

## REVIEW

By Assoc. Prof. Dr. Kremen Tsvetanov Tsvetkov, M.D.,

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## REGARDING

Acquisition of the academic degree "Doctor" by Dr. Tsvetomir Evgeniev Kachovski, field of higher education: 7. Health Care and Sports, professional field 7.1 Medicine and scientific specialty "Obstetrics and Gynecology"

Topic of the dissertation: "Correlation between Ultrasonographic Diagnosis and Immunohistochemistry in Early and Late Abortions"

Author of the dissertation - Dr. Tsvetomir Kachovski

Supervisors: Prof. Dr. Emil Kovachev, M.D., Ph.D.

Prof. Dr. Anton Tonchev, M.D., Ph.D.

### **Biographical Information of the Candidate:**

Dr. Tsvetomir Evgeniev Kachovski was born on January 5, 1989, in Vratsa. In 2014, he graduated in medicine from MU-Pleven.

During the period 2014 – 2020, he worked at CSMP – Varna, Dolni Chiflik Branch, while simultaneously specializing in Obstetrics and Gynecology at MU-Varna. From July 10, 2016, to January 20, 2020, he served as an honorary assistant at the Department of Obstetrics and Gynecology at MU-Varna. From July 17, 2018, to July 17, 2021, he was a regular doctoral student at MU-Varna. From October 1, 2018, to February 28, 2019, he worked as a resident physician at SBAGAL 'Prof. D. Stamatov' in Varna. Since 2020, he has been an assistant at the Department of Obstetrics and Gynecology at MU-Varna. From 2020 to the present, he has been a specialist doctor at SBAGAL 'Prof. D. Stamatov' in Varna, Gynecology Department.

The dissertation submitted for defense comprises a total of 161 standard pages and is illustrated with 62 tables and 36 figures. The bibliography includes 305 literary sources, with 32 in Cyrillic and 273 in Latin script.

### **Relevance of the Problem:**

The scientific problem addressed in Dr. Kachovski's dissertation, namely spontaneous abortions, can be defined as highly relevant, both medically and socially, on a global scale. According to the World Health Organization, one in four pregnancies worldwide ends in abortion. The frequency of spontaneous abortions in the first trimester ranges from 15-20%, but when clinical abortions are combined with preclinical ones, confirmed by positive b-hCG testing, the abortion rate reaches up to 50% of pregnancies. In our country, during the period 2019-2022, a total of 78,207 abortions occurred, out of which 23,561 were spontaneous abortions and 54,634 were therapeutic abortions.

Spontaneous abortions are becoming an increasingly pertinent issue for countries facing serious demographic crises like ours. Negative natural growth, an aging population, and a significant decline in people of childbearing age are just some of the problems. In recent years, the number of publications related to studying the causes of spontaneous abortions and the role of decidua NK cells has been increasing.

**The review of the literature** clearly demonstrates that the candidate has thoroughly examined a substantial number of contemporary literary sources, with 273 of them being in Latin script and 32 in Cyrillic, all from the last 10 years. The literature review possesses a valuable understanding, is logically structured, and can serve as a foundation for the development of the dissertation work. Correlations between ultrasonographic diagnosis and immunohistochemistry in early and late abortions have been described.

The formulation of **the aim and tasks** is clear and well-defined. The aim of the dissertation work is to discover a correlation between Doppler velocimetry of uterine arteries and the quantity of dNK (decidual natural killer) cells and the proliferative marker Ki 67, investigated in placental tissue through immunohistochemical analysis, in early and late spontaneous abortions and elective abortions. There are a total of **7 main tasks**.

In the "**Materials and Methods**" section, specific and precise delineations of the main groups of the working material have been made. The dissertation work is based on a study conducted at SBAGAL "Prof. D-r Dimitar Stamatov" EOOD, Varna, in the "Gynecology Department" from October 2018 to January 2023. The study is both retrospective and prospective in nature. The author has accurately identified the inclusion and exclusion criteria, which is an approach ensuring the maximum reliability of the subsequent conclusions. The statistical analysis is based on primary data processing, statistical analysis, and graphical

representation. Anamnestic, clinical, imaging, pathological, anatomical, and immunohistochemical data have been utilized. The scientific research was approved by the Ethics Committee for Scientific Research at MU-Varna, Protocol No. 78 dated October 25, 2018 (Appendix No. 1), and was funded by the "Science Fund," Project No. 19008.

The "**Results and Discussion**" section is presented in 4 subsections in accordance with the set tasks of the dissertation work, which provides a clear overview of the presented data. The results are summarized in tables and graphs, which illustrate the conclusions drawn by the author.

The doctoral candidate investigates the expression of the Ki67 proliferative marker according to the age of the patients, the gestational age of the abortions, the type of abortion, the mode of delivery, as well as the gestational age of the abortions and Ri and Pi of the uterine arteries in subgroups based on the intensity of Ki67. The candidate establishes that elective abortions have the lowest average abortion age at 6.8 weeks gestational age (g.s.) and the lowest Ri and Pi values of the uterine arteries, followed by early spontaneous abortions with an average abortion age of 8.4 weeks g.s. Abortions after 13 weeks g.s. are characterized by the highest gestational age at 10.5 weeks g.s. and the highest Ri and Pi values of the uterine arteries.

**In conclusion**, Dr. Kachovski emphasizes that Doppler velocimetry is a fundamental method for determining vascular changes in uterine and fetal vessels during pregnancy. Doppler velocimetry in elective abortions shows the lowest Ri and Pi values compared to the results obtained from spontaneous abortions. Immunohistochemistry with the Ki67 proliferative marker can determine the accumulation of syncytiotrophoblast in abortion specimens. In 17.3% of spontaneous abortion cases, Ki67 expression is absent, and in 45.4%, it is weak. Dr. Kachovski establishes a link between the activity of cytotrophoblast cells and maternal immune reactions, especially decidual NK cells. Significant correlation between Ki67 and CD56+ cells is found in spontaneous abortions. A connection exists between spontaneous abortions, trophoblastic invasion, and maternal immunity.

### **Comments and Recommendations**

Dr. Kachovski has taken into account the critical comments and recommendations I provided.

As a result of the analysis conducted in the submitted dissertation work, 11 conclusions have been formulated.

Regarding the original contributions of the dissertation, I believe the following should be highlighted:

An original screening algorithm for pregnant women with threatened abortion has been developed.

A study of abortion material through immunohistochemistry with the Ki67 proliferative marker and CD56+ in the first and second trimesters of pregnancy has been conducted.

An investigation and analysis of spontaneous abortions through a combination of immunohistochemistry and Doppler velocimetry have been carried out.

The other contributions mentioned by the candidate are of a confirmatory nature.

## **Conclusion**

The dissertation work presented by Dr. Tsvetomir Evgeniev Kachovski meets the criteria of a scientific work with clinical and practical orientation in terms of development, methods of execution, content, and presentation format.

The author's conclusions are well illustrated in the text with figures, diagrams, and tables.

The presented dissertation work of Dr. Tsvetomir Evgeniev Kachovski, "Correlation between Ultrasonographic Diagnosis and Immunohistochemistry in Early and Late Abortions," is relevant, timely, and meets scientific criteria, as well as the regulations for academic development at MU-Varna for the award of the academic degree "Doctor" in the scientific specialty of "Obstetrics and Gynecology."

After considering the critical comments, I respectfully recommend to the esteemed members of the Review Committee to vote positively for awarding the academic degree of "Doctor" in the scientific specialty of "Obstetrics and Gynecology" to Dr. Tsvetomir Evgeniev Kachovski.

August 18, 2023 Prepared by:

/Assoc. Prof. Dr. Kremen Tsvetanov Tsvetkov, M.D./