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The pups' endometrium morphology is affected by maternal malnutrition during suckling

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Abstract

Objectives: This study aims to determine the effects of maternal protein and energy malnutrition during lactation on the endometrial structures of the offspring at puberty.
Methods: At parturition, dams were randomly assigned to the following groups: control group (C), with free access to a standard laboratory diet containing 23% protein; protein-restricted (PR) group, with free access to an isoenergy and protein-restricted diet containing 8% protein; and energy-restricted (ER) group, receiving standard laboratory diet in restricted quantities. After weaning, all female pups had free access to standard laboratory diet. At puberty, the animals were sacrificed with pentobarbital and only females on the diestrum stage were used for the analyses. The stereological method used for quantifying the uterine endometrium was the M42 test system.
Results: When compared to C group, both PR and ER groups presented a significant reduction in the length density of the glands (PR = 53%, ER = 35.7%, $p < 0.001$), in the volumetric density of the epithelium (PR = 49%, ER = 38%, $p < 0.001$) and lumen (PR = 42.7%, $p < 0.001$; ER = 23.8%, $p < 0.001$) and in the surface density of the inner (PR = 22%, ER = 13.8%, $p < 0.001$) and outer (PR = 55.4%, $p < 0.01$; ER = 40.6%, $p < 0.001$) glands. The volumetric density of the stroma was significantly higher in both PR (114%, $p < 0.001$) and ER (117%, $p < 0.001$) groups. In all parameters studied, there was no significant difference between PR and ER groups.
Conclusions: Our results show that the protein and energy restriction during lactation leads to an atrophy of the uterine endometrial glands of the offspring at puberty.

Keywords: Nutrition disorders; Lactation; Puberty; Endometrium; Morphology

1. Introduction

Malnutrition is the most prevalent form of nutritional disorder among children in developing countries. Onís et al. [1], based on World Health Organization data, reported that, about 43% of children in developing

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countries suffer from malnutrition during some period in their lives. Protein malnutrition often occurs during gestation, lactation, and first 2 years of life [2].

Some authors showed that the nutritional status of the mother during gestational and lactational periods is essential to the normal growth and development in humans [3–5] or in experimental animals [6–10]. Also, some studies have shown that gestation and/or lactation could be a critical period for the future progeny's nutritional and hormonal status [8,11–13], a relationship that has been termed metabolic imprinting. This term is defined by Waterland and Garza [14] as the basic biological phenomena that putatively underlie relations among nutritional experiences of early life and early diseases.

The offspring of rats submitted to malnutrition during lactation presents at weaning alterations in neural development [15], insulin secretion [16] and thyroid function [17]. It was showed either that mother's nutrition during lactation could determine the body weight [17] and thyroid function [9,10] of her offspring in the adult life. Thus, lactation could be a critical period in determining the future endocrine status of the progeny.

Some authors showed that pre-pubertal malnutrition changes the serum and pituitary hormonal levels [18] morphological characteristics of gonadotrophs and mammotrophs [18], number of ovarian follicles [19], estrogen and progesterone receptors in the uterus [20,21] and the uterus collagen synthesis [22].

Recently, we have demonstrated that the female offspring whose mothers were submitted to protein and energy malnutrition during lactation, and had free access to a normal diet after weaning, presented at puberty a reduction in the body weight gain, linear growth, ovaries and uterus weight. Also, the onset of puberty was delayed, in spite of a normal estrous cycle [23].

Therefore, in the present study, we aim to evaluate, through a stereologic method, the effects of maternal protein and energy malnutrition during lactation on the uterine endometrial glands and stroma of the female offspring at puberty.

2. Methods

2.1. Protocol

Wistar rats were kept in a room with controlled temperature ($25 \pm 1\,^{\circ}C$) and with artificial dark–light cycle (lights on from 7:00 am to 7:00 pm). Three-month-old, virgin female rats were caged with one male rat at a proportion of 2:1. After mating, each female was placed in an individual cage with free access to water and food until delivery. The use and handling of experimental animals followed the principles described in the Guide for the Care and Use of Laboratory Animals [24] and the project was approved by the local Ethical Committee for care and use of laboratory animals.

Three dams were randomly assigned to one of the following groups: control group (C), with free access to a standard laboratory diet containing 23% protein; protein-restricted (PR) group, with free access to an iso-energy and protein-restricted diet containing 8% protein; and energy-restricted (ER) group, receiving standard laboratory diet containing 23% protein in restricted quantities, which were calculated according to the mean ingestion of the PR group. The low-protein diet was prepared in our laboratory and its composition is showed in Table 1. Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets [25].

Table 1
Composition of control and protein-restricted diets

	Control[b]	Protein-rstricted[c]
Ingredients (g/kg)		
Total protein[a]	230.0	80.0
Corn starch	676.0	826.0
Soybean oil	50.0	50.0
Vitamin mix[d]	4.0	4.0
Mineral mix[d]	40.0	40.0
Macronutrient composition (%)		
Protein	23.0	8.0
Carbohydrate	66.0	81.0
Fat	11.0	11.0
Total energy (kJ/kg)	17038.7	17038.7

[a] The principal protein resources are soybean wheat, steak, fish and amino acids.
[b] Standard diet for rats (Nuvilab-Nuvital Ltd., Paraná, Brazil).
[c] The protein-restricted diet was prepared in our laboratory by using the control diet, with 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.
[d] Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets [25].

Within 24 h of birth, excess pups were removed so that only six female pups were kept per dam, as it has been shown that this procedure maximizes lactation performance [26]. Malnutrition of the studied rats was started at birth, which was defined as day 0 of lactation (d_0), and was ended at weaning (d_{21}). After weaning, the female pups of the same treatment group were housed in group of three animals per cage, and given unlimited access to food and water until puberty (day 40), when the animals were anesthetized and sacrificed with a lethal dose of pentobarbital.

To evaluate the nutritional state, we monitored the food consumption, body weight and linear growth (nose–tail) throughout the experiment (data not shown).

At the slaughtered, cyclic stages of the ovaries were studied by vaginal smears and only rats on the diestrum stage were used for the analyses. The uterus was carefully removed, weighed, and fragments were obtained according to the vertical sections method [27].

Uterine fragments were placed for 24 h at room temperature immersed in a solution of 4% formalin in phosphate-buffer 0.1 mol%, pH 7.2, embedded in paraffin, and sections were systematically random-sampled, 5 μm in thickness and stained with Gomori's trichrome [28]. The analyzed fields were digitized with 400× final magnification using a video camera coupled to a light microscope.

Line and point probes are obtained in a field by superimposing a grid consisting of an arrangement of lines and points [27,29,30]. The M42 multipurpose test system was used where only well-preserved structures not crossing the test system forbidden line were considered. The short-line length (d) was used to calibrate the test system, the line length L_t is 21d, the test area AT is 36.36d^2, and it has 42 test points (P_p). The stereological methods were described in detail elsewhere [27,29–31].

Only the endometrial compartment of the uterus was quantified in this study. From each uterus, five different sections were selected from five fragments. Then, five random fields were evaluated from each section. Therefore, there were 25 test areas from each uterus.

2.2. Stereological parameters

The stereological parameters analyzed were: volumetric density (V_v) of the endometrial stroma; (2) volumetric density (V_v) of the glandular epithelium; (3) volumetric density (V_v) of the glandular lumen ($V_v = P_p/P_t$ (%), where P_p is the number of test points in the structure and P_t is the number of total test points); (4) surface density (S_v) of the inner and outer glands ($S_v = 2I/L_t$ (mm^2/mm^3), where I are intersections of the inner and outer glandular surfaces with the test line and L_t is the length of test line); (5) length density (L_v) of the glands ($L_v = 2QA$ (mm/mm^3), where QA is the number of the glandular profiles in the test area).

2.3. Statistical analysis

The data was reported as mean ± standard deviation. Statistical significance of experimental observations was determined by the Kruskal–Wallis test followed by Dunn's multiple comparison test. The level of significance was set at $p < 0.05$.

3. Results

The volumetric density of the glands (lumen and epithelium) is shown in Fig. 1. All these parameters were significantly reduced in both malnourished groups ($p < 0.001$) compared to the C group, and there was no difference between the PR and ER groups.

The V_v of the epithelium was significantly lower in both PR (49%, $p < 0.001$) and ER (38%, $p < 0.001$) groups when compared to the control group (Fig. 1a).

The V_v of the lumen area was significantly lower in both PR (42.7%, $p < 0.001$) and ER (23.8%, $p < 0.001$) groups when compared to the control group (Fig. 1b).

The S_v of the inner glands was significantly lower in both PR (22%, $p < 0.001$) and ER (13.8%, $p < 0.001$) groups, when compared to the control group (Fig. 1c).

The S_v of the outer glands was significantly lower in both PR (55.4%, $p < 0.01$) and ER (40.6%, $p < 0.001$) groups when compared to the control group (Fig. 1d).

The volumetric density of the stroma was significantly higher in both PR (114%, $p < 0.001$) and ER (117%, $p < 0.001$) groups when compared to the C group (Fig. 2).

The length density of the glands was significantly lower in both PR (53%, $p < 0.001$) and ER (35.7%, $p < 0.001$) groups when compared to the C group (Fig. 3).

The histological sections of uterus are showed in the Fig. 4.

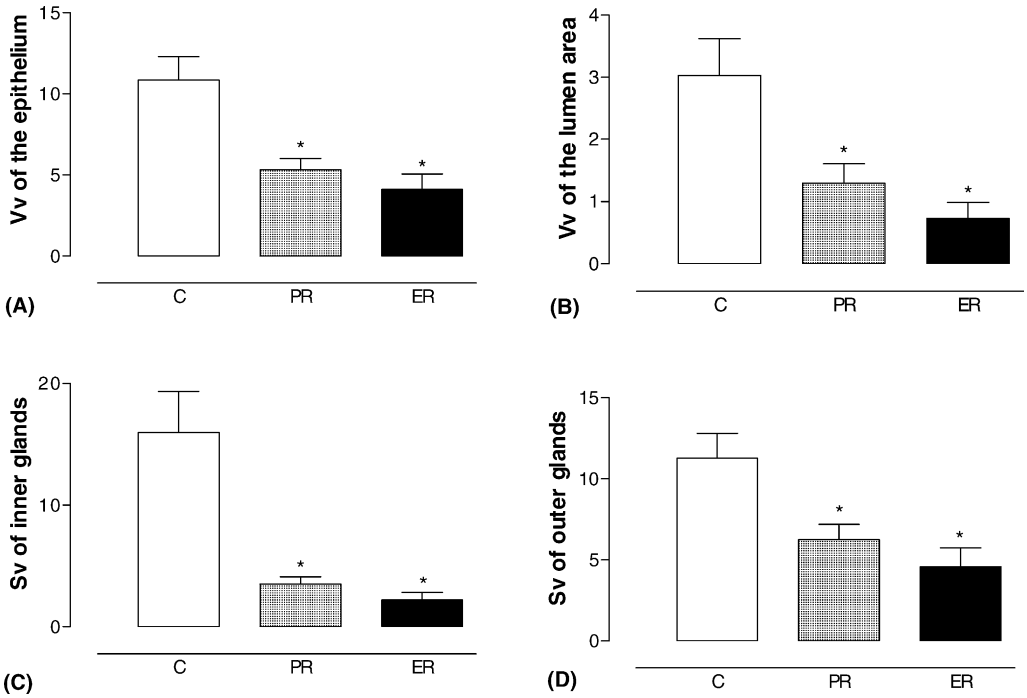

Fig. 1. Volumetric density, V_v, of the epithelium (A), V_v of the lumen area (B), surface density, S_v, of the inner glands (C) and S_v of the outer glands (D), in control group, C, protein-restricted group, PR, and energy-restricted group, ER. Values are given as mean ± S.D. of five animals. $^{*}p < 0.001$ compared with control group.

4. Discussion

The concept of programming associates physiological changes in adulthood with determined physiological changes in gestational or neonatal period [32]. Chronic diseases in the adulthood, as coronary insufficiency and the plurimetabolic syndrome (diabetes mellitus, hypertension and obesity) could be programmed in the initial stages of life [33]. Previous studies showed that maternal undernutrition during lactation causes alterations in the milk composition [8], body weight [17], thyroid function [9,10] and leptin serum concentration of pups at weaning [34]. When those animals reach the adulthood, the alterations in

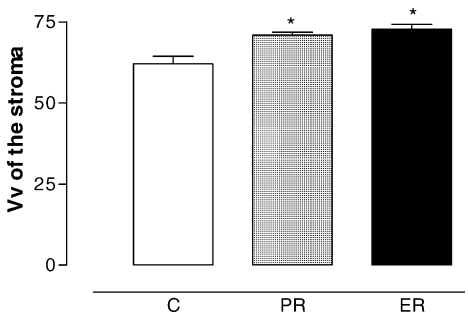

Fig. 2. Volumetric density, V_v, of the stroma of pups in control group, C, protein-restricted group, PR, and energy-restricted group, ER, at 40 days old. Values are given as mean ± S.D. of five animals. $^{*}p < 0.001$ compared with control group.

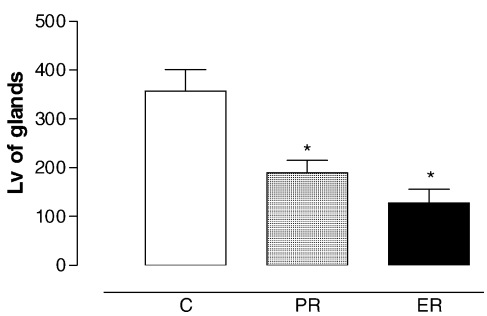

Fig. 3. Length density, L_v, of the glands of pups in control group, C, protein-restricted group, PR, and energy-restricted group, ER, at 40 days old. Values are given as mean ± S.D. of five animals. $^{*}p < 0.001$ compared with control group.

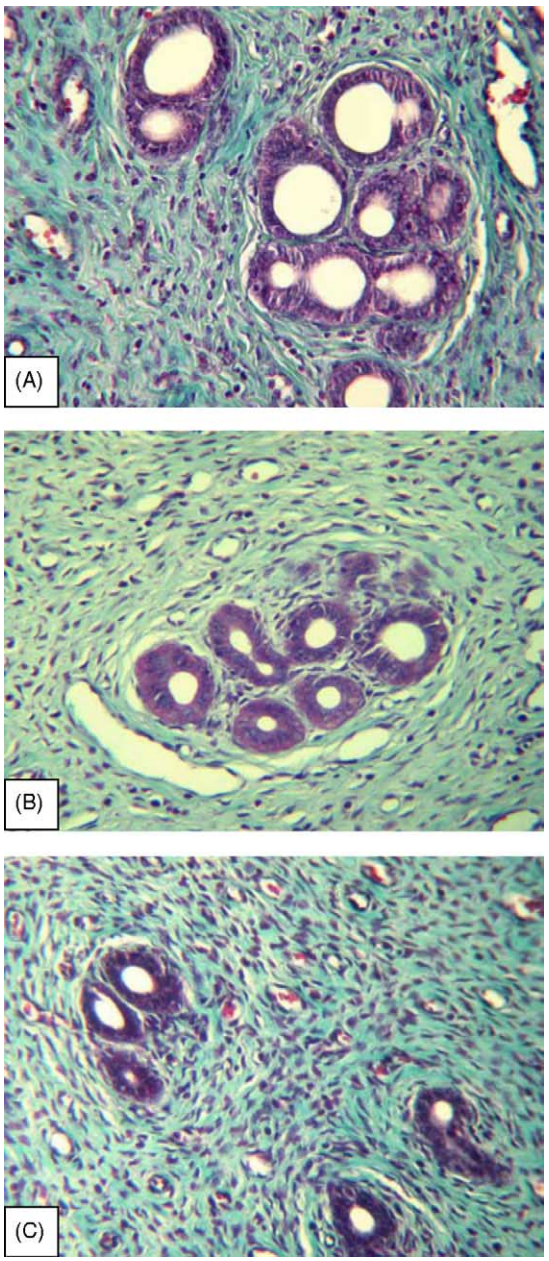

Fig. 4. Histological sections of uterus of control group (A), protein-restricted group (B) and energy-restricted group (C). The final magnification is 200×.

body weight and thyroid function are persistent [35], reinforcing the metabolic imprinting concept. Protein and energy restriction during lactation also leads to growth retardation, reduction in the ovaries and uterus weights and delayed on the puberty onset in pups [23].

The low gain in body weight observed in the undernourished female offspring could be due to lack of growth hormone (GH) of effects, since some authors described that food deprivation decreased GH-releasing hormone (GHRH) [36,37].

The morphometric results show that both groups on dietary restriction present a significant reduction in the number and lobular aspect of the endometrial glands, with a compensatory increment in the stroma, suggesting a glandular atrophy at puberty. As we know that endometrial development is dependent of gonadal hormones, lack of these hormones effects could result in endometrial atrophy.

The protein and energy malnutrition can alter the hypothalamo-hypophyseal-gonadal axis. It is clear that the GnRH pulse generator system and gonadotrophin release are inhibited by undernutrition [18,38–40] and that undernutrition causes morphological alterations in the mammotrophs and gonadotrophs that are typical of those cells whose secretory activities are suppressed [18,41]. Quantitative immunohistochemistry revealed a decrease in the number of gonadotrophs of undernourished adult monkeys that can be correlated with ultrastructural findings [37].

The delay on the onset of puberty observed in these animals, caused by maternal malnutrition [23], is probably due to an incomplete mature state of the hypothalamic-pituitary-gonadal axis. So, the low-hormonal stimulus could be insufficient to cause the normal development of the endometrial glands, and, probably, the uterus could not be mature enough to respond to the hormonal stimulus. This speculation could explain the atrophy of the endometrial glands.

Leptin, an adipocyte-secreted plasma hormone involved in the control of food intake and energy expenditure that plays a key role in body-weight homeostasis, has recently emerged as a pivotal signal in the regulation of fertility and in the control of pituitary gonadotrophins—LH and FSH [42]. It was related that energy restriction during lactation causes a drastic reduction in plasma leptin level in the weaned female offspring [43]. In vitro studies have shown that GH stimulates mitogenesis and amplifies the effects of FSH on the induction of LH receptors and on steroidogenesis by granulosa cells in numerous species [44]. So, the low leptin levels could contribute to alterations on

the hypothalamo-hypophyseal-gonadal axis, decreasing LH and FSH levels resulting in low estradiol serum levels.

Some authors showed that uterus' weight as well as estrogen and progesterone receptors are reduced in the uterus of protein and energy restricted pregnant and non-pregnant rats [20,21]. As these hormones are responsible for growing and development of endometrial glands, it is possible that lack of these receptors could lead to the reduction observed in the number and lobular aspect of endometrial glands in undernourished pups.

Although these authors have described that malnutrition leads to a state of hypofunction of the hypothalamic-pituitary-gonadal axis, the majority of them evaluated the effects of malnutrition in male animals. Besides, some authors described that in female rats, malnutrition during lactation time induces a drastic reduction of plasma leptin without affecting the circulating levels of LH, estradiol and progesterone [43]. Also, Abecia et al. [45] showed that the mean content of progesterone in the endometrium, the progesterone concentration in the ovarian vein, and the production of progesterone by corpus luteum are not affected by nutritional state. These data reinforce the hypothesis that the uterus is immature to respond to hormonal stimulus, which could explain the atrophy of the endometrial glands

Until this moment, we do not have knowledge on how malnutrition can affects the expression of estrogen, progesterone and androgen receptors in the uterus. Erα appears as the major regulator of estrogen function in the uterus and Erβ can modulate Erα activity in a response-specific and dose-dependent manner [46]. Estrogen increases progesterone receptor expression specifically in uterine stromal cells of the αERKO mouse, suggesting that Erβ may be capable of mediating some of estrogen effects in the uterus [47]. Although full response of the epithelium is dependent on an Erα-positive mesenchyme, stromal cell proliferation is independent of tissue Erα [48]. Also, diethylstilbestrol stimulates uterine progesterone expression in the glandular cell, but not in the luminal epithelial cells [49]. These results can probably explain our data of increase in volumetric density of stroma in face of reduction in glandular parameters.

Androgen can also be implicated in the uterine derangement responsiveness caused by maternal malnutrition during lactation since it was demonstrated that in the uterus, besides in other organs, there is nuclear staining for androgen receptors in glandular and luminal epithelial cells and in the stroma in which glands are embedded [50,51].

In conclusion, our results show that protein and energy restriction during lactation leads to an atrophy of the uterine endometrial glands in the offspring at puberty.

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References

[1] Onís M, Monteiro C, Glugston G. The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global database on child growth. Bull World Health Org 1993;71:703–12.
[2] Desai ID, Garcia Tavares ML, Dutra de Oliveira BS, Douglas A, Duarte FA, Dutra de Oliveira JE. Foods habits and nutritional status of agricultural migrant workers in Southern Brazil. Am J Clin Nutr 1980;33:702–4.
[3] Read WWC, Lutz PG, Tashjian A. Human milk lipids III. Short-term effects of dietary carbohydrate and fat. Am J Clin Nutr 1965;17:185–7.
[4] Phillips DI, Barker DJ, Osmond C. Infant feeding, fetal growth and adult thyroid function. Acta Endocrinol 1993;129(2):134–8.
[5] Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr 2000;71(5):1344S–52S.
[6] Naismith DJ, Richardson DP, Pritchard AE. The utilization of protein and energy during lactation in the rat, with particular regard to the use of fat accumulated in pregnancy. Brit J Nutr 1982;48:433–41.
[7] Ramos CF, Teixeira CV, Passos MCF, et al. Low-protein diet changes thyroid function in lactating rats. PSEBM 2000;224:256–63.
[8] Passos MCF, Ramos CF, Moura EG. Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. Nutr Res 2000;20:1603–12.
[9] Passos MCF, Ramos CF, Dutra SCP, Moura EG. Transfer of iodine through the milk in protein-restricted lactating rats. J Nutr Biochem 2001;12:300–3.
[10] Passos MCF, Ramos CF, Mouço T, Moura EG. Increase of T3 secreted through the milk in protein restricted lactating rats. Nutr Res 2001;21:917–24.

[11] Coleoni AH, Munaro N, Recupero AR, Cherubini O. Nuclear triiothyronine receptors and metabolic response in perinatally protein-deprived rats. Acta Endocrinol 1983;104:450–5.
[12] Jones AP, Simson EL, Friedman MI. Gestational undernutrition and the development of obesity in rats. J Nutr 1984;114:1484–92.
[13] Moura AS, Caldeira Filho JS, Mathias PC, Franco de Sá CCN. Insulin secretion impairment and insulin sensitivity improvement in adults rats undernourished during early lactation. Res Comm Mol Path Pharmacol 1997;96:180–92.
[14] Waterland RA, Garza C. Potencial mechanisms of metabolic imprinting that lead to chronic disease. Am J Clin Nutr 1999;69:179–97.
[15] Rocha-de-Melo AP, Guedes RCA. Spreading depression is facilitated in adult rats previously submitted to short episodes of malnutrition during the lactation period. Braz J Med Biol Res 1997;30:663–9.
[16] Moura AS, Carpinelli AR, Barbosa FB, Graverna C, Mathias PCF. Undernutrition during early lactation as and alternative model to study the onset of diabetes mellitus type II. Res Com Mol Pathol Pharmacol 1996;92:73–84.
[17] Ramos CF, Lima APS, Teixeira CV, Brito PD, Moura EG. Thyroid function in post-weaning rats whose dams were fed a low-protein diet during suckling. Braz J Med Biol Res 1997;30:133–7.
[18] Herbert DC. Morphology of the mammotrophs and gonadotrophs in the anterior pitutary gland of rats with protein-calorie malnutrition. Am J Anat 1980;158:521–31.
[19] Parshad RK. Effect of restriction in daily feeding periods on reproduction in the female rat. Acta Physiol Hung 1990;76:205–9.
[20] Singh RK, Roy RK. Studies on estrogen receptors in rat uterus during protein malnutrition and steroid-maintained pregnancy. J Nutr 1980;110(9):1745–53.
[21] Singh RK, Roy RK. Studies on progesterone receptors in rat uterus during protein malnutrition and steroid-maintained pregnancy in rat. Endocrinology 1981;77(1):13–22.
[22] Rao JS, Rao VH, Bose SM. Effect of protein malnutrition on the cross-linking of gingival and uterine collagen in rats. J Nutr 1983;113(8):1459–63.
[23] Faria TS, Ramos CF, Sampaio FJB. Puberty onset in the female offspring of rats submitted to protein or energy restricted diet during lactation. J Nut Biochem 2004;15:123–7.
[24] Bayne K. Revised Guide for the Care and Use of Laboratory Animals available. Am Physiol Soc 1996;38:208–11.
[25] Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76 rodent diet. J Nutr 1993;123:1939–51.
[26] Fishbeck KL, Rasmussen KM. Effect of repeated cycles on maternal nutritional status, lactational performance and litter growth in ad libitum-fed and chronically food-restricted rats. J Nutr 1987;117:1967–75.
[27] Mandarim-de-Lacerda CA. Stereological tools in biomedical research. Ann Acad Bras Ciênc 2003;75(4):469–86.
[28] Bradbury P, Rae K. Connective tissues and stains. In: Bancroft JD, Stevens A, editors. Theory and Practice of Histological Techniques. New York: Churchill Livingstone; 1996. p. 113–38.
[29] Weibel ER, Kistler GS, Scherle WF. Practical stereological methods for morphometric cytology. J Cell Biol 1966;30:23–38.
[30] Cruz-Orive LM, Weibel ER. Recent Stereological methods for cell biology: a brief survey. Am J Physiol 1990;258:148.
[31] Gundersen HJG, Bendtsen TF, Korbo L, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988;96:379–94.
[32] Lucas A. Programming by early nutrition: an experimental approach. J Nutr 1998;128:401S–6S.
[33] Barker DJP. In utero programming of cardiovascular disease. Theriogenology 2000;53:555–74.
[34] Teixeira CV, Passos MCF, Ramos CF, Dutra SP, Moura EG. Leptin serum concentration, food intake and body weight in rats whose mothers were exposed to malnutrition during lactation. J Nutr Biochem 2002;13:493–8.
[35] Passos MCF, Ramos CF, Dutra SCP, Bernardo-Filho M, Moura EG. Biodistribution of 99 mTc-O4Na changes in adult rats whose mothers were malnourished during lactation. J Nucl Med 2002;43:1–3.
[36] Brogan RS, Fife SK, Conley LK, Giustina A, Wehrenberg WB. Effects of food deprivation on the GH axis: immunocytochemical and molecular analysis. Neuroendocrinology 1997;65:129–35.
[37] Console GM, Jurado SB, Oyhenart E, Ferese C, Pucciarelli H, Gómez Dumm CLA. Morphometric and ultrastructural analysis of different pitutary cell populations in undernourished monkeys. Braz J Med Biol Res 2001;34:65–74.
[38] Glass AR, Mellitt R, Vigersky RA, Swerdloff RS. Hypoandrogenism and abnormal regulation of gonadotropin secretion in rats fed a low protein diet. Endocrinology 1979;104(2):438–42.
[39] Cameron JL. Regulation of reproductive hormone secretion in primates by short-term changes in nutrition. Rev Reprod 1996;1:117–26.
[40] Wade GN, Schneider JE, Li HY. Control of fertility by metabolic cues. Am J Physiol Endocrinol Metabol 1996;270(33):1–19.
[41] Heindel JJ, Berkowitz AS, Grotjan HE, Herbert DC, Klemckes HG, Bartke A. Pituitary and testicular function in the restricted rat. Int J Androl 1988;11:313–26.
[42] Rosenbaum M, Leibel RL. Leptin: a molecule integrating somatic energy stores, energy expenditure and fertility. Trends Endocrinol Metabol 1998;9:117–23.
[43] Léonhardt M, Lesage J, Croix D, Dutriez-Casteloot I, Beauvillain JC, Dupouy JP. Effects of perinatal maternal food restriction on pituitary-gonadal axis and plasma leptin level in rat pup at birth and weaning and on timing of puberty. Biol Reprod 2003;68:390–400.
[44] Booth PJ. Metabolic influences on hypothalamic-pituitary-ovarian function in the pig. J Reprod Fertil 1990;Suppl. 40:89–100.
[45] Abecia JA, Forcada F, Lozano JM. A preliminary reporto on the effect of dietary energy on prostaglandin F2 alpha production in vitro, interferon-tau synthesis by the conceptus, endometrial progesterone concentration on days 9 and 15 of pregnancy and associated rates of embryo wastage in ewes. Theriogenology 1999;52(7):1203–13.
[46] Frasor J, Barnett DH, Danes JM, Hess R, Parlow AF, Katzenellenbogen BS. Response-specific and ligand dose-dependent

modulation of estrogen receptor (ER) alpha activity by ERbeta in the uterus. Endocrinology 2003;144(7):3159–66.

[47] Kurita T, Lee K, Saunders PT, et al. Regulation of progesterone receptors and decidualization in uterine stroma of the estrogen receptor-α Knockout mouse. Biol Reprod 2001;64:272–83.

[48] Bigsby RM, Caperell-Grant A, Berry N, Nephew K, Lubahn D. Estrogen induces a systemic growth factor through an estrogen receptor-alpha-dependent mechanism. Biol Reprod 2004;70(1):178–83.

[49] Hughes CL, Liu G, Beall S, Foster WG, Davis V. Effects of genistein or soy milk during late gestation and lactation on adult uterine organization in the rat. Exp Biol Med 2004;229(1), 108–117.

[50] Pelletier G, Labrie C, Labrie F. Localization of oestrogen receptor α, oestrogen β and androgen receptors in the rat reproductive organs. J Endocrinol 2000;165:359–70.

[51] Hu YC, Wang PH, Yeh S, et al. Subfertility and defective folliculogenesis in female mice lacking androgen receptor. Proc Natl Acad Sci 2004;101(31):11209–14.