KARYOMETRY OF CELLS OF THE FETAL PART OF THE HUMAN PLACENTA AT THE END OF NORMAL AND DURING PATHOLOGICAL PREGNANCY

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UDC 611-013.85+618.36]-018.13

During differentiation of the placental tissues there is a decrease in size of the nuclei of the syncytium, fibrocytes, and endothelial cells. In the case of intrauterine death of the fetus, this decrease is more marked and the nuclei of the syncytium change their shape and become almost circular. The volume of the cytoplasm compared with the nuclei is reduced in the syncytium. These deviations in the size of the cell nuclei correspond to functional changes in the placenta during pathological pregnancy.

The size of the cells varies with the stage of individual development, and the changes correspond to functional activity of the cell and sometimes of the organ as a whole [2, 10-12, 16, 17]. The present investigation is a continuation of earlier work on the karyometry of the human placenta [4, 5]. Only isolated references to this problem can be found in the literature, mainly in connection with the chorionic epithelium [13].

In the present investigation all types of cells were studied in the fetal part of the human placenta and extreme functional states of the organ were compared: the placenta at the end of normal pregnancy and after intrauterine death of the fetus. No corresponding information could be found in the literature.

EXPERIMENTAL METHOD

The karyometric investigation was preceded by combined analysis of 205 human placentas, including 75 obtained at various stages of pathological pregnancy. The placenta was studied by histological and histochemical methods (nucleic acids, polysaccharides, hydrolytic and oxido-reductive enzymes, lipids, iron compounds, and sulfhydryl groups were demonstrated), from which the morphological and functional state of the organ as a whole could be deduced and chorionic villi typical of each stage of embryogenesis could be selected [7]. These tests had also to be preceded by measurement of the nuclei since evaluation of the results of karyometry without allowing for the cytological and cytochemical characteristics of the cells can lead to incorrect interpretations, as is shown by recent results for the functional morphology of the cell and, in particular, for the role of the nucleus in protein synthesis [1].

Karyometric examination of cells of the pathological placenta was carried out on the placenta of a woman whose 30-week pregnancy had terminated by intrauterine death of the fetus. The reasons for choice of this object were explained previously [8].

The cell nuclei were measured in sections obtained after fixation of pieces of the fetal part of the placenta in 10% neutral formalin and embedded in the usual way in paraffin wax. Sections 10 μ in thickness were stained with Böhmer's hematoxylin and eosin. The nuclei of the placental cells (at least 200 in each tissue) were measured after drawing on paper by means of a drawing apparatus [14, 15]. In connection with the structural peculiarities of the chlorionic villi, the nuclei of their syncytium and the nuclei of cells of

Department of Histology and Embryology, Crimean Medical Institute, Dimferopol'. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 6, pp. 96-99, June, 1972. Original article submitted August 6, 1971.

TABLE 1. Results of Statistical Analysis of Measurements of Cell Nuclei from Human Placenta at End of Normal Pregnancy (N) and after Stillbirth (SB)

Cells	Statistical index	Mean diameter		Smallest diameter		Largest diameter		Volume of nuclei	
		N	SB	N	SB	N	SB	N	SB
Syncytium	M	13,27	11,66	10,36	11,25	16,18	12,50	1239,0	856,0
	$\pm m$	0,11	0,09	0,08	0,10	0,17	0,11	31,5	21,7
	$CV(\%)$	12,10	10,50	10,90	12,50	14,80	12,10	35,8	35,7
	$V_{min}-V_{max}$	9,5—18,5	8,5—15,5	6,0—14,0	7,0—14,0	11,0—25,0	9,0—17,0	400,0—3000,0	200,0—2000,0
Fibrocyte	M	13,73	12,62	9,58	9,58	17,70	15,73	1283,0	1056,0
	$\pm m$	0,11	0,12	0,12	0,01	0,18	0,20	31,0	27,9
	$CV(\%)$	11,80	13,40	17,00	14,50	14,00	17,60	34,0	37,3
	$V_{min}-V_{max}$	10,0—18,0	8,5—17,0	6,0—13,0	6,0—13,0	11,0—24,0	10,0—22,0	400,0—2600,0	200,0—2400,0
Endothelial cells	M	12,62	11,69	8,00	7,41	17,32	16,02	953,0	757,0
	$\pm m$	0,12	0,12	0,11	0,12	0,18	0,18	28,8	24,3
	$CV(\%)$	13,50	14,70	19,30	22,30	14,30	16,20	42,2	45,2
	$V_{min}-V_{max}$	8,0—17,0	7,5—16,5	4,0—12,0	4,0—14,0	10,0—24,0	10,0—24,0	200,0—2600,0	200,0—1600,0
Syncytial appendages	M		8,72		7,29		10,17		360,0
	$\pm m$		0,11		0,11		0,13		13,0
	$CV(\%)$		16,90		21,20		17,80		50,0
	$V_{min}-V_{max}$		4,0—12,5		4,0—12,0		6,0—16,0		100,0—1000,0

Note: M arithmetic mean; m arithemtic mean error of M; CV) coefficient of variation of M; V_{min}, V_{max}) minimal variants; mean diameter is half the sum of the smallest and largest diameters of each nucleus measured perpendicularly to each other; arithmetic mean values of dimensions are given in conventional units (1 conventional unit is equivalent to 0.416 μ); syncytial appendages as a rule are absent at the end of normal pregnancy (for explanation, see text).

fibrocyte type in the stroma and of endothelial cells of the fetal vessels were measured during the last 3 months of pregnancy. The volume of the cell nuclei was determined from a table specially drawn up for this purpose [6] by means of the equation [18]:

$$V = 1.04 \cdot {}^1/_6 \pi \cdot a \cdot b \cdot \sqrt{ab},$$

where a, b, and $\sqrt{ab}$ are the axes of an ellipsoid.

Sections of the chorionic villi were drawn on squared paper and the ratio between the area occupied by the cytoplasm (S_c) and the area of the nuclei (S_n) was calculated. Since serial sections of constant thickness were compared, this ratio served to reflect the ratio between the volume of the cytoplasm and nuclei. The results of the measurements were subjected to statistical analysis by methods used constantly by the writer in biometrical investigations [6].

EXPERIMENTAL RESULTS

The results of the present investigation (Table 1) must be compared with those of other investigations published previously [4, 5]. At the end of normal pregnancy there is some decrease in the size of the nuclei of the syncytium in the villi (13.27 ± 0.11 compared with 14.15 ± 0.11 in the first 3 months). The nuclei remained of virtually the same shape, as is shown by the ratio between the arithmetic mean values of the smallest (D_s) and largest (D_l) diameters of the nuclei: 0.64 and 0.7 (it is evident that if this ratio is close to 1, the section through the nucleus is approximately circular in shape). The curve of variations in the dimensions of these nuclei is more asymmetrical at the end of the intrauterine period (A_s =0.25) than at its beginning (A_s = 0.16).* Toward the end of the intrauterine period the predominant cells in the stroma are those of fibrocyte type. The curve showing variation in size of their nuclei is practically symmetrical. Their mean diameter is smaller than during the first 3 months in cells of the fibroblastic series (13.73 ± 0.11 compared with 14.37 ± 0.01). The shape of the nuclei was almost unchanged, but the volume of the fibrocyte nuclei was considerably reduced. The greatest decrease in size took place in the nuclei of the endothelial cells: mean diameter 12.62 ± 0.12 compared with 14.65 ± 0.12; volume 953.0 ± 28.9 compared with 1536.0 ± 37.6. The nuclei of these cells are nearly ellipsoidal in shape, since

$$D_s : D_l = 0.46.$$

It was found that by the end of intrauterine development there was a statistically significant decrease in the S_c/S_n ratio to 2.04 ± 0.16 from 2.78 ± 0.16 in the initial stages of embryogenesis. At these

*A_s – coefficient of asymmetry.

times distinctive structures which most workers call syncytial nodules, with considerable preponderance of nuclear material ($S_c : S_n = 1.12$; $m = \pm 0.09$), appear in the chorionic villi.

Physiological aging of the placenta thus leads not only to a closer arrangement of the maternal and fetal circulations, as has been shown previously [8], but also to a decrease in size of the cell nuclei, accompanied by differentiation of the placental tissues which is complete by this period.

In pathological pregnancy the rate of differentiation of the chorionic villi in the placenta is sharply modified [9]. In cases of stillbirth the mean size of the nuclei of the syncytium is reduced and their shape is varied, for they become almost circular. The nuclei of the fibrocytes are also reduced in size and they become circular. Their range of variations is wider and their mean values more variable, indicating greater heterogeneity of these cells. The dimensions of the nuclei of the endothelial cells also are reduced, although their shape remains as before. In young villi found in the pathological placenta there are syncytial appendages, such as are characteristic of the chorionic villi in the earlier stages of pregnancy [5]. However, the nuclei of these appendages in cases of stillbirth were much smaller than during the first 3 months of pregnancy (8.72 ± 0.11 compared with 14.22 ± 0.12). Their volume was more than halved, and they became circular in shape, since $D_s : D_l = 0.72$. The ratio $S_c : S_n$ in the syncytium of the functioning villi (1.34 ± 0.20) was reduced by a statistically significant degree compared with its value at the end of normal pregnancy. Very characteristically this parameter was much more variable (57.4% compared with 31.9%).

The results of karyometric investigation of the placenta in this form of pathological pregnancy, compared with the histochemical findings [7], are evidence that a decrease in size of the syncytial nuclei of the villi is accompained by a decrease in intensity of cytochemical tests for nucleic acids, possibly reflecting disturbances in protein synthesis. The changes in size of the fibrocyte nuclei coincide with abnormalities in the biosynthesis of polysaccharides by stromal cells of the villi, giving rise to a disturbance of the ratio between hyaluronic acid and chondroitin sulfates, which alter the permeability of the human placental barrier. These results are consistent with the view that changes take place in the biometric characteristics of the placental barrier in cases of intrauterine fetal death [8].

It can be concluded from these results and the discussion that the changes discovered in the dimensions of the cell nuclei in the fetal part of the human placenta reflect morphological and functional properties of the placenta at the end of normal pregnancy and in cases of stillbirth.

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