



Citation: Zygmunt Domagała, Agnieszka Pinkowska, Aleksandra Piotrowska, Jurand Domański, Victoria Tarkowski, Aleksandra Zimmer-Stelmach, Jakub Śliwa (2022) Utility of the Movat pentachrome stain technique in the microanatomical analysis of the human placenta. *Italian Journal of Anatomy and Embryology* 126(2): 25-32. doi: 10.36253/ijae-13882

Copyright: © 2022 Zygmunt Domagała, Agnieszka Pinkowska, Aleksandra Piotrowska, Jurand Domański, Victoria Tarkowski, Aleksandra Zimmer-Stelmach, Jakub Śliwa. This is an open access, peer-reviewed article published by Firenze University Press (<http://www.fupress.com/ijae>) and distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID

ZD: 0000-0002-2317-1932
APin: 0000-0002-8918-0129
APio: 0000-0003-4093-7386
JD: 0000-0003-2416-8062
AZ-S: 0000-0003-1114-6980
JS: 0000-0001-5343-0707

Utility of the Movat pentachrome stain technique in the microanatomical analysis of the human placenta

ZYGMUNT DOMAGAŁA¹, AGNIEZKA PINKOWSKA¹, ALEKSANDRA PIOTROWSKA², JURAND DOMAŃSKI^{3,*}, VICTORIA TARKOWSKI³, ALEKSANDRA ZIMMER-STELMACH⁴, JAKUB ŚLIWA⁴

¹ Division of Anatomy, Department of Human Morphology and Embryology, Wrocław Medical University, Wrocław, Poland

² Division of Histology and Embryology, Department of Human Morphology and Embryology, Wrocław Medical University, Wrocław, Poland

³ Clinical and Dissecting Anatomy Students Scientific Club, Wrocław Medical University, Wrocław, Poland

⁴ II Department of Obstetrics and Gynaecology, Wrocław Medical University, Wrocław, Poland

*Corresponding author. E-mail: jurand.dom@gmail.com

Abstract. The efficacy and utility of pentachrome staining has been demonstrated in many studies on diverse human body tissues. Movat pentachrome technique is used for multicolor staining of tissue sections and vascular and stroma visualization. So far, the utility of this method for microanatomical evaluation of placental structures has not been demonstrated. The aim of this study is to evaluate the image of normal placenta and to develop some reference images for future evaluation of pathological tissues based on this technique. *Material and method.* The study was carried out on 21 paraffin slices taken from seven mature human placenta of single pregnant women without significant pathology and completed with a planned, elective Caesarean section. All paraffin slices were used for preparation of tissue microarrays and then performing a histochemical staining (HE and Movat). *Results.* On the basis of the collected material, microscopic analysis enabled the identification of normal placental villi - terminal villi, mature intermediate villi and stem villi. Moreover the maternal part of placenta was visualized. It is worth emphasizing that in each case not only the trophoblast but also the stroma structures were visualized. *Conclusions.* This study proved the effectiveness and usefulness of Movat imaging of placenta especially in visualization of the stroma.

Keywords: microanatomy, pentachrome staining, placenta.

INTRODUCTION

The placenta is an organ with a unique function and composition for the human body. The anatomical structure is relatively simple, consisting of two surfaces: fetal and maternal. On the fetal surface, which is covered by the amniotic membrane, there is the separation of the umbilical cord (Rome-

ro 2015). It is usually located in the central part of the placenta, but can be found marginally or eccentrically. There are vessels branching from the umbilical cord, which usually spread radially (Huppertz 2008). Underneath the fetal surface, there is a chorionic plate consisting of a fibrous mass and a thick layer of connective tissue in which various calibre blood vessels run: veins and chorionic arteries. From the plate, villous arteries descend deep into the placenta towards the intervillous space (Domagała et al. 2020). Small vessels diverge into the placental villi from the villous arteries. The villi tree itself grows from the chorionic plate starting from the broad stem villi, and is formed at a very early stage of placental development (Huppertz 2008). From the villi tree, successive generations of villi descend, which at the very end of the tree produce the intervillous space floating villi. Together the described elements form the structural unit of the placenta - the cotyledon (Otake et al. 2019) (Figure 1). There is a basal plate on the other side of the placenta on the maternal surface, which is a partially artificial structure created by the separation of the placenta from the uterus at birth (Kay, Nelson, and Wang 2011). It is therefore composed of maternal tis-

sues including the uterine arterial vessels. A thin layer of trophoblast separates it from the intervillous space, and is connected to the individual cotyledons by the anchoring villi (Salaria et al. 2005). Adequate, proper placental structure is critical for fetal welfare. Any disturbance of this structure translates into significant clinical abnormalities of both the fetus and the mother, e.g. pregnancy-related hypertension. Detailed knowledge of the placenta structure is important, not only from a cognitive perspective but also that of a clinical one (Kay, Nelson, and Wang 2011).

The understanding of the placenta microstructure requires, apart from *in vivo* and macroscopic analysis, a microscopic analysis. Immunohistochemical techniques, i.e. mono- or polyclonally labelled antibodies are most commonly used to visualise components of the examined tissue. In spite of this, modern diagnostics and scientific research still uses histochemical methods for research as the immunohistochemical technique is based mainly on the use of two primary colours: blue and brown. This is a stark contrast to the ability of dye-based methods to produce multi-coloured images. This type of staining allows for the simultaneous identification of

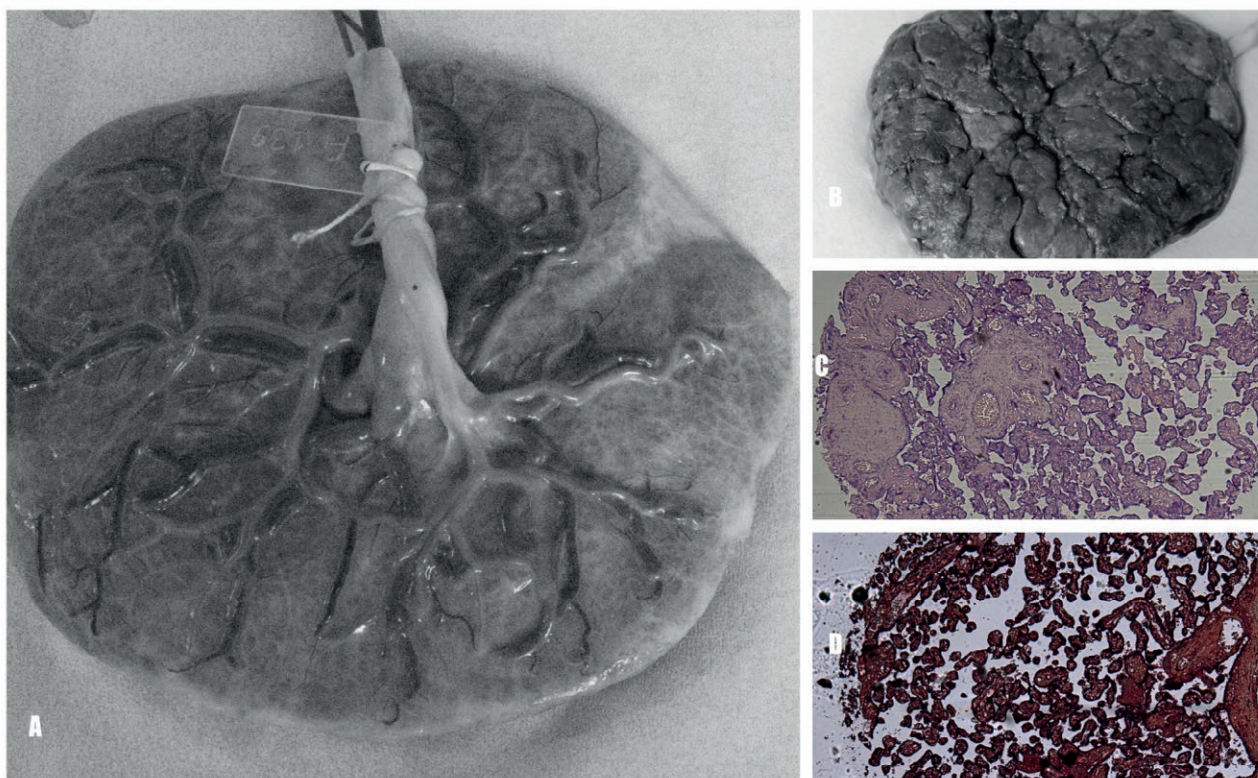


Figure 1. Human placenta (A – fetal side B – maternal side C – HE view of exemplary histospot, D – Pentachrome staining of exemplary histospot).

many tissue structures in order to provide a microscopic analysis of their coexisting morphological-functional interactions (Petrovic et al. 2011).

One such histochemical technique is Movat pentachrome staining (Felföldi et al. 2021; MOVAT 1955). In the case of the placenta, the authors believe that this technique can be useful to visualise parenchyma and stroma structures and, by simultaneously staining epithelia, also allow assessment of the interaction between trophoblast, stroma, and fetal vessels. The analysis of the available literature did not reveal the presence of any scientific work investigating the pentachrome staining image of normal placental tissues. Therefore, the aim of this study is to evaluate the normal microscopic analysis of the placenta using Movat staining. The definition of normal morphological images will then enable comparative studies comparing normal and pathological images to definitively confirm the usefulness of the histochemical technique in the diagnosis of placental pathology.

MATERIAL AND METHODS

The study was carried out on 21 paraffin slices taken from seven mature human placenta of single pregnant women without significant pathology and completed with a planned, elective Caesarean section (38-40 weeks of pregnancy).

The mean age of the mothers was 31.62 years, and mean neonatal weight was 3702 g, with a mean APGAR score of 9.86 points at the first minute after birth and 10 points at subsequent minutes (Table 1). All eligible mothers were healthy and the newborns did not show any abnormalities during the 24-hour clinical follow-up. Antenatal and postnatal assessment of the mothers and medical evaluation of the newborns were performed

Table 1. Basic clinical data and general characteristics of the material. SD – data in brackets, c-child/children; M- males, F-females; AG -Apgar points; BMI – body mass index; w – woman/women

Neonatus mass	Neonatus AG 1 min	Maternal BMI	Maternal parity	Maternal age
3702 (320.4) [g]	9,86	26.9 (2.4)	0c – 5 w 1c – 1 w 2c – 1 w	30,57 (1.6) [years]
Placenta mass	Placenta vertical diameter	Placenta horizontal diameter	Gestational age	Neonatus gender
560 (56) [g]	17.0(1.4) [cm]	18.5 (2.0) [cm]	40 (1.2) [weeks]	F-4 M-3

according to Polish medical standards (Zdrowia 2018). Material was collected from three placental sites (umbilical cord attachment area, paracentral zone and marginal zone of placenta) based on the technique proposed by Kay et al. (Kay, Nelson, and Wang 2011) using surgical clamps and dissecting forceps.

After sampling, the material was preserved in 4% buffered formalin. The paraffin blots were prepared from the obtained material in a typical manner. Hematoxylin and eosin stained slides were then made and, after initial evaluation in a Leica LD 5000HL light microscope (Leica Microsystems, Wetzlar, Germany), scanned using a Panoramic MIDI histology scanner (3DHistotech, Budapest, Hungary) to create virtual slides.

Based on these, representative 1.5mm histospots were selected for tissue microarrays using the automated TMA Gran Master system (3D Histtech, Budapest, Hungary).

Pentachrome Movat staining was carried out following a standard procedure. In the first step, staining was carried out in Alcian blue solution (20 minutes). This step was performed to buffer the basic substance. Next, the slides were rinsed under running water and then placed in alkaline alcohol. This part of the procedure was done to convert the Alcian blue into the insoluble pigment Monastral fast blue.

The next step was to rinse the material again with running water and then distilled water. Afterwards, staining in Verheoffs' haematoxylin solution was carried out to stain the cell nuclei and elastic fibres. This step was again followed by rinsing the material several times in distilled water. The next step was differentiation in a 2% aqueous solution of ferric trichloride. The differentiation process was interrupted by rinsing the preparation again. At this stage a microscopic check of the staining of the slide was carried out. If the result was satisfactory, the slides were placed in sodium trisulfate solution (1 min) and then rinsed again under running and distilled water.

The material was then stained in crocein scarlet acid fuchsin solution to stain fibrin and muscle. Slides were then washed in distilled water and, in the final step, in 0.5% ice-cold acetic acid. The washing was necessary to differentiate the slide in 5% hydrated phosphotungstic acid solution. Differentiation time was a maximum of 10 minutes. The staining effects were evaluated under the microscope. The slide was then rinsed again in acetic acid solution to remove phosphotungstic acid and the material was rinsed three times in 100% alcohol to prepare it for staining in alcoholic saffron solution. This staining took approximately 15-20 minutes to stain the collagen and reticulin fibres.

Finally, the slide was rinsed in alcohol solutions of decreasing concentrations, and covered with a coverslip.

The expression of the studied markers was evaluated independently by two independent observers (ZD, JD) using the Leica LD 5000HL light microscope. Identification of tissue types was based on colour evaluation of stained tissues derived from the available literature (Chu et al. 2009; Felföldi et al. 2021; Kajbafzadeh et al. 2017; Willershausen et al. 2019). Due to the small size of the material, statistical analysis was omitted and the results were presented as observational data.

RESULTS

Microscopic analysis based on the obtained material allowed us to identify normal villi of the placenta: terminal villi (Figure 2a), mature intermediate villi (Figure 3a,b), and stem villi (Figure 4a). Moreover, the maternal part of the placenta was visualised (Figure 5). Microscopic evaluation was conducted based on the acquired histospots. Qualification of tissue types was carried out independently based on the evaluation of the obtained multicolour image.

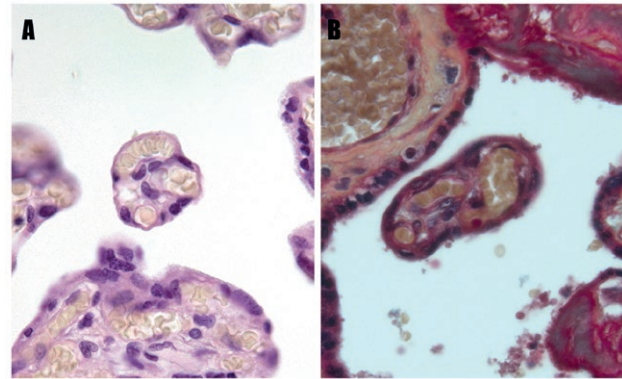


Figure 2. Terminal villus in HE (A) and MOVAT(B) staining (magnification 400x).

The evaluation of the terminal villi (Figure 2b) showed the presence of intensely black stained nuclei of trophoblast cells building the outer structure of the villi. The purplish-black colour is due to elastin fibres, which are present within the trophoblast, but also build the capillary walls and are found in the stroma. Inside the terminal villus, collagen fibres can also be seen (yellowish coloured tissue fragments).

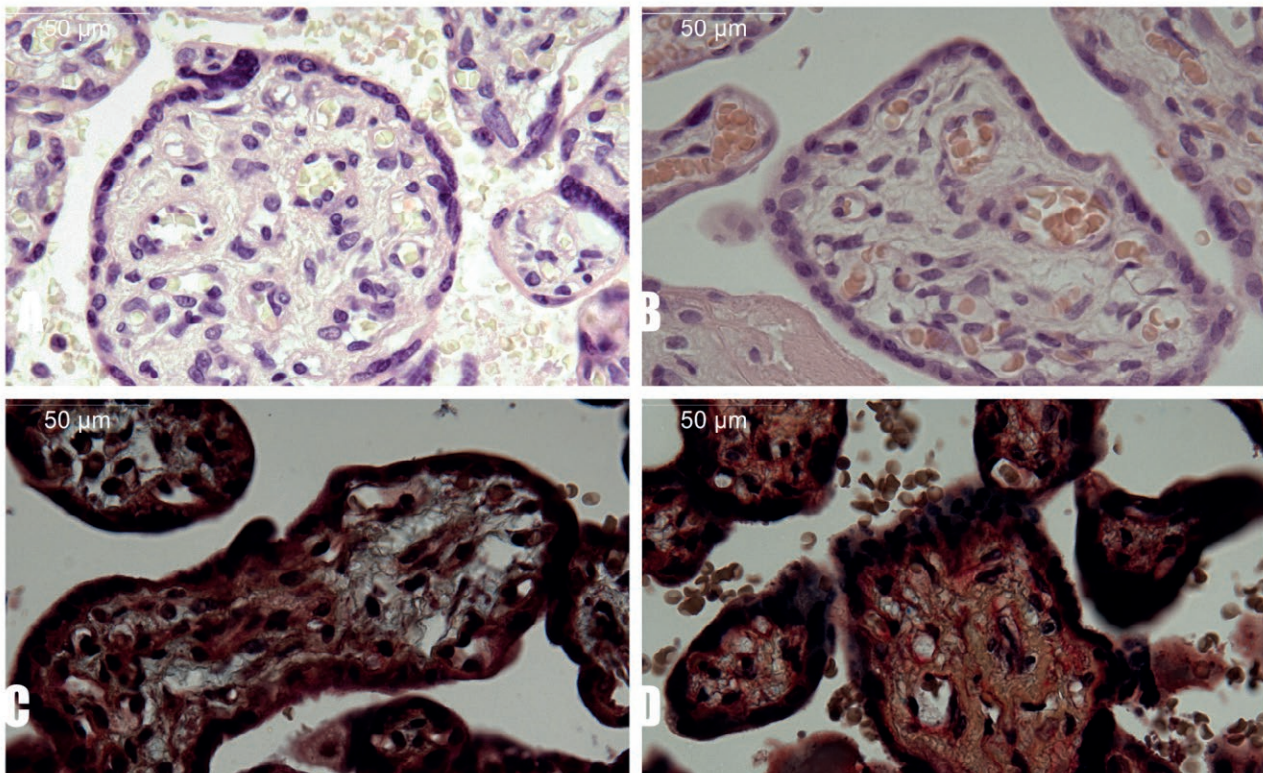


Figure 3. Human intermediate villus in HE(A,B) and MOVAT (C,D) staining.

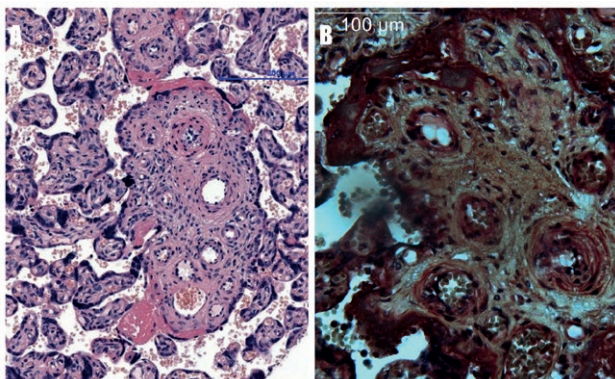


Figure 4. Human stem villus in HE (A) and Movat (B) staining.

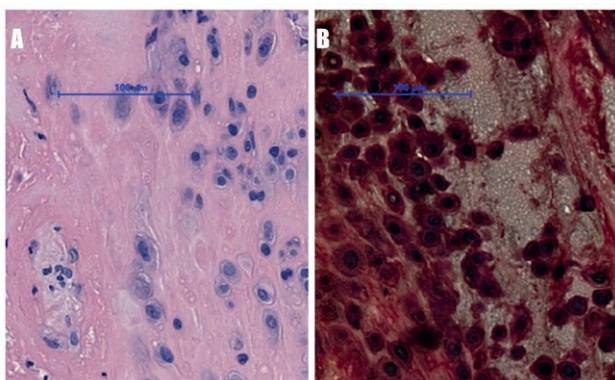


Figure 5. Extravillous trophoblast in HE (A) and Movat (B) staining.

In the case of the intermediate villi (Figure 3c,d), the presence of black-stained cell nuclei and black-purple elastin fibres present within the trophoblast and the central part is also demonstrated. In the central part of the villi, yellowish collagen fibres are visible. Around the few vessels red or orange coloured muscle fibres and in some places blue coloured reticular fibres can be seen.

In the case of stem villi (Figure 4), black stained cell nuclei and trophoblast cells were visualised. Around the vessels, reddish-brown stained muscle fibres were visualised and in the stroma blue stained collagen and brownish-yellow and brown proteoglycans and reticular fibres were visualised.

Assessment of maternal tissues stained with Movat demonstrated intense black colouration of villous and extravillous trophoblasts, red colouration of tissue surrounding villi and beige-yellow colouration of tissues in the stroma. Bluish staining was also found in limited areas corresponding to tissues containing large amounts of collagen and reticular fibres.

DISCUSSION

The reported data demonstrates the advantages of imaging the human placenta with Movat pentachrome staining techniques. Histochemical techniques (trichrome or pentachrome stain) are used relatively often for histochemical evaluation of tissues (Kara et al. 2020; Klećkowska-Nawrot et al. 2015; Skonieczna et al. 2021; Zaki and Youssef 2013). Despite its complexity, the Movat technique allows for simultaneous visualisation of multiple tissue structures such as muscles, cell nuclei or tissues containing elastin fibres or glycosaminoglycans (Wilson et al. 2021).

Its attractiveness lies primarily in the acquisition of a multicoloured image, which greatly facilitates the assessment and differentiation of individual placental structures by the consulting pathologist at a reasonable cost limit. Presently, most placentas in the postpartum period are not evaluated histopathologically. Reasons for this situation include staff shortages as well as the limitations associated with the high cost of histopathological examination (Hadravská et al. 2017).

In our opinion, a detailed microscopic evaluation of the placenta should be considered because the results may present clinically relevant data. Such data may indicate an association between certain placental features, assessed macroscopically and microscopically, and pathologies associated with the later development of the child as an independent organism from the mother (Barker et al. 2010; Barker et al. 2012; Cirillo and Cohn 2020; Hodyl et al. 2017). The human placenta is an organ that exists only during the developmental period and supports the growth and development of the fetus (Hupertz 2008). Its growth is associated with rapid proliferation of cytotrophoblast cells. This is accompanied by multistage differentiation into several subpopulations of cells with different anatomical localisation and function (Aplin et al. 2020). One such subpopulation, the syncytiotrophoblast, covers the outer side of the chorionic villi. It comes into direct contact with maternal blood and thus defines the nutritional, endocrine, and immunological interface between mother and fetus (Pijnenborg et al. 1981). Some trophoblast cells leave the placenta and enter the tissues of the mother; they are defined as extravillous trophoblasts. These cells penetrate the walls of the spiral arteries in such a way that they replace the endothelial cells of the mother's vessels. In addition, there is displacement of smooth muscle cells and breakdown of the associated extracellular matrix. This process induces remodelling of the spiral arteries, resulting in a change in vessel characteristics. The artery transforms from a narrow vasoreactive vessel into a wider low-

pressure tube that supplies the placenta and fetus with nutrient- and oxygen-rich maternal blood (Pijnenborg et al. 1981). The phenomenon described earlier defines placental tissues as “pseudomalignant tissue” because of the numerous similarities of trophoblast cells to tumour cells. Both cell types are characterised by a high tendency to proliferation and invasion (Wilczyński 2006). Scientific data suggest that, in their development, cancer cells revert to primitive developmental processes representative of trophoblast cells by taking over genes used by these cells to achieve metastatic potential (Koslowski et al. 2007). For example, the epithelial-mesenchymal transition (EMT) that is characteristic for neoplastic invasion occurs during early implantation/placentation (Roldán et al. 2020). Movat’s pentachrome technique is a useful tool in assisting physicians in the diagnosis of cancer. It supports the physician in assessing the degree of invasion of the proliferative process, and has been proven to confirm venous invasion in oesophageal adenocarcinoma (Castonguay, Li-Chang, and Driman 2014). It is also used to evaluate the stroma of tumours (Haeberle et al. 2018). For example, in pancreatic cancer, Movat’s method allows the probability of a successful tumour response to neoadjuvant chemotherapy to be determined (Haeberle et al. 2021). It is worth emphasizing that the assessment of the cell stroma is not only important in neoplastic diseases but also in the assessment of the physiology and pathology of the human placenta (Ji et al. 2021; Wang et al. 2011).

Pentachrome staining has also proven to be of value in the assessment of vascular remodelling associated with damage in the course of atherosclerosis. It allows a reliable qualitative and quantitative assessment of glycosaminoglycans, elastic fibres, proteoglycans, or collagen in tissues forming a blood vessel. For example, it has been shown that it can be used to assess the risk of cardiovascular events in patients after carotid endarterectomy.

In sum, the placenta is an organ whose main purpose is to ensure the exchange of nutrients between the developing fetus and its mother. This exchange is possible thanks to the characteristic structure of the organ, in which the blood vessels are a crucial element. The placenta is therefore characterised by a dynamic cellular development based on mechanisms typical for neoplastic tissues; simultaneously, it is an unusual “vascular” organ. The present work demonstrates that the use of histochemical techniques for tissue visualisation allows for a more comprehensive assessment of placental tissue in contrast to standard histochemical staining, and may be useful for both clinical and scientific evaluation of this tissue. In the future, it is planned to use this tool to assess placental tissue in health and disease research.

ETHICAL STATEMENT

The study was made according to the Declaration of Helsinki and approved by the Local Bioethics Committee.

ACKNOWLEDGEMENTS

This work was funded by grant SUB.A351.19.032.

REFERENCES

- Aplin, John D., Jenny E. Myers, Kate Timms, and Melissa Westwood. 2020. “Tracking Placental Development in Health and Disease.” *Nature Reviews Endocrinology*. doi:10.1038/s41574-020-0372-6.
- Barker, David J.P., Gail Larsen, Clive Osmond, Kent L. Thornburg, Eero Kajantie, and Johan G. Eriksson. 2012. “The Placental Origins of Sudden Cardiac Death.” *International Journal of Epidemiology* 41 (5): 1394–1399. doi:10.1093/ije/dys116.
- Barker, David J.P., Kent L. Thornburg, Clive Osmond, Eero Kajantie, and Johan G. Eriksson. 2010. “The Surface Area of the Placenta and Hypertension in the Offspring in Later Life.” *International Journal of Developmental Biology* 54 (2–3): 525–530. doi:10.1387/ijdb.082760db.
- Castonguay, Mathieu C., Hector H. Li-Chang, and David K. Driman. 2014. “Venous Invasion in Oesophageal Adenocarcinoma: Enhanced Detection Using Elastic Stain and Association with Adverse Histological Features and Clinical Outcomes.” *Histopathology* 64 (5): 693–700. doi:10.1111/his.12308.
- Chu, Kim E., Bettina G. Papouchado, Zhaoli Lane, and Mary P. Bronner. 2009. “The Role of Movat Pentachrome Stain and Immunoglobulin G4 Immunostaining in the Diagnosis of Autoimmune Pancreatitis.” *Modern Pathology* 22 (3): 351–358. doi:10.1038/modpathol.2008.196.
- Cirillo, Piera M., and Barbara A. Cohn. 2020. “Gestational Biomarkers of Daughter’s Breast Cancer in the Child Health and Development Studies.” *Reproductive Toxicology* 92: 105–111. doi:10.1016/j.reprotox.2019.05.057.
- Domagała, Zygmunt, Jurand Domański, Aleksandra Zimmer, Anna Tarczyska, Jakub Sliwa, and Bohdan Gworys. 2020. “Methodology of Preparation of Corrosive Specimens from Human Placenta — A Technical Note.” *Annals of Anatomy* 228 (March): 151436. doi:10.1016/j.aanat.2019.151436.

- Felföldi, Balázs, Ildikó Bódi, Krisztina Minkó, Zsófia Benyeda, Nándor Nagy, Attila Magyar, and Imre Oláh. 2021. "Infection of Bursal Disease Virus Abrogates the Extracellular Glycoprotein in the Follicular Medulla." *Poultry Science* 100 (4). doi:10.1016/j.psj.2021.01.023.
- Hadravská, Šárka, M. Dubová, P. Mukenšnabl, and O. Daum. 2017. "Complications Arising out of Insufficient Reporting of Clinical Data to the Pathologist Investigating Placentas." *Ceska Gynekologie* 82 (3): 197–201.
- Haerberle, Lena, Andrea Cacciato Insilla, Anne-Christine Kapp, Katja Steiger, Anna Melissa Schlitter, Björn Konukiewicz, Ihsan Ekin Demir, Helmut Friess, and Irene Esposito. 2021. "Stroma Composition and Proliferative Activity Are Related to Therapy Response in Neoadjuvant Treated Pancreatic Ductal Adenocarcinoma." *Histology and Histopathology*, 18332. doi:10.14670/HH-18-332.
- Haerberle, Lena, Katja Steiger, Anna Melissa Schlitter, Sami Alexander Safi, Wolfram Trudo Knoefel, Mert Erkan, and Irene Esposito. 2018. "Stromal Heterogeneity in Pancreatic Cancer and Chronic Pancreatitis." *Pancreatology* 18 (5): 536–549. doi:10.1016/j.pan.2018.05.004.
- Hodyl, Nicolette A., Natalie Aboustate, Tina Bianco-Miotto, Claire T. Roberts, Vicki L. Clifton, and Michael J. Stark. 2017. "Child Neurodevelopmental Outcomes Following Preterm and Term Birth: What Can the Placenta Tell Us?" *Placenta*. doi:10.1016/j.placenta.2017.06.009.
- Huppertz, B. 2008. "The Anatomy of the Normal Placenta." *Journal of Clinical Pathology*. doi:10.1136/jcp.2008.055277.
- Ji, Shuhan, Diane Gumina, Kathryn McPeak, Radu Moldovan, Miriam D. Post, and Emily J. Su. 2021. "Human Placental Villous Stromal Extracellular Matrix Regulates Fetoplacental Angiogenesis in Severe Fetal Growth Restriction." *Clinical Science* 135 (9): 1127–1143. doi:10.1042/CS20201533.
- Kajbafzadeh, Abdol Mohammad, Reza Khorramirouz, Seyede Maryam Kameli, Javad Hashemi, and Amin Bagheri. 2017. "Decellularization of Human Internal Mammary Artery: Biomechanical Properties and Histopathological Evaluation." *BioResearch Open Access* 6 (1): 74–84. doi:10.1089/biores.2016.0040.
- Kara, H., S. Gedikli, Z. Özudogru, D. Özdemir, and H. Balkaya. 2020. "A Morphological, Morphometrical and Histological Investigation of the Interdigital Gland in Hasmer and Hasak Sheep." *Folia Morphologica (Poland)* 74 (9): 742–747. doi:10.5603/FM.A2020.0014.
- Kay, Helen H., D. Michael Nelson, and Yuping Wang. 2011. *The Placenta: From Development to Disease. The Placenta: From Development to Disease*. doi:10.1002/9781444393927.
- Klećkowska-Nawrot, J., R. Nowaczyk, K. Goździewska-Harłajczuk, K. Krasucki, and M. Janeczek. 2015. "Histological, Histochemical and Fine Structure Studies of the Lacrimal Gland and Superficial Gland of the Third Eyelid and Their Significance on the Proper Function of the Eyeball in Alpaca (Vicugna Pacos)." *Folia Morphologica (Poland)* 74 (2): 195–205. doi:10.5603/FM.2015.0001.
- Kosłowski, Michael, Ugur Sahin, Rita Mitnacht-Kraus, Gerhard Seitz, Christoph Huber, and Özlem Türeci. 2007. "A Placenta-Specific Gene Ectopically Activated in Many Human Cancers Is Essentially Involved in Malignant Cell Processes." *Cancer Research* 67 (19): 9528–9534. doi:10.1158/0008-5472.CAN-07-1350.
- MOVAT, H. Z. 1955. "Demonstration of All Connective Tissue Elements in a Single Section; Pentachrome Stains." *A. M. A. Archives of Pathology* 60 (3): 289–295.
- Otake, Yuko, Hidenori Kanazawa, Hironori Takahashi, Shigeki Matsubara, and Hideharu Sugimoto. 2019. "Magnetic Resonance Imaging of the Human Placental Cotyledon: Proposal of a Novel Cotyledon Appearance Score." *European Journal of Obstetrics and Gynecology and Reproductive Biology* 232. doi:10.1016/j.ejogrb.2018.11.011.
- Petrovic, A., M. Abramovic, D. Mihailovic, J. Gligorijevic, V. Zivkovic, M. Mojsilovic, and I. Ilic. 2011. "Multi-color Counterstaining for Immunohistochemistry a Modified Movat's Pentachrome." *Biotechnic and Histochemistry* 86 (6): 429–435. doi:10.3109/10520295.2010.528026.
- Pijnenborg, R., W. B. Robertson, I. Brosens, and G. Dixon. 1981. "Review Article: Trophoblast Invasion and the Establishment of Haemochorial Placentation in Man and Laboratory Animals." *Placenta*. doi:10.1016/S0143-4004(81)80042-2.
- Roldán, Diana Barea, Matthias Grimmler, Christoph Hartmann, Stefanie Hubich-Rau, Tim Beißert, Claudia Paret, Giuseppe Cagna, et al. 2020. "PLAC1 Is Essential for FGF7/FGFR3b-Induced Akt-Mediated Cancer Cell Proliferation." *Oncotarget* 11 (20): 1862–1875. doi:10.18632/oncotarget.27582.
- Romero, Roberto. 2015. "Images of the Human Placenta." *American Journal of Obstetrics and Gynecology*. doi:10.1016/j.ajog.2015.08.039.
- Salaria, Carolyn M., John C. Pezzullo, Adrian K. Charles, Linda M. Ernst, Elizabeth M. Maas, Benita Gross, and Robert Pijnenborg. 2005. "Morphometry of the

- Basal Plate Superficial Uteroplacental Vasculature in Normal Midtrimester and at Term.” *Pediatric and Developmental Pathology* 8 (6): 639–646. doi:10.1007/s10024-005-0409-9.
- Skonieczna, Joanna, Jan P. Madej, Agnieszka Kaczmarek-Pawelska, and Romuald Będziński. 2021. “Histological and Morphometric Evaluation of the Urethra and Penis in Male New Zealand White Rabbits.” *Journal of Veterinary Medicine Series C: Anatomia Histologia Embryologia* 50 (1): 136–143. doi:10.1111/ah.12611.
- Wang, Y., J. Sun, Y. Gu, S. Zhao, L. J. Groome, and J. S. Alexander. 2011. “D2-40/Podoplanin Expression in the Human Placenta.” *Placenta* 32 (1): 27–32. doi:10.1016/j.placenta.2010.10.014.
- Wilczyński, Jacek R. 2006. “Cancer and Pregnancy Share Similar Mechanisms of Immunological Escape.” *Chemotherapy*. doi:10.1159/000092537.
- Willershausen, Ines, Christina Erbe, Sarah Al-Maawi, Anna Orłowska, Heiner Wehrbein, and Shahram Ghanaati. 2019. “Development of a Novel Histological and Histomorphometric Evaluation Protocol for a Standardized Description of the Mid-Palatal Suture – An Ex Vivo Study.” *Journal of Anatomy* 235 (1): 180–188. doi:10.1111/joa.12985.
- Wilson, Susan J., Jonathan A. Ward, Helen M. Pickett, Simonetta Baldi, Ana R. Sousa, Peter J. Sterk, Kian Fan Chung, et al. 2021. “Airway Elastin Is Increased in Severe Asthma and Relates to Proximal Wall Area: Histological and Computed Tomography Findings from the U-BIOPRED Severe Asthma Study.” *Clinical and Experimental Allergy* 51 (2): 296–304. doi:10.1111/cea.13813.
- Zaki, S. M., and M. F. Youssef. 2013. “Thyroid Hormone Dysfunctions Affect the Structure of Rat Thoracic Aorta: A Histological and Morphometric Study.” *Folia Morphologica (Poland)* 72 (4): 333–339. doi:10.5603/FM.2013.0056.
- Ministerstwo Zdrowia. 2018. “Rozporządzenie Ministra Zdrowia z Dnia 16 Sierpnia 2018 r. w Sprawie Standardu Organizacyjnego Opieki Okołoporodowej.” [Ministry of Health. 2018. “Decree of the Minister of Health dated August 16, 2018 on the Organizational Standard of Perinatal Care”] *Dziennik Ustaw*, poz. 1756.