Basic nutritional investigation

Maternal malnutrition during lactation affects folliculogenesis, gonadotropins, and leptin receptors in adult rats

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Objective: The goal of this study was to evaluate if maternal malnutrition during lactation could possibly program folliculogenesis, the ovarian expression of gonadotropins, leptin, and their receptors.

Methods: At parturition, dams were randomly assigned to a control group (C), with free access to a standard laboratory diet containing 23% protein, and a protein–energy-restricted group (PER), with free access to an iso-energy and protein-restricted diet containing 8% protein. After weaning, all female pups had free access to the standard laboratory diet until 90 d of age when they were euthanized in the diestrum stage.

Results: Maternal malnutrition caused decreases in the number of primordial (C 6.60 ± 0.24, PER 5.20 ± 0.20, P = 0.01), primary (C 5.80 ± 0.66, PER 4.00 ± 0.31, P = 0.04), and Graafian (C 2.18 ± 0.29, PER 1.08 ± 0.37, P = 0.05) follicle numbers. Maternal malnutrition led to a significant decrease in the aromatase mRNA expression (C 0.536 ± 0.008, PER 0.353 ± 0.041, P = 0.01) follicle-stimulating hormone receptor (C 1.25 ± 0.17, PER 0.75 ± 0.02, P = 0.04), luteinizing hormone receptor (C 0.93 ± 0.09, PER 0.54 ± 0.10, P = 0.03), leptin (C 0.55 ± 0.03, PER 0.42 ± 0.03, P = 0.04), Ob-R (C 1.05 ± 0.12, PER 0.64 ± 0.07, P = 0.03), and Ob-Rb (C 1.34 ± 0.21, PER 0.47 ± 0.10, P = 0.02) transcripts when compared with C.

Conclusion: Maternal malnutrition during lactation modulates folliculogenesis and the expression of the different isoforms of leptin and gonadotropin receptors and the aromatase enzyme. This probably is a consequence of alterations in perinatal leptin concentrations that may play a crucial role in determining the occurrence of long-term metabolic changes.

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Introduction

In rapidly growing organisms malnutrition in early life is a serious challenge to which the system will try to adjust to survive. The quantity or quality of nutrition at these critical periods has permanent consequences for later life. One of the mechanisms to adapt to an inadequate supply of nutrients is slowing down the rate of cell division in tissues and organs, which may lead to an altered “programming” of the structure and function of the system [1,2]. Malnutrition induced in early life is associated with an increased risk to develop type 2 diabetes, hypertension, and cardiovascular disease in the long term [3–6].

Follicular development begins during fetal life with the transformation of primordial germ cells into oocytes and their enclosure in structures called follicles. In most mammals, primordial follicles form before or in the first few days after birth. Primordial follicles give rise to primary follicles that transform into preantral, then antral follicles, and finally preovulatory Graafian follicles, in a co-ordinated series of transitions regulated by hormones, such as follicle-stimulating hormone (FSH) and local intraovarian factors, such as leptin and others. With the luteinizing hormone (LH) surge, Graafian follicles rupture and oocytes are released, leaving the follicular cells to luteinize and form a corpus luteum.

The gonadotropins, LH and FSH, act by binding to and activating their specific receptors, luteinizing hormone receptor (LHR) and follicle-stimulating hormone receptor (FSHR) [7–9].
Several local growth factors, insulin, and others.

These include specific nuclear hormone receptors as progesterone receptor, leptin, several local growth factors, insulin, and others.

Leptin, the product of the obese (ob) gene, secreted by adipose tissue, plays a role in the regulation of body weight and food intake. Leptin is synthesized in many other organs, including the ovary, and is involved in the control of reproductive function. Leptin exerts its effects by the leptin receptor (Ob-R), which is a transmembrane receptor found in many tissues, such as the hypothalamus, kidney, and many cells of the ovary, including thecal cells, granulosa cells, and oocytes. There are six known splice variants of the leptin receptor, all with the same extracellular domain, but with differing intracellular domains (Ob-Ra to Ob-Rf).

Leptin is essential in maintaining normal reproductive function, as mice deficient in leptin (ob/ob) are not only obese, but are also infertile. In addition to the effects on the hypothalamic–pituitary axis, some negative and positive actions are reported: acute administration of leptin to immature gonadotrophin-primed rats inhibits ovulation, and leptin accelerates the onset of puberty in rodents.

Several lines of evidence indicate that the leptin receptors are regulated by, and respond to, changes in circulating steroid hormones, leptin concentrations, and gonadotropins. Duggal et al. showed that ovarian leptin receptor expression vary throughout the estrous cycle in rats in response to the changing environment of the ovary. It was also shown that Ob-Ra and Ob-Rb expression patterns were similar and the maximum values were reached at diestrum stage I.

The maternal nutritional state during lactation is equivalent and possibly even more important than that during gestation, as evidenced by a study from Leonhardt et al. that showed that the offspring whose dams were malnourished during lactation had more drastic consequences on gonadal development when compared with the offspring whose dams were malnourished only during pregnancy or during pregnancy and lactation. Guzman et al. showed similar results. Based on those studies we decided to analyze the effects of malnutrition only during the lactation time.

The goal of this study was to evaluate if protein–energy malnutrition during lactation could possibly program folliculogenesis, the ovarian expression of gonadotropin receptors, and the different isoforms of leptin receptors of adult offspring.

**Materials and methods**

**Animals**

Wistar rats were kept in a room with controlled temperature (25 ± 1 °C) and an artificial dark–light cycle (lights on from 0700 to 1900 h). Virgin female rats of 3 mo of age were caged with one male rat at a proportion of 2:1. After mating, determined by the presence of a vaginal plug, each female rat was placed in an individual cage with free access to water and food until delivery. The handling of the animals was approved by the animal care and use committee of the Biology Institute of State University of Rio de Janeiro, which based their analysis on the Guide for the Care and Use of Laboratory Animals, and the study design was approved by the local ethical committee for the care and use of laboratory animals.

**Experimental design**

After delivery, six pregnant Wistar rats were separated into two groups: the control (C) group had free access to a standard laboratory diet containing (in grams per 100 g) 23 protein, 66 carbohydrate, and 11 fat and 17 038.7 total energy (kJ/kg), and the protein–energy–restricted (PER) group had free access to an iso-energy and protein-restricted diet containing 8% protein. The PER group, despite having free access to the diet, consumed about 60% of that consumed by the C group. The protein-restricted diet was prepared at our laboratory by using the control diet (Nuvilab–Nuvital Ltda., Paraná, Brazil), with the replacement of part of its protein content with cornstarch. The amount of the latter was calculated to replace the same energy content of the control diet. Vitamins and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93 G recommendation for rodent diets.

Within 24 h of birth, excess pups were removed so that only six female pups were kept per dam, because it has been shown that this procedure maximizes lactation performance. Malnutrition of the studied rats was started at birth, which was defined as day 0 of lactation and was ended at weaning (day 21). After weaning, female pups of the same treatment group were housed in groups of three animals per cage and given unlimited access to food and water until 90 d of age. Cyclic stages of the ovaries were studied by daily vaginal smears after vaginal opening until day 90. Then, only the animals in the diestrum stage were euthanized with a lethal dose of pentobarbital. To evaluate the nutritional state, food consumption of the offspring was monitored every day from weaning onward, and body weight and linear growth (nose to tail) were monitored every 5 d from birth until the end of experiment. The blood was collected by cardiac puncture and the serum kept at −20 °C for subsequent determination of hormonal parameters. Ovaries were excised and dissected. The right ovary was kept at −80 °C for subsequent measurements of FSHR, LHR, leptin receptor isoforms (Ob-R, Ob-Ra and Ob-Rb), leptin and aromatase transcripts by reverse transcriptase–polymerase chain reaction (RT-PCR). The left ovary was embedded in paraffin, sectioned at 5 μm thickness, and processed by routine histologic analyses.

**Morphologic classification of follicles**

Five ovaries randomly chosen from three different dams were processed and stained with hematoxylin and eosin for histologic examination of ovarian follicles as described previously.

**RNA extractions**

Total RNA from nine ovary tissues randomly chosen from three different dams were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. The quality of RNA samples was verified by determination of the ratio 260 nm/280 nm and by electrophoresis on a 1% agarose gel. The samples were treated to remove DNA contamination by using DNA-free reagents (Invitrogen) according to the manufacturer’s protocol. To quantify glyceraldehyde-3-phosphate dehydrogenase, FSHR, LHR, aromatase, Ob-R, Ob-Ra, and Ob-Rb transcripts, we determined the optimal number of amplification cycles for each gene. The applied PCR primers and the cycle profiles used are listed in Table 1. All amplified cDNA fragments were run on a 1.5% agarose gel stained with ethidium bromide, visualized under ultraviolet transillumination, and analyzed with Scion Image software (http://www.meyerinst.com/html/scion/scion_image_windows.htm).

**Semi-quantitative RT-PCR**

All RNA samples were rid of contaminating DNA by using DNA-free reagents (Invitrogen) according to the manufacturer’s protocol. To quantify glyceraldehyde-3-phosphate dehydrogenase, FSHR, LHR, aromatase, Ob-R, Ob-Ra, and Ob-Rb transcripts, we determined the optimal number of amplification cycles for each gene. The applied PCR primers and the cycle profiles used are listed in Table 1. All amplified cDNA fragments were run on a 1.5% agarose gel stained with ethidium bromide, visualized under ultraviolet transillumination, and analyzed with Scion Image software (http://www.meyerinst.com/html/scion/scion_image_windows.htm).

**Steroid determinations**

The estradiol, testosterone, and leptin serum concentrations were determined using a specific radioimmunoassay for each hormone (estradiol and testosterone from ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA; leptin from Linco Research, St. Charles, MO, USA). The intra- and interassay variation coefficients were 4.6% and 7.5% for testosterone, 6.4% and 5.9% for estradiol, and 2.4% and 4.8% for leptin. Sensitivity of the radioimmunoassay was 0.03 ng/mL for testosterone, 7.4 pg/mL for estradiol, and 0.5 ng/mL for leptin.

**Statistical analysis**

Statistical analysis was performed by Student’s t test. All results are means ± standard errors of the mean. P < 0.05 was considered statistically significant.
Table 1
Oligonucleotide sequences used for amplification of reverse transcriptase–polymerase chain reactions and cycling conditions for the different sets of pairs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5′-3′)</th>
<th>Cycle profile</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>5′-ACACAGCTCCATGGCCATCAG-3′, 5′-TCCACACCGTCCTGCT-3′</td>
<td>94°C 3 min, 94°C 30 s, 58°C 2 min, 72°C 2 min</td>
<td>30</td>
</tr>
<tr>
<td>Aromatase</td>
<td>5′-GCTTCGACTGCAGCAGTCCGG-3′, 5′-CAAGGTGAATATCATTGGGCGTGG-3′</td>
<td>94°C 5 min, 95°C 30 s, 60°C 30 s, 72°C 45 s, 72°C 15 min</td>
<td>33</td>
</tr>
<tr>
<td>FSHR</td>
<td>5′-CTCATAACGCGGGACACACAG-3′, 5′-GCAAGGATGTCCTGCCACAG-3′</td>
<td>94°C 2 min, 94°C 1 min, 60°C 50 s, 72°C 2 min</td>
<td>36</td>
</tr>
<tr>
<td>LHR</td>
<td>5′-ATGCCCACTTCATCTCCAC-3′, 5′-TTGATGGCAACAGTGAGA-3′</td>
<td>94°C 2 min, 94°C 1 min, 60°C 50 s, 72°C 2 min</td>
<td>33</td>
</tr>
<tr>
<td>Ob-R</td>
<td>5′-CTTCCGGACTACAGCCAGAACA-3′, 5′-TGGATGGCTGCCACACAA-3′</td>
<td>97°C 5 min, 96°C 1.5 min, 55°C 1.5 min, 72°C 3 min, 72°C 15 min</td>
<td>33</td>
</tr>
<tr>
<td>Ob-Ra</td>
<td>5′-CTAAAGTGATGACGGCACTTA-3′, 5′-GAGAAGGCTACGAGCAAA-3′</td>
<td>97°C 5 min, 96°C 1.5 min, 55°C 1.5 min, 72°C 3 min, 72°C 15 min</td>
<td>33</td>
</tr>
<tr>
<td>Ob-Rb</td>
<td>5′-TGGCATGATGACTTGCAGA-3′, 5′-CCAGAAAGAGTGACAAATAA-3′</td>
<td>97°C 5 min, 96°C 1.5 min, 55°C 1.5 min, 72°C 3 min, 72°C 15 min</td>
<td>33</td>
</tr>
<tr>
<td>Leptin</td>
<td>5′-GACATTTCACACACAGCAGTC-3′, 5′-GAGGAGTCTCAGGAGAT-3′</td>
<td>94°C 2 min, 94°C 1.3 min, 55°C 1.3 min, 72°C 1.3 min, 72°C 1.5 min</td>
<td>36</td>
</tr>
</tbody>
</table>

FSHR, follicle-stimulating hormone receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LHR, luteinizing hormone receptor

Results

Food consumption and body weight

Figure 1 shows food consumption and body weight of the groups throughout the experiment. Food consumption of the offspring was evaluated from weaning until sacrifice. The PER group had a lower food intake than the C group (P < 0.001) from weaning until 50 d of age (Fig. 1A). Overall, at each time point of measurement from day 4 after birth until 50 d of age, body weight in the PER group was significantly lower when compared with controls (P < 0.001; Fig. 1B). After this time there was no difference in food intake and body weight between groups.

Morphometry

The mean numbers of ovarian follicles per section classified by developmental stage are shown in Figure 2. The offspring whose dams were submitted to protein–energy-restricted diets during lactation presented a reduction in the number of all ovarian follicles, but this reduction was significant only in the primordial (C 6.60 ± 0.24, PER 5.20 ± 0.20, P = 0.01), primary (C 5.80 ± 0.66, PER 4.00 ± 0.31, P = 0.04), and Graafian (C 2.18 ± 0.29, PER 1.08 ± 0.37, P = 0.05) follicle numbers. Figure 3 shows photomicrographs of ovarian sections from female rats at 90 d of age.

Hormone concentrations

Serum testosterone concentrations were below the limit of sensitivity of the assay and therefore could not be measured. Estradiol (C 128.2 ± 11.49, PER 114.4 ± 12.79) and leptin (C 1.98 ± 0.18, PER 2.25 ± 0.15) concentrations did not differ significantly between the two groups (Table 2).

FSHR, LHR, and aromatase expression

Feeding of the maternal protein–energy-restricted diet led to significant decreases in the aromatase enzyme (C 0.536 ± 0.008, PER 0.353 ± 0.041, P = 0.01), FSHR (C 1.25 ± 0.17, PER 0.75 ± 0.02, P = 0.04), and LHR mRNA expression (C 0.93 ± 0.09, PER 0.54 ± 0.10, P = 0.03; Fig. 4).

Leptin and leptin isoform receptor expressions

Feeding of the maternal protein–energy-restricted diet led to significant decreases in leptin (C 0.55 ± 0.03, PER 0.42 ± 0.03, P = 0.04), Ob-R (C 1.05 ± 0.12, PER 0.64 ± 0.07, P = 0.03), and Ob-Rb mRNA expression (C 1.34 ± 0.21, PER 0.47 ± 0.10, P = 0.02). Despite the decrement in Ob-R mRNA expression, the difference was not significant (C 0.83 ± 0.26, PER 0.36 ± 0.07, P = 0.09; Fig. 5).
Discussion

Maternal protein malnutrition during lactation decreases the ovarian expression of FSHR, LHR, aromatase, leptin, Ob-R, and Ob-Rb. The follicular development of malnourished animals shows smaller numbers of primordial, primary, and Graafian follicles. This study is in agreement with others that provided further evidence that early malnutrition can program the function of several systems [4,28,34,35].

The dormant primordial follicles are recruited into the growing follicle pool in a continuous manner, a stage termed initial recruitment. During initial recruitment, intraovarian and/or other unknown factors stimulate a cohort of primordial follicles to initiate growth, whereas the rest of the follicles remain quiescent for months or years. Initial recruitment is believed to be a continuous process that starts just after follicle formation, long before pubertal onset. In contrast, cyclic recruitment starts after pubertal onset and is the result of the increase in circulating FSH during each reproductive cycle that rescues a cohort of antral follicles from atresia.

In rodents, the primordial follicles are formed by 3 d of age, and the first wave of follicles develops into antral follicles over the next 3 wk [36–38]. In agreement with the literature [39], we showed that in this period, the PER group presents significant alterations in body weight. Also, the literature shows that thyroid function and milk composition are altered at this time [39,40]. Thus, it is possible that the decrease observed in the number of primordial follicles could result from a direct action of malnutrition in the ovary of the pups in the first days of life when primordial follicles are being formed.

Although the exact mechanisms for the initial recruitment of follicles from the dormant primordial follicle pool are still unclear, FSH has been shown to be stimulated by estrogen and upregulates FSHR [41,42], resulting in a stimulus of growth and differentiation of primary and/or secondary follicles [43]. Then, the low expression of FSHR in the ovary of rats whose mothers were malnourished during lactation could also explain the decrease observed in the primary and preantral follicle numbers.

Fig. 2. Mean number per ovarian section of primordial follicles (A), primary follicles (B), preantral follicles (C), small antral follicles (D), Graafian follicles (E), and corpus luteum (F) in the C and PER groups at 90 d old. The total number of sections analyzed was 15–20 per ovary. Values are given as mean ± SEM of five animals obtained from three different dams. * P < 0.05 versus C. C, control; PER, protein–energy restricted.

Follicle-stimulating hormone is undoubtedly the primary stimulus for the cyclic recruitment [44]. LH is also important in promoting major changes in ovarian preovulatory follicles, including terminal differentiation of follicular cells and oocyte maturation. These events are required for ovulation of
a fertilizable egg [12]. Findings from the present study suggest that low expressions of FSHR and LHR in the ovary after maternal malnutrition during lactation could explain the reduction in the Graafian follicle and corpus luteum numbers. The low levels of FSH and LH in malnourished animals at 70 d old described in the literature [28] could also explain the decrease in the folliculogenesis.

As the result of follicle exhaustion, evidenced by the small number of all ovarian follicles, especially the primordial follicles, an earlier onset of senescence could be expected and is probably responsible for the decrease in the fertility rate showed in the 1-y-old rats whose mothers were malnourished during pregnancy and/or lactation [28].

Regulated production of estrogens by the ovary is essential in follicular development, ovulation, and luteal function. The protein-restricted diet given to dams during the lactation period resulted in low aromatase transcript levels that were not related to the estrogens levels. Ovaries are the primary source of estrogen, but it is known that extragonadal tissues, such as adipose tissue obtained from the subcutaneous abdominal wall, liver, adrenal glands [45,46], bone [45,46], and skin [45,46], are capable of producing estrogen by aromatization from androgens in normal and pathologic conditions [47], especially when the ovary production is reduced [45,46]. Therefore, we can suggest that an extragonadal aromatization could be important to keep the estradiol normal values in the PER group.

Fig. 3. Photomicrographs showing examples of ovarian sections from female rats at 90 d of age in the C and PER groups. (A) Primordial follicles (1) and primary follicles (2). (B) Preantral follicles (3) and antral follicles (4). (C) Graafian follicles (5) and corpus luteum (6). Magnifications 400× in A, 100× in B, 40× in C; scale bars 100 μm. C, control; PER, protein–energy restricted.
The aromatase activity in granulosa cells is stimulated mainly by FSH [48]. So, it is possible that the low expression of FSHR resulted in low aromatase expression. To our knowledge this is the first time that a reduction in FHSR, LHR, and aromatase expressions has been shown in the ovary of adult offspring after maternal malnutrition during lactation and can be related to alterations in the reproductive function of those animals.

Because the ovarian synthesis of leptin, acting by its receptors, is important to ovulation, oocyte maturation, and steroidogenesis, the reduction of leptin and its receptors mRNA expression could have contributed, together with the low expression of FSHR and LHR, to the decrease observed in the folliculogenesis and aromatase expression in the ovary of rats whose mothers were malnourished during lactation.

Leptin is essential in maintaining normal reproductive function. In this report we show that the adult animals whose dams were malnourished during lactation present unchanged food intake and body weight, which explains the normal serum leptin levels. Similar results have been shown in male adult animals whose mothers were subjected to the same experimental model [34].

Several lines of evidence indicate that the leptin receptors are regulated by, and respond to, changes in circulating steroid hormones [23,25,26], leptin concentrations [14,49], and gonadotropins [14]. Considering the regulation of leptin receptors by all these hormones, we can assume that the low expression of gonadotropin receptors seen in the adult offspring whose mothers were subjected to maternal malnutrition during lactation could have affected the expression of the different isoforms of leptin receptors.

Previously we reported that maternal malnutrition during lactation delayed the beginning of puberty [50]. We also showed that, around puberty, the malnourished animals presented an altered folliculogenesis characterized by a larger number of primary, preantral, and small antral follicles and a smaller number of Graafian follicles, corpus luteum, and primordial follicles. This alteration seems to be related to the high ovarian expression of gonadotropins, androgen, and estrogen isoform receptors [32]. However, the present results showed that in early adulthood, there is a reduction of all ovarian follicle number, especially in the primordial, primary, and Graafian follicles. This alteration seems to be related to the low ovarian expression of FSHR, LHR, leptin, and leptin receptor genes. We can hypothesize that the malnourished animals are immature compared with control animals and that the stimulatory effect observed at folliculogenesis around puberty could be a consequence of the release of gonadotropin-releasing hormone and of LH and FSH that are important factors to unchain puberty [51,52]. In early adulthood, the transitory peak in gonadotropin-releasing hormone release has ended and the negative effect of malnutrition on folliculogenesis becomes evident. It seems to be in agreement with Guzman et al. [28] who showed that 1-y-old rats whose mothers were

![Fig. 4. Expression of FSHR, LHR, and aromatase genes in ovaries of the C and PER groups. After reverse transcriptase–polymerase chain reactions, the amplified fragments were run on a 1.5% agarose gel and visualized by ultraviolet transillumination. (A) Graphic representation of the data. (B) Representative ethidium bromide–stained gel depicting products for expression of GAPDH, FSHR, LHR, and aromatase genes in ovaries. The ratios between the signals intensities (AU) of GAPDH, FSHR, LHR, and aromatase genes are represented as means ± SEMs of nine animals obtained from three different dams. *P < 0.05 compared with C. AU, arbitrary units; C, control; FSHR, follicle-stimulating hormone receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LHR, luteinizing hormone receptor; PER, protein–energy restricted.](image-url)

### Table 2

<table>
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<tr>
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<th>PER</th>
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<tbody>
<tr>
<td>Testosterone (ng/mL)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>128.2 ± 11.49</td>
<td>114.4 ± 12.79</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>1.98 ± 0.18</td>
<td>2.25 ± 0.15</td>
</tr>
</tbody>
</table>

C, control; ND, not detected; PER, protein–energy restricted

* All measurements reported are at the time of tissue collection. Values are means ± SEMs per animal (n = 7/group).
malnourished during pregnancy and/or lactation present a decrease fertility rate [28].

Data from epidemiologic and in vivo animal studies have given rise to the concept of developmental programming whereby the quantity or quality of nutrition at the perinatal periods has permanent consequences for later life [4,28,34]. Maternal malnutrition during the perinatal period is associated with reduced circulating concentrations of leptin in the first few days of life [34], at weaning [27,53], and in adult life [54]. Previous studies have suggested that leptin concentration during the neonatal period is critical in determining the structure and function of body tissues and the homeostatic mechanisms in adulthood, such as food intake and body weight [34,55], thyroid function [56], leptin resistance [57], and adrenal medullary function [58]. Vickers et al. [59] showed that several metabolic consequences of maternal malnutrition were reversed by neonatal leptin treatment in female rats and that alterations in perinatal leptin levels may play a crucial role in determining the occurrence of long-term metabolic changes.

In conclusion, maternal malnutrition during lactation modulates folliculogenesis and the expression of FSHR, LHR, aromatase, and leptin receptors probably as a consequence of alterations in perinatal leptin levels that may play a crucial role in determining the occurrence of long-term metabolic changes.

References

Fig. 5. Expression of Ob-R, Ob-Ra, Ob-Rb, and leptin genes in ovaries of the C and PER groups. After reverse transcriptase–polymerase chain reactions, the amplified fragments were run on a 2% agarose gel and visualized by ultraviolet transillumination. (A) Graphic representation of the data. (B) Representative ethidium bromide–stained gel depicting products for expression of GAPDH, Ob-R, Ob-Ra, Ob-Rb, and leptin genes in ovaries. The ratios between the signal intensities (AU) of GAPDH, Ob-R, Ob-Ra, Ob-Rb and leptin are represented as means ± SEMs of nine animals obtained from three different dams. * P < 0.05 compared with C. AU, arbitrary units; C, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PER, protein–energy restricted.


