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# Influence Of Ethnicity On The Human Term Placenta

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## Abstract

**Objective:** To perform a morphometric comparative analysis of placentas from Indigenous mothers (Guajajara communities) and non-Indigenous mothers, inhabitants in the rural areas of Imperatriz County, Maranhão State, Brazil.

**Methods:** Ten placentas from Indigenous mothers and 10 from non-Indigenous mothers, collected in a case-control study design, were submitted to stereological analysis.

**Results:** Several differences between Indigenous placentas and non-Indigenous placentas were demonstrated. The placentas from Indigenous mothers were oval-shaped and pale (50%), heavier and larger, with higher placental weight index, surface of insertion, diameter, ratio between diameters, eccentricity and index of contour, and smaller shape coefficient. Volumes occupied by syncytiotrophoblast, trophoblast and villi, the villous surface and the trophoblast thickness were all bigger in the Indigenous placentas.

**Conclusion:** The Indigenous placenta possesses a bigger functional reserve, being able, if necessary, to increase the number of vasculosyncytial membranes by the peripheral displacement of capillaries, facilitating the exchanges between maternal and fetal bloods.

## INTRODUCTION

Several studies were performed to assess the association between maternal factors and fetal growth [1,2,3,4,5]. Despite the fundamental role played by the placenta as a metabolic regulator of supply of all nutrients necessary to fetal growth [6], relatively fewer information is available about the influence of maternal factors on the development and structure of the placenta. Ethnicity is one of the maternal factors that can affect the fetal and placental development. However, its influence is frequently masked by socioeconomic and demographic disparities, as well as by the variable degree of miscegenation [3,4,7].

Many Brazilian Indigenous communities rarely have contact with the rest of the county population. They live on the land they were born (reservation), where still preserve the culture, religion and customs, relatively free of miscegenation. The study of placentas of indigenous mothers whom inhabit in reservations, using as controls placentas from non-Indigenous women resident in the rural area under similar environmental conditions, will contribute for the best

understanding of the ethnic influence on the placental structures.

Guajajara people are one of the most numerous Brazilian Indigenous communities. These natives live in 11 Indigenous lands at the eastern margin of the Amazon region, all of them located in the interior of the State of Maranhão. Traditionally, the Guajajara mothers gave birth in nets, mounts of sand covered with mat or even in the soil. Only in recent years, Guajajara pregnant has searched the health services for prenatal care and to give birth. By its privileged geographical location, the Women's and Children's Regional Hospital ("Hospital Regional Materno-Infantil" - HRMI) at the city of Imperatriz, Maranhão State, Brazil, is the most requested by the Indigenous women during pregnancy and parturition.

The objective of the present work is to evaluate comparatively the morphometric characteristics of placentas belonging to Indigenous mothers and non-Indigenous mothers proceeding from the rural area of Imperatriz County, who are assisted at the HRMI, by means of a case-

control study design.

### **MATERIAL AND METHODS**

All the placentas were obtained after spontaneous vaginal deliveries from uncomplicated term singleton pregnancies of healthy, non-smoking mothers. According to the case-control study design, a set of 10 placentas belonging to Guajajara Indigenous mothers was collected. Another set of 10 placentas from non-Indigenous mothers was selected, so that each of them matched to an Indigenous placenta as closely as possible on maternal age, weight, height, parity, socioeconomic status, gestational age, and newborn sex. Gestational age was calculated from the date of the last menses and confirmed by ultrasound examination.

Each placenta was examined immediately after delivery to ascertain its appearance, shape, color, consistency, as well as any alteration of the parenchyma, cord or membranes. The umbilical cord and free membranes were trimmed from the placental disc before it was weighed and its dimensions (length, width and depth) measured. The placental weight index was appraised by the placenta weight to newborn weight (both in g) ratio. The placental volume was determined by liquid displacement. Knowing the length and the width of the placental disc, it was calculated the area of the surface of placental insertion and assessed objectively the shape of the placenta by means of the length to width ratio, shape factor, eccentricity and contour index [8].

The placental disc was cut vertically into 1 cm thick slices, being each slice individually examined. Two fragments of placental tissue, one from the central region and another one from the intermediate region, involving from the fetal surface to the decidual floor, were isolated, fixed in 10 per cent formol saline for 12 h and embedded in paraffin wax. Five  $\mu\text{m}$ -thick sections were stained by the PAS-hematoxylin technique.

A correction factor for shrinkage due to histological processing was estimated for each placenta and introduced into the equations used for the stereological calculations [9].

The stereological analysis was performed with the C4 test-system [10]. Volume densities of intervillous space, fibrinoid, villi, trophoblast (cytotrophoblast and syncytiotrophoblast), villous stroma, conjunctive tissue, and capillaries, were estimated by the test-points counting method. Volume densities were converted into absolute volumes by multiplying them by the placental volume. Furthermore, cytotrophoblast to syncytiotrophoblast ratio (S/C) and the

vascularization index (VI) were determined.

Surface densities of villi, capillaries and vasculosyncytial membranes (VSM) were estimated by stereological methods [11]. Surface areas of these structures were obtained multiplying the respective surface densities by the placental volume. They were also determined the capillary-villous surface ratios (C/V) and the percentage of villous surface occupied by VSM. Mean diameters of villi and capillaries and the average thickness of the trophoblast were estimated employing suitable stereologic methods [12,13].

Data are presented as mean  $\pm$  SEM, and were analyzed using the Student's t-test for matched samples (for normally distributed continuous variables). The Fisher's exact test was used to assess categorical variables. All statistical tests were performed using the Prism 3.00 software for Windows (GraphPad Software, San Diego, CA). A two-tailed p value  $< 0.05$  was regarded as significant.

Approval from the Committee on Ethics in Research of the School of Dentistry of Ribeirão Preto, USP, was obtained prior to the commencement of this study.

### **RESULTS**

In each group, there were 5 male newborns and 5 female newborns. Birth weight showed no statistical differences between Indigenous newborns and non-Indigenous newborns ( $3422 \pm 176.8$  g and  $3235 \pm 183.4$  g, respectively), as well as the length at birth ( $48.8 \pm 0.7$  cm and  $49.6 \pm 0.6$  cm, respectively). On the other hand, the body mass index and the Rohrer's ponderal index were significantly higher ( $p < 0.01$ ) in the Indigenous infants ( $14.53 \pm 1.77$  kg/m<sup>2</sup> and  $29.29 \pm 0.93$  kg/m<sup>3</sup>, respectively) than in the non-Indigenous infants ( $13.05 \pm 1.33$  kg/m<sup>2</sup> and  $26.43 \pm 0.59$  kg/m<sup>3</sup>, respectively).

The shape of all the Indigenous placentas was oval, while among the non-Indigenous group there were 7 round placentas and 3 oval ones. The aspect of the Indigenous placenta was pale in 5 cases, while all the placentas from non-Indigenous mothers were red-alive. The insertion of the cord was central in 6 placentas and marginal in 4 placentas from Indigenous mothers, whereas among the non-Indigenous placentas the insertion of the cord was central in 7 and marginal in 3. The Fisher's exact test showed significant differences between the two groups both in placenta shape ( $p = 0.003$ ) and placenta color ( $p = 0.03$ ).

Except for the width and depth, all the macroscopic

morphometric parameters of the placenta showed significant differences between both groups. Thus, the Indigenous placentas were heavier and larger, with higher placental weight index, larger surface of insertion, higher length, length to width ratio, eccentricity and index of contour, and lower shape factor (Table 1).

**Figure 1**

Table 1: Macroscopic characteristics of the Indigenous placentas and non-Indigenous placentas (Mean ± SEM).

Parameter	Indigenous placenta	Non-Indigenous Placenta	Mean difference	Student's t-test	p
Weight (g)	509.5 ± 34.4	421.0 ± 38.1	88.5	t = 3.94	< 0.01
Volume (cm <sup>3</sup> )	453.6 ± 31.1	376.5 ± 32.4	77.1	t = 3.88	< 0.01
Placental weight index	0.15 ± 0.01	0.13 ± 0.01	0.02	t = 2.44	< 0.05
Surface (cm <sup>2</sup> )	308.4 ± 14.8	257.6 ± 18.2	50.8	t = 2.34	< 0.05
Length (cm)	23.0 ± 0.7	18.8 ± 0.7	4.2	t = 5.52	< 0.001
Width (cm)	17.0 ± 0.6	17.4 ± 0.6	- 0.4	t = - 0.46	n.s.
Depth (cm)	1.5 ± 0.1	1.5 ± 0.1	0.0	t = - 0.04	n.s.
Length/Width ratio	1.37 ± 0.05	1.08 ± 0.03	0.29	t = 4.34	< 0.01
Contour index	3.62 ± 0.01	3.55 ± 0.01	0.06	t = 3.71	< 0.01
Eccentricity	0.64 ± 0.04	0.29 ± 0.07	0.36	t = 3.84	< 0.01
Shape factor	0.96 ± 0.01	0.99 ± 0.01	- 0.03	t = - 3.60	< 0.01

The relative volumes occupied by the placental structures showed no significant differences between both groups, while the absolute volume of trophoblast, syncytiotrophoblast and villi were significantly higher in the Indigenous mother placentas (Table 2).

**Figure 2**

Table 2: Microscopic characteristics of Indigenous placentas and non-Indigenous placentas. Absolute volumes (cm<sup>3</sup>). (IVS: intervillous space; C/S: Cytotrophoblast to syncytiotrophoblast ratio; VI: vascular index) (Mean ± SEM).

Parameter	Indigenous placenta	Non-Indigenous Placenta	Mean difference	Student's t-test	p
Trophoblast	119.1 ± 7.1	89.4 ± 7.5	29.7	t = 2.95	< 0.05
Syncytiotrophoblast	106.0 ± 6.9	82.5 ± 7.4	23.5	t = 2.60	< 0.05
Cytotrophoblast	10.9 ± 2.2	6.8 ± 1.2	4.1	t = 1.55	n.s.
Fibrinoid	3.0 ± 0.7	1.5 ± 0.5	1.5	t = 1.67	n.s.
Stroma	188.4 ± 17.7	163.4 ± 12.0	25.0	t = 1.67	n.s.
Connective tissue	119.6 ± 12.6	111.2 ± 10.5	8.4	t = 0.88	n.s.
Capillaries	68.8 ± 8.2	51.9 ± 4.2	16.9	t = 2.06	n.s.
Villi	305.6 ± 21.8	252.9 ± 17.8	52.7	t = 3.07	< 0.05
IVS	145.3 ± 11.5	121.8 ± 14.7	23.5	t = 2.07	n.s.
C/S	0.11 ± 0.02	0.08 ± 0.02	0.03	t = 0.92	n.s.
VI	0.22 ± 0.01	0.21 ± 0.01	0.01	t = 0.62	n.s.

The superficial density of villi, capillaries and VSM, the capillary to villous surface ratio and the percentage of villi surface occupied by VSM showed no significant differences

between both groups. The villous surface area in the Indigenous mother placenta was significantly larger than in the non-Indigenous placenta (Table 3).

The average diameter of villi and capillaries showed no significant differences between the two groups, while the trophoblast was significantly thicker in the Indigenous mother placenta (Table 3).

**Figure 3**

Table 3: Microscopic characteristics of Indigenous placentas and non-Indigenous placentas Absolute surface (m<sup>2</sup>), diameter of villi and capillaries (Åµm) and trophoblast thickness (Åµm). (VSM: vasculosyncytial membranes; capillaries surface to villi surface ratio) (Mean Å± SEM).

Parameter	Indigenous Placenta	Non-Indigenous Placenta	Mean difference	Student's t-test	p
Villous surface	13.83 ± 1.46	11.00 ± 1.12	2.83	t = 2.29	< 0.05
Capillaries surface	13.48 ± 1.33	11.98 ± 0.61	1.50	t = 1.19	n.s.
VSM surface	2.91 ± 0.41	2.69 ± 0.48	0.28	t = 0.63	n.s.
Villous diameter	62.7 ± 3.7	60.9 ± 3.1	1.8	t = 0.38	n.s.
Capillary diameter	13.0 ± 0.7	11.1 ± 0.7	1.9	t = 1.93	n.s.
Trophoblast thickness	6.7 ± 0.3	5.9 ± 0.2	0.8	t = 4.01	< 0.01
C/V surface ratio	1.06 ± 0.11	1.14 ± 0.11	-0.08	t = -0.72	n.s.
VSM surface (%)	21.1 ± 1.8	25.3 ± 2.1	-4.2	t = -1.90	n.s.

**DISCUSSION**

All the placentas belonging to Indigenous mothers were oval, while among the non-Indigenous placentas there were only 3 oval-shaped. This difference was highly significant. Similarly, there was a significantly higher incidence of pale placentas (50%) between the Indigenous mothers. The color of the placenta normally is red-alive, and the pallor is generally associated to maternal anemia or infection of the villous parenchyma [14]. The Histopathological examination of all the placentas studied did not show the presence of infection, and the routine clinical and laboratory analysis showed no evidence of anemia among the Indigenous mothers.

No significant differences were found between the groups with relation to the site of implantation of the umbilical cord. In the present study, weight, volume and surface of implantation were larger in placentas from Indigenous mothers than in placentas from non-Indigenous mothers. Hendricks [15] and Freeman et al. [16] related that placentas from white mothers were significantly heavier than those from black mothers. Jackson et al. [17], however, found no significant difference between the weights of placentas from Indigenous and non-Indigenous mothers of Bolivia, at the

same conditions of altitude. Zhan [18] demonstrated that, in spite of heavier newborns, Tibetan mothers had lighter placentas than Han mothers, under the same environmental conditions, whereas Sivarao et al. [19] showed that placental surface area, placental weight and placental volume of Indian patients were significantly smaller than in Malayan and Chinese patients. Differences found by several authors were attributed mainly to ethnic and biological factors. However, Gruenwald [20] claimed that differences would be more socioeconomic than biological. Meanwhile, this author recognized to be impossible to separate clearly the effects of maternal factors of those due to the environment on the fetal supply line [21].

The increased weight, volume and surface of implantation of the Indigenous placentas, are justified by its enlarged length when compared with the non-Indigenous placentas.

The placental weight index was higher in the Indigenous than in the non-Indigenous mothers, due to the heavier placenta in the firsts, whereas the newborn weights were similar in both groups, resulting in the increased placenta weight to newborn weight ratio. Values of the placental weight index are higher in the non-whites than in whites until the 34th week, remaining afterwards similar, suggesting the absence of ethnic differences in the last trimester of pregnancy [22]. The placental weight index in the Tibetan newborn is lower than in the Han newborn, under the same environmental conditions [18]. The difference was attributed to the ethnic factor. For similar newborn weights, smaller placenta weight index would indicate better irrigation of the placenta and better supply of nutrients to the fetus [23]. A greater and paler placenta of newborns with similar weight and stature would reflect a less efficient uteroplacental circulation among the Indigenous mothers. Biochemical, ultrasonographic and uterine artery Doppler flow assessment would be necessary to evaluate this hypothesis.

All the objective parameters that assess the shape demonstrated that Guajajara placentas have a more prolonged shape than the non-Indigenous ones. Normally, the placenta is discoid, being considered this the better shape to develop a more efficient function [24]. In pregnancies developed in the altitude, with a less favorable situation of oxygenation, the placenta shape is most frequently oval [25].

The relative volumes of the placental structures were similar in both groups. Jackson et al. [26] found no differences in the relative volumes of the placenta structures belonging to Indigenous and non-Indigenous of Bolivia. Meanwhile,

Mayhew et al. [27] found increased relative volume of fibrinoid in placentas belonging to Bolivian Indigenous mothers when compared with placentas of non-Indigenous mother, at the same altitude.

Significant increased volumes of villi, trophoblast and syncytiotrophoblast were demonstrated in the Indigenous placentas. Moreover, Jackson et al. [17] demonstrated more trophoblast and villous stroma in the Bolivian Indigenous placentas than in the non-Indigenous placentas.

No significant differences were demonstrated in the surface density of villi, capillaries and VSM, as well as in the capillary surface to villi surface ratio and in the percentage of villous surface occupied by VSM in the Indigenous placenta and in the non-Indigenous one. In contrast, Zhan [18] demonstrated a higher frequency of VSM and vascular proliferation in the placentas belonging to Tibetan mothers than in those belonging to Han mothers.

The analysis of the areas of surface of villi, capillaries and VSM in placentas from the two groups demonstrated a significant increase of 2.83 m<sup>2</sup> in the villous surface of the placenta belonging to the Indigenous mothers. Jackson et al. [26] observed no differences in the superficial areas of villi and capillaries in placentas belonging to Bolivian Indigenous mothers when compared with placentas of non-Indigenous mothers, in the same conditions of altitude.

The analysis of diameters of villi and capillaries demonstrated no differences between the placentas of our two groups. In Bolivia, according to Jackson et al. [17], the villous capillaries were longer and thinner in the Indigenous placenta than in the non-Indigenous placenta, in the same environmental conditions.

Statistical analysis demonstrated that the trophoblast was significantly thicker in the villi from Guajajara placentas than in non-Indigenous placentas.

The results allow affirm that the placenta belonging to Indigenous mothers is heavier, greater, with higher placenta index and larger surface of insertion, with a higher volume occupied by villi, trophoblast and syncytiotrophoblast, greater villous surface and thicker trophoblast layer. These findings suggest the existence of a bigger functional reserve in the Indigenous placenta, being able, when necessary, to decrease the trophoblastic membrane thickness, and to increase the number of VSM by capillary marginalization, improving diffusive conductance between maternal and fetal

bloods [28].

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