Stereological analysis of alpha and beta zones of syncytiotrophoblast in human term placenta

BOSILJKA DURST-ŽIVKOVIĆ* and DURDICA GRBEŠA

Department of Histology and Embryology, Faculty of Medicine, University of Zagreb, Zagreb, Republic of Croatia, Yugoslavia

ABSTRACT **The investigations were carried out to attempt to define and analyze various quantitative structural parameters of syncytiotrophoblast in human term placenta, especially of its functionally active parts, that is alpha and beta zones. The results demonstrated the following: The arrangement of alpha and beta zones in the placenta as a whole is even and regionally independent. Beta zones prevail quantitatively. Alpha zones make up only 8% of the total volume, 18% of the total surface and 39% of the thickness of beta zones.Sexual dimorphism is shown by a significantly higher volume density (V_{Va}) (P<0.002), total volume (V_a) (P<0.05) and surface density (S_{Va}) (P<0.025) of alpha zones in placentas of female newborns. The fetoplacental index is higher in male newborns. During the tenth lunar month the structure of syncytiotrophoblast is changed. Between the 38th and 39th week the volume and surface densities of alpha zones are significantly higher (P<0.01), and in the 40th week the volume density and total volume of beta zones prevail significantly (P<0.01).**

KEY WORDS: *placenta, syncytiotrophoblast, stereology*

Introduction

Human placenta is a highly complex organ with multiple functions of synthesis and transport of substances due to its endocrine, immunological and metabolic function. During pregnancy, it becomes structurally differentiated and adapts to the increased needs of the growing fetus. Thus, the nutritional function of the placenta becomes adequate to the infant's needs during the final weeks of pregnancy. The prenatal morphological and functional degree of placental maturity influences the child during delivery and its postnatal development. The most important knowledge of the metabolic function in placenta has been obtained through studies of the mature organ.

Transport of substances between the blood of the mother and the fetus takes place in the metabolically active syncytial layer of the trophoblast. This main component of the placental barrier in the term placenta is not structurally and functionally homogeneous (Amstutz, 1960). During development it becomes differentiated into functionally specialized and morphologically different parts (Baker *et al.*, 1944; Getzowa and Sadowsky, 1950; Wislocky and Dempsey, 1955; Becker and Bleyl, 1961; Clavero-Nuñez, 1962; Boyd and Hamilton, 1967; Dearden and Ockleford, 1983) (Fig. 1). Light-microscopically, it has already been observed (Bremer, 1916; Getzowa and Sadowsky, 1950) that some very thin parts of cytoplasm of the syncytiotrophoblast lie directly over the capillaries of chorionic villi. The two components are not fused. They remain separate, but functionally they make one whole, the so-called vasculo-syncytial membrane.

Ultrastructural analysis of the syncytiotrophoblast of term placenta has shown that it contains two structurally and functionally different parts, alpha and beta zones (Burgos and Rodríguez, 1966). These zones are scattered and blended within the syncytiotrophoblast. Therefore the free surface of chorionic villi assumes the form of a mosaic.

In the alpha zone, which corresponds to the syncytial part of the vasculo-syncytial membrane, the syncytiotrophoblast does not usually contain nuclei, it is thinner, 2-14 μm thick (Burgos and Rodríguez, 1966), covered with microvilli among which are pinocytotic vacuoles, vesicles, apical canaliculi, phagosomes and lysosomes. On the basal surface there are numerous twists like those on nephron epithelia reflecting absorption. Alpha zones are more numerous on the top of the free chorionic villi.

Light-microscopically, the beta zone is the thicker layer of cytoplasm (16-60 μm thick) (Burgos and Rodríguez, 1966) with a larger number of nuclei and usually well-defined nucleoles. The nuclear membrane has pores. Under this zone lie just a few cytotrophoblast cells. Microvilli varying in number and size protrude from the surface of the syncytium into intervillous space (Burgos and Cavicchia, 1974). In contrast, the cellular surface of the cytotrophoblast is smooth (Dearden and Ockleford, 1983).

In our previous research we attempted to define and analyze various quantitative structural parameters of syncytiotrophoblast in human term placenta, especially of its functionally active parts, *i.e.* alpha and beta zones. Morphometric analyses were performed on normally structured term placentas of physiological pregnancy and delivery. Quantitative parameters of the mentioned zones were

***Address for reprints**: Department of Histology and Embryology, Faculty of Medicine, University of Zagreb, Salata 3, 41000 Zagreb, Republic of Croatia, Yugoslavia. FAX: 38-41-424.001

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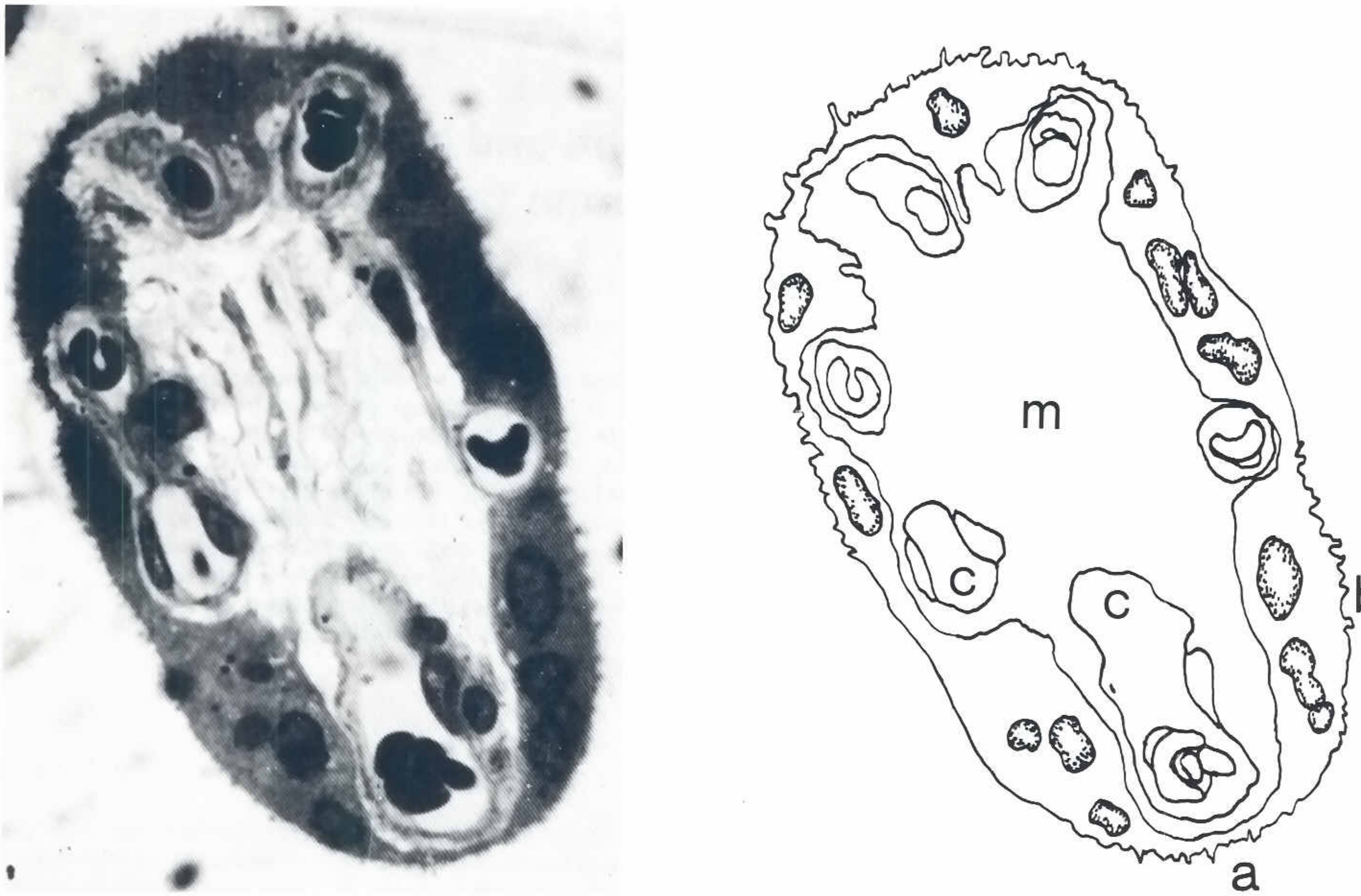

Fig. 1. Terminal chorionic villus. *Touluidine blue, x1000. a, alpha zone; b, beta zone; c, capillary; m, mesenchyme.*

defined by stereological methods from different points of view. Therefore in this work we describe procedures and results from our various research.

Results and Discussion

A few authors have shown by morphological analyses of placental structure that placenta is not homogeneous, *i.e.* that certain regions of the organ differ structurally (Boyd *et al.*, 1980; Bacon *et al.*, 1986; Schuhmann and Wynn, 1980; Teasdale, 1978; Matheus and Sala, 1989; Pivalizza *et al.*, 1990). We are in agreement because in our past research we found differences in the quantitative parameters of some structural components between the region of umbilical cord insertion and placental periphery (Grbesa, 1984; Grbesa and Durst-Zivkovic, 1989; Rajkovic *et al.*, 1990).

Prompted by these results we have performed a systematic quantitative analysis of alpha and beta zones of syncytiotrophoblast in the human term placenta from two points of view: comparatively, between the region of umbilical cord insertion and placental periphery, and then in the organ as a whole. Comparatively, values of the volume (VV) and surface (SV) density both in alpha and beta zones did not differ significantly. We conclude that syncytiotrophoblast in placenta as a whole is structurally homogeneous with regard to alpha and beta zones. In both regions the volume (V_{Vb}) and surface (S_{Vb}) density of the beta zone are significantly higher than those of the alpha zone ($P<0.0005$) (Table 1).

In placenta as a whole, values of the total volume (V) and total surface (S) are significantly higher for beta zones. So the total volume of the alpha zone (Va) makes up only 8% of the total volume of the beta zone (V_b), and the total surface of the alpha zone (S_a) only 18% of the total surface of the beta zone (S_b) (Table 2).

The mean thickness of alpha and beta zones was stereologically estimated. The arithmetical thickness of zones was defined by measuring the length of intercepts (Gundersen *et al.*, 1978).

TABLE 1

VOLUME (V_V) AND SURFACE (S_V) DENSITY OF ALPHA (a) AND BETA (b) ZONES AT THE PERIPHERY OF PLACENTA AND IN THE REGION OF UMBILICAL CORD INSERTION

Stereological variable	Periphery $x \pm 1$ SE	Insertion $x \pm 1$ SE	Whole placenta $x \pm 1$ SE
$V_{Va}/mm^0/$	0.012 ± 0.001	0.012 ± 0.001	0.012 ± 0.001
$V_{Vb}/mm^0/$	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
$S_{Va}/mm^{-1}/$	3.57 ± 0.31	4.14 ± 0.50	3.94 ± 0.29
$S_{Vb}/mm^{-1}1/$	25.58 ± 0.66	24.29 ± 0.55	24.93 ± 0.43

TABLE 2

TOTAL VOLUME (V), TOTAL SURFACE (S) AND MEAN THICKNESS OF ALPHA (a) AND BETA (b) ZONES

Stereological variable	V/cm³/ x ±1 SE	S /m²/ x ± 1 SE	Thickness /µm/ x ± 1 SE
a	6.85± 0.73	2.26 ± 0.31	1.81 ±0.10
b	87.87± 6.34	12.62 ± 0.45	4.59 ±0.15

Measuring was performed on 1 µm thick sections, colored with toluidine. Mean values of the thickness of alpha zones were significantly lower ($P<0.0005$) (Table 2).

Numerical values of structural parameters show that functional activity of syncytiotrophoblast is even in the whole placenta. This refers both to biosynthesis in beta zones and to transport of gases through alpha zones. The quantitative prevalence of beta zones manifests the predominance of biosynthesis. The functional characteristics of each zone are manifested in differences of their thickness: the thinner alpha zone is primarily the place of transsyncytial transport, and in the thicker beta zones cellular organelles and products of synthesis are accumulated.

There has been very little research done on the structural characteristics of the placenta depending on the newborn's sex, and quantitative results did not show any sex-dependent differences (Jackson *et al.*, 1987a,b). We have performed research on sexual dimorphism of the placenta at the level of syncytiotrophoblast on 16 term placentas of female and 14 term placentas of male newborns. The gestational age of both groups did not differ significantly. Female newborns had lower body weight but higher placental weight so that the fetoplacental index (newborn's body weight/placental weight) was significantly higher ($P<0.025$) in male newborns. Values of the fetoplacental index indicate that a gram of placenta provides the statistically significant larger mass in male newborns (7.92) (Table 3).

Results of analyses of alpha and beta zones indicate significantly higher values of volume density (V_{Va}) ($P<0.002$), total volume (V_a) ($P<0.05$) and surface density (S_{Va}) ($P<0.025$) of the alpha zone in female newborn placentas (Table 4).

The established sex dependent quantitative differences of placental index and the structure of syncytiotrophoblast should be functionally compensated on the assumption that newborns of both sexes have identical metabolical needs. This should refer to increased activity and more effective physiological transport of substances, especially in the alpha zone in placentas of male newborns.

Placenta finishes its development at the end of the 36th week. Up to the end of the 40th week there is the period of maturation when the weight of the fetus increases without growth of placental parenchyma including placental membrane, *i.e.* the area for exchange of substances. This concept of maturation is based on morphological, biochemical and physiological data obtained from human and other mammalian placentas. They show that towards the end of pregnancy the efficiency of substance exchange increases per gram of placental weight in spite of decreasing cellular content of the placenta (Teasdale, 1980; Boyd, 1984; Teasdale and Jean-Jacques, 1985).

TABLE 3

THE WEIGHT OF NEWBORNS AND PLACENTAL INDEX IN RELATION TO THE NEONATAL SEX

Stereological variable	Male	Female
Gestational age/days/	280.00 ± 2.12	276.00 ± 2.52
Newborns' weight/g/	3727.14 ± 87.97	3517.81 ± 130.65
Placental weight/g/	479.43 ± 22.29	496.25 ± 22.02
Placental index	7.92 ± 0.27*	7.15 ± 0.18

*P<0.025

TABLE 4

VOLUME DENSITY (V_V) AND TOTAL VOLUME (V), SURFACE DENSITY (S_V) AND TOTAL SURFACE (S) OF ALPHA (a) AND BETA (b) ZONES IN TERM HUMAN PLACENTAS OF FEMALE AND MALE NEWBORNS

Stereological variable	Female x ±1 SE	Male x ± 1 SE
VVa/mm⁰/	0.015± 0.001***	0.010± 0.001
VVb/mm⁰/	0.16 ± 0.01	0.18 ± 0.001
Va/cm³/	7.38 ± 0.60*	5.14 ± 0.80
Vb/cm³/	82.26 ± 7.09	89.12 ± 6.28
SVa/mm⁻¹/	4.18 ± 0.30**	3.17 ± 0.29
SVb/mm⁻¹/	25.34 ± 0.53	24.47 ± 0.70
Sa/m²/	1.97 ± 0.20	1.87 ± 0.35
Sb/m²/	12.77 ± 0.56	12.44 ± 0.74

*** P<0.002
** P<0.025
* P<0.05

It seemed interesting to analyze quantitatively the correspondence of the above with structural characteristics of syncytiotrophoblast. Therefore, we investigated placentas in the tenth lunar month divided into two age groups: 12 placentas aged 38 to 39 weeks, and 11 placentas aged 40 weeks. We found that between the 38th and 39th week the volume (V_{Va}) and surface (S_{Va}) density of the alpha zone was significantly higher than in the 40th week when the volume density (V_{Vb}) and total volume (V_b) of the beta zone prevailed significantly ($P<0.01$) (Table 5).

From the functional point of view the obtained results support the theory that in the last four weeks of pregnancy the growing physiological needs of the fetus correspond to the structural changes in syncytiotrophoblast. At the beginning of this period of maturation differentiation of trophoblast is indicated by domination of alpha zones, the areas responsible for transplacental gas exchange. The end of this period is characterized by the increased value of the beta zone, *i.e.* with a metabolically active syncytial zone.

The results of morphological and histochemical research on syncytiotrophoblast show that from the functional point of view it is not a simple semipermeable membrane with passive transcellular

TABLE 5

VOLUME DENSITY (V_V), TOTAL VOLUME (V), SURFACE DENSITY (S_V) AND TOTAL SURFACE (S) OF ALPHA (a) AND BETA (b) ZONES IN TWO PERIODS OF THE 10th LUNAR MONTH: 38th-39th WEEK AND 40th WEEK OF PREGNANCY

Stereological variable	38th-39th week x ± 1 SE	40th week x ± 1 SE
V_{Va} /mm°/	0.015± 0.001*	0.011± 0.001
V_{Vb} /mm°/	0.148± 0.008*	0.187± 0.010
V_a /cm³/	7.77 ± 0.95	6.17 ± 0.67
V_b /cm³/	72.14 ± 4.27*	100.28 ± 8.89
S_{Va} /mm⁻¹/	4.45 ± 0.35*	3.34 ± 0.26
S_{Vb} /mm⁻¹/	25.72 ± 0.71	24.08 ± 0.64
S_a /m²/	2.55 ± 0.36	1.88 ± 0.18
S_b /m²/	12.60 ± 0.48	12.82 ± 0.69

*$P<0.01$

transport of substances (Hamilton and Boyd, 1966), but also a structurally highly differentiated, functionally specialized syncytium. Its beta zones are responsible for biosynthesis (steroids, proteins), which quantitatively show the general synthetic potential of the placenta (Anderson and McKay, 1966; Challis and Mitchell, 1983). Alpha zones, the structural component of functionally active syncytiotrophoblast, are its considerably thinned part designed for transport of substances of small molecular weight and gas transport. In the literature it is often referred to as a part of the vasculo-syncytial membranes. They appear relatively late during development, as late as the 33rd week of pregnancy, and become more numerous until term. Their incidence then makes up 6-30% of the chorionic villi. In case of prolonged pregnancy their incidence suddenly falls. The capacity of placental gas exchange grows in parallel with the number of vasculo-syncytial membranes (Fox, 1967). Therefore, the number of vasculo-syncytial membranes is considered to be the indicator of optimal structural maturity of chorionic villi, and additionally the indicator of potentials of O_2 transplacental transport. A small number of vasculo-syncytial membranes can indicate deficient development and immaturity of chorionic villi or regressive changes that occur in case of prolonged pregnancy, preeclampsia and hypoxia (Fox, 1967). The results of morphometric research show that in gestoses the size of chorionic villi as well as the length of epithelial plates is considerably decreased (Hitschold *et al.*, 1989).

Materials and Methods

This study was performed on 30 human term placentas of physiological pregnancies and deliveries. The embryonic membranes and umbilical cord were trimmed from placentas. Each placenta was weighed and its volume determined by water displacement. The tissue samples were cut on a vertical plane through the full thickness and in different parts of the organ, from its periphery to the region of umbilical cord insertion. Some samples were immersed in glutaraldehyde and embedded in Durcupan. Semi-thin sections were stained with toluidine blue. The greater part of tissue was fixed in 10% neutral formaldehyde and embedded in paraffin. The 8 µm-thick sections were stained by standard histological methods, and analyzed stereologically using a x40 lens and x10 projection screen of a microscope with a multipurpose test system after Merz. The area referred to was the parenchyma of placenta. The alpha and beta zones of syncytiotrophoblast on the terminal chorionic villi were estimated.

Statistical evaluation of the data included calculation of arithmetic means of results obtained for each component, standard error of means and Student's *t* test.

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