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Histometric evaluation of dental alveolar repair in malnourished rats in the intrauterine or postnatal phase

Fábio A.S. Sartorato *, *Cláudia A.M. Mura*, *Sandra R.R. Lucas*

Laboratory of Developmental Biology, Department of Morphology and Genetics, Federal University of São Paulo (UNIFESP), São Paulo, SP, Brazil

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ABSTRACT

Objective: Nutritional aggravations during pregnancy or during the early stages of postnatal development can impair bone development; thus, we aimed to assess the effects of food restriction on the dental alveolar bone repair process using histometric analysis.

Design: Thirty-six Wistar rats were divided into three groups: (C) 12 pups were obtained from control mothers with food intake at ease; (GR) 12 pups from mothers subjected to 70% food restriction during pregnancy; (PNR) 50% of maternal food restriction during lactation and 50% of restriction for the 12 pups after weaning. At three months of age, the upper right incisor was extracted from the pups. After 14 or 28 days, the pups were sacrificed for evaluation of newly formed bone area (NB) and total bone area (TA) in the medial and apical thirds of the alveolus.

Results: In the apical third of the alveolus, the ratio of NB/TA was greater at 28 days for all groups and there was no damage to any of the groups. In the medial third, the ratio was higher at 28 days for the C and GR groups. The PNR group did not show an evolution of alveolar dental repair. Compared between the thirds, all groups exhibited a higher percentage of newly formed bone in the medial third area, at any time point after surgery.

Conclusions: The percentage of the total alveolar area covered by newly formed bone (NB/TA) revealed a late preference in the process of alveolar repair in the medial third, although only in the PNR group.

1. Introduction

Annually, approximately 30 million children are born with low weight, representing approximately 24% of all births during this period. These children often face serious short- and long-term health problems because low birth weight is the greatest determinant of mortality or morbidity and disability in childhood with repercussions extending into adult life.[1]

Foetal programming is the phenomenon by which changes in foetal growth and development in response to the intrauterine environment generate permanent effects.[2] Thus, several clinical studies have shown that certain parameters, such as bone structure, physiology and metabolism,[3,4] bone mass,[5,6] its growth[7] and its mineral content,[8] can be programmed by environmental influences during intrauterine life. Experimental models used for the study of foetal programming theory have demonstrated that nutrition is an important modifiable factor because it determines significant

* *Corresponding author at*: Departamento de Biologia do Desenvolvimento, Universidade Federal de São Paulo, Rua Botucatu, 740, Edifício Leitão da Cunha, 2° andar, São Paulo, SP, CEP: 04023-900, Brazil. Tel.: +55 11 55764262; fax: +55 11 55764848.
E-mail address: fabiosigs@uol.com.br (Fábio A.S. Sartorato).
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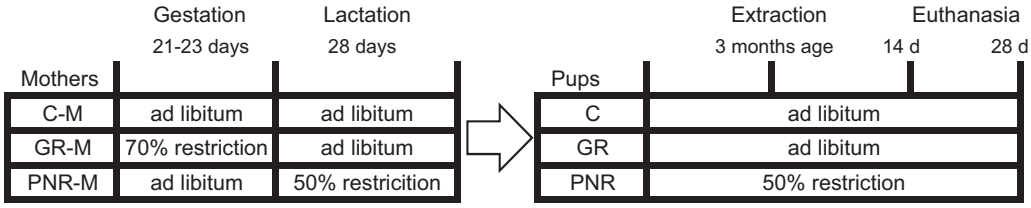

Fig. 1 – Nutritional scheme imposed to the pregnant rats and offspring.

changes in bone parameters. Nutritional aggravations arising from protein, energetic or protein-caloric restrictions imposed on animals during pregnancy or during the early stages of their postnatal development can impair bone development.[9–14]

In addition, specific phenomena of the alveolar bone repair process after tooth extraction have been elucidated using experimental models.[15–17]

Thus, by imposing a food restriction at different time periods of offspring development, we aimed at evaluate distinctly the potential repercussions of this nutritional aggravation on bone formation and development, which can compromise the dental alveolar repair process of the adult rat using histometric analyses.

2. Materials and methods

All procedures were approved by the institutional animal research committee. Twelve-week-old Wistar male and female rats were used for breeding. Pregnant animals were transferred into individual plastic cages, enabling the manipulation on their diets during the different developmental phases of their offspring.

First, the control group consisting of mothers (C-M, control mother) was established. Control mothers received commercial feed ad libitum throughout gestation and lactation. Once the average daily dietary intake of these animals was determined, some of the animals were subjected to a dietary restriction according to their experimental group. In the group of rats subjected to constraint only during pregnancy (GR-M, gestational restriction mother), the diet was reduced by 70% compared to the normal average daily ingestion of the C-M group throughout the time points. On the day of birth, these mothers received feed ad libitum during lactation. In addition to this restricted group, a group of mothers with postnatal restriction (PNR-M, postnatal restriction mother) was established, in which the rats were deprived of 50% of the average normal diet ingested by C-M animals, but only for 28 days of lactation.

At birth, the groups of pups were divided according to the group of pregnant mother rats: Group C, puppies of control mothers, which continued to receive feed ad libitum after weaning; GR group, pups from mothers who have a 70% food restriction only during gestation and after weaning received feed ad libitum; Group PNR; pups of mothers that were subjected to 50% food restriction during the entire lactation period, and continued to be subjected to this restriction until the time of euthanasia.

To avoid differences in the nutritional status of the offspring resulting from differences in the number of animals per litter, only six young males pups were left with each mother. If the litter generated was less than 6 males, then the females of this litter were used to obtain the minimum number of animals until the end of lactation, thus ensuring the same amount of breast milk for all animals. After weaning, only males were used in this experiment.

This nutritional scheme was maintained until the animals were sacrificed at either 14 or 28 days after tooth extraction, which was performed when the animals reached adulthood (Fig. 1). All animals used during the experiment were maintained under controlled conditions of temperature ($23 \pm 1\ ^{\circ}C$) and relative humidity ($55 \pm 5\%$), with artificial lighting via a fluorescent lamp with a photo-period of 12 h of light and 12 h of dark. The animals were fed commercial Labina–Purina chow, which consisted of ingredients listed in Table 1.

2.1. *Surgical procedure*

After general anaesthesia with ketamine (10% Dopalen, Vetbrands, Brazil) and xylazine (Anasedan 2%, Vetbrands, Brazil), the male rats had their upper right incisor extracted, with respective to the dental germ. The tooth avulsion was achieved with the aid of a chisel and forceps adapted for such purpose. At the end of the procedure, after applying simple gingival using a mononylon suture wire 5–0, all of the animals received a single dose of antibiotic (Veterinarian Pentabiotic Small Animals, Fort Dodge Animal Health Ltd.).

2.2. *Histological procedures*

After 14 or 28 days of post-operative care, six animals from each group (C, GR and PNR) were sacrificed using excessive anaesthesia. After soft tissue dissection, the maxilla was separated from the skull via a crosscut surfacing the distal face of the last upper molar. The samples were fixed in 10% formalin for 48 h and decalcified in a solution of formic acid and sodium citrate.[18] During decalcification, the samples were

Table 1 – Commercial chow composition.

Moisture not more than	13%
Crude protein not less than	23%
Crude fat not less than	4%
Crude fibre not more than	5%
Minerals not more than	10%
Calcium not more than	1.3%
Phosphorus not more than	0.85%