

Effects of Cow's Milk on Reproduction in ICR Male Mice

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Objective To study the effects of Cow's milk on the reproduction in male mice. **Methods** Twenty-four male mice were divided randomly into two groups: milk group (M) and control group (C). Each mouse was given 10 mL milk per day from 4 to 16 weeks in the group M. At the age of 17 weeks, all the mice were sacrificed. **Results** Serum testosterone was decreased in the group M ($P=0.037$). No significant difference was found in weight of testes, seminal vesicle or adrenal gland of mice between the groups C and M. However, the weight of seminal vesicle decreased when expressed in g/100 g body weight in the group M. Epididymal sperm concentration, motility, morphology, and sperm head number were not affected by milk. **Conclusion** Cow's milk has adverse effects on the reproductive system in ICR male mice. Further studies are needed to clarify the specific effects of milk on reproductive health.

Key words: Cow's milk; Male mice; Reproductive health

INTRODUCTION

Milk, usually cow's milk, is a good source of protein, calcium, vitamins A and D, and some other bioactive components such as insulin-like growth factor-I (IGF-I) in human dietary, all of which facilitate bone growth. In China and the United States, milk is a mandatory element in their nutrition policies. In Japan, the average daily milk consumption in children aged 7-14 years reaches 334 g/day^[1] and pregnant women are also encouraged to consume milk and dairy products to meet their calcium requirements. Most researchers often pay attention to the benefits of milk. However, the adverse effects of milk have become a concern recently. Several epidemiologic reports indicate that milk consumption is a risk factor for prostate and testicular cancers^[2-4]. Larsson *et al.*^[5] stated that a high intake of dairy foods and lactose increases the risk of developing ovarian cancer.

In 2004, the effect of modern milk on reproduction in rats was assessed by Ganmaa^[6]. The results showed that milk have some adverse effects in both parents and their offsprings. The present study

was designed to confirm whether cow's milk can affect the reproduction in experimental animals.

MATERIALS AND METHODS

Three-week-old Kud: ICR male mice were purchased from Kyudo Co., Ltd., Fukuoka, Japan. The mice were housed in cages ($n=6$ per cage) in an air-conditioned room (24 ± 2 °C) with a 12-h light and 12-h dark cycle. After a 1-week acclimation, the animals were randomly assigned to 2 groups: control group (C) and milk group (M) ($n=12$ mice in each group). The mean weight of the mice was not significantly different at beginning of the study. Mice in the group C did not receive any treatment while each mouse in the group M received 10 mL milk per day. All mice had free access to food (CE-2 feed, purchased from CLEA Inc., Tokyo, Japan) and tap water. Clinical changes in the animals were observed daily. Body weight and food consumption were measured once a week. After 13 weeks of treatment, all the mice were anaesthetized with diethyl ether, weighed, and sacrificed.

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Blood was collected from the heart and separated serum was stored at $-80\text{ }^{\circ}\text{C}$ until analysis for estradiol (E_2) and testosterone (T). Essays for E_2 and T were carried out in duplicate with a Diagnostic Product Corporation (DPC) 17β -estradiol kit and a DPC testosterone kit, respectively, according to the manufacturers' protocols.

The reproductive organs (the testes, epididymides, seminal vesicle, and adrenal gland) in the mice were separated from adhering fats and weighed immediately. The left testis was fixed in 10% formalin for histopathologic examination.

The left epididymis was excised and placed in a pre-warmed petri dish containing 5 mL saline at $37\text{ }^{\circ}\text{C}$. The tissue was minced with scalpels for approximately 1 min and placed in a $37\text{ }^{\circ}\text{C}$ incubator for 5 min, prior to determining sperm motility. The suspension was stirred, one drop was placed on a warmed microscope slide which was covered with a $22\text{ mm} \times 22\text{ mm}$ cover-slip, and observed under a standard optical microscope (Nikon, Eclipse, E600, Japan) at $400\times$ magnification. The percentage of motile sperms was calculated after 200 or more sperm cells on each slide were analyzed. For morphological examination, another drop of the sperm cell suspension was taken for a thin smear, and stained with 1% eosin Y, and then 200 sperm cells on each slide were assessed at $400\times$ for morphologic abnormalities.

The right epididymis and testis were frozen immediately after weighed until evaluation. After thawing at room temperature, the whole epididymis and testis specimens were homogenized in 1 mL of a 0.9% NaCl solution containing 0.01 mL Triton X-100. Ten strokes of a manual homogenizer were used for each sample. The sperm cells were counted at $200\times$ magnification in a Neubauer hemocytometer, and further diluted if necessary. The number of sperm cells was expressed as 10^6 per mL. Three counts per sample were averaged.

The left testis and adrenal gland were fixed in a 10% formalin-buffer solution. After dehydration in alcoholic series and cleaning in xylol, the tissues were embedded in paraffin wax and cut into $5\text{-}\mu\text{m}$ thick sections. The sections were stained with hematoxylin-eosin and examined under a light microscope.

The data were analyzed with SPSS statistical software (SPSS, Inc., Chicago, IL). A *t*-test (parametric) analysis was used to assess the differences between the groups M and C; $P < 0.05$ was considered statistically significant.

RESULTS

No death occurred during the study and no

changes were found in the appearance or behavior of the mice in groups both M and C. In the group M, one male mouse had only one testis.

The food consumption in the group M was significantly decreased ($2.68 \pm 0.39\text{ g}$ vs $5.10 \pm 0.57\text{ g}$, $P = 0.000$), but there was no difference in calorie intake between the 2 groups after the milk consumption was increased in the group M ($73.59 \pm 8.28\text{ kJ}$ vs $65.48 \pm 5.56\text{ kJ}$, $P > 0.05$).

Statistically significant decrease in serum testosterone was found in male mice of the M group ($P = 0.037$). The serum hormone levels were not different between the groups M and C ($P > 0.05$, Table 1).

TABLE 1

Serum Hormone Levels in Male Mice			
	Samples	Group C	Group M
Estradiol (pg/mL)	12	5.92 ± 1.26	5.48 ± 1.58
Testosterone (ng/mL)	12	15.38 ± 4.24	$3.95 \pm 10.70^*$

Note. * $P < 0.05$ vs group C.

The weights of testes ($0.30 \pm 0.06\text{ g}$ vs $0.33 \pm 0.04\text{ g}$), seminal vesicle ($0.31 \pm 0.05\text{ g}$ vs $0.36 \pm 0.05\text{ g}$) and adrenal gland ($6.15 \pm 2.40\text{ mg}$ vs $5.31 \pm 2.33\text{ mg}$) in the group M were not significantly different from those in the group C. However, the weight of seminal vesicle decreased significantly when expressed in g/100 g body weight ($6.64 \pm 0.76\text{ g}/1000\text{ g BW}$ vs 7.61 ± 1.08 , $P < 0.05$).

Sperm cell count in the epididymis (33.46 ± 8.24 vs 33.94 ± 6.52 , unit: 10^6 /cauda) or testis (23.04 ± 4.63 vs 22.29 ± 3.58 , unit: 10^6 /testis) in the group M was not significantly different from those in the group C. There was no difference in the sperm motility between the groups M and C.

There were no obvious pathologic changes in the reproductive organs of male or female mice in the two groups.

The study so far represents merely a preliminary study about the effects of cow's milk on the reproductive system in male mice. Further study is needed to clarify such effects in a more intensive manner.

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