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Protein- and tryptophan-restricted diets induce changes in rat gonadal hormone levels

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Abstract

The release of gonadotrophic hormones starts at puberty and, along with the subsequent estral cyclicity, is subject to hormonal feedback systems and to the action of diverse neuroactive substances such as gamma amino butyric acid and catecholamines. This study shows the effect of the administration during 40 days of protein-restricted and corn-based (tryptophan- and lysine-deficient) diets on the serotonin concentration in medial hypothalamic fragments as well as in follicle-stimulating luteinizing hormones, 17-β-estradiol and progesterone serum levels, and estral cyclicity in 60- and 100-day-old rats (young, mature, and in gestation). In young rats, a delay in vaginal aperture development, and a lengthening of the estral cycle to a continuous anestral state was observed, mainly in the group fed corn. This group showed a 25% decrease in the serotonin concentration compared with the protein-restricted group, which exhibited an increase of 9% over the control group. Luteinizing hormone levels decreased in 16% and 13%, whereas follicle-stimulating hormone increased in 13% and 5% in the young animals of restricted groups, respectively, compared with the control group. Serum progesterone levels decreased only in young restricted versus control animals, and no differences were seen among adult and gestational rats. Serum levels of 17-β-estradiol in restricted animals showed different concentration patterns, mainly in the corn group, which was higher at the 20th gestational day, falling drastically postpartum. The results obtained in this study show serotonin to be a very important factor in the release of gonadotrophic hormones and the start of puberty. © 2001 Elsevier Science Inc. All rights reserved.

Key words: protein restriction; corn; serotonin; gonadotrophic hormones

1. Introduction

The effects of food restriction, mainly in young animals, has been manifested by alterations in reproductive function inducing a delay in the start of puberty, estral acyclicity, weight loss, and uterine atrophy [1–5]. These events are associated with a decrease in the levels and actions of anabolic hormones including growth hormone, growth factors similar to insulin-1 and steroid hormones, and also with a decrease in the release of gonadotrophic hormones [6–8].

The gonadotrophic hormone-releasing system in the brain constitutes a common pathway for the releasing of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the hypophysis. A number of neurons from this system send fibers from the medial eminence (ME) where the gonadotrophin-releasing hormone (GnRH) is released to the portal circulation9. Similarly, it has been suggested that almost all neurons releasing GnRH are of a neuroendocrine nature10. In females, the synthesis and release of GnRH from the ME and, consequently, the preovulatory release of LH, are under control by a positive feedback system with the gonadal steroids progesterone and 17-β-estradiol (E_2) [11–13]. However, it has been shown that neurotransmitters affect the release of both GnRH and the gonadotrophins. The neuroactive substances best studied in the regulation of the expression of GnRH are glutamate, gamma amino butyric acid and catecholamines (noradrenaline and dopamine) [9,14–16].

Previous studies conducted in our laboratory showed

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changes in estral cyclicity of the rats as well as an increase or decrease in the synthesis of brain serotonin (5-HT) [17–19], in animals fed with protein-restricted (PR) or corn-based (C) diets, respectively.

In several studies, a significant increase in hypothalamic 5-HT has been seen in preovulatory stages (diestrous and proestrous). Similarly, stimulating effects of the serotoninergic system on developing neuroendocrine mechanisms have been observed. Hence, 5-HT has been suggested as an in vivo modulator involving the release of GnRH [9,20–23].

The aim of the present study was to evaluate the changes in the serum levels of LH, FSH, progesterone, and E_2 of those female rats having reduced concentrations of 5-HT in the medial hypothalamic region, induced by feeding them a corn-based diet (restricted in tryptophan and lysine). This was correlated with changes in the start of puberty, sexual maturity, and the final period of gestation delivery.

Table 1
Composition of the diets

Components	Control	Protein restricted	Corn
Corn meal (g/kg diet)	—	—	860
Purina chow (g/kg diet)	980	340.4	—
Vegetable oil (g/kg diet)	20	31.3	20
Dextrose* (g/kg diet)	—	190	—
Saccharose (g/kg diet)	—	201.0	—
Dextrin* (g/kg diet)	—	126.7	—
Vitamin mix* (g/kg diet)	—	10	10
Mineral mixture RH* (g/kg diet)	—	10	21
Non-nutritive fiber* (g/kg diet)	—	90.6	89.0
Protein content (%)	23	8	8
Energy (kcal/100 g)	350	350	346.5

* Mixtures were obtained by International Customer Service, Costa Mesa, CA USA (cat. No. 105594; 960376; 904654; 902842, and 900453, respectively).

2. Material and methods

2.1 Materials

The ingredients used for the preparation of the diets were Purina rodent chow (Purina Mills, St. Louis, MO USA), vitamin, mineral, alphacel, glucose, saccharose, and dextrin mixtures obtained from the International Customer Service (Costa Mesa, CA USA), and corn flour and corn oil obtained from a commercial source.

The salts and solvents used were obtained from Merck (Mexico, D.F.) and Sigma Chemical Company (St. Louis, MO USA). The reagent kits for hormone testing were obtained from Amersham Life Sciences (Buckinghamshire, UK).

2.2 Experimental animals

For the biological study, 200 nulliparous female Wistar rats were obtained at 21 and 60 days old from an outbred colony maintained at the Centro de Investigación Biomédica de Occidente, Instituto Mexicano de Seguro Social (Guadalajara, Mexico). Animals were divided into three groups based on the type of food administered. The control (CTL) group was fed commercial Purina rodent chow with 23% protein, the PR group was fed a diet containing 8% protein in a Purina rodent chow base, and the C group was fed with 8% protein based on corn flour. All diets were isocaloric (*Table 1*). Food consumption was registered every second day throughout the study in all groups of animals.

The females were given free access to the diets for 40 days and kept under automatically controlled 12-hr light/dark cycles, with a room temperature of 22 ± 2°C and a relative humidity of 40–50%. Their body weights were recorded weekly and 10 days before being killed by cervical dislocation (at 50 and 90 days old). Vaginal smears were taken daily from all females with vaginal apertures (VA) and stained according to Papanicolaou [24]. At the end of their feeding period during the metaestrous-diestrous phase (60 and 100 days old), the animals were killed by cervical dislocation and blood samples were taken immediately through direct heart puncture. The samples were later centrifuged to obtain serum and for testing for LH, FSH, progesterone and E_2 levels with the use of radioimmunoassay techniques [25,26]. Afterwards, the entire brain was extracted and the medial hypothalamic fragments (MHFs) dissected out from which 5-HT concentrations were measured [27–28].

Three subgroups of 40 animals each were fed the three types of diet, starting their feeding at 60 days old, and were then mated between 90 and 100 days old with males of the same strain without any feeding restriction. Day 0 of gestation was defined as when spermatozoa were found in the vaginal smear. The animals in gestation from each group were killed at 18 and 20 days of gestation and between 2 and 7 seven hr after delivery. Blood samples were taken for assessing progesterone and E_2 hormone concentrations using radioimmunoassay techniques [25].

2.3 Statistical analysis

Data analysis was performed using analysis of variance and Student paired *t*-test using the Biostat program. A statistically significant difference was accepted at $P < 0.05$.

3. Results

There were no significant differences in the food consumption of adult animals belonging to all groups; the CTL group consumed 16.87 ± 0.41 g/day (4.83 ± 0.11 kcal/day), whereas rats from PR and C groups consumed 16.59 ± 1.14

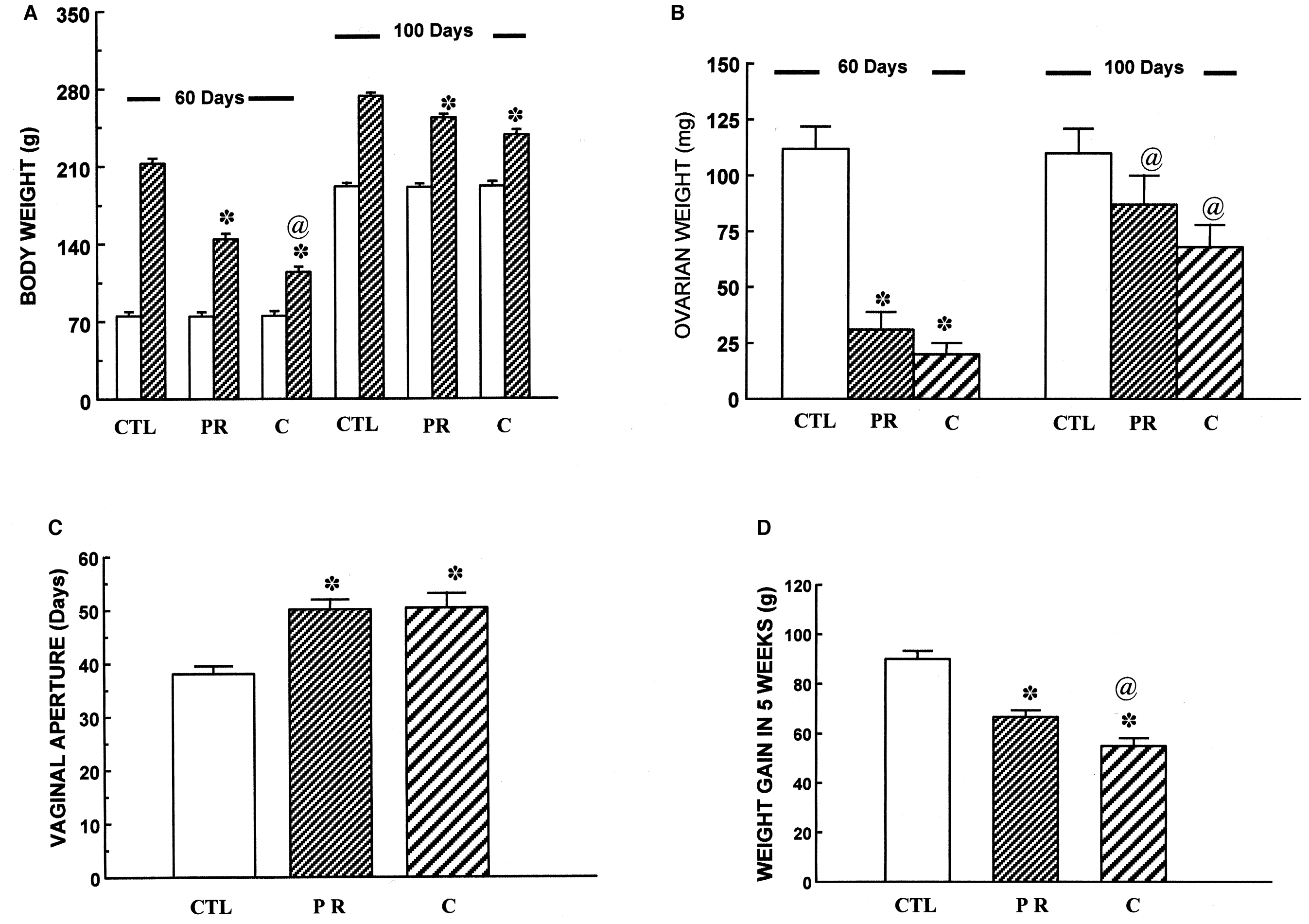

Figure 1 Body (A) and ovarian weight (B) of young (60-day-old) and mature (100-day-old) female rats fed with the various diets. In 1A, the open bars represent the weight at the beginning of the study and the closed bars represent the weight at the end of the study. (C) Age when vaginal aperture appears in the three groups of animals studied. (D) Body weight gain of the female animals from the three groups studied during the 5 weeks before mating. Data represent the means ± standard error of the means. Statistically different from CTL at $*P < 0.001$; and different from PR values at $@P < 0.01$. CTL, control diet; PR, protein-restricted diet; C, corn-based diet.

g/day (4.74 ± 0.32 kcal/day) and 14.75 ± 1.86 g/day (4.25 ± 0.53 kcal/day), respectively.

3.1 Young females

The restricted animals exhibited a significantly lower weight gain when compared with that of the CTL group (32% for PR, 46% for C; *Figure 1A*). They also showed VA at 50 days old with a 12-day delay with respect to the control group. In the group fed with corn, VA was seen in only 70% of the animals (*Figure 1C*). The ovarian weights showed a decrease of 72% and 82% in PR and C groups, respectively (*Figure 1B*). The sexual cycles in the PR group were markedly prolonged (8-day cycles) and the group C females with VA remained in a continuous anestrous phase (*Figure 2A*). The 5-HT content in the MHFs of restricted animals was significantly increased in the PR group (9% in relation to the CTL group), and over 25% with respect to the C group, which was 16% less than that seen in the CTL group ($<P < 0.01$; *Figure 3A*).

The LH serum concentrations were decreased by 16% and 13% in animals of the PR and C groups, respectively, in comparison with those seen in rats of the CTL group ($P < 0.02$; *Figure 3B*). The FSH serum concentration in animals of the PR group was 13% ($P < 0.02$) higher than that seen in the CTL group, and a nonsignificant 9% over that found in rats of the C group (*Figure 3C*). The progesterone concentration was 28% and 90% less in animals of the PR and C groups, respectively, than that seen in rats of the CTL group ($P < 0.001$; *Figure 3D*). Serum estrogens in this study were not detectable with the methods used, although they had a sensitivity of 1.4 pg/mL [25].

3.2 Adult females

The body weight gain in animals of the PR and C groups was decreased by 7% and 13%, respectively, compared with that recorded in rats of the CTL group (*Figure 1A*). Estral cycles of between 6 and 8 days were seen in animals of the restricted groups (2 to 4 more days than in rats of the CTL group; *Figure 2B*). In addition, a decrease in ovarian weight of 21% and 61% for the PR and C groups, respectively, was seen when compared with that of the CTL group (*Figure 1B*). The changes in concentration of 5-HT in MHFs and in serum LH, and of FSH and progesterone levels in rats of the restricted groups were not statistically significant with respect to data obtained from animals of the CTL group (*Figure 3A–D*).

3.3 Gestating females

As in the previous experiments, weight gain during the 5 weeks before gestation in the restricted animals decreased significantly compared with animals of the CTL group (*Figure 1D*). E_2 serum levels in animals of the restricted groups showed differences in their patterns in comparison with what was found in animals of the CTL group. In the latter, E_2 decreased from day 18 to day 20 of gestation, increasing two-fold the value recorded for day 20 immediately after delivery. In animals of the PR group, a progressive decrease of E_2 values was seen from the 18th gestational day to the postpartum period. On the other hand, E_2 levels in rats of C group were significantly reduced at day 18 of gestation, compared with the values for the CTL and PR groups, increasing considerably at the 20th gestational day, and falling drastically immediately after delivery (*Figure 4A*).

Neither the weight of the ovaries nor the progesterone serum concentration exhibited any significant differences when those values were compared among the three groups of animals in the three periods evaluated, with the exception of data from rats of the PR group in the postpartum period (*Figure 4B*).

4. Discussion

A decrease in body weight gain seen in experimental animals in some models of malnutrition is a response to protein restriction; for instance, in the current study, only 8% protein was administered, whereas the minimum protein requirement is 12%1.

On the other hand, carbohydrate content in the restricted diets increased 20%, which stimulates the release of insulin and favors protein synthesis and amino acid uptake within muscles. When there is a decrease of serum amino acids, the organism tends to obtain them from the degradation of body tissues, inducing growth delay in young rats and the loss of body protein in adults7. In addition, the decrease in tryptophan in the diet reduces the secretion of growth hormone and protein synthesis in the liver [6], findings reflected in weight decreases and seen mainly in those animals fed corn.

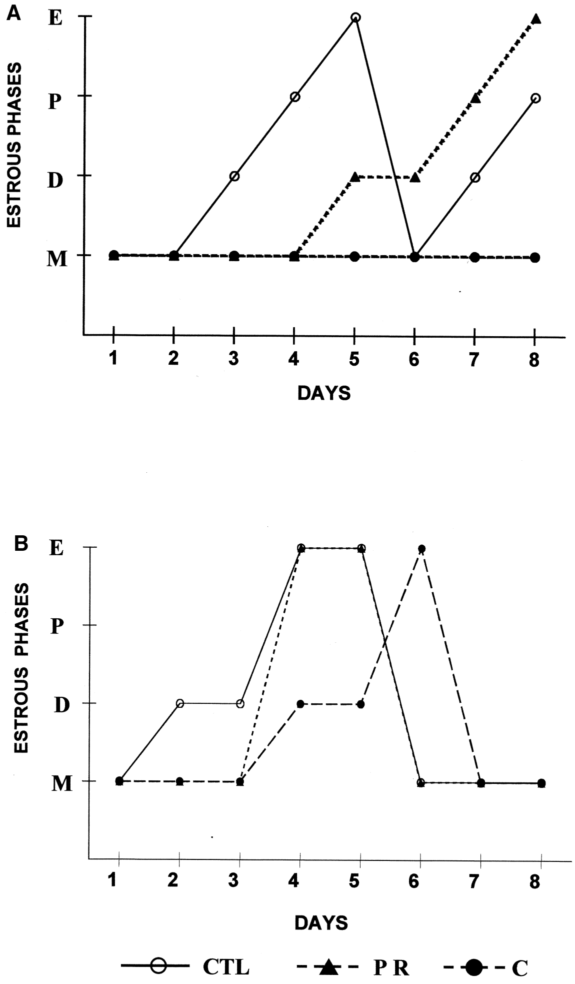

Figure 2 Representative estral cycles of young (60-day-old) (A) and mature (100-day-old) (B) rats fed with the various diets employed. CTL, control diet; PR, protein-restricted diet; C, corn-based diet; E, estrous; P, proestrous; D, diestrous; M, metaestrous.

Low body weight was accompanied by a severe decrease in ovarian weight, both in the PR animals and in those fed with a diet based on corn. These findings are in agreement with data reported by Youg et al.29, who established that the most severe effect caused by malnutrition seems to be at an ovarian level, where changes in estrogen metabolism occur. These studies showed a decrease in the activity of 3-hydroxysteroid dehydrogenase and an increase in the level of 2-hydroxyestrone, which apparently is an inhibitor of LH secretion30. Low ovarian weight has also been observed in rats with persistent estrous, attributed to those ovaries only containing immature Graff follicles and no corpora lutea responsible for the first estral cycle in pubescent rats [31].

On the other hand, evidence has shown that there is a close relationship between body weight and the appearance of menarche in prepubescent women [3,4]. Similarly, in diet-restricted rats, a 10–15-day delay has been seen in VA and the later start of the estral cycle, which under normal conditions usually occurs between 30 and 50 days old [1,3,

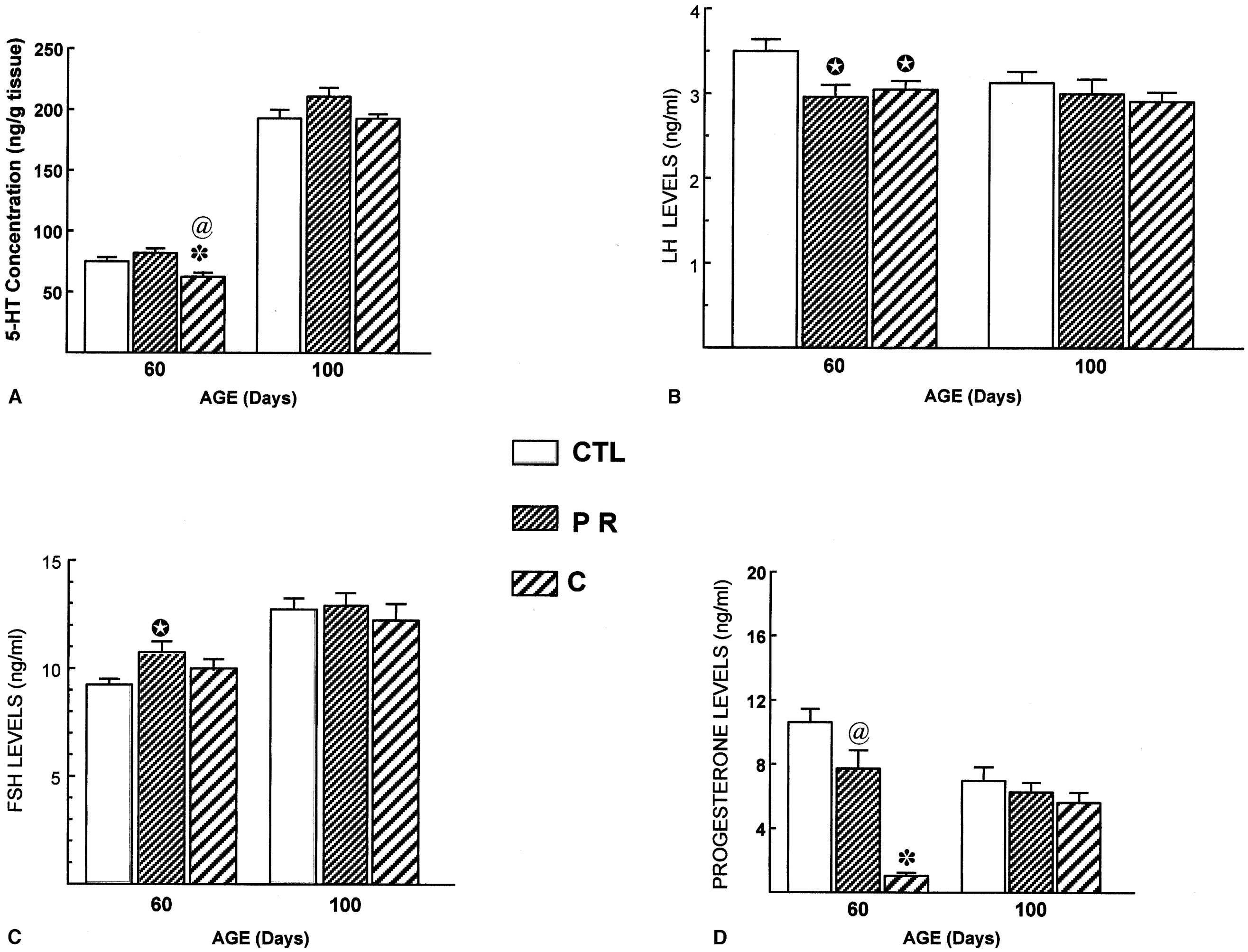

Figure 3 5-HT concentration in MHFs (A) in young (60-day-old) and mature (100-day-old) female rats fed with the control (CTL), protein-restricted (PR), and corn-based (C) diets. Serum levels of LH (B), FSH (C), and progesterone (D) in young (60-day-old) and mature (100-day-old) female rats fed with the various diets employed. Data represent the means ± standard error of the means. Statistically different from CTL at $*P < 0.001$, from PR at $@P < 0.01$, and from CTL at ✪ $P < 0.02$.

32]. In our study, those animals fed with corn had a 12-day delay in the appearance of VA and remained in continuous anestrous, whereas those animals fed a PR diet showed only irregularities and lengthening of the estral cycle. However, the changes observed in vaginal smears indicate irregularities in hormonal release or an atrophy of one of the hypothalamic–hypophysial–gonadal axis elements based on findings by Sprangers and Piacsek [33] in ovariectomized rats with estradiol capsule implants. Those rats exhibited cytologies with numerous leukocytes and a decrease in LH concentration with a 50% dietary restriction.

The delay in VA can be related to a failure in estradiol synthesis by the ovary because, for puberty to start, the ovary must first produce E_2 and stimulate the hypothalamus through positive feedback for the production of GnRH and, later, the release of LH and FSH by the hypophysis for the first ovulation and normal estral cyclicity. However, in the present study, when working with anestral phase animals, it was not possible to detect E_2 serum levels, although the sensitivity of the method used was 1.4 pg/mL [25].

The low serum progesterone concentration observed in this study, especially in young female rats fed with corn, can be due to a decrease in the synthesis of 3-β-hydroxysteroid in thecal, granulous, and luteal cells, provided that these enzymes catalyze the oxidation of pregnenolone as pointed out by Nakanishi et al. [34]. Similarly, the decrease of progesterone may indicate the absence of a functional corpus luteum, which leads to the assumption that there is dysfunctionality at an ovarian level that does not allow for the normal pulsatility of LH [35,36]. Under these conditions, in the presence of ovarian atrophy, the existing progesterone could be provided from other sources such as the adrenal cortex [37].

The response of gestating animals in relation to diet and hormonal release can be due because at this stage, the ovary rests in terms of estrogen synthesis, which is mainly regu-

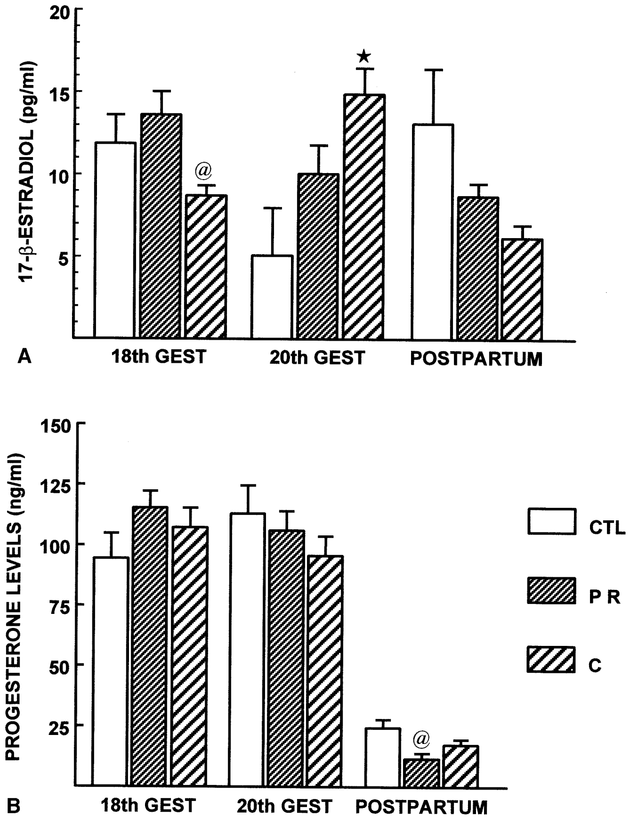

Figure 4 Serum levels of 17-β-estradiol (A) and progesterone (B) of rats at days 18 and 20 of gestation and immediately postpartum, fed with control (CTL), protein-restricted (PR), and corn-based (C) diets. Data correspond to the means ± standard error of the means. Statistically different from CTL at @$P < 0.01$, and at ★ [1]0$P < 0.05$.

lated by placental-type factors [37–40]. In this way, the rise of E_2 on day 20 of gestation may be a result of a greater response to the synthesis of E_2 by the corpus luteum, which during this stage is regulated by the chorionic gonadotrophic hormone whose main function in the rat after day 12 of gestation is luteotrophic in nature [39,41].

Rasby et al. 42 also observed an increase in the concentration of estradiol and estrone in the last stage of gestation in bovines under diet restriction, and concluded that this could be a response to malnutrition for maintaining an adequate hormonal environment and maintaining gestation.

The reduction in E_2 observed in our results postpartum decreases the luteotrophic effect [41,43–45], which would lead to a decrease in the release of LH and FSH normally occurring 4 and 7 hours after delivery. This is of the utmost importance for the first follicular maturation, which would consequently increase the synthesis of E_2 [7].

In addition to their importance in the establishment and maintenance of the estral cycle, gonadal steroids play an important role in modulating the morphology and synaptic density of structures responding to sexual steroids in the central nervous system in such a way that they affect synaptic development and plasticity, as has been described by García-Segura et al. [46–48]

In the same way, Pérez-Vega et al. [49] showed a decreased number of spines in basal dendrites of hippocampal CA1 of animals on a tryptophan-restricted diet, in a similar manner to the model used in the present study.

Our results show both an increase and a decrease in 5-HT in MHFs in animals fed PR and C diets, respectively. The increase may be explained by the fact that there is a high carbohydrate content in the diet that causes the release of insulin, which induces uptake of neutral amino acids (leucine, isoleucine, valine) into the muscle. As a result, the plasma levels of neutral amino acids (LNAA) decline, the plasma tryptophan/LNAA ratio increases and the brain levels of tryptophan and 5-HT rise [7,50].

However, Manjarrez et al. [51] proposed a possible modification of the tryptophan-hydroxylase enzyme structure under restricted food conditions, which favors its affinity for tryptophan and an increase in the synthesis of 5-HT.

On the other hand, corn-based food combines both protein restriction with 8% protein as well as the tryptophan-based meal, providing only 0.048 g of tryptophan/100 g of protein in comparison with the CTL diet, which provides 1.5 g of tryptophan/100 g of protein and an elevated content of the neutral amino acids leucine and isoleucine [17,52], inducing a decrease in 5-HT levels in MHFs, especially in young animals. This fact, together with a decrease of LH and progesterone levels observed in a tryptophan-restricted diet, makes 5-HT a very important factor, along with noradrenaline, in the release of gonadotrophic hormones and steroids as well as in the start of puberty.

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