MORPHOLOGY AND PATHOMORPHOLOGY

Morphofunctional Changes in the Placenta after Ozone Therapy

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Macro- and microscopic aspects in the morphology of placenta after ozone therapy were studied in pregnant women with placental insufficiency and the results were compared with the control group. In the main group, the placentas were smaller and weighed less. After ozone therapy enlargement of terminal villi with true syncytiocapillary membranes was noted. A stimulating effect of ozone therapy on the growth and differentiation of terminal villi was established.

Key Words: *ozone therapy; placenta; terminal villi; hypoxia*

Ozone therapy is widely used in obstetrics and gynecology in the treatment of acute and chronic inflammations of the genitals, viral diseases, threatened abortions, fetoplacental insufficiency (FI) caused by various pathological states, and other female genital diseases [2].

Fine molecular mechanisms of the effect of the ozone are not clearly understood, but the available reports indicate that biomembranes are not permeable for ozone molecules [3,4]. Therefore, the effect of ozone on cells is mediated via products of its reactions with components of cell plasma membranes and/or receptor on sensitive cells. The effect of ozone on protein-lipid components of cells is observed at very low doses. Lipid oxidation proceeds via direct ozonolysis of unsaturated fatty acids. Ozone molecule binds to double bonds with the formation of peroxides and ozonides. Chain lipid peroxidation plays a minor role in this process [5]. The presence of ozonolysis products and active oxygen forms in the microenvironment activates the antioxidant system, including superoxide dismutase, glutathione peroxidase, catalase, *etc.* [6]. Under these conditions activation of the pentose-phosphate pathway of glucose metabolism results in the formation of ribose-5-phosphate and reduced NADPH. This latter compound serves as the proton donor for NAD^+ and oxidized glutathione, whose reduced form acts as a potent antioxidant.

Stimulating effect of ozone therapy on the fetoplacental complex in pregnant women with threatened abortions during the first and second trimesters was confirmed by increased levels of hormones (estradiol, progesterone, placental lactogen) in comparison with the control [1]. It seems that this therapy improves peripheral circulation and normalizes microcirculation, thus improving oxygen delivery to tissues and has a favorable impact on the functioning of organs suffering from hypoxia (placenta, myometrium).

Hence, the efficiency of ozone therapy in pregnancy abnormalities caused by FI is obvious.

We investigated the morphofunctional changes in the placenta after ozone therapy in pregnant women with intrauterine fetal hypoxia.

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MATERIALS AND METHODS

Macro- and microscopic characteristics of 12 placentas from patients treated by ozone therapy for FI of different origin and 20 placentas from healthy women with normal pregnancy were studied. The study and control groups were matched for the age and somatic health. The mean age in the main and control groups was 29.0±0.7 and 30.0±0.5 years, respectively.

Pregnancy terms in both groups were 39-40 weeks. Macroscopic characteristics of placentas were described, their size and weight were measured. For histological analysis fragments from the central, paracentral, and peripheral zones were taken. For morphometry the preparations were stained with hematoxylin and eosin.

Quantitative assessment of morphometric structures (stem, intermediate, and terminal villi) was carried out on similar sites of micropreparations under a Reichert microscope (×40) in 10 visual fields.

The significance of differences was evaluated using Student's *t* test.

RESULTS

In the main group the placentas were smaller and weighed less than in the control. The mean diameter of placentas in the main group was 17.2 cm *vs.* 18.4

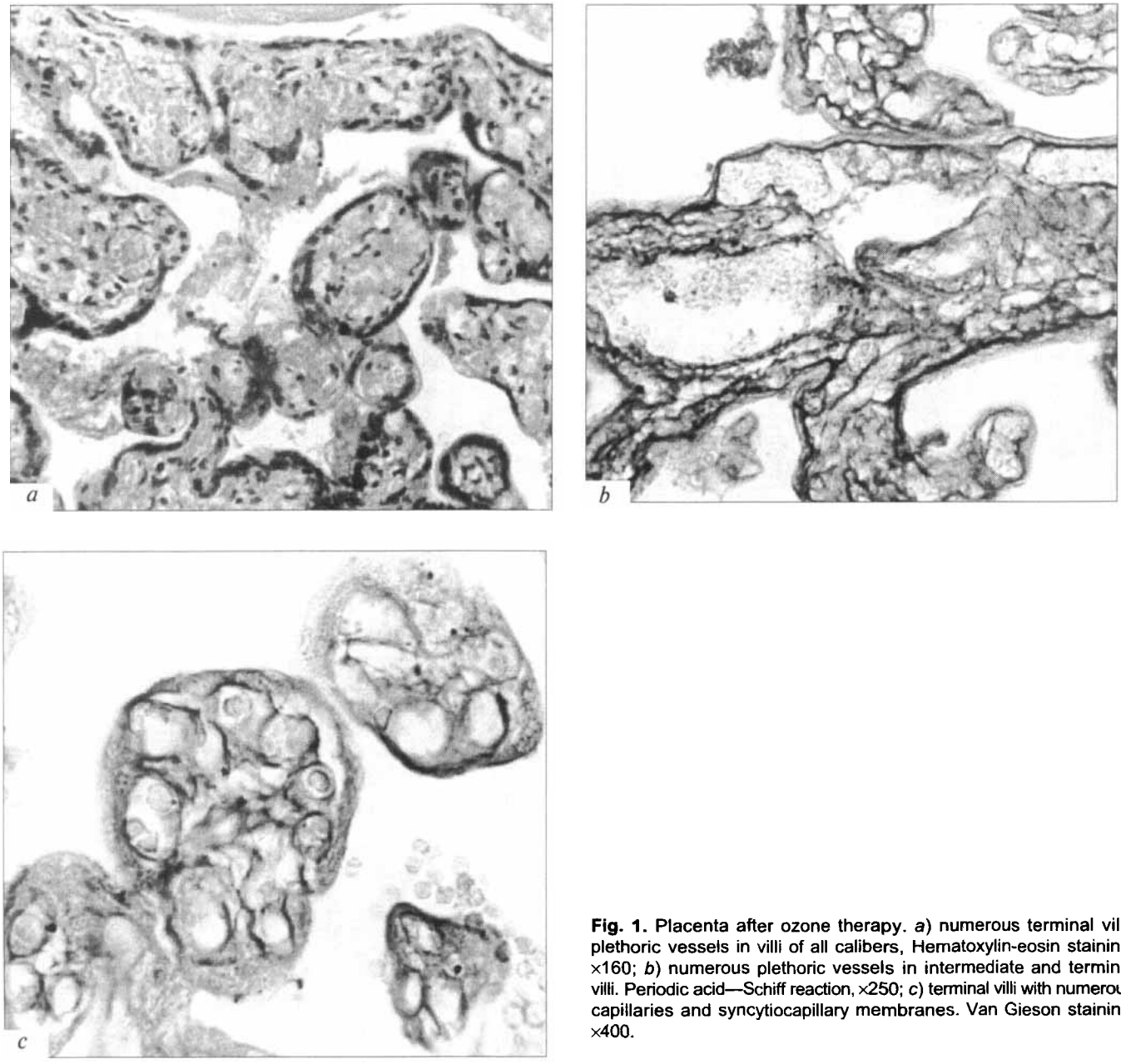

Fig. 1. Placenta after ozone therapy. *a*) numerous terminal villi, plethoric vessels in villi of all calibers, Hematoxylin-eosin staining, ×160; *b*) numerous plethoric vessels in intermediate and terminal villi. Periodic acid—Schiff reaction, ×250; *c*) terminal villi with numerous capillaries and syncytiocapillary membranes. Van Gieson staining, ×400.

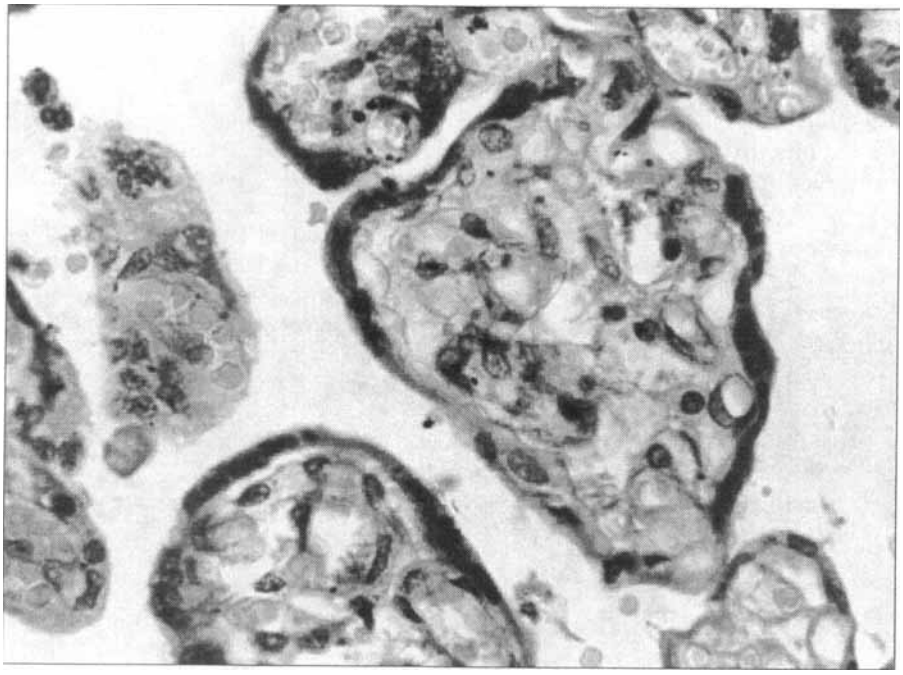

Fig. 2. Fragment of villous tree of the placenta in the control. Normal vascularization of intermediate and terminal villi. Hematoxylin and eosin staining, ×400.

cm in the control, weight of the placentas was 401.3± 20.0 and 524±25 g, respectively ($p<0.001$), and hence, ozone therapy did not stimulate the growth of placenta. The percentage of extrachorial placentas was 38% in the main group and 22% in the control. The mean incidence of extrachorial placentas is 19-24%; it increases essentially in cases with small-for-date fetuses and intrauterine fetal hypoxia.

Microscopic examination of the placentas in the main group showed signs of chronic placental insufficiency, which manifested by disorders of the maternal (a history of infarctions of different times, narrowing of the intervillous space) and fetal bloodflow (sites of chaotic villi, predominance of intermediate villi, arteriopathies – sclerosis, stenosis of the vessels). These changes were paralleled by pronounced strain of all compensatory processes. The number of terminal villi with true syncytiocapillary membranes and numerous plethoric vessels was increased in the majority of cases, there were plethoric vessels in the stem and intermediate villi (Fig. 1). In the control group the structure of placentas corresponded to the age norm (Fig 2).

TABLE 1. Effect of Ozone Therapy on Number of Villi in 10 Visual Fields ($M\pm m$)

Type of villi	Control	Main group
Stem	1.7	1.5
Intermediate	45.5	411.7
Terminal	68.3±4.0	101±4*

Note. *$p<0.001$ *vs.* the control.

In the group treated with ozone, the number of terminal villi per 10 visual fields was significantly ($p<0.001$) higher than in the control, the number of stem and intermediate villi being the same in both groups (Table 1).

Signs of chronic placental insufficiency were paralleled by drastic activation of compensatory processes, which manifested by increased number of terminal villi and vessels in the villi, plethoric vessels in the villi of all calibers.

The results indicate a selective effect of ozone on placental vessels and its stimulating effect on differentiation of terminal villi.

Ozone therapy was prescribed for correction of placental insufficiency and fetal hypoxia. The terminal parts of the vascular bed presented by sinusoids and capillaries proved to be most sensitive to ozone. These vessels have the thinnest walls and actively grow under the effect of ozone. This growth manifested by increased number of plethoric dilated vessels and their length. Ozone does not affect the growth of stem and intermediate villi, and when longitudinal growth of vessels surpasses the growth of villi, they form surface loops and protrusions, which represent future terminal villi. This is confirmed by increased number of terminal villi in the main group in comparison with the control.

Hence, active structural changes in the placenta induced by ozone therapy and leading to an increase in the number of terminal villi are due to primary growth of the distal parts of the vascular bed in comparison with villous growth. The number and vascularization of terminal villi are the main parameters

characterizing the degree of fetoplacental transport, because metabolic processes between the maternal and fetal blood are realized in the terminal villi. Great number of terminal villi and abundant vascularization promote intensive metabolism, gaseous exchange, release of metabolites and hormones, which eventually has a favorable impact on the fetus.

Microcirculation in the intervillous space is normalized at the expense of morphofunctional changes (increase in the number of terminal villi and decrease in the number of fibrinoid sites) in the placenta under the effect of ozone.

Transformations induced by ozone lead to normalization of microcirculation, improvement of tissue oxygen supply, and favorably affect the functions of organs suffering from hypoxia (myometrium and placenta). Ozone-induced changes have a favorable impact on the fetus and are important for prevention and treatment of FI of different origin.

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