nature are more considerable among elements intensive involved in inorganic and organic world circulation of substances [4]. According to recent publications, the concentration of deuterium in drinking water plays an important role in metabolic processes [2, 5-7]. For example, depressions number in the population was shown to be correlated with the concentration of deuterium in drinking water [8] as well as low concentrations of deuterium in drinking water caused stress resistance in mammals [9].

Deuterium concentration in blood plasma is higher in several times than potassium, calcium, magnesium and trace elements concentrations (fluorine, iodine, copper, manganese, and cobalt). Water with modified isotope composition (deuterium depleted water - DDW) is water isotopologue $^{1}H_{2}{}^{16}O$ formed by light stable oxygen and hydrogen isotopes. DDW content in natural water is 99.73–99.76 mol. %. Natural water is multicomponent mixture of isotopologues: 311 molecules of $^{1}HD^{16}O$ are presented in 10^{6} molecules of water. The concentration of water molecules containing heavy hydrogen isotopes in natural water varies in the limits mentioned in the international standard for the isotopic composition of the hydrosphere – (Standard Mean Ocean Water). Deuterium content is 155.76 ppm in SMOW which is defined according to isotopic composition of the deep water in oceans [10].

Lobyshev V. I. *et al* [11] pronounced that deuterium concentration in human plasma is higher than in drinking water, which is the main source of heavy hydrogen isotopes. Proteins, fats and carbohydrates are formed by organic molecules; thereby D is also incorporated in its structure and may accumulate in tissues by trophic way with food consumption. A number of biological effects are caused by variation of D and H consumption ratio and therefore led to arisen interest of the biomedical scientific community to this fact [5, 12, 13]. Growth of microorganisms by stimulating metabolic activity and, accordingly, the quantitative growth of bacterial cells are induced by addition of DDW in culture medium [14, 15]. According to recent publications DDW consumption can increase adaptive capacities and structural alterations of immune organs (thymus and spleen) in experimental animals [16], as well as radioprotective effect induction [17] and prooxidant-antioxidant system activation during pathological processes [18, 19]. However,

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despite on wide attention of scientists to the unique properties of DDW, so far there is no clear explanation of it influence on biological objects.

The aim of this work was a comparative study of the reproductive function and postnatal development in rats treated with DDW.

MATERIALS AND METHODS

Seventy five outbred adult mature males and females Wistar rats (265±15g, F0) and five hundred thirty three Wistar pups (F1, F2, F3) were kept in standard conventional conditions (temperature 20±3^{o}C, humidity 48±2%, day/night (from 6:00 to 18:00/from 18:00 to 6:00); no more than six rats were placed in plastic cages (TECNIPLAST type IV S) [23], water and feed were available ad libitum. All procedures were performed according to EU Directives 86/609EEC.

Parent generation of rats (F0) was obtained from the "Andreevka" FGBU "NCBMT" RAMN (Moscow region, Solnechnogorsky district, Andreevka). Rats were randomly divided into 2 groups: experimental group consumed DDW ad libitum and control group consumed filtered standardized tap water ad libitum.

DDW (40 ppm) was obtained on the plant created at Kuban State University [3]. Mineralization of DDW was conducted by addition of mineral salts in it in order to obtain the physiologically full mineral composition (mineralization 314-382 mg/l: hydrocarbonates 144-180, sulfates < 1, chlorides 60-76, calcium 6, magnesium 3, sodium 50-58, potassium 50-58) the same as for water with a deuterium content of 150 ppm. Filtered standardized tap water (150 ppm) prepared on water treatment plant EMD Millipore RiOs™ 50 (Merk Millipore, Germany).

Experiment consisted of adaptation to water changing, examination of reproductive function in rat generations F0, F1, F2 and postnatal development of rat generations F1, F2, F3. Adaptation to water changing to DDW was carried out during 20 days before mating in order to receive following generation (F1).

For mating females and males aged 100-110 days placed together in a ratio of 2:1 for 14 days. Litters were transferred from parent animals on 30th day of life. Rats were selected from different females for further mating.

Reproductive parameters including fertility index and offspring viability were evaluated in generations F0, F1 and F2. Fertility index was evaluated in the percentage of pregnant females per impregnated males to the total number females and males placed together for mating. In this case, the pregnancy one or both the females confirmed the fertility of the male; in the case that none of the females became pregnant, the male is considered as infertile, and both females as potentially fertile. Postnatal F1, F2 and F3 progeny development were evaluated by several landmarks (body mass index, opening of eyes, eruption of teeth, hair growth, descend of testes, vaginal opening) and reflexes (grip reflex, cliff avoidance, emotional and motor behavior) within the 1st month of life [19, 20].

Body weight of rat pups was measured on 2, 7, 14, 21, 28 and 30th day of life using an electronic technical scales (Ohaus, Adventurer Pro, USA) with accuracy (±0.1). Offspring size and survival rate from 0 to 5th day of life (the ratio of the number of pups surviving till 5 day to the number of newborn rat pups) and from 6th to 25th day of life (the ratio of the number of pups surviving till 25 days to ones surviving till 6 days), the ratio of males to females [21, 22] were determined. Consumption of DDW was also measured.

Data are presented as mean±s.e.m. and min-max in absolute value and percentage ratio. Statistical analysis was carried out on Software "SPSS Statistics 17.0". The distribution of quantitative features was determined using the $\chi 2$-criteria, equality of dispersion was determined using the Leuven criteria. The significance of mean differences satisfied to conditions of normal distribution and equality of variances were assessed using one-factor analysis of variance (ANOVA). Nonparametric analogue to the independent samples (Mann-Whitney U-criteria) was used to compare quantitative features placed out of conditions of normal distribution and equality of variances (the difference was considered significant at $p < 0.05$) [22].

RESULTS AND DISCUSSION

Feed consumption was 13.5-29.8g/day per rat (males) and 12.6- 25.8g/day per rat (females). During the period of F0 adaptation, DDW consumption was 24.8±2.2 ml/day per rat (females) and 27.4±1.8 ml/day per rat (males) in experimental group, filtered tap water consumption was 22.9±3.4 ml/day per rat (females) and of 25.8±2.4 ml/day per rat (males) in control group. During F0 pregnancy and lactation, water consumption was increased up to 32.9 and 43.6 ml/day per rat in the experimental group and up to 35.6 and 44.7 ml/day per rat in control group. Consumption of DDW in F1 and F2 generations ranged from 32.9 to 33.1 ml/day per rat

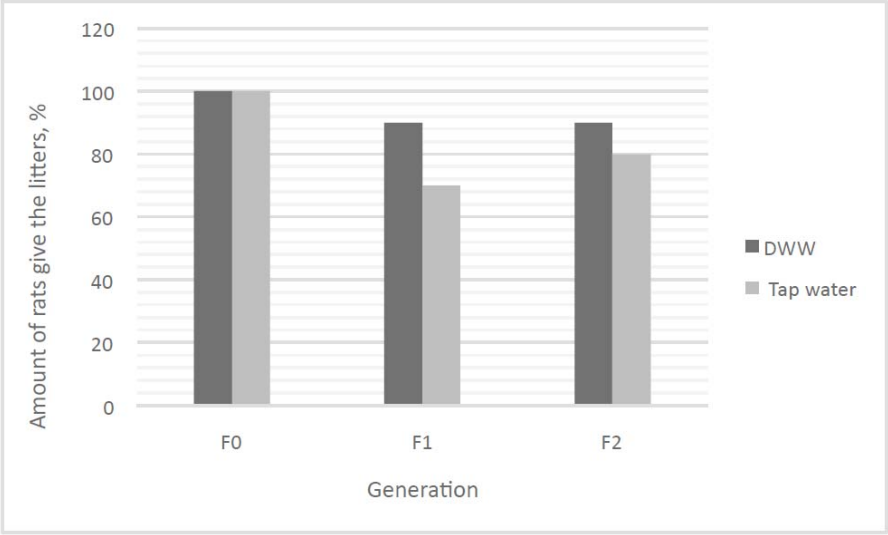

Figure 1: The fertility index.

during pregnancy and from 44.9 to 46.5 ml/day per rat during lactation. Filtered tap water consumption in F1 and F2 generations ranged from 33.9 to 34.7 ml/day per rat during pregnancy and from 45.5 to 46.7 ml/day per rat during lactation in control group. In the period of adaptation to DDW experimental animals gained weight on average 0.8-1.9 g/day, control animals gained weight on average 1.2-2.2 g/day.

Fertility index (Figure **1**) of F0 experimental animals was 100% (females), 99% in F1 and F2 generation (females), 89-100% in F0, F1 and F2 generations (males). Certain trends weren't note, therefore the data were random, not related to the impact of any external factors. Mated females in F0, F1 and F2 generations mortality weren't also recorded.

High survival rate in F1, F2 and F3 generations were revealed during postnatal progeny development in both experimental and control groups. Survival in F1 was 95% from 1st to the 5th day of life and 99% from 6th to 25th day of life in control group. Survival in F1 was

Table 1: Survival and Postnatal Development of the Offspring

Generation	Register indicators	Group of animals	
		Experiential	Reference/ Control
F1	The total number of pups	102	102
	Litter size, M±m max-min	10,1±5,8 14-7	10,2±2,0 15-7
	Sex ratio (♂/♀), %	46/54	47/53
	Survival from 1st to 5th day of life, %	89	95
	Survival from 6th to 25th day of life, %	99	99
F2	The total number of pups	96	53
	Litter size, M±m max-min	10,7±2,9 16-6	8,8±2,3 13-6
	Sex ratio (♂/♀), %	52/48	35/65
	Survival from 1st to 5th day of life, %	96	97
	Survival from 6th to 25th day of life, %	99	99
F3	The total number of pups	98	86
	Litter size, M±m max-min	10,9±3,6 16-6	8,2±5,6 15-6
	Sex ratio (♂/♀), %	42/58	47/53
	Survival from 1st to 5th day of life, %	95	93
	Survival from 6th to 25th day of life, %	98	97

89% from 1st to the 5th day of life and 99% from 6th to 25th day of life in experimental group (Table **1**). Survival in F2 was 97% and 96% from 1st to the 5th day of life in control and experimental groups, respectively. Survival in F2 was 99% from 6th to 25th day of life in both control and experimental groups. Survival in F3 was 93% and 95% from 1st to the 5th day of life and 97% and 98% day of life from 6th to 25th in control and experimental groups, respectively.

Averaged litters size was 10.1 to 10.9 in experimental group, the maximum number of pups in the litter were observed in F2 and F3 generations. Averaged litters size was 8.2 and 8.8 in control group (Table **1**). The ratio of males and females in each generation weren't significantly different in both control and experimental groups.

Body weight gains in F1, F2 and F3 were not significantly different between control and experimental groups during 14 days of postnatal progeny development. Despite of less body weight gains in F1, F2 and F3 experimental pups from 1st to 21st days of life comparison with control pups, the final weight of the animals in experimental group was higher than in control group on 4-6% from 25th 30th days of life (Figure **2**).

Postnatal F1, F2 and F3 progeny development were evaluated by opening of eyes, eruption of teeth, hair growth, descend of testes, vaginal opening (Table **2**). Slight acceleration of reflexes (grip reflex, cliff avoidance, emotional and motor behavior) of rat pups in experimental group F1, F2 and F3 generations were also revealed.

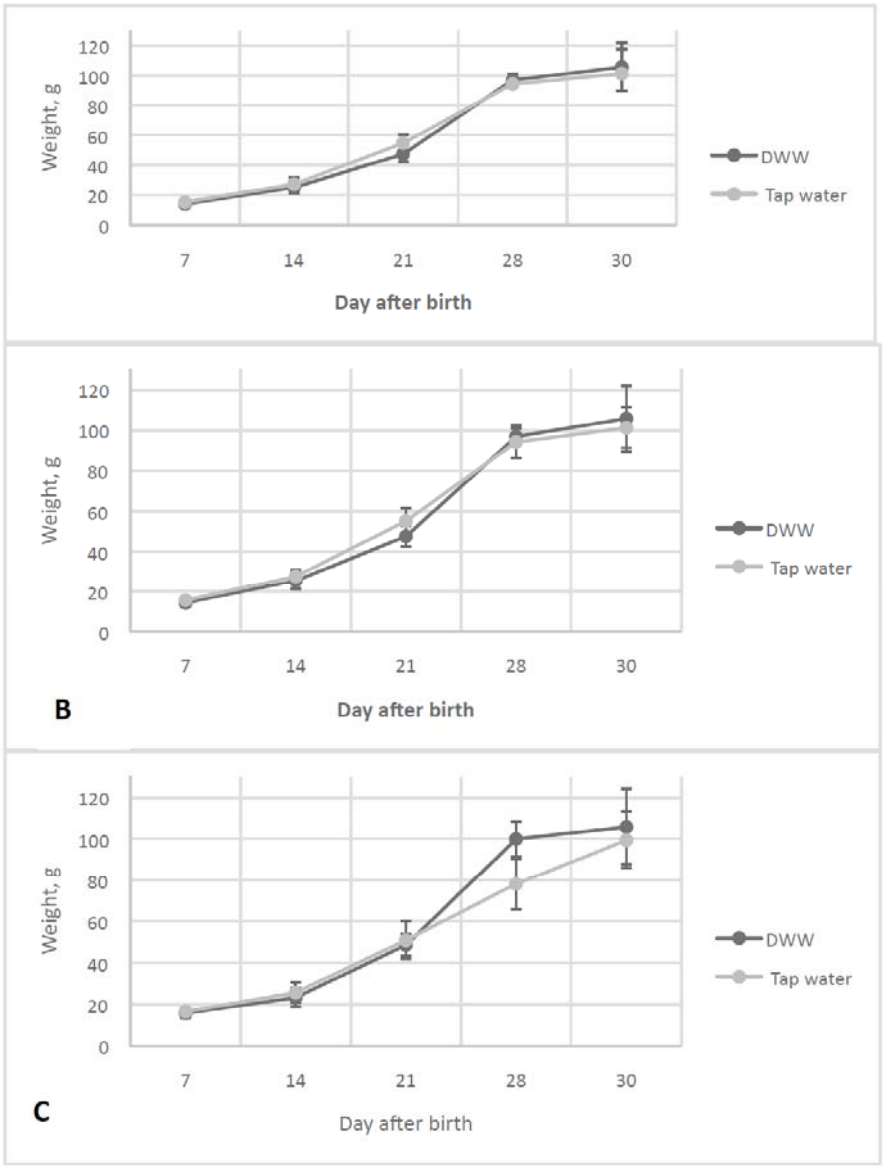

Figure 2: Dynamics of change of rats weight F1 (**A**), F2 (**B**) and F3 (**C**).

Table 2: Physiological Formation Pups

Generation	Register indicators (M±m, сутки)	Group of animals	
		Experiential	Reference/ Control
F1	Hair growth	5,2±0,9	5,7±0,8
	Eruption of teeth	8,9±0,6	9,4±0,7
	Opening of eyes	15,9±0,5	16,2±0,4
	Descend of testes	24,4±0,7	24,8±0,4
	Vaginal opening	28,4±0,7	28,7±0,9
F2	Hair growth	5,4±0,7	5,8±0,8
	Eruption of teeth	8,8±0,3	9,3±0,9
	Opening of eyes	16,0±1,3	17,6±2,3
	Descend of testes	24,1±0,6	24,4±0,1
	Vaginal opening	28,2±0,9	28,6±0,3
F3	Hair growth	5,3±0,5	5,7±0,5
	Eruption of teeth	8,3±0,5	9,0±0,6
	Opening of eyes	15,7±0,4	15,7±0,5
	Descend of testes	24,1±0,9	24,8±0,8
	Vaginal opening	28,3±0,1	28,8±0,6

Cliff avoidance and grip reflex were formed significantly faster in pups of experimental group than in the control group. Acoustic startle response was completely developed until 9th day of postnatal development in experimental offspring and until 10th day in control group. Pupillary light reflex was fully formed until 15th day of postnatal development in experimental offspring and until 16th day in control group. Cliff avoidance was completely formed in pups both control and experimental groups till 18th day of life while olfactory response in experimental offspring, especially in F2 and F3 generations, was more remarkable than in the control. Muscle strength in experimental pups was more intensive than in control animals during all observation period as well as free-fall righting and negative geotaxis were noticed in pups of both groups by 16th day of life.

CONCLUSION

Received results indicated that the DDW consumption do not influence on fertility index, survival and postnatal progeny development. Reproductive function, physical parameters and reflexes development in rats and pups consumed DDW was similar or more intensive in comparison with control group. Therefore, DDW consumption did not possess any toxic effects and may enhance general postnatal development.

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