



J. Dairy Sci. 99:1–9

<http://dx.doi.org/10.3168/jds.2015-10610>

© American Dairy Science Association®, 2016.

## Effect of dietary estrogens from bovine milk on blood hormone levels and reproductive organs in mice

N. Grgurevic,\*<sup>1</sup> J. Koracin,\*<sup>1</sup> G. Majdic,\*<sup>†</sup> and T. Snoj\*<sup>2</sup><sup>\*</sup>Institute for Preclinical Sciences, Veterinary Faculty, University of Ljubljana, Gerbiceva 60, 1000 Ljubljana, Slovenia<sup>†</sup>Institute of Physiology, Medical Faculty, University of Maribor, Taborska 8, 2000 Maribor, Slovenia

### ABSTRACT

Cows are often milked until 60 d before their next expected calving. Milk from cows in the third trimester of pregnancy contains up to 20 times more estrogens than milk from nonpregnant cows. The aim of this study was to evaluate whether exposure to known doses of estrogens from bovine milk could affect blood hormone levels in mice and influence their reproductive organs. This study was performed with 30 intact male and 30 ovariectomized female mice. Mice of each sex were randomly divided into 3 experimental groups, each with 6 animals of each sex, and a control group with 12 animals of each sex. The first experimental group received 4 mL of milk each day from a pregnant cow with natural estrone (E1) and 17 $\beta$ -estradiol (E2) in concentrations 0.093 and 0.065 ng/mL, respectively. The second experimental group received 4 mL of the same milk each day, with an added 10 ng/mL of both E1 and E2. The third experimental group received 4 mL of the same milk each day, with an added 100 ng/mL of both E1 and E2. The control group received no milk. After 8 d of treatment, mice were euthanized, blood was collected, and the uteruses, testes, and seminal vesicles were weighed. The results of our study demonstrated that consumption of native milk from a pregnant cow did not affect plasma E1 and E2 levels in either sex; uterine weight in females; or testosterone levels and testes and seminal vesicle weights in males. Similarly, we found no changes in the group that received the milk with an added 10 ng/mL of E1 and E2. We did observe elevated plasma estrogens in both sexes, increased uterus weight in females, and decreased plasma testosterone levels in males from the group that received milk with an added 100 ng/mL of E1 and E2. However, concentrations in the third group exceeded the physiological concentration of milk estrogens by

1,000 times, so it would be extremely unlikely to find such concentrations in native cow milk.

**Key words:** cow milk, estrogens, lactation, mice

### INTRODUCTION

In milk production, dairy cows are usually milked until the seventh or eighth month of pregnancy. The period between calving and first insemination is approximately 60 d, and cows are usually dried 2 mo before the next expected calving. During pregnancy, the concentration of estrogens in the body increases, mainly because of increased production in the placenta, and this increase is believed to play an important role in preparation for parturition (Davidson and Stabenfeldt, 2013). Pape-Zambito et al. (2008) have shown that estrogen levels in milk are correlated with estrogen levels in blood, suggesting that milk from cows in late pregnancy may contain increased estrogen concentrations. The average levels of estrone (**E1**) in milk from cows that are more than 6 mo pregnant could be up to 20 times higher than those in milk from nonpregnant cows. According to Pape-Zambito et al. (2008), levels of E1 and 17 $\beta$ -estradiol (**E2**) in the milk of cows in the third trimester of pregnancy are approximately 25 and 5 pg/mL, respectively. Malekinejad et al. (2006) reported even higher levels: up to 118  $\pm$  17 pg/mL for E1 and 21  $\pm$  3 pg/mL for E2. It has also been reported that levels of milk estrogens correlate with milk fat content (Pape-Zambito et al., 2007, 2010; Farlow et al., 2012; Macrina et al., 2012). These levels reportedly decrease with processing (Wolford and Argoudelis, 1979), although Pape-Zambito et al. (2010) did not find a reduction in milk E2 levels during pasteurization and homogenization.

The role of milk consumption in the incidence of some cancers in humans has been described in several epidemiologic studies, but the results have not been conclusive. Consumption of milk with relatively high levels of estrogens is a source of concern. It has been suggested that estrogens from bovine milk could contribute to some endocrine-related cancers and male

Received November 9, 2015.

Accepted April 21, 2016.

<sup>1</sup>These 2 authors contributed equally to the manuscript.

<sup>2</sup>Corresponding author: tomaz.snoj@vf.uni-lj.si

reproductive disorders in humans (Ganmaa et al., 2001; Ganmaa and Sato, 2005), but this hypothesis has not been tested or proven experimentally. Some other experiments and meta-analyses have shown that milk consumption is not a risk factor for cancer development (Davoodi et al., 2013; Bernichtein et al., 2015; Larsson et al., 2015), and some of them have even shown protective effects for milk (Cho et al., 2004; Elwood et al., 2008; Davoodi et al., 2013). However, Maruyama et al. (2010) have shown that the consumption of cow's milk could significantly increase serum levels of E1 and progesterone in humans. Additionally, in the same experiment, the authors observed a significant decrease in levels of LH, FSH, and testosterone. Rich-Edwards et al. (2007) reported higher values of IGF-1 and growth hormone after long-term intake of milk in 1 of 2 tested groups of children. In rodent models, some studies have reported uterotrophic effects after milk intake in prepubertal ovariectomized rats (Ganmaa et al., 2006; Zhou et al., 2010), but other studies have not found significant effects of commercial milk consumption on female rats' reproductive system (Li et al., 2005), uterotrophic activity in mice (Nielsen et al., 2009), or changes in behavior and uterine weights in rats (Furnari et al., 2012).

The aim of our study was to evaluate whether the consumption of milk with known doses of estrogens (both naturally presented and added in concentrations 100 and 1,000 times higher) could affect blood hormone levels and reproductive organs in mice.

## MATERIALS AND METHODS

### *Animals and Housing*

We used inbred BALB/c mice, reared in the Center for Animal Genomics at the Veterinary Faculty, University of Ljubljana. Animals were housed under standard conditions ( $22 \pm 2^\circ\text{C}$ , 12:12 h light:dark regimen) with regular rodent chow (phytoestrogen-free diet; Teklad 2016, Harlan, Milan, Italy) and water ad libitum. All female mice were ovariectomized bilaterally at 60 d of age (after puberty) to eliminate fluctuations in endogenous gonadal steroids. The mice were anesthetized with a mixture of ketamine (100  $\mu\text{g/g}$  of BW; Vetoquinol Biowet, Gorzow, Poland), acepromazine (2  $\mu\text{g/g}$  of BW; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (10  $\mu\text{g/g}$  of BW; Chanelle Pharmaceuticals Ltd., Loughrea, Ireland), and the gonads were excised through small incisions. The incisions were stitched with absorbable sutures (Braun, Tuttlingen, Germany) and mice received 2 injections of butorphanol (2  $\mu\text{g/g}$  of BW; Fort Dodge Animal Health) after surgery to alleviate potential pain. Animals were allowed at least 14 d to recover from the ovariectomies. One week be-

fore starting the experiment, animals were placed in individual polycarbonate cages with a surface area of 300  $\text{cm}^2$ . The age of the mice at the beginning of the experiment was 80 to 105 d. All animal experiments were approved by the Administration of the Republic of Slovenia for Food Safety, Veterinary, and Plant Protection, license number 34401–45/2011/6, and all experiments were done according to European Union directives and National Institutes of Health guidelines.

### *Assay Characteristics of E1 and E2 Measurements in Milk*

Milk E1 and E2 concentrations were determined using an estrone ELISA kit and an estradiol-sensitive ELISA kit (catalog numbers DE4174 and DE4399; Demeditec Diagnostics, Kiel-Wellsee, Germany). Because these commercial ELISA kits were validated for the detection of E1 or E2 in serum or plasma, we performed partial validation for the detection of E1 and E2 in milk.

The milk sample (1.5 L) used for partial validation originated from a 201-d pregnant Holstein cow. Milk was collected during morning milking and immediately transported to the laboratory. Validation for E1 was performed the same day, and validation for E2 was performed the next day. The milk sample was stored at  $4^\circ\text{C}$ . We used native, unprocessed milk to obtain assay characteristics of E1 and E2 measurements. The assay procedure was performed following original instructions from the E1 and E2 ELISA kits. We used pure milk (without extractions) as a matrix for determining assay characteristics.

We assessed the accuracy of the test (recovery) by measuring known amounts of E1 and E2 added to milk; E1 (Fluka, St. Galen, Swiss) and E2 (Sigma-Aldrich, St. Louis, MO) were separately dissolved in dimethyl sulfoxide (Merck, Darmstadt, Germany) in concentrations of 300  $\text{ng/mL}$ . After that, 10, 20, 30, and 40  $\mu\text{L}$  of dissolved E1 or E2 (as well as 50 and 60  $\mu\text{L}$  for E1 only) were added to 100 mL of milk to reach concentrations of 0.03, 0.06, 0.09, and 0.12  $\text{ng}$  of added E1 or E2 per mL of milk (as well as 0.15 and 0.18  $\text{ng/mL}$  for E1 only). Every spiked sample was measured in 6 replicates.

We determined the precision of the test using intra- and interassay CV. Intraassay CV were determined by measuring natural and added E1 and E2 into milk. Interassay CV were determined by measuring native E1 and E2 in milk in 2 assays, with 8 replicates in each assay. Table 1 presents the details of assay characteristics.

The average recovery rates for E1 and E2 measurements were 100 and 107%, respectively. Interassay CV for E1 ( $n = 16$ ) and E2 ( $n = 16$ ) were 12.40 and 14.26%, respectively.

Download English Version:

<https://daneshyari.com/en/article/10973085>

Download Persian Version:

<https://daneshyari.com/article/10973085>

[Daneshyari.com](https://daneshyari.com)