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Original Article

The prostate of weaned pups is altered by maternal malnutrition during lactation in rats

Cristiane da F. Ramos, Marcio A. Babinski, Waldemar S. Costa, Francisco J.B. Sampaio

Urogenital Research Unit, State University of Rio de Janeiro, UERJ, Rio de Janeiro 20551–030, Brazil

Abstract

The aim of this study was to evaluate the effects of maternal malnutrition during lactation on prostate growth and estradiol serum concentration in the prostate of pups. At delivery, nine Wistar rats were separated into three groups: control group (C) with free access to a standard laboratory diet containing 22% protein; protein–energy-restricted group (PER) with free access to an isoenergy and protein-restricted diet containing 8% protein; and energy-restricted group (ER) receiving standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PER group. All pups were killed at weaning. PER and ER groups presented a significant reduction in estradiol serum concentration (C = 73.8 ± 4.6, PER = 48.7 ± 3.2, ER = 59.7 ± 5.5 pg mL^{-1}, $P<0.01$), total prostatic acini (C = 24 190.0 ± 716.5, PER = 20 290.0 ± 631.4, ER = 19 550.0 ± 759.1; $P<0.01$), lumen of the prostatic acini (C = 5 590.0 ± 165.4, PER = 3 776.0 ± 251.3, ER = 4 658.0 ± 198.1; $P<0.01$) and epithelial area of the prostate dorsal lobe (C = 18 120.0 ± 391.4, PER = 16 520.0 ± 799.2, ER = 14 890.0 ± 589.8; $P<0.01$). Testosterone concentration was significantly increased only in the PER group when compared with the C group (C = 0.09 ± 0.01, PER = 0.44 ± 0.04, ER = 0.15 ± 0.03 ng mL^{-1}, $P<0.001$). An adequate nutritional state in early life is important for normal growth of the prostate gland, which seem to be related to serum levels of estradiol.

1 Introduction

Malnutrition is known to have a wide variety of effects on the endocrine system [1, 2]. With regard to the reproductive system, it has been shown that food restriction can inhibit both the maintenance and onset of reproductive capability [3], and reduce the androgen receptor protein level in the testis of adult rats [4]. In addition, in adult rats, food restriction can reduce the body weight [4, 5], as well as testes, epididymis and prostate weights [4]. Serum concentration of luteinizing hormone, follicle-stimulating hormone and testosterone are reduced by undernutrition [4–7], which also leads to alterations in gonadotroph morphology that are typical of those found in cells whose secretory activities are suppressed [6].

Nutritional status of the mother during gestation and lactation is essential to the normal growth and development in humans and experimental animals [8]. Throughout life, normal development, growth

Correspondence to: Dr Francisco J.B. Sampaio, Urogenital Research Unit, State University of Rio de Janeiro, UERJ, Avenue. 28 de Setembro, no. 87 – fundos – FCM – térreo, Rio de Janeiro 20551–030, RJ, Brazil.
Fax: +55-21-2587-6133
E-mail: sampaio@urogenitalresearch.org

and function of the prostate gland are dependent on androgens, which act in synergy with other modulating hormones such as estrogen and prolactin [9]. In rats, prostate development is initiated late in the fetal period and undergoes extensive branching morphogenesis and cellular differentiation during the neonatal period [10]. It is during this early developmental period that hormonal modulation can have a permanent and irreversible effect on the prostatic morphology, cellular organization and function.

Exposure of neonatal mice to the nonsteroidal estrogen, diethylstilbestrol, disturbs prostatic development, alters epithelial cell differentiation, and predisposes the animal to prostatic hyperplasia and dysplasia, analogous to human prostatic intraepithelial neoplasia [9,11].

Despite the fact that malnutrition-related reproductive suppression in rats is a well-documented phenomenon, we do not have any previous knowledge to date on how maternal malnutrition during lactation affects the prostate growth of pups. Therefore, the present study aims to evaluate the effects of protein and energy maternal malnutrition during lactation on the prostate growth of the offspring and to correlate it with estradiol serum concentrations.

2 Materials and methods

2.1 Animals

Wistar rats were kept in a room with controlled temperature (25 ± 1 ℃) and with artificial dark-light cycle (lights on from 07:00 hours to 19:00 hours) all through the experiment. Virgin female rats (3-month old) were caged with one male rat at a proportion of 2:1. After mating, 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 State University of Rio de Janeiro, which based their analysis on the Guide for the Care and Use of Laboratory Animals [12], and the study design was approved by the local Ethics Committee for the care and use of laboratory animals.

2.2 Experimental model

Nine pregnant Wistar rats were separated at delivery into three groups: control group (C)—with free access to a standard laboratory diet; protein–energy-restricted group (PER)—with free access to an isoenergy and protein-restricted diet containing 8% protein; and energy-restricted group (ER)—receiving standard

laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PER group. In spite of having free access to diet, the PER group consumed ~60% of that consumed by the control group. Therefore, the amount of food consumed in both ER and PER groups was almost the same.

The low-protein diet was prepared in our laboratory, and vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets [13]. The composition of both diets is shown in Table 1.

To evaluate the nutritional state, the food consumption and body weight were monitored throughout the experiment.

Within 24 h of birth, excess pups were removed, so that only six male pups were kept per dam, because it has been shown that this procedure maximizes lactation performance. At weaning (21-day old), seven pups from three different dams of each group were killed under thiopental anesthesia (0.10 mL per 100g body weight), always in the morning. Blood was collected by cardiac puncture and the serum was kept at –20 °C. The prostates were excised, dissected, weighed, fixed in formalin in phosphate buffer (4% in 0.1 mol L^{-1}; pH 7.4),

Table 1. Composition of control and protein–energy restricted diets

	Control[a]	Protein restricted[b]
Ingredients (g kg^{-1})		
Total protein[c]	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^{-1})	17 038.7	17 038.7

[a]Standard diet for rats (Nuvilab-Nuvital Ltd, Paraná, Brazil).
[b]The protein-restricted diet was prepared in our laboratory using the rol 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.
[c]The principal protein resources are soybean wheat, steak, fish and amino acids.
[d]Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (Reeves *et al.* [13]).

paraffin-embedded and sectioned at 5 μm and processed by routine histological methods .

2.3 Hormonal evaluation

The serum hormonal concentrations were determined by using specific radioimmunoassay (ICN Pharmaceuticals Inc, CA, USA). The intra- and inter-assay variation coefficients were 6.4% and 5.9% for estradiol, and 4.6% and 7.5% for testosterone, respectively.

2.4 Histological and morphometric procedures

For morphometric analysis, five samples from each prostate were randomly excised from the dorsal lobe, and from each sample, five different sections were obtained. The sections were then stained using hematoxylin-eosin. From each section, all acini of five different random fields were analyzed and quantified, totaling upto 25 test areas in each prostate and 125 fields in each group. The analyzed images were obtained at × 40 using a video camera coupled with a light microscope. The images were converted to digital signals and transferred to a computer.

The morphometric features assessed were the delineation of the luminal surface of the acini, which provided the total area of the lumen, and the basal limit of the acini, which provided the total acinar area. On the basis of these two measurements, the software Image Pro (Media Cybernetics, Bethesda, MD, USA) was used to estimate the total area of the epithelium (μm^2).

2.5 Statistical analysis

The data was reported as mean ± SD. The parameters were determined by the Kruskal–Wallis test followed by Dunn's multiple comparison test. The level of significance was set at $P < 0.05$.
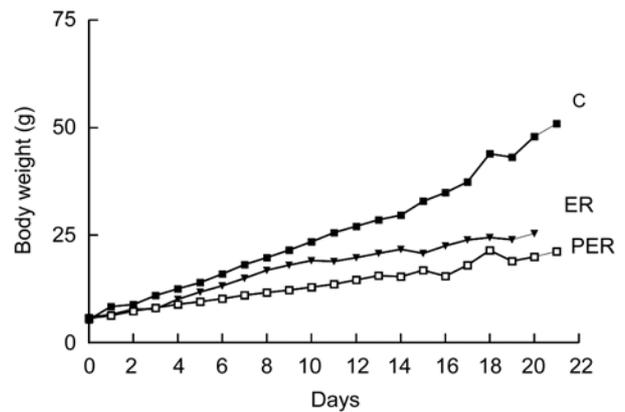

Figure 1. Body weight of 21-day-old rats whose dams were fed with a diet containing 23% of protein—control group (C), a diet with 8% of protein—protein-restricted group (PER) or a diet with 23% of protein but in restricted quantities—energy-restricted group (ER), during lactation period, $P < 0.01$, ER *vs.* C; $P < 0.01$, PER *vs.* C. Values are shown as mean ± SD of seven animals per group.

3 Results

When compared to controls, the mean body weight of pups whose mothers were submitted to PER or ER diet, was lower during the entire lactation period. The mean weight decrease reached 58% and 46% in the PER and ER groups, respectively, at the end of lactation ($P < 0.01$, Figure 1).

Table 2 shows the results of absolute and relative prostate weights and acinar morphometric analysis. All parameters, but epithelium and relative prostate weights, were reduced in both PER and ER groups ($P < 0.01$).

The serum estradiol concentrations were decreased in both PER and ER groups compared with controls

Table 2. Absolute and relative weights, areas of the acini, lumen and epithelium of the prostate in 21-day-old rats whose dams were fed a diet consisting of 23% of protein—control group (C), a diet with 8% of protein—protein-restricted group (PER) or a diet with 23% of protein but in restricted quantities—energy-restricted group (ER).

Group	C	PER	ER
Prostate weight (g)	0.060 ± 0.005	0.035 ± 0.003[*]	0.042 ± 0.001[*]
Prostate weight/body weight (mg g^{-1})	1.250 ± 0.050	1.750 ± 0.090	1.650 ± 0.070
Total acini area (μm^2)	24 190.000 ± 716.500	20 290.000 ± 631.400[*]	19 550.000 ± 759.1000[*]
Total lumen area (μm^2)	5 590.000 ± 165.400	3 776.000 ± 251.300[*]	4 658.000 ± 198.100[*]
Total epithelial area (μm^2)	18 120.000 ± 391.400	16 520.000 ± 799.200	14 890.000 ± 589.800[*]

Values are shown as mean ± SD of seven animals per group.
[*]$P < 0.01$, compared with C.

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Maternal malnutrition and prostate in rats
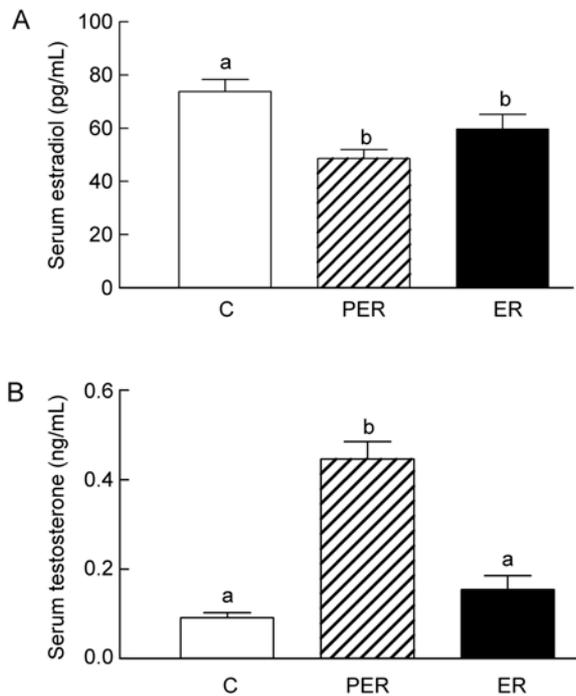Cristiane da F. Ramos *et al*.

4

Figure 2. Serum estradiol (A) and testosterone (B) concentration of 21-day-old rats whose dams were fed with a diet consisting of 23% of protein—control group (C), a diet with 8% of protein—protein-restricted group (PER) or a diet with 23% of protein, but in restricted quantities—energy-restricted group (ER), during lactation period. Values are shown as mean ± SD of seven animals per group. Different letters mean statistical difference.
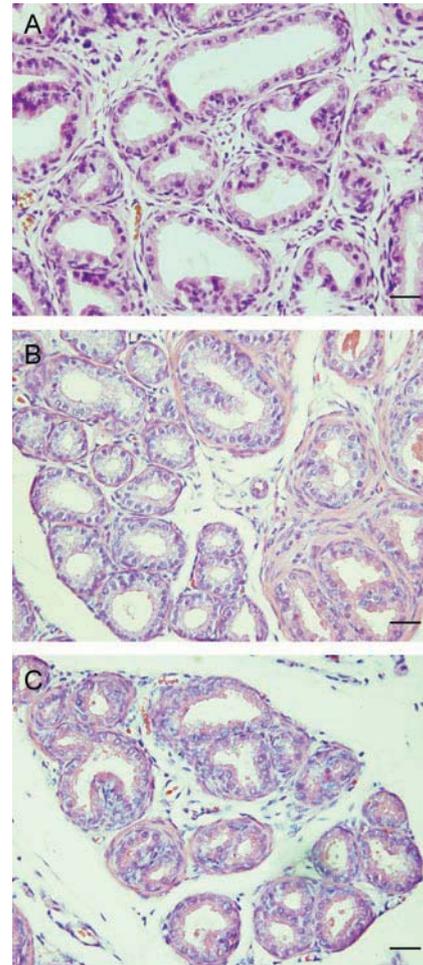

Figure 3. Histological sections of the dorsal lobe of the prostate of 21-day-old rats. (A): Control group; (B): protein–energy-restricted group; (C): energy-restricted group. There was a reduction in the total acini, total lumen and total epithelial areas in both malnourished groups (B and C). The sections were stained using hematoxylin-eosin. Bars = 50 μm.

(PER = 34%, ER = 9%; $P < 0.01$) (Figure 2A). Compared with the control group, the serum testosterone concentration was significantly increased in the PER group (390%; $P < 0.001$). Serum testosterone concentration in the ER group was slightly, but not significantly, higher than that in controls (Figure 2B).

The histological sections of the dorsal lobe of the prostate gland are shown in Figure 3. Both malnourished groups presented a reduction in the total acini, total lumen and total epithelial areas.

4 Discussion

The prenatal and early postnatal nutritional status has a critical role in postnatal growth and development. Early malnutrition may change the original programming of organs, especially those involved in development,

which can result in long-term changes in metabolism [14, 15]. Recently, we have shown that maternal protein and energy malnutrition during lactation, in additional to growth retardation, leads to alteration in testicular morphology, testis steroid hormone genes expression, uterine endometrial glands number and folliculogenesis [16].

The maternal nutritional state during lactation is equivalent and possibly even more important than that during gestation, as evidenced by a study by Leonhardt *et al*. [17], which 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.* [18] showed similar results.

On the basis of these papers and the lack of studies regarding the effects of malnutrition on the prostate gland, we decided to analyze the effects of maternal malnutrition during the lactation time on the prostate morphology of the weaned pups and its correlation with estradiol serum levels.

Some reported studies have shown that food deprivation decreased growth-hormone (GH)-releasing hormone and consequently the GH serum levels, which could easily explain the low weight gain of these animals [1]. We have also shown that pups whose mothers were subjected to protein or energy malnutrition during lactation present an alteration in the leptin serum concentration [2], which could also be related to the low weight gain of the animals.

The rodent prostate has a complex structure, consisting of a ventral prostate (VP), lateral prostate (LP), dorsal prostate (DP) and anterior prostate, or coagulating gland [19]. Although most studies have focused on the VP, this lobe is considered to have no counterpart in the primates, whereas the LP and the DP are histologically similar to the human prostate [20], and for this reason, we decided to evaluate the morphology of the dorsal lobe instead of others.

Our study shows, for the first time to our knowledge, that a protein and energy maternal malnutrition during lactation leads to a reduction in the total acini, lumen and epithelial areas of the dorsal prostate lobe. This result corroborates the reduction observed in the prostate weight, which is also in agreement with the literature [21].

After normalization of the body weight, the prostates sizes were not statistically different. We know that malnutrition does not have a direct effect on prostate size or on any other organ. It could possibly be a multifactorial disease, affecting several organs and functions, which may not be related to each other. Previous works have shown that malnutrition can affect several systems [1, 2].

Prolactin and GH belong to the same protein family sharing genomic, structural and biological features. The presence of GH and its receptor [22], as well as prolactin and prolactin receptor [23] in the prostate gland has been previously shown. There are some evidences correlating both prolactin [24] and GH [25] with prostate cancer, but there is clearly a need for more work to confirm this hypothesis. However, we cannot discard the fact that the effect of malnutrition on the prostate gland can also be mediated by GH and prolactin, although no data related to malnutrition and both hormones in the prostate have been reported to date.

Some of the effects of estrogen may be indirectly mediated through the hypothalamic–pituitary–testicular axis, although a direct response at the prostate level has also been documented [26]. In our study, maternal malnutrition resulted in low serum estradiol concentration in the pups of both malnourished groups, whereas testosterone concentration was higher only in the PER group. Hence, the prostate growth does not seem to be under testosterone control on the malnourished groups, as the testosterone alterations were different between these two groups while they showed the same growth retardation, a possibility related to the estrogen serum levels.

We can hypothesize that whether or not early maternal malnutrition causes a programming of the prostate, this offspring could develop some prostate alteration in adulthood as a result of the changes in serum levels of estradiol. Some reported results have supported this theory [28, 29]. Bianco *et al.* [27] showed that estradiol administration stimulates growth and expansion of the stromal, epithelial and luminal compartments of the mouse prostate lobes, showing characteristics of squamous metaplasia [28]. In the absence of estrogen, there is a lifelong elevation of androgens in male ArKO (aromatase knockout) mice and benign prostatic hyperplasia develops in maturity, suggesting that, in combination, androgens and estrogens can induce dysplasia, premalignant and malignant changes in the prostatic cells [29]. Both protein–energy restriction and energy restriction resulted in dorsal lobe prostate growth retardation, probably related to estradiol serum alteration. As there was no statistical difference between the results of the two malnourished groups, we can hypothesize that energy restriction, but not protein restriction, is the determinant factor in this study.

In conclusion, our results suggest that an adequate nutritional state in the early life is important for normal development of the prostate gland. We have shown that maternal malnutrition during lactation can affect the development of pups' prostate dorsal lobe by

presumably altering the serum estradiol concentration. It is possible that malnutrition during lactation can program the prostate gland and could be responsible for some prostate alteration in the adulthood.

Acknowledgment

References

1   Console GM, Jurado SB, Oyhenart E, Ferese C, Pucciarelli H, *et al.* Morphometric and ultrastructural analysis of different pituitary cell populations in undernourished monkeys. Braz J Med Biol Res 2001; 34: 65–74.

2   Teixeira C, Passos M, Ramos C, Dutra S, Moura E. Leptin serum concentration, food intake and body weight in rats whose mothers were exposed to malnutrition during lactation. J Nutr Biochem 2002; 13: 493.

3   Desjardins C, Lopez MJ. Environmental cues evoke differential responses in pituitary-testicular function in deer mice. Endocrinology 1983; 112: 1398–1406.

4   Santos AM, Ferraz MR, Teixeira CV, Sampaio FJ, da Fonte Ramos C. Effects of undernutrition on serum and testicular testosterone levels and sexual function in adult rats. Horm Metab Res 2004; 36: 27–33.

5   Chik CL, Ho AK, Brown GM. Effect of pinealectomy on the undernutrition-induced suppression of the reproductive axis in rats. Acta Endocrinol (Copenh) 1989; 120: 569–73.

6   Herbert DC. Morphology of the mammotrophs and gonadotrophs in the anterior pituitary gland of rats with protein-calorie malnutrition. Am J Anat 1980; 158: 521–31.

7   Young KA, Zirkin BR, Nelson RJ. Testicular regression in response to food restriction and short photoperiod in white-footed mice (peromyscus leucopus) is mediated by apoptosis. Biol Reprod 2000; 62: 347–54.

8   da Silva Faria T, da Fonte Ramos C, Sampaio FJ. Puberty onset in the female offspring of rats submitted to protein or energy restricted diet during lactation. J Nutr Biochem 2004; 15: 123–7.

9   Prinsac GS, Birch L, Habermann H, Chang WY, Tebeau C, *et al..* Influence of neonatal estrogens on rat prostate development. Reprod Fertil Dev 2001; 13: 241–52.

10   Prins GS, Birch L. The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen. Endocrinology 1995; 136:

1303–14.

11   Strauss L, Makela S, Joshi S, Huhtaniemi I, Santti R. Genistein exerts estrogen-like effects in male mouse reproductive tract. Mol Cell Endocrinol 1998; 144: 83–93.

12   Bayne K. Revised guide for the care and use of laboratory animals available. American physiological society. Physiologist 1996; 39: 199, 208–11.

13   Reeves PG, Nielsen FH, Fahey Jr 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-76a rodent diet. J Nutr 1993; 123: 1939–51.

14   Lucas A. Programming by early nutrition: an experimental approach. J Nutr 1998; 128: 401–6S.

15   Barker DJ. *In utero* programming of cardiovascular disease. Theriogenology 2000; 53: 555–74.

16   Faria Tda S, Brasil Fde B, Sampaio FJ, Ramos Cda F. Maternal malnutrition during lactation alters the folliculogenesis and gonadotropins and estrogen isoforms ovarian receptors in the offspring at puberty. J Endocrinol 2008; 198: 625–34.

17   Leonhardt M, Lesage J, Croix D, Dutriez-Casteloot I, Beauvillain JC, *et al.* 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.

18   Guzman C, Cabrera R, Cardenas M, Larrea F, Nathanielsz PW, *et al.* Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. J Physiol 2006; 572: 97–108.

19   Hayashi N, Sugimura Y, Kawamura J, Donjacour AA, Cunha GR. Morphological and functional heterogeneity in the rat prostatic gland. Biol Reprod 1991; 45: 308–21.

20   Rennie PS, Bruchovsky N, Leco KJ, Sheppard PC, McQueen SA, *et al.* Characterization of two cis-acting DNA elements involved in the androgen regulation of the probasin gene. Mol Endocrinol 1993; 7: 23–36.

21   Engelbregt MJ, Houdijk ME, Popp-Snijders C, Delemarre-van de Waal HA. The effects of intra-uterine growth retardation and postnatal undernutrition on onset of puberty in male and female rats. Pediatr Res 2000; 48: 803–7.

22   Weiss-Messer E, Merom O, Adi A, Karry R, Bidosee M, *et al.* Growth hormone (gh) receptors in prostate cancer: gene expression in human tissues and cell lines and characterization, gh signaling and androgen receptor regulation in lncap cells. Mol Cell Endocrinol 2004; 220: 109–23.

23   Nevalainen MT, Valve EM, Ingleton PM, Nurmi M, Martikainen PM, *et al.* Prolactin and prolactin receptors are expressed and functioning in human prostate. J Clin Investig 1997; 99: 618–27.

24   Nakamura A, Shirai T, Ogawa K, Wada S, Fujimoto NA, *et al.* Promoting action of prolactin released from a grafted transplantable pituitary tumor (mtt/f84) on rat prostate carcinogenesis. Cancer Lett 1990; 53: 151–7.

25   Fuhrman B, Barba M, Schunemann HJ, Hurd T, Quattrin T, *et al.* Basal growth hormone concentrations in blood and the risk for prostate cancer: a case-control study. Prostate 2005; 64: 109–15.

Maternal malnutrition and prostate in rats
Cristiane da F. Ramos *et al*.

npg

7

26 Prins GS. Neonatal estrogen exposure induces lobe-specific alterations in adult rat prostate androgen receptor expression. Endocrinology 1992; 130: 3703–14.

27 Bianco JJ, Handelsman DJ, Pedersen JS, Risbridger GP. Direct response of the murine prostate gland and seminal vesicles to estradiol. Endocrinology 2002; 143: 4922–33.

28 Cunha GR, Wang YZ, Hayward SW, Risbridger GP. Estrogenic effects on prostatic differentiation and carcinogenesis. Reprod Fertil Dev 2001; 13: 285–96.

29 Risbridger GP, Bianco JJ, Ellem SJ, McPherson SJ. Oestrogens and prostate cancer. Endocr Relat Cancer 2003; 10: 187–91.