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Effects of induced precocious puberty on cranial growth in female Wistar rats

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SUMMARY This investigation examined the effects of pharmacologically induced precocious puberty on cranial growth in Wistar rats. Forty-eight female newborn Wistar rats were divided into two groups: a control group (C) and an experimental group (E), with four subgroups of six animals each. The time interval from birth until sacrifice differed between the subgroups, and was set at 30, 60, 90, and 120 days. An intramuscular single dose (300 g) of steroid hormone danazol was administered on day 5 after birth, as a means of inducing precocious puberty. Alizarin (2 mg/100 g) was administered to three animals in each subgroup three days prior to sacrifice. Body mass and dates corresponding to the beginning of the oestrous cycle were recorded. Craniometric measurements were undertaken. Histological analysis using light and fluorescence microscopy was then carried out to qualitatively and quantitatively evaluate the spheno-occipital synchondrosis and to visualize bone deposition patterns. The results were analysed with a Student's *t*-test and analysis of variance.

Precocious puberty was effectively induced and differences between groups denoted an earlier maturation in the experimental rats. In qualitative analysis, a significant increase of total synchondrosis width was noted only in group E60, in comparison with C60, and an increase in the E90 subgroup cortical bone width compared with the C90 subgroup. Histomorphometrically, a statistical difference between total width values of subgroups E60 (434.3 m) and C60 (323.5 m) was detected. However, body mass and macroscopic measurements did not show statistically significant differences. An appropriate model for studying bone growth associated with precocious puberty in Wistar female rats was not achieved using steroid hormone danazol, when evaluated at 30 day intervals.

Introduction

Precocious puberty can be identified by the appearance of any physical or hormonal signs of pubertal development earlier than that considered 'normal'. Affected individuals have premature growth spurts associated with advanced skeletal age and epiphyseal ossification, causing an overall reduction in their final adult height; the more intense or the earlier puberty starts, the more severe are the resulting effects (Carel *et al.*, 2004; Brown and Warne, 2006).

Advanced skeletal maturity suggests a chronic increase in sex hormone release. Individuals usually need psychological support, especially males due to higher social implications of a short overall height (Taranger and Hägg, 1980; Sierra, 1987; Phinney *et al.*, 1990; Chemaitilly *et al.*, 2001; Ge *et al.*, 2001).

There are reports of a decrease in bone density during the clinical evolution of precocious puberty. No differences were, however, found in the same individuals in adulthood when compared with those with normal pubertal development (Van der Sluis *et al.*, 2002).

Despite the fact that most of these changes take place during a period of special interest to the orthodontist, there are few studies concerning the effects of precocity on craniofacial growth. The aim of this study was to analyse the effects of pharmacologically induced precocious puberty on craniofacial dimensions, as well as the histological characteristics at the spheno-occipital synchondrosis in female Wistar rats.

Materials and methods

All procedures in this research were approved by the Ethics Committee for Animal Research (CAUAP) of the Federal University of Rio de Janeiro (UFRJ).

Forty-eight newborn female Wistar rats (*Rattus norvegicus*) weighing between 12 and 14 g were used in this study. The animals were housed in standard cages, six per cage, in a controlled temperature (22°C) with a 12 hour light/dark cycle, and chow and mineral water were available *ad libitum*. There was no need for acclimatization

since the birth of the animals and the experiment were carried out within the same conditions. The experiment lasted 120 days. The animals were randomly divided into two groups: a control (C) and an experimental group (E), each with 24 rats, subdivided into four subgroups of six animals. Each subgroup differed in the time interval from birth until sacrifice, set at 30, 60, 90, and 120 days (Figure 1).

The dates for sacrifice was 30 days after birth as there would be pharmacologically induced alterations passive of evaluation, even before the oestrous cycle; at 60 and 90 days after birth, when changes of a larger magnitude would be expected when comparing both groups at each date, based on the expected sexual maturity stage discrepancy; and that at 120 days, females of this species are considered adults, with little growth expression.

The control group consisted of animals that were administered 25 l of normal saline solution on day 5 of life. In the experimental group, steroid hormone danazol (Ladogal; Sanofi-Synthelabo, São Paulo, Brazil) was used to induce precocious puberty. An intramuscular single dose of 300 g, corresponding to 25 l was administered at day 5 of life (Morishita *et al.*, 1993; Roth *et al.*, 2004; Tian *et al.*, 2004, 2005).

It was possible to monitor hypothalamo-pituitary-gonadal axis activity through specimen cycle control. This procedure made it possible to determine if females had started their reproductive cycle, that is, the oestrous cycle, which represents the beginning of sexual maturity. The oestrous cycle may be identified according to the proportion of cell types observed in vaginal smear cytology. At the same hour of every day, vaginal fluid was collected from each female rat, from day 21 of life until the observation of three regular consecutive oestrous cycles (Marcondes *et al.*, 2002). A pro-oestrus smear consisted of a predominance of nucleated epithelial cells; an oestrous smear primarily presented enucleated cornified cells; a metoestrus smear comprised the same proportion of leukocytes, cornified cells and nucleated epithelial cells; and a dioestrus smear primarily consisted of a predominance of leukocytes (Marcondes *et al.*, 2002; Figure 2).

Three animals from each control and experimental subgroup (30, 60, 90, and 120 days) underwent alizarin administration (Sigma-Aldrich, St. Louis, Missouri, USA) to visualize bone deposition in the spheno-occipital synchondrosis. The concentration was 2 mg/100 g of body mass, three days prior to sacrifice (Roberts, 2002). Body mass was registered at birth and immediately before sacrifice of all animals. Sacrifice was carried out through ether asphyxiation followed by decapitation. The cranium was then dissected and fixed in 4 per cent paraformaldehyde.

Total cranium length (TCL) and cranium height (CH) measurements were taken with a digital paquimeter (Mitutoyo no. 500-14313, Kawasaki, Japan). CH is the

Groups \ Days	5	30	60	90	120
Control (24)	Θ(24)	†(6)	†(6)	†(6)	†(6)
Experimental (24)	Φ(24)	†(6)	†(6)	†(6)	†(6)

Figure 1 Chronological description of the experiment. θ = saline solution, Φ = danazol, † = sacrifice. The number of animals in each subgroup are given in parentheses.

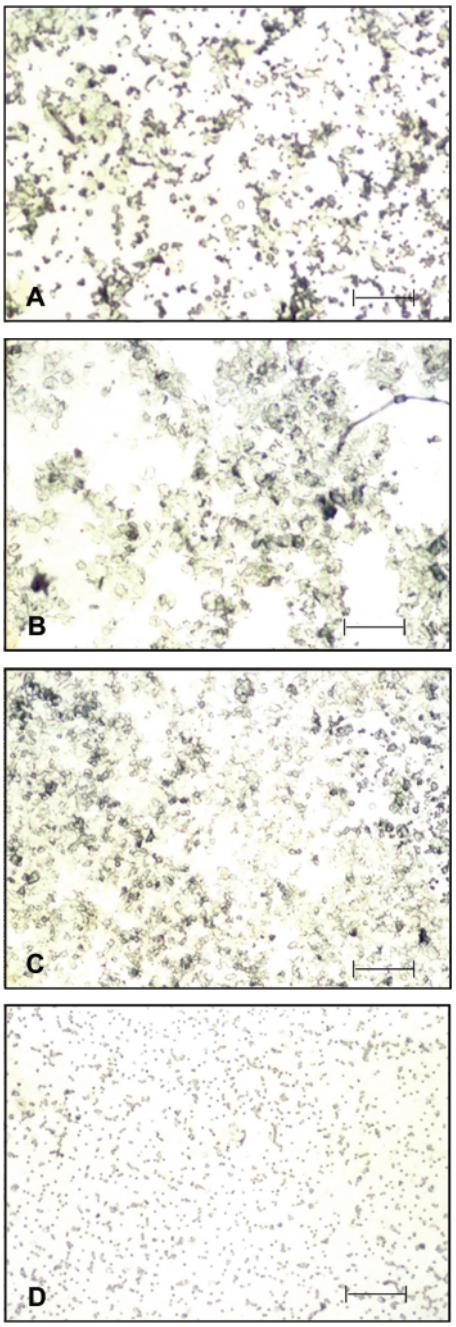

Figure 2 Photomicrographs showing the oestrous phases. (A) Pro-oestrus; (B) oestrus; (C) metoestrus; (D) dioestrus. Unstained vaginal wash. Scale = 100 m.

distance between the sagittal suture and spheno-occipital synchondrosis in the vertical axis, and TCL the distance measured from the anterior portion of the nasal bones to the most posterior part of the occipital bone (Figure 3).

Three specimens from each control and experimental subgroup were prepared as decalcified sections for light microscopy observation of the spheno-occipital synchondrosis. Tissues were fixed in 4 per cent paraformaldehyde 0.1 M phosphate buffer solution for 48 hours and then decalcified in 10 per cent ethylenediamine tetraacetic acid solution for 10–16 weeks. Tissue block preparation followed until 6 m sections were obtained and subsequently stained with haematoxylin–eosin or Gomori's trichrome. Histological section readings were carried out blind with a HM-LUX E600 microscope (Nikon, Tokyo, Japan).

Histomorphometric evaluation was performed on 15 decalcified sections for each subgroup presenting the greater widths of cartilaginous tissue (total cartilage width), encompassing the distance from one hypertrophic zone to the other, at the spheno-occipital synchondrosis (Figure 4). At each section, five equidistant measurements were taken so that an average value could be obtained, from the lowest to the highest portion of the suture. A total of 75 measurements (15 averages) were obtained for each of the eight subgroups. Section images were registered with an AxioCam MRc digital camera (Carl Zeiss, Oberkochen, Germany) at the Axioskop2 plus microscope (Carl Zeiss). The total cartilage width was measured using ImageTool Software 3.0 (San Antonio, Texas, USA).

Descriptive analysis was performed by observation of cartilaginous tissue of the spheno-occipital synchondrosis regarding the following features: number and arrangement of chondroblasts (columns or not); presence and pattern of deposition of the cartilagenous matrix; and presence and amount of bone marrow, trabeculae, and cortical bone.

Fluorescence microscopy was applied to the alizarin-stained sections in order to observe bone mineralization in the period before sacrifice, due to its calcium affinity. The specimens were prepared as ground sections of approximately 70 m using a low speed diamond wheel (model 650, South Bay Technology, Inc. San Clemente, California, USA) and filed at a polisher (Politrix APL-02; Arotec S/A, Cotia, São Paulo, Brazil). The section surfaces were refined with 400, 600, and 1200 grit sandpaper. The readings were taken with Axioskop2 plus microscope with a Fluorescein Fs 09 filter.

Statistical analysis

Rigorous control of sections and micrometer positioning was performed. Any disagreements were discussed between evaluators in order to achieve reliability of the data. One examiner (AMI) blinded to which group the slides belonged, conducted the analysis. Craniometric and histomorphometric, as well as body mass values, were subjected to analysis of variance and *post-hoc* Tukey multiple comparisons testing using the Statistical Package for Social Sciences version 13.0 (SPSS Inc., Chicago, Illinois, USA). Data concerning the beginning of the oestrous cycle were analysed with a *t*-test. As the data were normally distributed, parametric statistics were used.

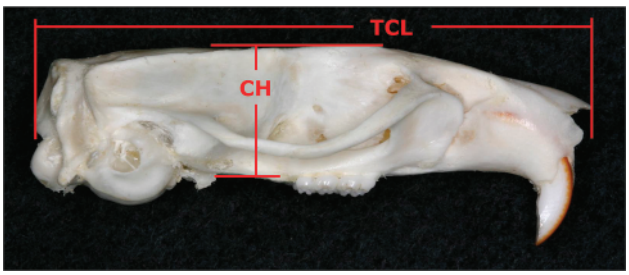

Figure 3 Photograph of a rat skull illustrating: total cranium length (TCL) and cranium height (CH).

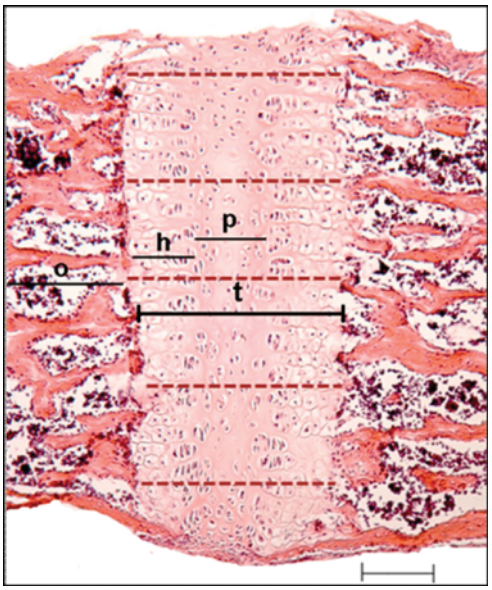

Figure 4 Photomicrograph of the spheno-occipital cartilage of an experimental rat at day 3 illustrating total cartilage width (t), proliferative layer (p), hypertrophic layer (h) and osseous layer (o). Pink dotted lines represent the five measurements taken. Stain: haematoxylin–eosin. Bar = 100 m.

Results

The animals started the oestrous cycle at an average of 51.2 days (SD = 5.9) in the control group and at an average of 41.8 days (SD = 4.1) in the experimental group. Vaginal opening did not occur in animals sacrificed on day 30, obstructing material collection. Data were statistically evaluated using a Student's *t*-test, separately, for each time period (60, 90, and 120). A statistical difference was found between the control and experimental subgroups regarding

the beginning of the cycle (Table 1). The results confirmed the effect of danazol in inducing precocious puberty, validating the sample for continuation of the study.

The weight of the animals at birth and before sacrifice showed no statistically significant difference when the dates were evaluated separately (Table 2). The average values found are shown in Figure 5A.

Craniometric analysis

The average macroscopic measurements of CH and TCL were similar (Figure 5B and 5C). The experimental group had, in all cases, a greater tendency for growth to stop before the control group. No statistical difference was found between the control and experimental groups when compared at each time point (Tables 3 and 4).

Table 1 Statistical values of Wistar rats related to the beginning of oestrous cycle (days).

Group	Subgroup	First Oestrus	SD	Significance
C	60	52.33	4.50	0.012*
E		45.83	2.64	
C	90	53	5.10	<0.001**
E		41	2.19	
C	120	48.33	7.45	0.018*
E		38.83	3.78	
C	All	51.22	5.87	<0.001**
E		41.83	4.13	

*$P < 0.05$; **$P < 0.001$.

Table 2 Body mass of the Wistar rats. Mean (g) at birth and sacrifice periods.

Group	Subgroup	Body mass	SD	Significance
C	0	13	0.63	1.000
E		13.33	0.51	
C	30	80.5	5.99	1.000
E		81.33	3.77	
C	60	192.5	10.89	0.073
E		219	31.06	
C	90	234.83	21.99	1.000
E		237.66	17.06	
C	120	262.5	9.66	0.998
E		269.33	10.46	

*$P < 0.05$.

Qualitative and histomorphometric analysis

At 30 days, synchondrosis was similar in both groups. Chondroblasts organized in columns with matrix interposition were observed in the experimental group; column organization was poor in control group. In the medium portion (junction), cartilaginous matrix was seen, indicating good growth potential despite the low numbers of chondroblasts. Cortical and bone trabeculae were thin with large bone marrow areas (Figure 6A and 6B). No statistical difference was found histomorphometrically between the 30 day controls and the 30 day experimental groups (343.4 and 358.6 m; Figure 7 and Table 5).

For the 60 day specimens, a significant increase in total synchondrosis width was noted but only in the 60 day experimental group, with numerous chondroblasts in all layers and well-defined columns, indicating high growth potential. In the control group, no notable altered layers were observed compared with the first observation at 30 days. The cortical and trabeculae bone marrow areas maintained a similar aspect when compared with the first month, except for the increase in cortical bone in the control group (Figure 6C and 6D). Total width values were in accordance with the descriptive analytical findings, evidencing a statistical difference between the subgroups E60 (434.3 m) and C60 (323.5 m) (Figure 7 and Table 5).

In the 90 day control and experimental subgroups, total width values became equivalent due to the diminished total width in the experimental group. The columns in the control group were better organized; this pattern was not as evident in the experimental group, in which cartilaginous matrix interposition was noted. The experimental group showed an increase in cortical bone width (Figure 6E and 6F). Quantitative analysis again showed values in accordance with the descriptive analysis, with no statistical difference between the 90 day control and experimental subgroups (354.2 and 367.4 m, respectively; Figure 7 and Table 5).

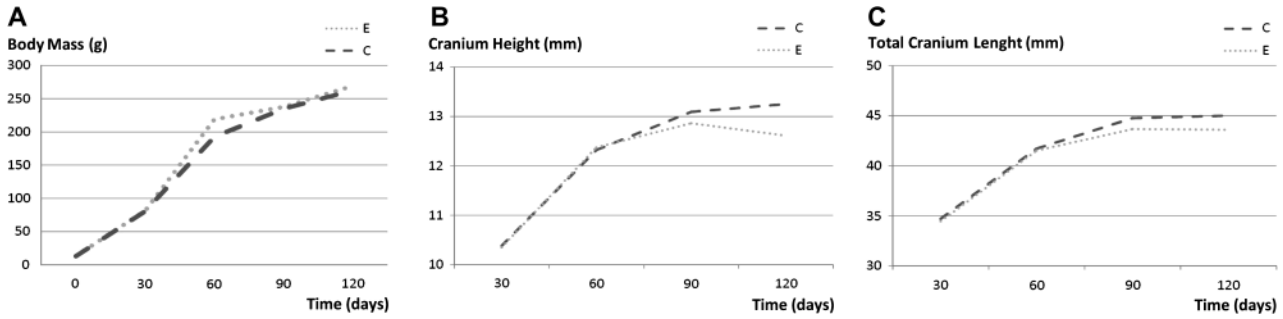

Figure 5 Graph showing (A) body mass; (B) cranium height; and (C) total cranium length of the animals during observation in the control (C) and experimental (E) groups.

Table 3 Paired comparisons of cranium height between the eight subgroups using Tukey's test after one-way analysis of variance.

Subgroups	C30	E30	C60	E60	C90	E90	C120	E120
C30		0.03	−1.94	−1.99	−2.23	−2.48	−2.88	−2.71
E30	**1.000**		−1.97	−2.02	−2.26	−2.51	−2.91	−2.74
C60	<0.001	<0.001		−0.05	−0.29	−0.54	−0.94	−0.77
E60	<0.001	<0.001	**1.000**		−0.24	−0.49	−0.89	−0.72
C90	<0.001	<0.001	0.747	0.883		**−0.25**	−0.64	−0.47
E90	<0.001	<0.001	0.086	0.155	**0.868**		−0.39	−0.22
C120	<0.001	<0.001	<0.001	<0.001	0.023	0.405		**0.17**
E120	<0.001	<0.001	0.003	0.008	0.193	0.922	**0.981**	

Above the diagonal line shows the average differences (mm) and below the diagonal line, its significance (*P* value).

Table 4 Paired comparisons of total cranium length between eight subgroups using Tukey's test after one-way analysis of variance, showing average differences and statistical significances.

Subgroups	C30	E30	C60	E60	C90	E90	C120	E120
C30		**0.25**	−7.01	−6.82	−8.87	−8.96	−10.32	−10.06
E30	**0.997**		−7.26	−7.07	−9.12	−9.21	−10.57	−10.31
C60	<0.001	<0.001		**0.19**	−1.86	−1.95	−3.31	−3.05
E60	<0.001	<0.001	**0.999**		−2.05	−2.14	−3.49	−3.24
C90	<0.001	<0.001	<0.001	<0.001		**−0.09**	−1.44	−1.19
E90	<0.001	<0.001	<0.001	<0.001	**1.000**		−1.35	−1.10
C120	<0.001	<0.001	<0.001	<0.001	0.006	0.011		**0.26**
E120	<0.001	<0.001	<0.001	<0.001	0.038	0.070	**0.996**	

Above the diagonal line shows the average differences (mm) and below the diagonal line, its significance (*P* value).

At 120 days in both groups, a few chondroblasts were seen, which were not arranged in columns; cartilaginous matrix was abundant and advanced ossification was noted; the osseous layer seemed aged, with diminished growth potential due to thick bone corticals and trabeculae (Figure 6G and 6H). No statistically significant difference was found between the average total width in the 120 day control and experimental subgroups (353.1 and 303.2 m, respectively; Figure 7 and Table 5).

In spheno-occipital synchondrosis, a more linear mineralization was identified because of the better organization of cell columns. No difference in deposition was found between the groups. The only alteration seen was in columnar cell quantity, involving bone deposition, throughout time (Figure 8).

Discussion

Despite following the recommended application of danazol (Morishita *et al.*, 1993), the average dates of the first oestrus in this study (51.2 versus 41.8 days) differed from those found by Morishita *et al.* (1993) (38.2 versus 29.1 days) but were in agreement with veterinarian findings, where puberty has been reported to start between 50 and 60 days (Harkness and Wagner, 1993; Mezadri *et al.*, 2004). This is justifiable since there are other factors associated with the establishment of puberty besides the endocrinological component, such as heritage, diet, and body mass (Roberts and Blackwood, 1983).

Based on this knowledge, it was important to divide the litter similarly between control and experimental groups and between subgroups with the same sacrifice date, as well as providing similar conditions of development. Statistical analysis was limited to each specific date in order to validate the sample with regard to the start of the cycle. Since advancement of puberty was little more than 1 week, mixing distinct litters in subgroups with different sacrifice dates could interfere in the results (Roberts and Blackwood, 1983).

The spheno occipital synchondrosis was selected for microscopic investigation due to the clinical importance of such structures for growth of the mid and lower face, functioning as a growth site affecting neighbouring bone dimensions. Another reason is that the cranial base bones associated with the cartilage are not involved in any joint movements and therefore the effect of any muscular forces is limited (Roberts and Blackwood, 1983; Proffit, 2002).

The sacrifice dates selected were chosen to reflect the period before the first oestrus, a time in close proximity, and the period of up to 4 months, when growth is significantly reduced (Roberts and Blackwood, 1983; Tanaka, 1998). Sacrifice dates were based on these principles. However, data collection of groups C60 and E60 was undertaken to

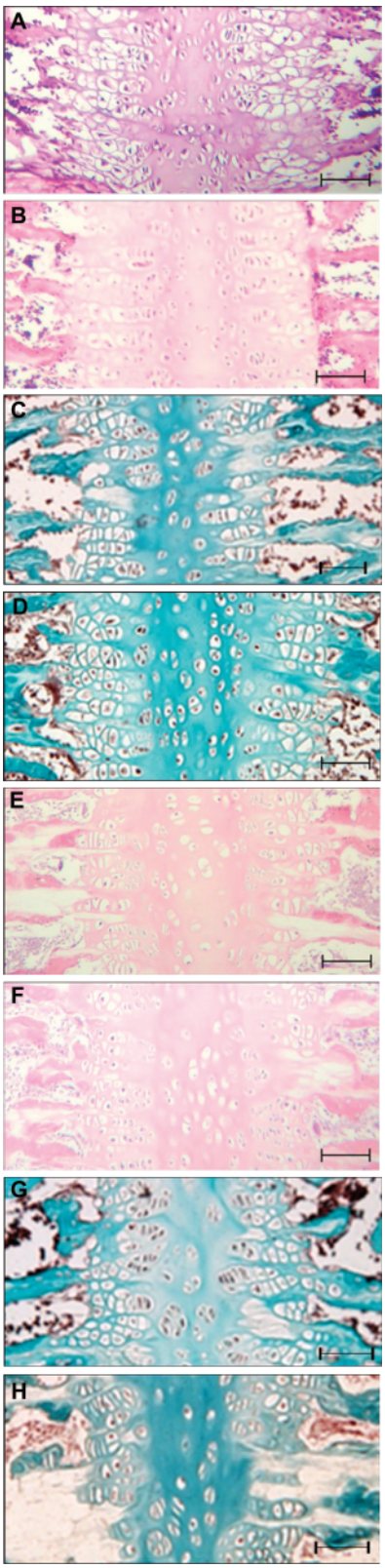

Figure 6 Decalcified sections illustrating morphological characteristics of the spheno-occipital synchondrosis for the control and experimental groups. (A) C30. (B) E30. (C) C60. (D) E60. (E) C90. (F) E90. (G) C120. (H) E120. Stain: haematoxylin–eosin (A, B, E, F). Gomori's trichrome (C, D, G, H) Bar = 100 m.

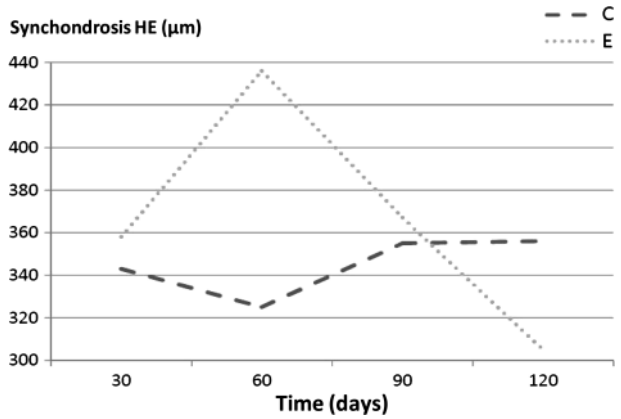

Figure 7 Graph showing total cartilage width of the spheno-occipital synchondrosis of the animals during the observation period in the control (C) and experimental (E) groups.

confirm the beginning of the cycle prior to sacrifice, which served to support the chosen dates.

The beginning of puberty, starting from the central nervous system, leads to a sequence of events that affect bone growth and increase body mass. Although hormonal indicators showed statistically significant values regarding the beginning of the oestrus cycle, in the control and experimental subgroups, no statistical differences were noted when body mass, TCL, and CH were evaluated between the subgroups. Despite the unexpected findings, these results are in agreement with those of Flor-Cisneros *et al.* (2004), who stated that the magnitude of advance or delay in puberty is related to an advance or delay in skeletal maturation. In contrast, maturation of the hypothalamus-hypophisis-gonad axis is not synchronous to other maturation processes, such as body mass and height (Flor-Cisneros *et al.*, 2004).

Despite the lack of synchrony, no differences were found between subgroups of the same age. A possible explanation for this finding could be the time between sacrifice dates, if the metabolic speed of these animals and the short difference in days corresponding to the beginning of the cycles between subgroups of the same date (from 7 to 12 days) are considered. This might be of importance not only in the establishment of shorter time intervals but adding a new subgroup between dates corresponding to the beginning of the cycle for the control and experimental groups.

Histological light microscopy findings showed similar descriptive and histomorphometric differences (Luder, 1994) compatible with the occurrence of precocious puberty in the experimental as opposed to control group, for all time periods and some of the subgroups. However, microscopic alterations for each date were not evidenced macroscopically.

Light microscopy of the spheno-occipital synchondrosis showed minimal differences in total cartilage width, between subgroups for each date, and longitudinally, except at 60 days, when the value in the experimental subgroup was

Table 5 Paired comparisons of spheno-occipital synchondrosis total width between the eight subgroups using Tukey's test after one-way analysis of variance.

Subgroups	C30	E30	C60	E60	C90	E90	C120	E120
C30		−15.3	19.8	−91.0	−10.8	−24.0	−9.8	40.2
E30	0.988		35.1	−75.7	4.4	−8.8	5.5	55.4
C60	0.950	0.554		−110.8	−30.7	−43.8	−29.6	20.3
E60	0.003	0.013	<0.001		80.1	66.9	81.2	131.1
C90	0.998	1.000	0.697	0.008		−13.2	1.1	51.0
E90	0.879	1.000	0.301	0.033	0.995		14.2	64.2
C120	0.999	1.000	0.730	0.007	1.000	0.992		49.9
E120	0.398	0.107	0.944	<0.001	0.163	0.044	0.179	

The upper triangle presents average differences (m) and the lower one, its significance (*P* value).

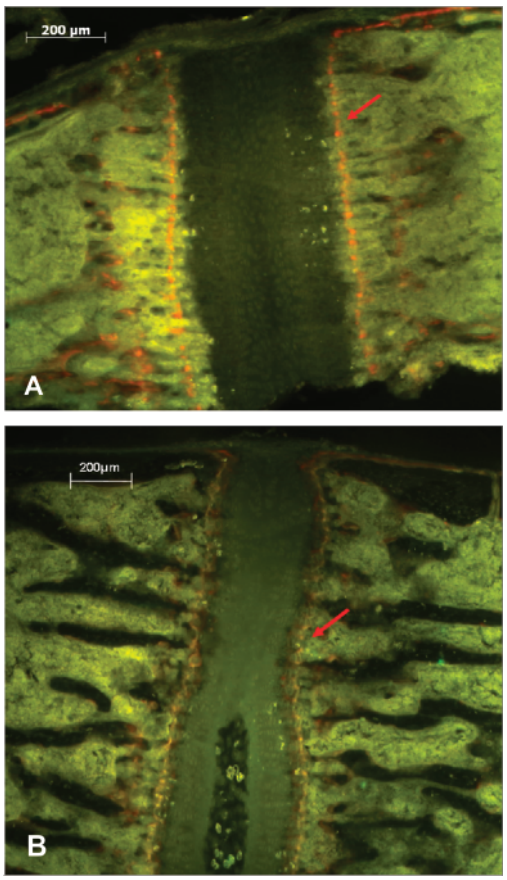

Figure 8 Ground sections of spheno-occipital synchondrosis illustrating bone mineralization (arrow). (A) Thirty day control group. (B) Ninety day experimental group. Bar = 200 m.

larger than in the control subgroup ($P < 0.05$). This confirms that the spheno-occipital synchondrosis is a typical primary cartilage; it does not demonstrate significant changes in width from external stimuli and its growth potential ceases simultaneously with the end of overall growth, through gradual sutural ageing (Roberts and Blackwood, 1983; Byers *et al.*, 2000).

The fluorescence microscopy findings were in accordance with descriptions of bone mineralization for cartilage, showing a linear pattern in synchondrosis due to cell column organization. No evident difference was noted regarding mineralization between the subgroups. The width of the cell column involved in mineralization could have provided evidence of increasing and decreasing speed, differentiated among subgroups, had different sacrifice dates been chosen for this study.

Conclusions

An appropriate model for studying bone growth associated with precocious puberty in Wistar female rats was not achieved using danazol steroid hormone. When evaluated at 30 day intervals, these effects did not show statistically significant differences regarding body mass and anatomical height and length of the cranium, between subgroups for each sacrifice date.

Qualitative and histomorphometric light microscopy analysis showed characteristics in accordance with a stage of advanced maturity of cartilaginous tissue in the experimental group. However, bone mineralization under fluorescence microscopy did not demonstrate representative alterations between subgroups at any sacrifice date.

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References

Brown J J, Warne G L 2006 Growth in precocious puberty. Indian Journal of Pediatrics 73: 81–88

Byers S, Moore A J, Byard R W, Fazzalari N L 2000 Quantitative histomorphometric analysis of the human growth plate from birth to adolescence. Bone 27: 495–501

Carel J C, Lahlou N, Roger M, Chaussain J L 2004 Precocious puberty and statural growth. Human Reproduction Update 10: 135–147

Chemaitilly W, Trivin C, Adan L, Gall V, Sainte-Rose C, Brauner R 2001 Central precocious puberty: clinical and laboratory features. Clinical Endocrinology 54: 287–288

Flor-Cisneros A *et al.* 2004 In boys with abnormal developmental tempo, maturation of the skeleton and the hypothalamic-pituitary-gonadal axis remains synchronous. Journal of Clinical Endocrinology and Metabolism 89: 236–241

Ge X, Conger R D, Elder Jr G H 2001 The relation between puberty and psychological distress in adolescent boys. Journal of Research on Adolescence 11: 49–70

Harkness J E, Wagner J E 1993 Biologia e Clínica de Coelhos e Roedores. Livraria Roca Ltda, São Paulo

Luder H U 1994 Perichondrial and endochondral components of mandibular condylar growth: morphometric and autoradiographic quantitation in rats. Journal of Anatomy 185: 587–598

Marcondes F K, Bianchi F J, Tanno A P 2002 Determination of the estrous cycle phases of rats: some helpful considerations. Brazilian Journal of Biology 62: 609–614

Mezadri T J, Tomáz V A, Amaral V L L 2004 Animais de Laboratório. Cuidados na Experimentação Animal. Editora da UFSC, Florianópolis, Santa Catarina

Morishita H, Takemoto M, Kondo H, Higuchi K, Aono T 1993 Induction of true precocious puberty by neonatal treatment with Danazol in female rats. Neuroscience Letters 157: 33–36

Phinney V G, Jensen L C, Olsen J A, Cundick B 1990 The relationship between early development and psychosexual behaviors in adolescent females. Adolescence 25: 321–332

Proffit W R 2002 Ortodontia Contemporânea. Editora Guanabara Koogan S. A, Rio de Janeiro

Roberts G J, Blackwood H J 1983 Growth of the cartilages of the mid-line cranial base: a radiographic and histological study. Journal of Anatomy 136: 307–320

Roberts W E 2002 Fisiologia do Osso, Metabolismo e Biomecânica na Prática Ortodôntica. In: Graber T M, Vanarsdall R L Jr (eds). Ortodontia—Princípios e Técnicas Atuais. Guanabara-Koogan, Rio de Janeiro, p. 920

Roth C *et al.* 2004 Pituitary and gonadal effects of GnRH (gonadotropin releasing hormone) analogues in two peripubertal female rat models. Pediatric Research 55: 126–133

Sierra A M 1987 Assessment of dental and skeletal maturity. A new approach. Angle Orthodontist 57: 194–208

Tanaka O M 1998 Estudo dos efeitos da aplicação de hormônio de crescimento em ratos Wistar. Thesis, Universidade Federal do Rio de Janeiro

Taranger J, Hägg U 1980 The timing and duration of adolescent growth. Acta Odontologica Scandinavica 38: 57–67

Tian Z, Zhao H, Sun Y, Cai D, Chen B 2005 Evaluation of the true precocious puberty rats induced by neonatal administration of danazol: therapeutic effects of nourishing "Yin"-Removing "Fire" Chinese herb mixture. Reproductive Biology and Endocrinology 3: 38

Tian Z Z, Zhao H, Chen B Y 2004 Decreased hypothalamic aromatization in female rats of true precocious puberty. Neuroscience Letters 366: 92–96

Van der Sluis I M, Boot A M, Krenning E P, Drop S L, de Muinck Keizer-Schrama S M 2002 Longitudinal follow-up of bone density and body composition in children with precocious or early puberty before, during and after cessation of GnRH agonist therapy. Journal of Clinical Endocrinology and Metabolism 87: 506–512