

Statement

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Member of the Scientific Jury appointed by Order No. R-109-282 of 30.07.2026 of Prof. Dr. Dimitar Raykov, Rector of the Medical University of Varna, Minutes No. 62/27.07.2026 of the Faculty Council, and report Ref. No. 103-5069/27.07.2026.

Regarding: Procedure for the award of the scientific degree “Doctor” to Dr. Vilhelm Ruslanov Mladenov

Dr. Vilhelm Ruslanov Mladenov was born on 06.02.1970 in Varna.

Education:

- 1989 – graduated from the First English Language School, Varna.
- 1995 – graduated from the Medical University “Paraskev Stoyanov” – Varna.

Qualifications:

- 2002 – acquired a specialty in Pediatrics.
- 2007 – acquired a specialty in Pediatric Endocrinology and Metabolic Diseases.

Career development

- 2002–2003: Regional Health Insurance Fund, Varna (expert);
- 2003–2009: St. Anna Multiprofile Hospital for Active Treatment, Varna, Pediatric Department (pediatrician);
- 2009: St. Marina University Hospital, Varna, First Pediatric Clinic (pediatric endocrinologist).

Academic development:

- Since 2017: Medical University of Varna, Assistant Professor at the Department of Pediatrics.

Courses and professional skills:

- Certificate in ultrasound of the endocrine system;

- DEXA scanning of bone and adipose tissue;
- Certificate in insulin pump and CGM therapy; GCP.

Invited speaker:

- 2013 – National Conference on Pediatric Endocrinology with international participation, Timișoara, Romania;
- The VIth ISRAD/VAPES Conference “Diabetes and rare endocrine diseases”, 20–22 April 2023, Pomorie, near Varna.

Professional organizations:

- Bulgarian Society of Endocrinology;
- Varna Society of Pediatric Endocrinology;
- Bulgarian Society of Human Genetics and Genomics;
- ESPE;
- ISPAD;
- Bulgarian Medical Association.

Scientific publications, presentations and participations related to the dissertation:

- Full-text scientific publications: 4;
- International participations: 1;
- Participations in Bulgaria: 3.

Publication activity: More than 30 publications in Bulgarian and international journals, with more than 200 citations.

Languages:

- Bulgarian;
- English.

Computer skills: MS Office

The topic of the dissertation of Dr. Wilhelm Ruslanov Mladenov for the award of the educational and scientific degree “Doctor” is:

“Polycystic Ovary Syndrome in Adolescence – Contemporary Aspects and Diagnostic Approaches”.

The doctoral candidate examines contemporary aspects of polycystic ovary syndrome (PCOS), which is a serious challenge for modern pediatric endocrinology. Although 91 years have passed since it was first described, it remains topical to this day, as its onset is often in adolescence. Beginning mainly as ovarian dysfunction and hyperandrogenism in adolescence, it is very often accompanied by obesity, insulin resistance, impaired glucose and lipid metabolism, and increased cardiovascular risk. Of extreme importance are also the reproductive changes associated with the syndrome, such as impaired fertility, pregnancy complications, preterm births and spontaneous abortions, as well as an increased risk of developing certain carcinomas. Mental disorders such as anxiety, depression, emotional stress and eating disorders should not be overlooked either.

The relevance of the chosen topic is determined by the need to enrich the diagnostic criteria in adolescence with new, highly specific biomarkers. In this way, the application of personalized early interventions will ensure effective prevention of late complications, which in turn will improve the quality of life of the patients.

The dissertation comprises a total of 173 pages, illustrated with 25 tables and 16 figures. A total of 180 literature sources are cited, 4 in Cyrillic and 176 in Latin script.

The aim of the dissertation is precisely and clearly formulated. It emphasizes the importance of improving the diagnostic criteria for patients with PCOS in adolescence.

Five objectives have been set to achieve the aim. They cover not only the assessment of the clinical and laboratory profile of adolescent patients with PCOS, but also the investigation of some new diagnostic biomarkers for PCOS in adolescence, such as anti-Müllerian hormone (AMH) and the free androgen index (FAI), as well as the determination of their cut-off values that are specific and sensitive for the syndrome. The main purpose of the fourth and fifth objectives is to determine the prevalence of metabolic and cardiovascular disorders (dyslipidemia, impaired glycemic control, insulin resistance, hyperinsulinism, arterial hypertension) in patients with PCOS, as well as the sensitivity and specificity of selected miRNAs and their correlation with these disorders.

The inclusion and exclusion criteria for the participation of girls with PCOS are clearly and precisely defined.

The following methods were used in the study:

- Questionnaire method;
- Anthropometry;
- Clinical method;
- Laboratory biochemical and hormonal tests (blood glucose, lipid profile, CRP, insulin, homeostasis model assessment of insulin resistance – HOMA-IR, hormonal parameters (luteinizing hormone, follicle-stimulating hormone, estradiol, testosterone, androstenedione, 17-OH progesterone, sex hormone-binding globulin (SHBG), AMH, prolactin, cortisol, FAI), and miRNA testing.
- Ultrasound examination for the presence of polycystic ovarian morphology (PCOM).

An extremely precise specialized statistical software package was used for the medical-statistical processing of the data. The following methods were applied:

- Statistical grouping of data;
- Statistical estimation;
- Graphical method;
- Descriptive analysis;
- Analysis of variation;
- Non-parametric analysis;
- Correlation analysis;
- Linear regression analysis;
- ROC curve analysis was used to determine the sensitivity and specificity of the potential diagnostic biomarkers for PCOS, and cut-offs were calculated using the Youden index according to a defined formula.

The study includes 59 participants aged 12.9–17.9 years. They are divided into three groups: patients with PCOS diagnosed according to the selected criteria, a group of healthy controls, and a group of controls with idiopathic hyperandrogenism. The clinical characteristics and manifestations of hyperandrogenism are described: oligomenorrhea, hirsutism, acne, alopecia, hair loss and oily hair. Oligomenorrhea is a mandatory criterion for the diagnosis; it was found in 100% of the patients studied. The prevalence of excess weight in the present study was 63.9%, with 25% of participants being overweight and 38.9% obese. Manifestations of clinical hyperandrogenism – hirsutism and cystic acne –

are a main diagnostic criterion for PCOS. Hirsutism was the most frequent in the study – 88.9%; acne occurred in 69.4% and oily hair in 72.2%. The latter symptom is rarely described as a separate sign of hirsutism and is usually included among the manifestations of seborrhea.

The hormonal results of the study participants were also analyzed. Significantly higher levels of testosterone (T), androstenedione (A4) and FAI were found in girls with PCOS compared with the healthy control group. AMH is considered a potential diagnostic biomarker because of its involvement in the pathogenesis of the disease. Blood pressure (BP) values were also analyzed, and higher values were found in the patients participating in the study, in contrast to published literature data. The influence of various factors on BP is noted, and its monitoring in patients with PCOS is recommended. Glucose homeostasis – fasting blood glucose and HOMA-IR – was also studied. No significant differences were found with respect to impaired fasting glycemia, fasting hyperinsulinemia and insulin resistance. Impaired fasting glycemia was recorded in 5.3% of the girls, a frequency comparable to that found in other studies. A parallel is drawn between impaired fasting glucose and impaired glucose tolerance because of the difference in their pathophysiology. It is noted that the predominant metabolic defect in PCOS affects peripheral insulin sensitivity rather than hepatic gluconeogenesis, so most carbohydrate metabolism disorders in this disease involve impaired glucose tolerance (IGT) rather than isolated impaired fasting glucose (IFG). An OGTT is recommended for inclusion in the diagnostic algorithm for PCOS in adolescence, regardless of body mass index (BMI). Lipid metabolism disorders, which are common and result from the combined effects of obesity, insulin resistance and hyperandrogenism, are also reported. A frequent disorder in the study is hypertriglyceridemia as a manifestation of impaired lipid metabolism. In the study, elevated CRP, found in 19.4% of girls with PCOS, is considered a second marker of increased cardiovascular risk. Polycystic ovarian morphology (PCOM) was also assessed. It is not recommended as a diagnostic criterion because: a transvaginal transducer cannot be used in adolescent girls; ovarian volume in adolescence is larger than in adult women; and a multicystic ovarian appearance is regarded as a variant of normal up to 8 years after menarche and is not associated with hyperandrogenism. The international guideline for the diagnosis of PCOS in adolescents is cited, which recommends avoiding ultrasound examination until 8 years after menarche because of the overlap with normal pubertal physiology and the low specificity of PCOM for the diagnosis of PCOS in this age group. An extremely important part of the dissertation is the study of 6 preselected miRNAs as biomarkers for PCOS. Four miRNAs were found to be significantly expressed in girls with PCOS. MiR-320-5p and miR-

103a-5p showed optimal characteristics as diagnostic markers. MiR-15a-5p showed a very good sensitivity profile in distinguishing patients with PCOS from girls with other hyperandrogenic conditions. To clarify the role of miRNAs in the pathogenesis of PCOS and related pathogenetic conditions, they need to be studied further.

Reviewing the answers to the objectives, the following is established:

- *First objective*

- Oligomenorrhea is the main clinical manifestation of PCOS in adolescence and is observed in all patients.
- In patients with overt manifestations of hyperandrogenism, the most common symptom is hirsutism, followed by seborrhea and acne.
- Overweight and obesity are found in 2/3 of girls with PCOS.
- Acanthosis nigricans is common in girls with PCOS and correlates with obesity, clinical hyperandrogenism, blood pressure and dyslipidemia.
- Girls with PCOS have significantly higher levels of T and A4 and a significantly higher FAI than healthy girls.

- *Second objective*

- FAI is the marker with the highest specificity and sensitivity in distinguishing patients with PCOS from healthy girls.
- FAI, A4 and AMH are suitable for inclusion in the general panel of diagnostic criteria for PCOS in adolescence.

- *Third objective*

- Total ovarian volume is a predictor of AMH levels.
- No correlations were found between AMH levels, androgens, and metabolic and cardiovascular parameters.
- No correlations or causal relationships were found for A4 and FAI with metabolic and cardiovascular parameters.

- *Fourth objective*

- Almost half of the girls have elevated systolic and/or diastolic BP, which correlates positively with obesity, acanthosis nigricans,

HOMA-IR and insulin levels, and negatively with sex hormone-binding globulin (SHBG) levels.

- Girls with PCOS have a high prevalence of impaired glucose homeostasis, expressed as frequent insulin resistance and fasting hyperinsulinemia.
- The most common lipid metabolism disorder, found in 1/4 of girls with PCOS, is hypertriglyceridemia, followed by low HDL cholesterol, hypercholesterolemia and elevated LDL cholesterol.
- No statistically significant effect of androgens on lipid disorders was found.

- *Fifth objective*

- In girls with PCOS, miR-320-5p and miR-103a-5p show the highest specificity (100%) and a sensitivity of 76% for diagnosing the disease, while miR-15a-5p distinguishes patients from healthy girls with 91% specificity and 76% sensitivity. MiR-15a-5p also distinguishes PCOS from other hyperandrogenic conditions.
- It is also extremely important that these studies establish that, in combination, these miRNAs can be used as independent diagnostic markers in patients with PCOS.
- The miRNAs of diagnostic significance for PCOS in the study also show positive associations with systolic blood pressure.

The contributions of the dissertation demonstrate the scientific and practical value of the study of polycystic ovary syndrome in adolescence. It defines contemporary aspects and approaches in the diagnosis of one of the most common endocrine disorders and the most common hyperandrogenic disease in women of reproductive age.

- This is the first study in Bulgaria to comprehensively examine PCOS in adolescence, with a detailed clinical and laboratory assessment.
- The prevalence of overweight, obesity, and metabolic and cardiovascular complications in girls with PCOS has been determined for the first time.
- This is the first research project in Bulgaria, and the second worldwide, investigating the expression of selected microRNAs in girls with PCOS and control groups.
- In the present study, the most sensitive and specific diagnostic cut-off value of AMH in adolescent girls with PCOS has been determined for the first time in Bulgaria.

- For the first time in Bulgaria, the present study has determined the most sensitive and specific cut-off values of A4 and FAI in adolescent girls with PCOS.
- The present work enriches the diagnostic algorithm for PCOS in girls under 18 years of age with new diagnostic and sensitive biomarkers.

The materials submitted for the procedure by Dr. Vilhelm Ruslanov Mladenov reflect his thorough clinical work, his scientific inquiry and his theoretical preparation. The dissertation confirms all of this. It investigates an extremely important problem in pediatric endocrinology: polycystic ovary syndrome, its contemporary aspects and diagnostic approaches. The development of endocrinology and scientific research into many diseases in this field of pediatrics have answered many questions. This shapes both the clinical and the diagnostic approach to diseases such as PCOS. It is extremely important that innovations in clinical practice and scientific research have not only a theoretical but also an applied character.

I believe that the dissertation of Dr. Vilhelm Ruslanov Mladenov fills a gap that needs to be filled for the prevention of an endocrine disease with many complications, and that it will be used in clinical practice by endocrinologists, pediatricians and general practitioners alike.

Dr. Vilhelm Ruslanov Mladenov set himself an ambitious goal requiring dedication, hard work and competence. My review of the materials submitted gives me grounds to give a positive assessment, for knowledge is a treasure that follows its owner everywhere.

17.09.2026

Prof. Dr. D. Bliznakova, MD, PhD

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