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EFFECT OF ALKALINE IONIZED WATER ON REPRODUCTION IN GESTATIONAL AND LACTATIONAL RATS

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ABSTRACT — Alkaline ionized water (AKW) produced by electrolysis was given to gestational and lactational rats, and its effect on dams, growth of fetuses and offsprings were investigated. The results showed that the intake of food and water in dams increased significantly when AKW was given from the latter half of the gestation period and from the former half of the lactation period. Body weight of the offsprings in the test group, both males and females, increased significantly from the latter half of the lactation period. During the lactation period and after weaning, the offsprings in the test group showed significantly hastened appearance of abdominal hair, eruption of upper incisors, opening of eyelids and other postnatal morphological developments both in males and females, as well as earlier separation of auricle and descent of testes in males compared with the control was noted.

As mentioned above, it was suggested from the observations conducted that the AKW has substantial biological effects on postnatal growth, since intake of food and water and body weight of the offsprings increased and postnatal morphological development was also accelerated.

KEY WORDS : Alkaline ionized water, Influence, Reproduction, Rats.

INTRODUCTION

Equipment for producing ionized water is commercially available for health purposes. Alkaline ionized water (AKW) is produced by electrolysis by attracting the cations in the water toward the cathode. Recently, AKW is used in Japan as drinking water, for cooking, green tea, coffee, and black tea and dilution of whisky and distilled sake. Meat and other phosphate-containing acid foods tend to make blood and body fluid more acidic. To maintain alkali in living organisms and improve body disposition, attention has been directed to AKW.

Watanabe and Shirai (1990) reported that body weight of the littermates 3 weeks after being borne by the dam given AKW throughout her gestation and lactation periods increased significantly compared with that of the control. Kuchida *et al.* (1993) gave AKW to the cattle and reported that the color of the meat was much brighter. Literature on the safety of AKW in the reproduction of perinatal and postnatal rats is very few.

Thus, in this report, the effect for AKW given to gestational and lactational dam rats, their fetuses and offsprings regarding their growth was investigated.

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MATERIALS AND METHODS

Animals, housing and feeding : Female rats of Spraque-Dawley strain, weighing 182–197 g (Jcl. SD, Clea Japan Inc., Tokyo, Japan) were purchased at 8 weeks of age and maintained at 23± 1°C, humidity of 40–60%, 14 hour illumination, housed 5 rats in each plastic cage with wood chips as bedding, and food (CE–2, Clea Japan Inc.) and tap water (TPW) intake *ad libitum*. After 3 week-period of acclimation, animals without any abnormal findings were used. Copulation was induced by placing an experienced male rat of the same strain in one cage made of aluminum with 10 female rats above 12 week-old with regular estrous cycle by prior vaginal smears. A single male rat was used throughout the experiment. Smears were studied daily under microscope to confirm copulation. On the day sperm appeared on the smear, females were separated from the male and this day was called day zero of gestation. All pregnant rats were individually housed in polycarbonated cages. AKW was subsequently given to gestational rats (test group, n=10). In the control, the day of appearance of sperm on the smear was defined as day zero of gestation, but TPW was given as before (control group, n= 10). Copulated females were divided daily into test and control groups with essentially the same number. The removed female rats were replaced by new female rats so that there would always be 10 female rats per 1 male rat.

Electrolytic water ionizer : AKW was obtained using an apparatus for the production of ionized water (Minekaru TBC-R 6103, Tokyo Seiden Co., Ltd., Tokyo, Japan). This apparatus is based on the principle of electrolysis of an electrolyte solution, bringing cation to the cathode and anion to the anode. The amount of ions transferred varies with the amount of reacting substances, hydrogen ion concentration, and flow speed. The pH of AKW was 9.0, as measured by pH meter (M-7EII, Hitachi-Horiba Co., Tokyo, Japan), maximum flow-speed was 140 l/h. Acidic water made by the flow of anions to the anode was discarded. For the purification of AKW, TPW was electrolyzed without drugs.

Water quality test : The Japan Food Hygiene Association, a food-testing organization recommended by the Minister of Health and Welfare according to the Japan Food Hygiene Act and the Japan Drug Act, measured pH, degree of alkalinity and electrolyte concentration of AKW. The methods and items of the tests are summarized in Table 1.

Observation of gestational rats and fetuses : For 20 days starting from the day zero, animals in the test group were given AKW only, while those in the control were given TPW *ad libitum*. Body weight was measured on the 0, 5, 10, 12, 14, 16, 18 and 20th day of gestation. Intake of food and water was measured on the 0, 4, 7, 10, 12, 14, 16, 18 and 20th day of gestation. Body weight of a fetus on the 20th day was measured by an electronic reading balance (Libror ED-200, Shimadzu Co., Tokyo, Japan). On the 20th day of gestation, animals were autopsied under anesthesia with ether, and various organs in the thoracic and abdominal regions were observed in gross, with the ovary and uterus being extracted. Using these ovaries, the number of corpora lutea was counted, while using the uterus obtained by a cesarean operation, the numbers of surviving fetuses, implantation sites and resorbed embryos, dead fetuses, were counted, from which the implantation rate and intrauterine mortality were calculated. Regarding the surviving fetuses, anomaly of the external form and sex were observed in gross, and the body and placenta were weighed by an electronic reading balance.

Observation of delivery and nursing state : Animals in the test group were given AKW only from the 17th day of gestation until the 21st day after delivery, while those in the control were given TPW *ad libitum*. The general and nursing state of the dams were observed daily. The gestational rats, after spontaneous delivery, were observed as to their state of delivery, from which the gestation index and gestation period were calculated. Body weight was measured on the 0, 5, 10, 15, 17, 19 and 21th day of gestation and the 0, 4, 7, 14 and 21th day after delivery. Body weight during the gestation and lactation periods was measured using an automatic balance for rat (Shin-maiko, Yamato Co., Tokyo Japan). Intake of food and water was measured on the 0, 4, 7, 10, 13, 15, 17, 19 and 21th day of gestation and after delivery, respectively. After weaning 21 days after delivery, the dams were autopsied

under anesthesia with ether, and various organs in the thoracic and abdominal regions were observed in gross, and after extraction of uterus, the number of implantation sites was counted.

Observation of offsprings: At the time of delivery, the litter size (a total number of newborns surviving and dead), number of stillbirths, sex, and external anomaly were examined, while the birth index, viability index on the date of birth and that on the 4th day after birth were calculated. Four days after birth the number of littermates was adjusted at random so that there were 4 males and females each, 8 offsprings in total, and they were raised for 21 days. Body weight of the offsprings was measured on the 0, 4, 7, 14 and 21th day after birth. On the 21st day of lactation, the weaning index was calculated. During the lactation period and after weaning, separation of auricle, appearance of abdominal hair, eruption of upper incisors, opening of eyelids, descent of testes and opening of vaginal orifice, and other postnatal morphological developments were observed. TPW was supplied to offsprings of the test group and the control after weaning, and observations were continued until when descent of testes in males and opening of vaginal orifice in females were noted.

Following completion of all tests, fetuses and offsprings, were autopsied under anesthesia with ether after being weighing, various organs were fixed in formalin to prepare histological sections after being weighing.

RESULTS

Qualities of AKW and TPW: pH, alkalinity and calcium, sodium, potassium, magnesium, zinc, iron and chloridion concentrations were measured in AKW and TPW given to the rats by the Japan Food Hygiene Association. The results are shown in Table 1. Concentrations of electrolytes other than chloridion and iron in AKW were higher than in TPW.

Effects on gestational rats and their fetuses: Fig. 1 shows changes of the mean body weight of the rats given AKW starting from the day when the sperm was found on the smear until the 20th day of gestation. No significant difference was noted between the test group and the control as far as the body weight was concerned. Fig. 2 shows the changes in the mean intake of food and water until the 20th day of gestation. A significant increase of food intake was noted from the 16th to the 18th day of gestation, and that of water intake from the 14th to the 18th day of gestation in the test group when compared with the control. The animals on the 20th day of gestation were autopsied under anesthesia with ether, and various organs in thoracic and abdom-

Table 1. Results of testing qualities of alkaline ionized water and tap water.

	Control (Tap water)	Alkaline ionized water
pH [a,e]	7.3	8.7
Alkalinity (mg/l) [b]	38	50
Calcium (mg/l) [c]	17.5	20.1
Sodium (mg/l) [c]	7.8	8.6
Potassium (mg/l) [c]	1.7	2.1
Magnesium (mg/l) [c]	4.1	4.4
Zinc (mg/l) [c,e]	0.03	0.04
Iron (mg/l) [c,e]	0.05 (less than)	0.05 (less than)
Chloridion (mg/l) [d,e]	9.9	7.8

Water quality was assessed by the Japan Food Hygiene Association, as recommended by Minister of Health and Welfare based on the Japan Food Hygiene Act and the Japan Drug Act.
[a] : pH meter method.
[b] : Sulfuric acid neutralization titrimetry method.
[c] : Atomic absorption spectrophotometry method.
[d] : Silver nitrate titrimetry method.
[e] : Indicates use of the method for standardization of water quality based on the Japan Waterworks Act.

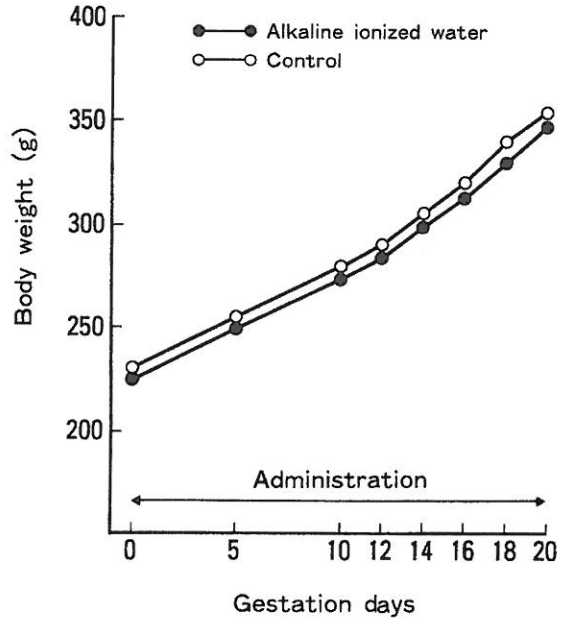

Fig. 1. Changes in body weight during gestation in 10 mother rats given alkaline ionized water from day zero to day 20 of pregnancy. Results essentially the same as in the control.

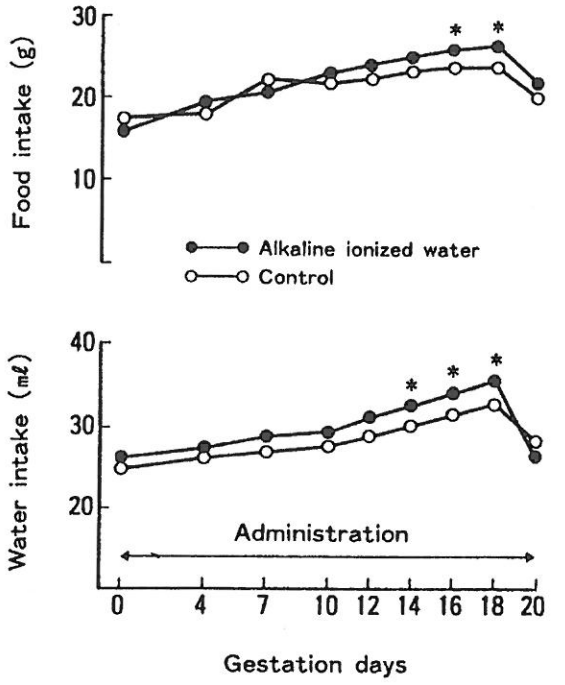

Fig. 2. Changes in food and water intake during gestation in 10 mother rats given alkaline ionized water from day zero to day 20 of pregnancy. * : Significant difference from control by Student's *t (Welch)*-test, with $P<0.05$.

inal regions as well as the presence of anomaly regarding the fetuses extracted by a cesarean operation were observed in gross. Table 2 shows survival of the fetuses at 20 days, together with weights of the body and the placenta. No significant difference was noted in the results between the test group and the control.

Effects on delivery and nursing state : AKW was given to the dams from the 17th day of gestation until the 21st day after delivery, and the state of gestation, delivery and nursing state were observed. No abnormal findings were found. Fig. 3 shows the changes in the mean body weight of the animals during the gestation and lactation periods. Compared with the control, no significant change in body weight was noted. Fig. 4 shows the mean intake of food and water of the animals in the gestation and lactation periods. Intake of food and water increased significantly from the 7th day after delivery to the 21st day of the weaning period in the test group. Table 3 shows the state of delivery and nursing regarding the dams. The gestation index was 100% both in the test group and the control. Gestation period and the number of implantation sites showed no statistical difference for either group.

In addition, the mess and smell in the cage (due to the use of wood chips) was far less for the test animals than the control.

Effect on the offsprings : Table 3 shows the survival and body weight of the offsprings during their nursing period. No significant difference was noted for either the test group or the control regarding the birth index, viability index and weaning index. However, the mean body weight of the offsprings increased significantly after being given AKW from the 14th day of lactation in males and from the 21st day of weaning in males and females. Table 4 shows the results of postnatal morphological development in the offsprings during the lactation period and after weaning. The appearance of abdominal hair, eruption of upper incisors and opening of eyelids both in males and females, and separation of auricle and descent of testes in males were hastened significantly in the test group compared with the control.

Meanwhile, the hair on the offsprings in the test group was much brighter and more lustful than that of the control. Especially, the density

Table 2. Viability, body weight and placental weight of fetuses from mother rats given alkaline ionized water from day zero to day 20 of gestation.

Group	Control (Tap water)	Alkaline ionized water
No. of pregnant females	10	10
No. of corpora lutea	14.9±1.9	15.6±1.5
No. of implantation sites	13.8±1.6	14.3±1.4
Implantation rate (%) [a]	90.0±4.0	88.2±3.9
No. of resorbed embryos	6	7
No. of dead fetuses	0	0
Intrauterine mortality (%) [b]	4.2±3.4	4.8±4.4
No. of live fetuses	12.0±1.7	12.0±1.4
Sex ratio (Male/Female)	0.98 (59/61)	0.99 (58/62)
Body weight of live fetuses (g)		
Male	3.5±0.5	3.6±0.5
Female	3.3±0.4	3.4±0.4
Placental weight (g)		
Male	0.44±0.06	0.44±0.05
Female	0.42±0.04	0.41±0.03

Values represent the mean ± S.D.
[a] : Implantation rate=(number of implantation sites / number of corpora)×100%.
[b] : Intrauterine mortality=(number of deaths / number of implantation sites)×100%.

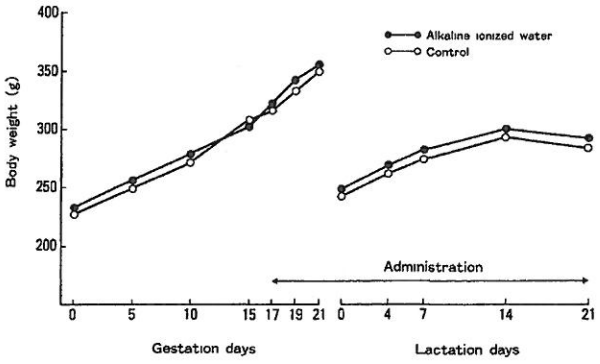

Fig. 3. Changes in body weight during gestation and lactation in 10 mother rats given alkaline ionized water from day 17 of pregnancy to postpartum day 21. There were no significant difference with the control.

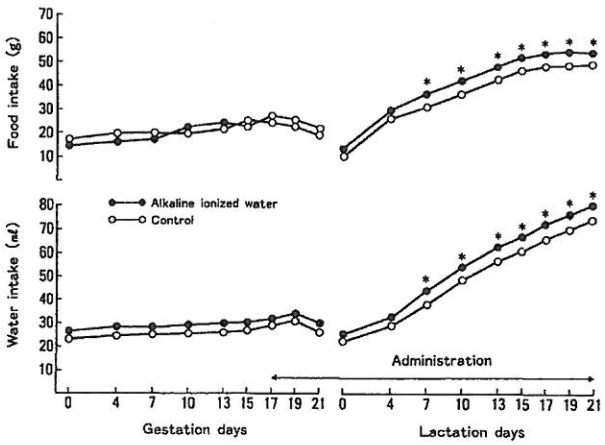

Fig. 4. Changes in food and water intake during gestation and lactation in 10 mother rats given alkaline ionized water from day 17 of pregnancy to postpartum day 21. * : Significant difference from control by Student's *t (Welch)*-test, with $P<0.05$.

Table 3. Reproductive performance of mother rats given alkaline ionized water from day 17 of gestation to postpartum day 21 and viability of offspring.

Group	Control (Tap water)	Alkaline ionized water
No. of pregnant females ; A	10	10
No. of females having live newborn ; B	10	10
B/A (%)	100	100
Duration of pregnancy in days	21.4±0.5	21.3±0.4
No. of implantation sites ; C	14.3±1.8	13.9±1.7
Day 0		
No. of newborns ; D	13.1±1.4	13.3±1.5
D/C (%)	95.0±4.5	95.7±4.8
No. of live newborns ; E	13.1±1.4	12.8±1.3
E/C (%)	91.6±6.1	92.0±6.3
E/D (%)	97.0±3.5	96.2±2.8
Live offspring weight (g)		
Male	5.4±0.7	5.4±0.6
Female	5.2±0.6	5.3±0.7
Day 4		
No. of live offspring ; F	11.8±1.1	11.5±1.0
F/E (%)	90.1±7.9	90.0±6.5
No. of live offspring after culling ; G	8	8
Live offspring weight after culling (g)		
Male	8.2±0.5	8.3±0.6
Female	8.0±0.5	8.1±0.6
Day 7		
Live offspring weight (g)		
Male	13.8±1.3	14.3±0.9
Female	12.9±1.4	13.3±1.1
Day 14		
Live offspring weight (g)		
Male	26.4±1.7	29.2±1.5*
Female	24.2±1.6	28.1±1.7
Day 21		
No. of offspring ; H	8	8
H/G (%)	100	100
Live offspring weight (g)		
Male	40.6±2.2	44.5±2.6*
Female	37.3±2.2	42.5±2.1*

Values represent the mean ± S.D.
* : Significant difference from control by Student's *t (Welch)*-test, with $P<0.05$.

Table 4. Postnatal morphological development of offspring from mother rats given alkaline ionized water from day 17 of gestation to postpartum day 21.

Group	Male		Female	
	Control (Tap water)	Alkaline ionized water	Control (Tap water)	Alkaline ionized water
Physical development				
No. of offspring observed (before culling)	62 (10) [b]	62 (10) [b]	69 (10) [b]	66 (10) [b]
Separation of auricle [a]	2.7±0.4	2.5±0.5*	2.5±0.5	2.6±0.4
No. of offspring observed (after culling)	40	40	40	40
Emergence of abdominal hair [a]	9.6±0.7	8.5±0.8*	9.8±0.7	8.4±0.5*
Eruption of upper incisor [a]	11.2±0.8	10.2±0.8*	11.4±0.9	10.3±0.8*
Opening of eyelid [a]	15.7±0.6	14.9±0.8*	16.4±0.5	15.5±0.7*
Sexual differentiation				
Descent of testis [a]	27.0±0.7	25.9±0.8*		
Opening of vagina [a]			37.3±2.2	36.2±1.7

[a] : Mean days of age ± S.D.
[b] : No. of litters examined (No. of dams examined).
* : Significant difference from control by Student's *t (Welch)*-test, with $P<0.05$.

of the hair covering the body was noteworthy.

DISCUSSION

As the results of body weight measurement of the young born from 10 mother rats given AKW during gestation and lactation indicated a significant increase in both male and female offspring compared to the corresponding value in the young born from mothers given TPW. Further, the postnatal morphological developments in males and females were significantly hastened. This may be explained as follows. AKW is produced by the movement of cations in TPW toward the cathode in electrolysis. The amount of transferred ion depends on the quantity of electrolytes, pH, and flow rate. All cations are transferred to alkaline water, increasing the total amount of the cations in AKW considerably. Increased alkalinity of AKW compared to that of TPW may support such hypothesis. Consequently, AKW given to a female animal contains a higher concentration of cations. This is in agreement with the actual measurement of electrolytes in AKW and TPW. Since most of the cations transferred to the AKW are hydrated with water molecule through electrolysis, they are rapidly absorbed and readily utilized as a physiologically active form. The pH of AKW adjusted to 9.0 at the time of electrolysis of TPW was measured as 8.7 by the Japan Food Hygiene Association. This discrepancy may be explained by the time elapsed before measurement. In any case, the difference would not be significant.

The intake of food and water by the dams given AKW starting from the date when the sperm was noted on the smear until the 20th day of gestation, increased significantly compared with that in the control during the latter half of gestation period. However, no significant difference was noted between the test group and the control regarding changes in the body weight of the dams upto the 20th day of gestation, survival of the fetuses extracted on the 20th day of gestation, as well as weight of the body and placenta. Regarding the various organs in the thoracic and abdominal regions of the gestational rats and external form of fetuses, no abnormality was noted macroscopically. Accordingly, the effect of AKW given to the gestational rats regarding their fetuses is considered to be comparatively less.

The intake of food and water in the dams of the test group given AKW from the 17th day of gestation until the time of weaning increased significantly compared with the control starting from the 7th day of lactation. However, changes in the body weight in the gestational and lacta-

tional rats, gestation index, gestation period, sex ratio, birth index, viability index and weaning index were within the range of the results already reported (Kawai *et al.*, 1985;Kaneko *et al.*, 1993;Matsuura *et al.*, 1993;Matsuura *et al.*, 1993), showing no significant difference compared with that of the control.

On the other hand, the appearance of abdominal hair, eruption of upper incisors, opening of eyelids, separation of auricle and descent of testes, and other postnatal morphological developments in the offsprings born from the dams administered AKW during the lactation period and after weaning, were hastened significantly compared with the control. Earlier appearances of these changes were confirmed by consulting the time table of postnatal morphological developments reported by Ninomiya *et al.* (1989), and Shirota *et al.* (1993) using the same type of SD strain rats. Further, the body weight of the offsprings in the test group increased significantly starting from the latter half of the lactation period. The increased body weight among the offsprings agreed with the previous report of Watanabe and Shirai (1990) describing that the body weight of a litter of offsprings 3 weeks after birth, when their dams were given AKW from the date of gestation to the time of weaning, increased significantly compared with the control.

Also, the body weight increase of the offspring, however, may occur either by the placental transfer to the fetus of water-hydrated cation produced by electrolysis, or by nutritional supplementation through the mother's milk up to 3 weeks after birth. It is unclear at present whether the increase in body weight of the offspring was caused by increased production and secretion of milk by the mammary gland and improvement of the quality of milk in response to the supply of AKW with high cation concentration, or a pathological increase in body weight induced by the supply of AKW to the mothers during gestation and lactation. The body weight increase in the offspring is important enough to deserve further study. Moreover, the significant increase of both food and water intakes in the test group of dams is another question remained for answer.

As stated above, by giving AKW to the gestational and lactational rats, increased intake of food and water, increased body weight of the offsprings and hastened postonatal morphological development could be observed.

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