

Two-Year Experience Of a Formalin-Free Preservation Of The Human Organs And Total Corpses For Anatomical Dissection In Kaunas University of Medicine

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According to EU norms for workplace air (DIN EN 689: 1994-04-E), no inhalation of dangerous vapours from chemical agents utilizing routinely for embalming of organs and corpses in the human anatomy is allowed. Therefore, a formalin-free preservation of the human organs and total corpses for anatomical dissection has been launched in Kaunas University of Medicine since 2007. The aim of the study is to prospect a suitability of the commercially available FineFix fixative (Milestones, Sorisole, Italy) for preservation of dissecting material in laboratories of study of the human anatomy.

Material and methods. The human organs and corpses consigned for embalming were carefully washed from blood by a cool saline via main arteries and/or veins employing a perfusion apparatus (Dodge, USA). The ready to use fixative (RTU) was *ex tempore* prepared diluting the concentrated FineFix in 96% ethanol in a ratio 3:7. After the perfusion, the organs were stored in the RTU FineFix fixative until their dissections, on average 2-3 months. The blood from the total human corpses was washed out via a perfusion of saline through common carotid arteries, internal jugular veins, femoral arteries and veins applying a pressure up to 3 atm, when it was necessary in order to achieve a proper body lavage from blood. The blood and washing saline drainage was performed via the openings and/or ligatures of the above-mentioned great veins and arteries. Following perfusion of the total corpses with the RTU FineFix fixative via both the common carotid and femoral arteries, the perfused bodies were entirely immersed into a cuvette with the RTU FineFix fixative for 2-3 days, after which the embalmed bodies were carefully enveloped by a thin plastic film in order to avoid any desiccation and stored in a refrigerator at 0° - +4°C for at least 6 months. To prevent desiccation of the exposed muscles and fascia during dissections a vaseline or silicon oil was used.

Results. Preservation of internal organs (liver, stomach, intestines, larynx) and the brain was deep and/or transmural. The consistence of parenchymal organs was lightly stiffened, but its color - rather similar to the natural one. No unpleasant odor was floated around the embalming organs and corpses. Slightly irritating smell of the ethanol could be easily abolished by a washing of the embalmed organs and bodies in a flow of pipe water before their dissection. In the embalming human corpses, the skin, subcutaneous fatty tissue and superficial fasciae were sufficiently preserved and easily dissectible. Comparing with a former formalin fixation, the fasciae, skeletal muscles and aponeuroses were evidently softer and preserved their natural color and typical consistence. However, some skeletal muscles (*m. biceps brachii*, pectoral or thigh adductor muscles) had focal points that may be considered as the poorly embalmed ones. In these foci, the muscular tissues ruptured during muscular prosecutions. Gross anatomical structures within the axillary, popliteal, and elbow fossae were perfectly preserved, flexible and clearly discernible during their dissections. The content of the radial, adductor, and cruro-popliteal canals was also perfectly preserved and all arteries, veins and peripheral nerves looked similarly as in a fresh corpse without chemical fixation. The preservation of the heart, kidneys, spleen and lungs was comparatively the weakest because their consistence was too

yielding for their good dissections and demonstration *in situ*.

Conclusions. The RTU FineFix fixative can be successfully used for embalming of the human organs and corpses consigned for studies of gross anatomy. Further technical improvements and practice are necessary in order to solve the above-mentioned problem associated with insufficient preservation of lungs, heart, spleen and kidneys inside the human corpses embalmed via vascular perfusion.

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Oral presentations

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Metabolism, Growth Factors, Apoptosis And Cell Totipotency In Growth Retarded, Different N And Re-Growing Invalid For Implantation Human Embryo

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Many factors have been shown to affect rate of embryo preimplantation development, the conceptus cell number, metabolism and apoptosis. The good quality embryo and its viability are influenced by glycolysis, adhesion, methylation, expression of the same genes and growth factors, apoptosis. Aim of this work was detection of growth factors, metabolic enzymes, genes and cell death in invalid for implantation human conceptus to clear out the possible reasons for embryo retardation.

Materials. 73 invalid for implantation embryo were donated by their 19 mothers. Different n embryo (n=3), retarded in growth and those - "re-growth stage" were 2-6 days old after *in vitro* fertilization (IVF) procedure. Immunohistochemistry was used for detection of embryo metabolism regulatory enzymes lactate dehydrogenase (LD), hexokinase, growth factors and their receptors - bFGF, FGFR1, IGF, IGF1R, TGF α , stem cell marker Oct 3/4, genes wnt and barx1. Caspase 6 and TUNEL were used for detection of apoptosis.

Results showed variable expression of LD in 3rd day and stabilization until the moderate number of LD positive cells in the 4th day. Only few cells were hexokinase positive in the 3rd and 6th day. Despite variable bFGF expression in 3rd day, FGFR1 were richly expressed in all developmental days. IGF was seen only in occasional cells of one case of the 4 days old embryo, but IGF1R was found in blastomeres in all developmental time, except the 4th day. Few cells showed also Oct3/4 and TGF α expression in 3 days old embryo. Caspase 6 marked blastomeres in the 3rd and 4th day with increase of embryo developmental time. barx1 and wnt were expressed from the 4th day from 4-cell embryos.

Conclusions. Variable expression of LD, bFGF, rich for FGFR1 and limited for hexokinase and TGF α between days 3 and 4 possibly is the reason for growth retardation in invalid for implantation human embryo. However, sudden evaluation of LD and bFGF expression in retarded embryos probably is the reason for re-growth and raises the question about the initiation mechanism. Retarded embryos show down regulation of IGF1R expression and apoptosis via caspase 6 mechanism. First gene expression starts from 4-cell stage in different n embryos. Expression of Oct3/4 in retarded embryo demonstrates still kept cell totipotency.