Annals of Anatomy 200 (2015) 79–87

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Research Article

High salt intake negatively impacts ovarian follicle development

Guang Wang[a,d,1], Cheung-Kwan Yeung[a,1], Jing-Li Zhang[b,1], Xi-Wen Hu[b], Yu-Xiang Ye[b], Yong-Xia Yang[b], Jiang-Chao Li[b], Kenneth Ka Ho Lee[c], Xuesong Yang[a,d,**], Li-Jing Wang[b,*]

[a] Key Laboratory for Regenerative Medicine of the Ministry of Education, Medical College, Jinan University, Guangzhou 510632, China
[b] Institute of Vascular Biological Sciences, Guangdong Pharmaceutical University, Guangzhou 510006, China
[c] Key Laboratory for Regenerative Medicine of the Ministry of Education, School of Biomedical Sciences, Chinese University of Hong Kong, Shatin, Hong Kong
[d] Institute of Fetal-Preterm Labor Medicine, Jinan University, Guangzhou 510632, China

ARTICLE INFO

Article history:
Received 27 August 2014
Received in revised form 6 February 2015
Accepted 9 February 2015

Keywords:
High salt
Infertility
Ovarian follicle
Apoptosis
Proliferation

ABSTRACT

Many human disorders induce high salinity in tissues and organs, interfering with their normal physiological functions. Using a mouse model, we demonstrated that high salt intake caused infertility. Specifically, we established that high salinity dramatically affects ovarian follicle development and the extent of follicular atresia. However, it did not significantly influence the primordial follicles. TUNEL assays revealed that high salt intake inhibited follicle development by inducing the granulosa and theca cells that surround the oocytes to undergo apoptosis. Furthermore, immunohistological staining for the proliferation markers Ki67 and PH3 showed that high salt intake also repressed granulosa cell proliferation. *In vitro* testing of granulosa cells also confirmed that high salt significantly repressed cell proliferation and promoted cell apoptosis. In summary, high salt consumption negatively impacts reproductive functions in female mice by interfering with ovarian folliculogenesis.

1. Introduction

The ovaries are mainly composed of dormant primordial follicles made up of oocytes surrounded by granulosa cells. At puberty, some of the primordial follicles are hormonally induced to develop and mature. In histological sections of these ovaries, it is possible to see follicles at different stages of development (McGee and Hsueh, 2000). The basic function of the ovary is to protect and support the oocytes. At birth, the ovary contains millions of primordial follicles, most of which eventually undergo atresia. It is only under appropriate levels of follicular stimulating hormone (FSH) and luteinizing hormone that the primordial follicles undergo folliculogenesis and ovulation (Bertoldo et al., 2013; Matsuda et al., 2012; Raju et al., 2013). FSH stimulates the proliferation of granulosa and theca cells, induces the oocytes to re-enter meiosis and maintains the survival of the developing follicles (Fauser, 1994; Hsueh et al., 2000). Correspondingly, the theca cells secrete keratinocyte growth factor (KGF) and granulosa cells secrete c-kit, which act in a paracrine fashion to control preantral follicle growth and development (Hsueh et al., 2000).

High salt intake disrupts normal physiological functions, causing hypertension, cardiovascular and chronic kidney diseases (Appel et al., 2011; Mohan and Campbell, 2009). Strazzullo et al. (2009) demonstrated that excess salt consumption is related to enhanced risk of stroke and cardiovascular disease. They also showed that reducing salt intake suppressed cardiovascular disease. Another negative impact of excess salt intake, an increase in extracellular osmolarity, has been demonstrated in epidemiological and clinical studies. In monkeys, one year of high salt intake significantly increased extracellular osmolarity (Cherchovich et al., 1976). High osmolarity levels, in turn, may damage the arteries, kidneys, eyes and heart prior to a hypertension diagnosis (Borghi et al., 2002; Devine et al., 1995; Whelton et al., 2002). Furthermore, excess consumption of salt and fructose beverages significantly reduced fertility (Gray et al., 2013). However, it is still not known how high salt intake affects reproductive function and the mechanisms involved, especially on folliculogenesis in the ovaries.

The mouse is the most common experimental animal model in life science studies. As a mammal, the mouse is physiologically similar to and shares a high degree of homology with human beings (Paigen, 2002). Mice have been used in reproductive

* Corresponding author at: Vascular Biology Research Institute, Guangdong Key Laboratory of Pharmaceutical Bioactive Substances Guangdong Pharmaceutical University, 280 Waihuandonglu, Guangzhou, Guangdong 510006, China.
Tel.: +86 20 39352231.
** Corresponding author at: Department of Histology and Embryology, School of Medicine, Jinan University, No. 601 Huangpu Road West, Guangzhou 510632, China.
Tel.: +86 20 85228316.
E-mail addresses: yang_xuesong@126.com (X. Yang), wanglijing62@163.com (L.-J. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aanat.2015.02.009
0940-9602/

biological research since the 16th Century. Mice are frequently used for research in genetics, psychology, medicine and other scientific disciplines (Hedrich, 2004). In this context, we examined the effect of high salt intake on ovarian follicle development in a mouse model.

2. Materials and methods

2.1. Mice

Five-week-old C57BL/6 mice were obtained from the Guangdong Province Animal Centre (Guangzhou, China) and maintained at 25 °C on a 12 h light/dark cycle. The mice were housed in a pathogen-free animal facility at the Institute of Vascular Biological Sciences, Guangdong Pharmaceutical University. The mice were allowed free access to food and water. Female mice were divided into control and experimental groups, with the latter maintained on 4% NaCl water and control mice on pure water. Male mice were provided with pure water. When the female mice reached 9 weeks of age, one male was mated with every two females. From this point on, all of the mice were provided with pure water. The females were checked for the presence of vaginal plugs each morning. Pregnant females were killed by cervical dislocation when the embryos they carried reached approximately 10 days old. The control groups were not pregnant, and there were four mice in each group. The animals were used in experiments according to the guidelines of the animal experimental ethics committee of Guangdong Pharmaceutical College.

2.2. Hormone test

Serum hormone levels were determined using chemiluminescence immunoassays. The measurement was carried out in the Clinic Laboratory of Reproductive Medicine Centre, Guangdong General Hospital.

2.3. Ions test

Ion concentrations in serum (the control groups – not pregnant and four mice in each group) were determined with an electrode method (Dimeski et al., 2006; Levy, 1981), which was performed in the Clinic Laboratory of Reproductive Medicine Centre, Guangdong General Hospital.

2.4. Histology

Briefly, ovaries were harvested from the mice and fixed in 4% paraformaldehyde at 4 °C for 12 h. The ovaries were then dehydrated, cleared in xylene and embedded in paraffin wax. The embedded ovaries were serially sectioned at 5 μm using a rotary microtome (Leica, RM2126RT). The sections were mounted onto glass slides and used for haematoxylin and eosin (H&E) or immunohistochemical staining.

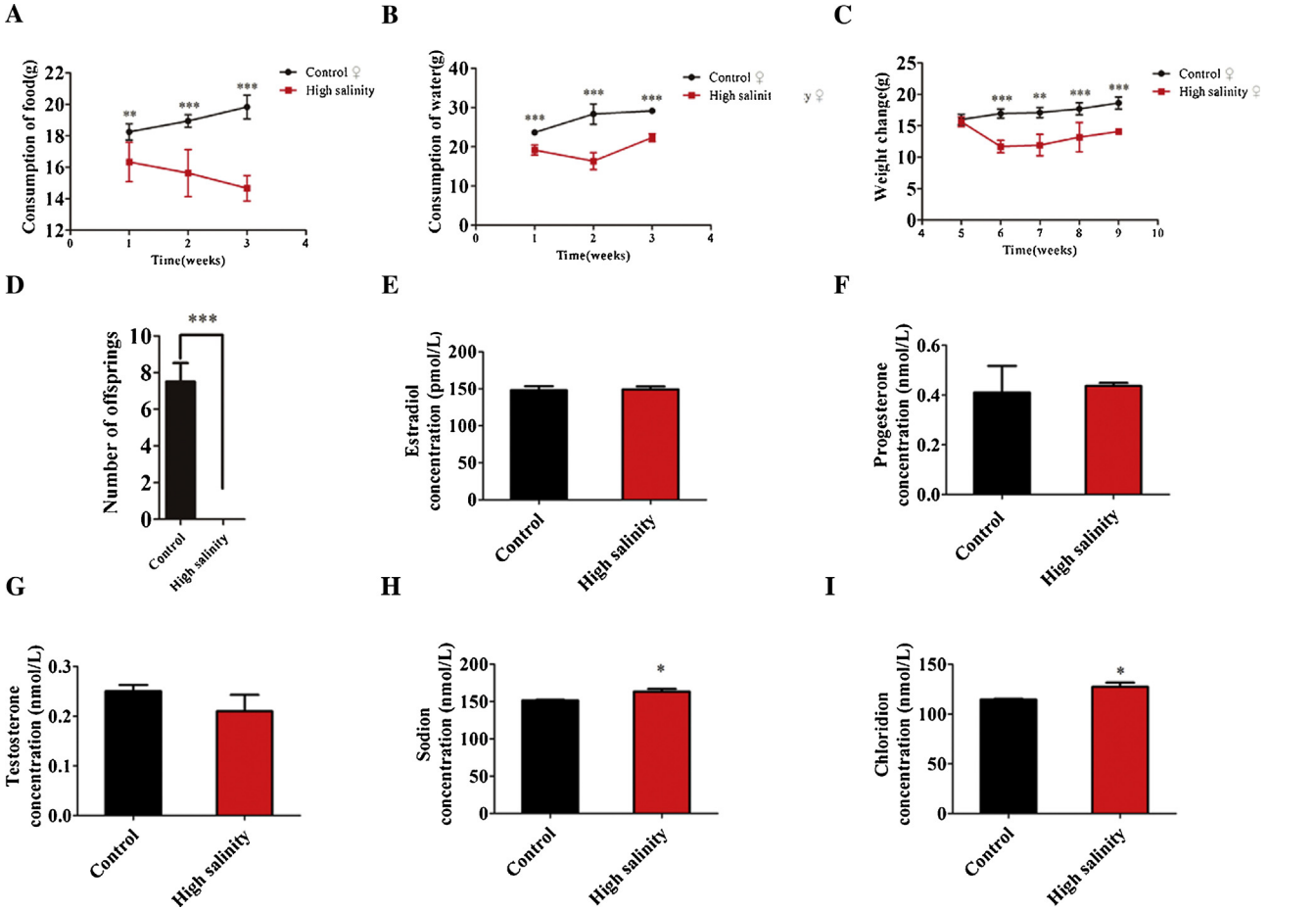

Fig. 1. Effects of high salinity on mice. Charts showing the effect of high salt intake on food consumption (A), water intake (B), and weight of mice (C). (D) Bar chart showing the number of offspring born to control and high salt treated mice. $N = 6$ mice in control group and $N = 8$ mice in high salinity group. (E–G) Bar charts showing the serum hormone levels (E is estradiol, F is progesterone, G is testosterone) in control and high salt treated mice. $N = 4$ mice in each group. (H,I) Bar charts showing the ion concentrations (H is Sodium, I is Chloride) in control and high salt treated mice. $N = 4$ mice in each group. $^{*}p < 0.05$ and $^{***}p < 0.001$ indicate significant differences between control and experimental animals (independent samples T test).