ELSEVIER

Archives of Medical Research 45 (2014) 294–300

Archives of Medical Research

ORIGINAL ARTICLE

Maternal Protein Restriction During Pregnancy and/or Lactation Negatively Affects Follicular Ovarian Development and Steroidogenesis in the Prepubertal Rat Offspring

Carolina Guzmán,[a] Rocío García-Becerra,[b] Marco Antonio Aguilar-Medina,[b] Isabel Méndez,[c] Horacio Merchant-Larios,[d] and Elena Zambrano[b]

[a]*HIPAM, Unidad de Medicina Experimental, Hospital General de México, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), México, D.F., México*

[b]*Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición SalvadorZubirán, México, D.F., México*

[c]*Instituto de Neurobiología, UNAM, Campus UNAM-Juriquilla, México*

[d]*Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, UNAM, México, D.F., México*

Received for publication November 12, 2013; accepted March 14, 2014 (ARCMED-D-13-00640).

Background and Aims. Maternal protein restriction during rat pregnancy and lactation is associated with alterations in reproductive function of female offspring including delayed onset of puberty, decreased fertility and premature reproductive aging. These alterations may be related to ovarian prepubertal development, distribution of follicle populations and their steroidogenic capacities. We undertook this study to evaluate the ovarian function of prepubertal female offspring exposed to maternal protein restriction during pregnancy and/or lactation.

Methods. Adult female Wistar rats were fed a control (C-20% casein diet) or restricted isocaloric diet (R-10% casein) during pregnancy—first letter—and lactation—second letter, to form four groups, CC, RR, CR, RC. Ovaries were collected from 21-day-old female offspring. Preantral and antral follicles were quantified and mRNA expression of key genes involved in follicular development and steroidogenesis (gonadotropin receptors, StAR, P450scc and P450 aromatase) was evaluated. Serum gonadotropin levels were measured.

Results. Significantly decreased numbers of preantral and antral follicles were observed in CR and RC ovaries compared with CC. LH levels were lower and FSH higher in CR pups. mRNA expression of LH receptor (LH-R) was decreased in RR in comparison with the other groups. CR and RC expressed higher StAR, RC increased and RR decreased P450scc, whereas RR and CR decreased aromatase expression in comparison with CC.

Conclusions. Maternal protein restriction influences prepubertal ovarian follicular number and steroidogenic function in the rat offspring, although RR and CR nutritional schemes have similar outcomes, the mechanisms affecting ovarian function are at different levels of the hypothalamic-pituitary-ovarian axis. © 2014 IMSS. Published by Elsevier Inc.

Key Words: Maternal protein restriction, Ovary, Prepubertal development, Developmental programming.

Introduction

Programming of reproductive function has been demonstrated in response to exposure to a suboptimal environment during fetal and postnatal development (1–7). Maternal

Address reprint requests to: Dr. Elena Zambrano, Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Tlalpan 14000, México, D.F., México. Phone: 52 55 5487 0900; E-mail: zamgon@unam.mx

0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2014.05.005

protein restriction during rat pregnancy alters key components of maternal steroid function increasing fetal exposure to estradiol, testosterone, progesterone and corticosterone (2,8). In the female offspring, onset of puberty and estrous cycles are delayed (1,8), fertility rates decreased (3,8), and reproductive lifespan shortened (8).

Nutritional challenges during early life have been associated with altered pubertal development. Maternal protein restriction during lactation in the rat delays vaginal opening and first estrous, independently of maternal nutrition during pregnancy (8). In contrast, females fed high fat from weaning have advanced onset of puberty (4,9). In humans, migration has allowed researchers to identify the developmental effects of environmental changes during early life. For example, Bangladeshi girls raised in the UK have higher salivary progesterone levels if they migrate earlier than menarche. When migration occurs at a later age, they show lower progesterone salivary levels and later pubertal maturation (10).

Sexual maturation and fertility depend on an adequate ovarian function, which is based on the availability of a pool of follicles for recruitment and ovulation (11) as well as an appropriate hormonal environment. In rats, follicular development begins in neonatal life when follicular structures are established in numbers that will progressively decrease through the reproductive lifespan (11,12).

Ovarian follicles contain an oocyte surrounded by follicular cells. The numbers of follicular cell layers depends on the stage of follicular development (11,13,14). Follicles are classified according to the layers of granulosa cells as well as presence or absence of the antral cavity (15). In the rat, well-developed antral follicles are present by post natal day 15−17 (12).

Ovarian steroidogenesis starts before puberty. Prepubertal estradiol levels regulate the establishment of pulsatile LH secretion (16). Both gonadotropins, LH and FSH, regulate expression of their receptors and the enzymes involved in sex steroid synthesis (17).

Antral follicles express LH and FSH receptors and are capable of aromatization (14). Most of the prepubertal antral follicles undergo atresia, only a few reach the preovulatory stage under gonadotropin stimulation (11,14,18,19). FSH prevents programmed demise in early antral follicles (14).

We hypothesized that assessment of prepubertal ovarian development and function could provide an early indicator of programming of sexual development due by maternal protein restriction during pregnancy and/or lactation. We assessed the number of the ovarian preantral and antral follicles in ovaries of 21-day-old offspring exposed to maternal protein restriction during pregnancy and/or lactation. Expression of ovarian proteins involved in follicular development and steroidogenesis (LH receptor (LH-R), FSH receptor (FSH-R), steroidogenic acute regulator (StAR), P450 side chain cleavage (P450scc) and P450 aromatase), as well as LH and FSH serum levels were measured.

Materials and Methods

Care of Animals

Details of protein restriction and generation of pups have been published previously (2). Briefly, 3-month-old virgin female albino Wistar rats (240−260 g) were obtained from the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (Mexico City, Mexico) and maintained under controlled lighting and temperature (lights on 07:00 h to 19:00 h at 22−23°C). All procedures were approved by the Institutional Animal Experimentation Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City and are in accordance with Mexican and international laws for laboratory animal care.

Rats were mated overnight with proven male breeders and the day spermatozoa were present in the vaginal smear was designated day of conception, day 0. Only rats becoming pregnant within 5 days of mating were studied. Pregnant rats were transferred to individual cages and allocated at random to one of two groups fed either 20% casein (control diet−C) or 10% casein isocaloric diet (restricted diet−R) (2). Food was provided as large flat biscuits retained behind a grill through which rats nibbled the food. Food and water were available ad libitum.

Vaginal delivery occurred spontaneously in the early daylight hours between 09:00 and 12:00 h on postconceptual day 22. Day of delivery was considered as day 0 of postnatal life. Timing of delivery, litter size and pup weight were recorded at birth. Anogenital distance was assessed to determine pup sex using our previous criteria (8). Sex was judged according to whether anogenital distance was lower (female) or larger (male) than 2.5 mm. To ensure homogeneity of study subjects, litters over 14 pups were not included. Litters of 12−14 pups were adjusted to 12 pups for each dam, maintaining as close to a 1:1 sex ratio as possible. Four groups were established CC, RR, CR and RC. First letter refers to maternal diet in pregnancy and second diet in lactation−C control, R restricted. We report data on female offspring only.

At 21 days of postnatal life, one female pup per litter was rapidly euthanized by decapitation by experienced personnel trained in the use of the rodent guillotine (Thomas Scientific, Swedesboro, NJ), serum and ovaries were collected. Right ovaries were stored at −80°C until assayed. Left ovaries were fixed in 4% paraformaldehyde-PBS (pH 7.4) and embedded in paraffin. Trunk blood was collected and allowed to clot at 4°C. Serum was obtained by centrifugation at 3500 g for 15 min at 4°C and stored at −20°C until assayed.

Follicular Count

Ten micrometer serial sections were obtained from the complete ovary and stained with hematoxylin-eosin. Each preantral and antral follicle was quantified throughout the