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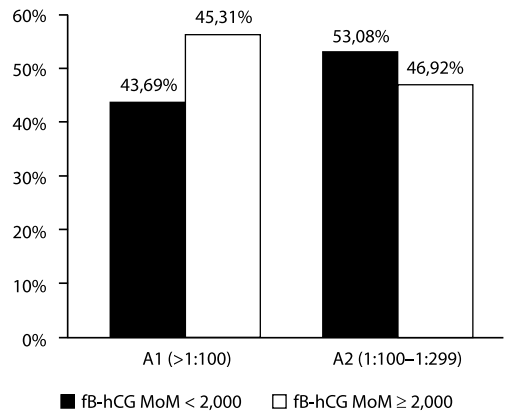

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A prospective self-controlled study on shortening the time before taking delayed radiographs with iodized oil hysterosalpingography

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ABSTRACT

Objectives: To verify the feasibility of walking to shorten the time before obtaining delayed radiographs after iodized oil hysterosalpingography (HSG).

Material and methods: One hundred women with infertility were selected for HSG from June 2018 to December 2018 at the Women's Hospital of Nanjing Medical University; the subjects were randomly divided into walking and control groups. The walking group was required to walk more than 12,000 steps within 6 hours after HSG, while the control group was prohibited from performing high-intensity exercise. The degree of pelvic adhesion was diagnosed with delayed radiographs acquired at 6 and 24 hours, and the diagnostic consistency of the radiographs at the two time points was evaluated.

Results: No significant difference was observed in the baseline data between groups ($p > 0.05$). The delayed radiograph results in the walking group showed good agreement ($p = 0.255 > 0.05$, Kappa value $0.781 > 0.75$), while those in the control group showed general agreement ($p = 0.002 < 0.05$, Kappa value $0.493 > 0.40 < 0.75$).

Conclusions: The time for acquiring delayed radiographs can be shortened by instructing patients to walk after HSG. This method improves the diagnostic efficiency of Iodized oil, saves time and costs, and may contribute to the popularization of HSG for female infertility screening, while offering good clinical application prospects.

Key words: hysterosalpingography; Iodized oil; delayed radiographs; pelvic adhesions; self-controlled study

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INTRODUCTION

Hysterosalpingography (HSG), a long-standing diagnostic imaging technology, plays an important role in preliminary screening for female infertility due to its simplicity, convenience, minimal trauma and image quality. Oily contrast agents have obvious advantages in showing details of uterine tubal lesions and pelvic adhesions. With continuous improvements in equipment and technology, the risk of serious complications such as pulmonary embolism is decreasing. Recent studies have shown that lipiodol contrast agents improve patient pregnancy rates [1], which has increased interest in HSG. However, delayed films should be taken at 24 hours after initial HSG to evaluate the presence of pelvic adhesions, which significantly reduces diagnostic efficiency and speed and increases the time cost for patients. Especially for nonlocal patients, it also increases the cost of transportation or accommodation, which greatly hinders the popularization of iodized oil HSG. The aim of this study was to promote pelvic iodized oil diffusion through appropriate exercise after HSG. This method could shorten the time before delayed films can be obtained, improve diagnostic efficiency, and reduce the time and economic costs for patients without affecting diagnostic accuracy.

MATERIAL AND METHODS

Clinical material

From June 2018 to December 2018, one hundred female infertility patients who underwent HSG at the Women's Hospital of Nanjing Medical University, aged 33.54 ± 6.41 years, with an infertility duration of 4.80 ± 3.08 years, were se-

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lected. All subjects were randomly divided into walking and control groups (n = 50 each). The inclusion, exclusion and removal criteria were shown in Table 1. And this study was approved by the Medical Ethics Committee of The Women's Hospital of Nanjing Medical University.

Equipment and drugs

A Shimadzu Digital Gastrointestinal System (FLEXAVISION, Japan) was used for imaging during the HSG procedure. Shida disposable hysterography tubes (type 12B, China) were the main consumable items used. Papaver Ethyl Iodine Oil Injection (Hengrui Medicine, China) was used as a contrast agent.

HSG operation process

The procedure was performed 3 to 7 days after menstruation. First, the perineum was disinfected, and surgical towels were spread out. Second, an angiographic catheter was inserted into the uterine cavity under X-ray guidance. Third, the balloon was filled to seal the cervical opening. Fourth, iodized oil was gently injected into the uterine cavity. Images of uterine filling, tubal filling and pelvic overflow were recorded. Finally, the balloon was withdrawn, and the catheter was removed.

Exercise guidance and delayed radiographs obtained

After HSG, the researchers instructed the walking group patients to walk at a prescribed intensity to accelerate pelvic contrast medium diffusion. These subjects were instructed to walk more than 12,000 steps within 6 hours after HSG. The control subjects were asked to maintain normal daily activity intensity and avoid strenuous exercise post-HSG. "WeChat" (Tencent Inc, China) was used to count the steps walked and quantify exercise intensity post-HSG.

Delayed radiographs were obtained for the two groups at 6 ± 0.5 and 24 ± 2 hours post-HSG to evaluate pelvic dispersion of the contrast material.

Randomization

The delayed radiographs taken at 6 and 24 hours post-HSG were randomly assigned to five diagnostic physicians (A to E) for evaluation of the degree of pelvic adhesion, which was divided into four grades (grade 0 to 3), as shown in Table 2.

All operators, imaging technicians and imaging diagnosticians involved in the clinical trial had more than 5 years of experience in HSG-related work. Before initiation of this

Table 1. Inclusion, exclusion and removal criteria

	Criteria
Inclusion	1. Women aged 21 to 45 years 2. Conformity with HSG indications 3. Voluntary participation in the trial with informed consent
Exclusion	1. History of iodine allergy, hyperthyroidism, or thyroid tumors 2. Trichomonas vaginitis, candida vaginitis, vaginal secretion cleanliness over II degrees 3. Acute/subacute pelvic inflammation, chronic pelvic inflammation, active pelvic/uterine/fallopian tuberculosis 4. Uterine or cervical bleeding 5. Less than 6 months postpartum or uterine cavity operation less than 4 weeks 6. Infertility due to lack of ovulation 7. Copulation or vaginal administration of semen within 3 days before HSG 8. Body temperature of higher than 37.5°C on the day of HSG 9. Inability to diagnose the degree of pelvic adhesion because iodized oil could not flow into the pelvic cavity through any side of the fallopian tube after intrauterine injection
Removal	1. Incorrect diagnosis at admission 2. HSG was immediately terminated because of serious complications, such as venous/lymphatic reflux or allergic reaction 3. The subject asked to withdraw from the study 4. Researchers deemed a subject no longer suitable for inclusion in the study for medical safety reasons 5. The patient failed to fulfill or seriously violated the research plan

Table 2. Imaging grading of pelvic adhesions

Classification	Diagnostic criteria
Grade 0 (No clear pelvic adhesions)	Wide and uniform contrast agent distribution in the pelvic cavity
Grade 1 (Mild pelvic adhesions)	The pelvic contrast medium diffusion range was slightly smaller than normal, and the distribution was generally uniform
Grade 2 (Moderate pelvic adhesions)	The contrast medium diffusion range was obviously limited, and the medium was strongly localized
Grade 3 (Severe pelvic adhesions)	The contrast medium was concentrated in the pelvic region and had not disseminated, resulting in a mass image

study, a coordination meeting was held to standardize the processes followed by the operation and technical groups and to standardize the pelvic adhesion evaluation criteria used by the diagnostic group.

The MedSci medical randomization tool was used for randomization. One hundred subjects (50 each in the walking and control groups) were included, with 200 delayed film images.

Blinding

All diagnosing doctors were blinded. After the quality controllers removed the subjects' identifying information and times that the imaging data were obtained, the delayed films taken 6 and 24 hours post-HSG in the walking and control groups were randomly sent to the five diagnostic physicians. These physicians evaluated the degree of pelvic adhesiveness (grade 0 to 3) on the pelvic images. To eliminate interference of the diagnostician's residual image memory on the diagnosis (for example, if the same subject was assigned to the same diagnostician at different times after randomization), the interval between reading the two sets of imaging data was required to be longer than 14 days.

Data analysis

IBM SPSS version 20 software was used to analyze the data collected in this trial. Measurement data are expressed as $\bar{x} \pm s$, and numerical data are expressed as percentages (%). One-way ANOVA was used to compare the normally distributed measurement data that passed the homogeneity of variance test. The rank sum test was used to compare the non-normally distributed grade data and measurement data that did not pass the homogeneity of variance test. The chi-square test was used for non-grade count data. A value of $P < 0.05$ indicated a significant difference.

The 6- and 24-hour standard delayed radiographs of the walking and control groups were compared. McNemar-Bowker test was used to evaluate the statistical difference between 6-hour and 24-hour delayed radiograph. And the diagnostic consistency between the two groups was evaluated using the Kappa test. If the Kappa value was higher than 0.75, the diagnosis based on the 6-hour delayed film was considered consistent with that of the traditional 24-hour delayed film; therefore, the 6-hour film could be used for diagnosis instead of the 24-hour film. If the Kappa value was lower than 0.40, the diagnostic consistency between the 6- and 24-hour films was considered poor. If the Kappa value was between 0.40 and 0.75, the diagnostic consistency between the 6- and 24-hour films was intermediate, making it necessary to analyze the clinical trial design or to increase the sample size for further observation.

RESULTS

No significant differences in age, number of pregnancies, production time or number of years of infertility were observed between the walking and control groups ($p > 0.05$), as shown in Table 3.

There were no significant differences in the histories of pelvic inflammation, endometriosis, appendicitis, tubal pregnancy, cesarean section and other pelvic operations between the walking and control groups ($p > 0.05$), as shown in Table 4.

The McNemar-Bowker test showed no significant difference between the 6- and 12-hour data in the walking group ($p = 0.255 > 0.05$). There was no significant difference between the results of the 6- and 24-hour delayed radiographs in the diagnosis of pelvic adhesion. The Kappa value was 0.781, which exceeded 0.75. The results suggested that the diagnosis of pelvic adhesion based on the 6-hour delayed radiograph was consistent with that based on the traditional 24-hour delayed radiograph. Therefore, it is feasible to use a 6-hour delayed film instead of a 24-hour delayed radiograph to diagnose pelvic adhesion.

According to the McNemar-Bowker test, $p = 0.002 < 0.05$ for the 6- and 24-hour radiographs in the control group. A significant difference was observed between the 6- and 24-hour delayed radiographs in the diagnosis of pelvic adhesion. The Kappa coefficient was 0.493, which was between 0.40 and 0.75. For the control group, the diagnosis of pelvic adhesion based on the 6-hour delayed radiograph was somewhat consistent with that based on the 24-hour delayed radiograph. Therefore, the evidence did not support replacing the diagnosis based on a 24-hour delayed radiograph with that based on a 6-hour delayed radiograph.

DISCUSSION

According to recent reports, the incidence of female infertility in the general population ranges from 9 to 18%

Table 3. Comparison of measurement indexes between the two groups of subjects($\bar{x} \pm s$)

Measurement indexes	Walking group (n = 50)	Control group (n = 50)	t value	p value
Age (years)	34.52 ± 6.57	32.56 ± 6.16	1.539	0.127
Number of pregnancies	1.72 ± 1.11	1.42 ± 1.11	1.354	0.179
Production times	0.80 ± 0.61	0.78 ± 0.68	0.155	0.877
Number of years of infertility (years)	4.82 ± 2.90	4.78 ± 3.27	0.065	0.949

Table 4. Comparison of count data between the two groups of subjects

Count indexes	Walking group (n = 50)		Control group (n = 50)		χ^2	p value
	Cases	Percentage (%)	Cases	Percentage (%)		
Pelvic infection					0.219	0.640
Yes	37	74.0	39	78.0		
No	13	26.0	11	22.0		
Endometriosis					0.298	0.585
Yes	43	86.0	41	82.0		
No	7	14.0	9	18.0		
Appendicitis					0.000	1.000
Yes	46	92.0	47	94.0		
No	4	8.0	3	6.0		
Tubal pregnancy					0.271	0.603
Yes	42	84.0	40	80.0		
No	8	16.0	10	20.0		
Cesarean section					0.480	0.488
Yes	39	78.0	36	72.0		
No	11	22.0	14	28.0		
Other pelvic surgery					0.233	0.629
Yes	40	40.0	38	76.0		
No	10	20.0	12	24.0		

[2], and the incidence is increasing [3]. With the liberalization of the national fertility policy, the diagnosis and treatment of female infertility has become a prominent clinical issue in China [4]. The main causes of female infertility are related to the fallopian tubes [5], ovaries [6] and uterus [7]. In addition, pelvic adhesion [8] is considered an important cause of female infertility. Among the above factors, the diagnosis of ovarian factor infertility mainly relies on laboratory examinations to detect and analyze reproductive endocrine hormones [9]. Tubal factor, uterine factor and pelvic adhesion infertility can be diagnosed by hysteroscopy, laparoscopy [10] and imaging [11] because they can cause morphological changes.

The main imaging methods currently used to diagnose female infertility are hysterosalpingo contrast sonography (HyCoSy) [12], MR [13] and HSG [14]. Among these methods, HSG has the advantages of minimal trauma, low cost, convenience, clear results, and the ability to show tubal, uterine and pelvic adhesion at the same time; thus, it is widely used for assessing female infertility.

According to the different contrast agents used, HSG can be further divided into iodine water radiography and iodine oil radiography. The advantages of iodine hydrography are mainly safety (no risk of serious complications such as pulmonary embolism) and convenience (short time prior to the delayed radiograph). However, iodized oil radiography has obvious advantages in the level of detail shown of uterine and fallopian tube lesions and pelvic adhesions. In addition, recent studies have revealed that lipiodol angiography can improve pregnancy rates due to its immunomodulatory effects [15]. Therefore, HSG with iodized oil has attracted the attention of clinicians and has been increasingly applied for the screening of female infertility.

Inflammation, surgery and pelvic endometriosis are common causes of pelvic adhesions, which are closely related to female infertility [16]. Different degrees of pelvic adhesions have varying impacts on the patency of fallopian tubes and the prognosis of treatment [17]; thus, increased attention should be paid to the diagnosis of pelvic adhesions.

The fimbriated extremity of fallopian opens in the peritoneal cavity. During HSG, the contrast agent is injected into the uterine cavity through the cervix via a contrast catheter, moves into the fallopian tube cavity, and finally exits into the peritoneal cavity through the opening of the fimbriated extremity of fallopian. After the contrast agent enters the peritoneal cavity, it continuously diffuses to distant locations due to body movement and intestinal peristalsis. If pelvic peritoneal adhesions exist, the diffusion process is hindered, and the contrast agent accumulates in the region with adhesions. Imaging diagnosticians evaluate pelvic adhesions by observing contrast media diffusion. When a water-based contrast agent is used for HSG, the delayed radiograph can be obtained after approximately 15 minutes [18]. As the dispersion and absorption of water-based

contrast agents occur very quickly, the details of the diffusion image are difficult to capture, making the diagnostic value of the test for pelvic adhesions relatively low. The diagnostic accuracy of HSG for pelvic adhesions is much higher when oil-based contrast agents are used than when water-based contrast agents are used because of the slow diffusion and absorption of the former. Therefore, to ensure the complete diffusion of iodine oil throughout the pelvic cavity, a longer interval of approximately 24 hours is needed before re-examination [19].

After the oil contrast agent enters the peritoneal cavity from the opening of the fimbriated extremity of fallopian, it spreads continuously around the spillover point and eventually reaches diffusion equilibrium, i.e., the point at which the diffusion range no longer expands. This process is usually completed approximately 24 hours after iodized oil injection. The diffusion rate and range of iodized oil are not only related according to the degree of pelvic adhesion but also affected by body movement and intestinal peristalsis. This principle is similar to that affecting cancer patients, who are required to change their posture frequently after intraperitoneal infusion chemotherapy to promote the uniform distribution of the drugs in their abdominal cavities [20]. This phenomenon provided us with an idea: after iodized oil angiography, increasing patient movement could accelerate the spread of iodized oil, thereby reducing the time needed to achieve diffusion equilibrium and ultimately shortening the interval before the delayed radiograph can be obtained.

Although MRI, contrast-enhanced ultrasound and laparoscopy are all used to diagnose uterine and fallopian tubal infertility, HSG still plays an irreplaceable role, especially in screening. Compared to water-based contrast media, oil-based contrast media have obvious advantages when used for diagnosis and could promote pregnancy by modulating immunity after HSG. Therefore, the use of HSG with oil-based contrast medium has become increasingly widespread in recent years. With the improvement of surgical instruments, operative technology and the technology involved in contrast agent production, the risk of serious complications of lipiodol radiography has significantly decreased, and there is now no obvious safety disadvantage compared to iodized water radiography. Currently, as the pace of life is clearly accelerating, the 24-hour interval before obtaining a delayed radiograph has become an important hindrance to the further promotion of HSG with oil-based contrast medium.

The shortcomings of our study included the small sample size and use of only one additional time point for the delayed radiograph (6 hours), which prevented precise determination of the time required to reach dispersion equilibrium. Additionally, the stride length, stride frequency and walking duration differed among the subjects. Simply analyzing the number of steps did not accurately reflect the exercise intensity.

The results of this exploratory research, which aimed to maintain diagnostic accuracy, revealed that the diagnostic efficiency of HSG with an oil-based contrast agent could be improved and the time required could be reduced by instructing patients to exercise appropriately. Especially for nonlocal patients, this new method could reduce the amount of time needed for delayed radiographs to less than one day, reducing the costs associated with transportation and accommodation. These findings suggest that HSG is clinically applicable and should be further promoted for use in female infertility screening.

Conflicts of interest

No conflict of interests to declare.

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Comparison of Anti-müllerian Hormone (AMH) and Hormonal Assays for Phenotypic Classification of Polycystic Ovary Syndrome

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ABSTRACT

Objectives: The aim is to compare the hormonal status and anti-müllerian hormone (AMH) levels of patients who have different polycystic ovary syndrome (PCOS) phenotypes, polycystic ovarian morphology (PCOM) and healthy women.

Material and methods: A total of 350 PCOS women, 71 women with PCOM and 79 healthy women with normal ovarian morphology (NOM) were observed. PCOS patients were divided into groups according to the phenotypes. Phenotype A- characterized by anovulation, hyperandrogenism and PCOM; phenotype B- defined as anovulation, hyperandrogenism; Phenotype C- identified as hyperandrogenism and PCOM; Phenotype D- outlined as anovulation and PCOM. AMH levels were compared for each group.

Results: Among 350 PCOS patients the highest number belonged to phenotype A (n = 117, 33.4%). The rest were distrubuted as follows: phenotype B (n = 89, 25.4%), phenotype C (n = 72, 20.6%), phenotype D (n = 72, 20.6%). Phenotype A (9.17 ± 4.56) had the highest mean AMH levels in our study. Comparison of AMH levels showed a statistically significant difference between phenotypes A and D. There was a statistically significant difference on comparison of AMH between NOM, PCOM and all PCOS phenotypes.

Conclusions: Phenotype A is the most serious form of PCOS and these patients has all three features which are hyperandrogenism, anovulation and ultrasound findings of polycystic ovary (PCO). AMH reflects the severity of PCOS and patients with Phenotype A have higher AMH levels.

Key words: polycystic ovary syndrome; phenotype; polycystic ovarian morphology; anti müllerian hormone; hyperandrogenism

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the highest prevalent hormonal disturbance among reproductive age women. PCOS affects 4–21% of women worldwide [1]. The symptoms of PCOS include menstrual irregularity, clinical or biochemical hyperandrogenism findings and obesity. The definition and diagnosis of PCOS have long been debated. In 1990, National Institute of Child Health and Human Disease (NICHD) congress participants agreed that the major diagnostic criteria for PCOS should include hyperandrogenism and menstrual dysfunction and the exclusion of other endocrine disorders [2]. On the other hand, the ultrasound has been widely used in Europe for the diagnosis of PCOS [3]. In 2003, a meeting was held in Rotterdam to provide consensus for PCOS diagnostic criteria. According to the Rotterdam classification in 2003, when two or more of the following aspects are existent, PCOS can be determined: anovulation, hypernadrogenism and polycystic ovaries [4, 5]. In the Rotterdam consensus conference, it was decided that the following sonographic descriptions of polycystic ovarian morphology (PCOM) should be included: enlarged volume of the ovaries (≥ 10 cm^3) or either ≥ 12 follicles per ovary sized between 2–9 mm [5]. The Rotterdam consensus decisions generated the possibility of four phenotypes of PCOS. For the diagnosis of PCOS, only the presence of PCOM on ultrasound is not acceptable [5]. In addition, according to Azziz, the definition of PCOM should be made very carefully and in the abscence of any other sign or symptom of PCOS, PCOM should not be accepted as PCOS [6]. The clinical significance of the polycystic presentation of the ovaries on ultrasound still continues to be unclear. Indeed, signs and symptoms are heterogeneously combined in each affected

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woman. There is a wide spectrum of clinical and biochemical differences between PCOS patients [7]. The classification of such a varied pathology has presented dilemma for the gynecologist and therefore set up the potentiality of four different phenotypes of PCOS.

According to the Rotterdam criteria, there are four major phenotypes defined on the symptoms and clinical findings of PCOS. In patients with PCOS, phenotypes should be determined by following the correct algorithm in order to obtain healthier results [8]. Phenotype A- identified by anovulation, hyperandrogenism and polycystic ovaries on ultrasound; phenotype B- identified with anovulation, hyperandrogenism; Phenotype C- defined as hyperandrogenism and polycystic ovaries on ultrasound; Phenotype D- diagnosed as anovulation, polycystic ovaries on ultrasound [5]. The utmost serious form of PCOS is phenotype A and these patients have all three features which are hyperandrogenism, anovulation and ultrasound findings of polycystic ovary (PCO).

Anti-müllerian hormone (AMH), a polypeptide, which is synthesized from the granulosa cells of the preantral and early developing antral follicles, belongs to the transforming growth factor beta superfamily [9]. The major physiological act of AMH in the ovary is the prevention of primordial follicles recruitment and the regulation of FSH secretion in the early follicular phase [10]. Antral follicule count is associated with serum AMH levels. PCOS patients display an elevated number of antral follicles; therefore AMH levels are 2–3-fold augmented in PCOS patients [11–14]. As a result of a recent study in animals, AMH acted a significant part in the pathogenesis of PCOS starting from intrauterine life [15]. Pigny et al. [16] found that there was a significant relation between follicle count and AMH values in PCOS patients. Although the AMH is elevated in patients with PCOS, it is still not recommended to use serum AMH measurement alone to diagnose PCOS [17, 18]. Since the phenotypes of polycystic ovary syndrome has different clinical and biochemical features, it has been claimed that the serum levels of AMH may vary between these phenotypes. In a recent study it is shown that serum AMH levels were higher in hyperandrogenic PCOS phenotypes and in PCOS patients that have all three Rotterdam criterias [19].

PCOS is a heterogenous clinical condition and the four major phenotypes show different clinical presentations. Investigation of the disparities between women with different phenotypes of PCOS, women with polycystic appearance only (PCOM) and healthy women with normal ovarian morphology (NOM) enhance the understanding of the pathophysiology of PCOS. Our aim is to observe and compare the hormonal status and AMH levels of patients with different PCOS phenotypes, PCOM and healthy women with normal ovarian morphology. Our hypothesis is that PCOS phenotypes with anovulation may produce elevated levels of AMH compared to phenotypes with regular ovulatory cycles or only polycystic ovarian morphology (PCOM) on ultrasound.

MATERIAL AND METHODS

The indicated retrospective study was planned in the Obstetrics and Gynecology Clinic of Dokuz Eylul University Hospital, Faculty of Medicine, Izmir/Turkey, during the time period of January 2012 to July 2015. The research customs was authorized by Dokuz Eylul University local review board. Enlightened approval form was collected from all participitants of the study. In date range we specified, 1871 patients who underwent serum anti-mullerian hormone tests in our hospital were identified by scanning the database and patient files of the hospital. The flow chart of the study is shown in Figure 1. A total of 500 eligiable patients were recruited, including 350 PCOS women, 71 women with polycystic ovarian morphology on ultrasound (PCOM) and 79 healthy women with normal ovarian morphology (NOM) which were suitable for our study. PCOS patients were divided into groups according to the phenotypes defined in Rotterdam criteria [5].

In our hospital, anamnesis, gynecological examination and pelvic ultrasonography are standard practice for all patients who apply to the gynecology outpatient clinic. These history and examination findings were documented from hospital database and patients' files. Calculations were done for Ferriman-Gallwey score (FGS) and Body Mass Index (BMI). Hirsutism was represented when FGS was ≥ 8. PCOS diagnosis was made according to the Rotterdam Consensus [5]. The polycystic ovarian morphology was described as reported by the Rotterdam criteria [5, 6]. All healthy women had regular monthly menstrual cycles and initial examination by transvaginal ultrasound showed normal uteruses and ovaries. Exclusion principles were as follows; age up 35 years,

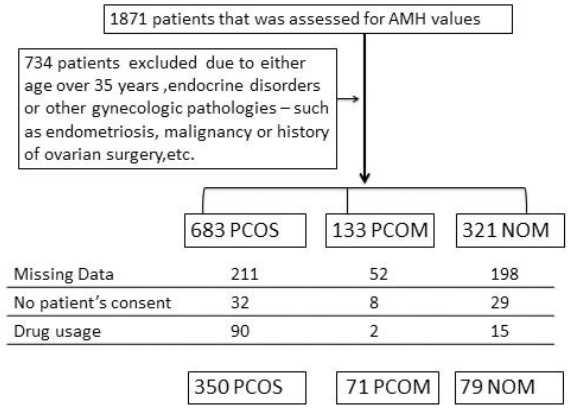


Figure 1. Flow chart of the study; PCOS — polycystic ovary syndrome; PCOM — polycystic ovarian morphology on ultrasound; NOM — normal ovarian morphology

ovarian surgery history, thyroid disease, systemic diseases, hyperprolactinemia, congenital adrenal hyperplasia, or drug usage effecting androgen metabolism, glucose/insulin metabolism and hypothalamus-hypophysis-ovary axis. In addition, the women who had used contraceptives or hormonal treatment up to 6 months were also considered as exclusion factors. If any existence of lesions, cysts or follicles greater than 10mm on the ovaries, it was accepted as an exclusion feature. Weight and height were measured in the initial check-up. The formula weight (kg)/height $(m)^2$ was used to analyze BMI. Biochemical and hormonal assesments were done for anti-müllerian hormone (AMH), androstenodione, thyroid stimulating hormone (TSH), luteinizing hormone (LH), total testosterone, follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), prolactin (PRL), insulin and fasting plasma glucose. Homeostatic model of insulin resistance (HOMA-IR) was used to evaluate normal insulin sensitivity. Determination of HOMA-IR was by the equation: HOMA-IR = fasting blood glucose (mg/dL) X fasting insulin (mIU/mL)/405. Definition of insulin resistance was made in the presence of HOMA-IR being equal or greater than 2.5. Formula for the calculation of free androgen index (FAI) was 100x (Total testosterone/SHBG). Between the second and fifth days of the menstrual cycle, patients' blood samples were collected in the morning following 8 hours fasting. For amenorrhoeic women, pregnancy was eliminated then, medroxyprogesterone acetate (TARLUSAL; Deva Holding A.Ş. Istanbul, Turkey) was initiated 5 mg twice a day for a 5 day period to promote uterine bleeding.

The commercial kit as reported by the manufacturer's guidance depend on the settlements of the competitive enzyme linked immunosorbent assay (ELISA) approach (catalog number: CSB-E12756h, CUSABIO Biotech Co., USA) was used to evaluate serum anti-müllerian hormone (AMH). A basic curve of known concentration (0, 0.375, 1.31, 4.69, 28.12, and 150 ng/mL) of AMH was certified and the concentration of analyte in the fragments was adjusted correspondingly. The sensitivity of the ELISA assays of AMH was 0.375 ng/mL; the detection range was 0.375–150 ng/mL and intraassay coefficient of variation was < 10%, and interassay coefficient of variation was < 15%.

Statistics are demonstrated as mean ± standard deviation except elseways declared. Calculations were done by the program Statistical Program for Social Sciences (SPSS) version 16. (SPSS, Chicago, IL). To check for the homogeneity of the study group, the Kolmogorov Smirnov test was conducted. Continuous variables (normally distributed) were compared by one-way ANOVA (> 2 groups) and then Bonferroni test was used for post hoc comparison. Tamhane's test was performed for continous variables that were not following normal distribution. $P < 0.05$ was accepted as the level of significance.

RESULTS

An overall of 500 women's results were evaluated. 350 PCOS patients, 71 women with polycystic ovarian morphology on ultrasound (PCOM) and 79 healthy women with normal ovarian morphology (NOM) were assessed. Among the 350 PCOS patients the highest number of patients belonged to phenotype A (n = 117, 33.4%). The rest were distrubuted as follows: phenotype B (n = 89, 25.4 %), phenotype C (n = 72, 20.6%), phenotype D (n = 72, 20.6%). A comparison of the demographic and hormonal status of NOM, PCOM and PCOS phenotypes is shown in Table 1. The mean BMIs of all groups were similar and the mean BMI of groups were less than 25 kg/m^2. Comparison of the LH/FSH ratio showed that all PCOS phenotypes were found to be statistically similar. In fact, there was a statistically significant difference

Table 1. Comparison of demographic and hormonal status of NOM, PCOM and PCOS phenotypes

Variables	NOM (n = 79)	PCOM (n = 71)	PCOS A (n = 117)	PCOS B (n = 89)	PCOS C (n = 72)	PCOS D (n = 72)	P
Age [years]	24.05 ± 4.59	24.86 ± 4.17	23.54 ± 4.15	23.99 ± 4.35	23.40 ± 4.84	24.81 ± 5.05	NS
BMI [kg/m^2]	24.70 ± 3.35	24.93 ± 3.49	24.77 ± 4.77	23.02 ± 4.71	24.03 ± 4.48	23.06 ± 4.11	NS
LH/FSH ratio	1.01 ± 0.42	1.11 ± 0.56	1.70 ± 1.08	1.84 ± 1.49	1.44 ± 0.72	1.71 ± 0.95	< 0.001*, **
AMH [ng/mL]	3.65 ± 2.42	2.99 ± 2.00	9.17 ± 4.56	8.15 ± 4.85	7.30 ± 4.13	6.18 ± 5.46	< 0.001*, **,a
SHBG [nmol/L]	67.76 ± 24.55	69.52 ± 23.29	42.18 ± 26.02	46.44 ± 24.75	44.34 ± 38.81	55.73 ± 30.81	< 0.001a, b, c
Androstenodione [ng/mL]	1.68 ± 1.05	1.60 ± 0.93	6.09 ± 5.14	4.56 ± 4.04	4.76 ± 3.73	1.95 ± 0.60	< 0.001b, c, d
FAI	1.48 ± 0.70	1.43 ± 0.66	7.28 ± 5.37	6.14 ± 4.92	6.81 ± 5.65	3.48 ± 3.50	< 0.001*, **, e
Total testosterone [ng/dL]	0.90 ± 0.32	0.90 ± 0.31	2.15 ± 0.75	1.97 ± 0.60	1.87 ± 0.66	1.29 ± 0.40	< 0.001 *, **, e
HOMA-IR	1.60 ± 1.67	1.20 ± 1.29	2.91 ± 2.31	2.63 ± 2.14	2.70 ± 2.35	2.75 ± 2.20	< 0.001*, **

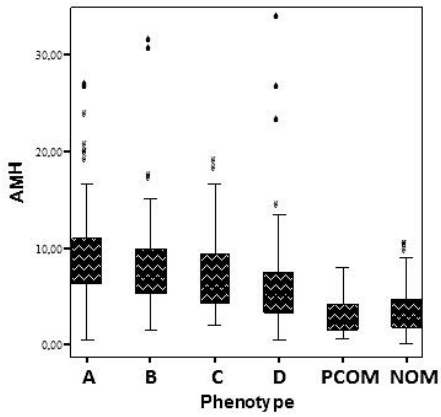
*Significant difference in NOM and PCOS A, PCOS B, PCOS C, PCOS D; **Significant difference in PCOM and PCOS A, PCOS B, PCOS C, PCOS D; [a]Significant difference in PCOS A vs. PCOS D; [b]Significant difference in NOM vs. PCOS A, PCOS B, PCOS C; [c]Significant difference in PCOM vs. PCOS A, PCOS B, PCOS C; [d]Significant difference in PCOS D vs. PCOS A, PCOS B, PCOS C; [e]Significant difference in PCOS D vs. PCOS A, PCOS B, PCOS C, NOM, PCOM; BMI — body mass index; LH — luteinizing hormone; FSH — follicle stimulating hormone; Total T — total testosterone; FAI — free androgen index; AMH — antimüllerian hormone; HOMA-IR — homeostatic model for assessment of insulin resistance; NS — nonsignificant; *p < 0.05 is statistically significant. One-way ANOVA test

between PCOM, NOM and all PCOS phenotypes in terms of the LH/FSH ratio ($p < 0.001$). The mean highest AMH levels were in phenotype A (9.17 ± 4.56 ng/mL) which was followed by phenotype B (8.15 ± 4.85 ng/mL), phenotype C (7.30 ± 4.13 ng/mL), and phenotype D (6.18 ± 5.46 ng/mL). Comparison of AMH levels showed a statistically significant difference between PCOS phenotypes A and D. Table 2 demonstrates the AMH levels of each group. There was a statistically significant difference on comparison of AMH between NOM, PCOM and all PCOS phenotypes (Fig. 2). A graphic of free androgen index is shown in Figure 3. In the comparison of HOMA-IR, there was no statistically significant difference between PCOS phenotypes. HOMA-IR showed a statistically significant difference between PCOM and all phenotypes of PCOS patients. NOM participants showed markedly lower HOMA-IR levels compared to all PCOS phenotypes (Fig. 4).

Table 2. AMH levels of groups

Phenotype	OA	HA	PCO	Frequency [%]	AMH [ng/mL]
A	+	+	+	% 23.4 (n = 117)	9.17 ± 4.56
B	+	+	–	%17.8 (n = 89)	8.15 ± 4.85
C	–	+	+	%14.4 (n = 72)	7.30 ± 4.13
D	+	–	+	%14.4 (n = 72)	6.18 ± 5.46
PCOM	–	–	+	%14.2 (n = 71)	2.99 ± 2.00
NOM	–	–	–	%15.8 (n = 79)	3.65 ± 2.42

OA — oligoanovulation; HA — hyperandrogenism; PCO — polycystic ovary appearance; AMH — anti müllerian hormone; PCOM; polycystic ovarian morphology; NOM — normal ovarian morphology

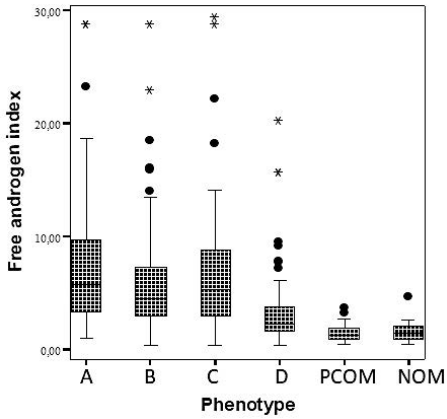


NOM vs PCOM $p = 0.65$
NOM vs PCOS phenotype D $\mathbf{p = 0.007}$
NOM vs PCOS phenotype A,B,C $\mathbf{p < 0.001}$
PCOM vs PCOS all phenotypes $\mathbf{p < 0.001}$
PCOS A vs B $p = 0.997$
PCOS A vs C $p = 0.968$
PCOS A vs D $\mathbf{p = 0.002}$
PCOS B vs C $p = 0.663$
PCOS B vs D $p = 1.0$
PCOS C vs D $p = 0.442$

Figure 2. Mean values of AMH for PCOS phenotypes, PCOM and NOM; NOM — normal ovarian morphology; PCOM — polycystic ovarian morphology on ultrasound; PCOS — polycystic ovary syndrome

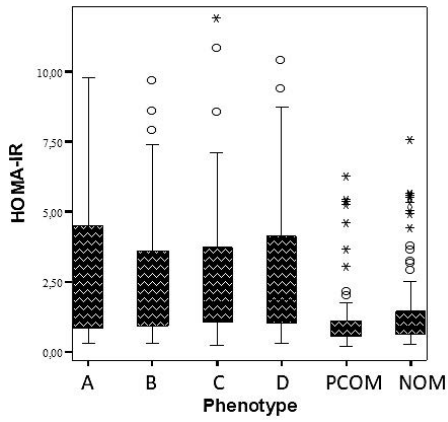
DISCUSSION

We investigated the clinical, hormonal and AMH levels and this study maintains knowledge on the diversity in these factors among the four phenotypes of PCOS according to the Rotterdam criteria [5], PCOM and healthy women with normal menstrual cycle. Mostly, AMH is produced from the small antral follicles and it's levels in circulation resemble to total antral follicle count , granulosa cell action and ovarian volume [20]. Our research supported previous studies in terms of high serum AMH levels in PCOS women compared to healthy ones and women with polycystic ovarian morphology only [14, 16, 19–21]. Ovulatory disfunctions in PCOS are generated by two mechanisms [22]. Firstly, there is an increased early follicular growth which results in an increased number of follicles. Secondly, there is an abnormal pick of dominant follicle from this increased follicular pool, leading to follicular cessation [22]. It is also known that the excess AMH origination by polycystic ovaries is an outcome of the increased follicle number [22]. Bhide et al. [23] suggested that for the assesment of antral follicle pool, AMH is a good tool because serum AMH concentrations have a powerful link with the antral follicle count (AFC). Although AMH is a good indicator in testing ovarian reserve, there is not enough data to determine a cut off value in PCOS diagnosis and dif-



NOM vs all PCOS phenotypes $\mathbf{p < 0.001}$
PCOM vs all PCOS phenotypes $\mathbf{p < 0.001}$
PCOS D vs A, $\mathbf{p < 0.001}$
PCOS D vs B $\mathbf{p < 0.001}$
PCOS D vs C $\mathbf{p < 0.001}$

Figure 3. Mean values of free androgen index for PCOS phenotypes, PCOM and NOM; NOM — normal ovarian morphology; PCOM — polycystic ovarian morphology on ultrasound; PCOS — polycystic ovary syndrome



NOM vs all PCOS phenotypes ***p < 0.001***
NOM vs PCOM p = 0.793
PCOM vs PCOS all phenotypes ***p < 0.001***

Figure 4. Mean values of HOMA-IR for PCOS phenotypes, PCOM and NOM; NOM — normal ovarian morphology; PCOM — polycystic ovarian morphology on ultrasound

ferentiating between the phenotypes of PCOS [17, 18, 24]. The relation between AFC and AMH predicts that AMH values may be higher in PCOM than NOM, but in our study, serum AMH values were similar for PCOM and NOM group. This situation shows that the increased number of 2–9 mm diameter follicles, is not the only determinant of serum AMH [2]. LH is also involved in higher AMH production [25]. When granulosa cells are exposed to LH, there is an increased secretion of AMH in anovulatory polycystic ovaries, which is not seen in normal ovaries [25]. In our study group, AMH levels were statistically lower in PCOM patients compared to all phenotypes of PCOS. In our study, the most common PCOS phenotype was A, similar to previous studies in the literature [26–28]. In the current study, hyperandrogenic PCOS phenotypes (A, B, C) presented higher AMH levels as compared with normoandrogenic phenotype D. The highest AMH levels were observed in phenotype A which shows all three components of the syndrome. AMH levels of PCOS phenotype A were statistically significantly higher compared to PCOS phenotype D. Piouka et al. showed that anti-müllerian hormone levels indicate the severity of PCOS, which was negatively correlated with BMI, and they found that phenotype A has the highest levels of AMH [29]. In addition, Piouka et al. [29] determined that AMH concentrations were 65% lessened in women with high BMI compared to women of normal BMI and this outcome was supported by the study of Freeman et al. [30]. Our study group was formed from patients with similar mean BMIs and therefore we did not observe any correlation between BMI and AMH.

In our study, the highest FAI was detected in Phenotype A, while the lowest value was observed in phenotype D among PCOS patients. There was a statistically significant difference in FAI between phenotype D vs other PCOS phenotypes. In addition, in the phenotype D group, although the mean of FAI was in the normal range, it was significantly higher than the NOM and PCOM groups. Romualdi et al. found a markedly difference in terms of FAI, between phenotype A vs phenotype D and phenotype A vs healthy controls, similar to our study findings [31]. However, in contrast with our results, they did not find a statistically significant difference between phenotype D vs phenotype B and C [31]. Polak's study showed that the mean of FAI in hyperandrogenic phenotypes of PCOS was markedly higher than control group while phenotype D and control groups were similar [32]. In the comparison of PCOS subgroups in terms of FAI, there was a significant increase only in phenotype A compared to the phenotype D [32]. Whereas in our research, FAI was significantly higher in hyperandrogenic PCOS phenotypes (A, B, C) than in phenotype D.

Although, insulin resistance (IR) has been very popular in the evaluation of PCOS, it is not included in the diagnostic criteria just like AMH. High insulin resistance in women with PCOS is considered as an alternate factor to assess the severity of the disease. Therefore, in most severe phenotype, insulin resistance is expected to be higher . We found that HOMA-IR values were statistically higher in all PCOS phenotypes compared to PCOM and NOM. Between the phenotypes, phenotype A had the highest HOMA-IR level, but this finding was not statistically significant. We can conduct that the HOMA-IR values have same distribution among the four phenotypes. Gupta et al. observed the relationship between insulin resistance, AMH and BMI among the four phenotypes of PCOS and their study results showed no differences in terms of IR among the different phenotypes of PCOS [33]. About the relation between IR and AMH, there is no concensus with some declaring a positive correlation [28, 34], while others stating there is no association [27, 29], or a negative correlation [35]. Piouka et al. found that IR indexes and AMH does not have any direct link [29]. Also, our study outcomes did not show any correlation between AMH and HOMA-IR. This result may be due to our sample's similar BMI values.

The most important strength of this study is that it is one of the largest studies in the literature that evaluates serum AMH among PCOS phenotypes. The homogenous study population and well defined PCOS, PCOM patients and healthy control groups are the advantages of this work. Although the similar characteristics of PCOS and healthy groups in terms of body mass index makes the study more homogeneous, the lack of comparison in obese PCOS patients can be considered as the limitation of the study. The other limitation of the study is that it was performed retrospectively. Additionally, the ultrasound examinations of the

patients were done by different clinicians and this is another limitation to the study.

CONCLUSIONS

In conclusion, according to our results, the prevalence of the four phenotypes of PCOS varies and Phenotype A is the most severe and common form. The literature shows controversial results on the relationship between hormonal and metabolic aspects in various phenotypes. AMH reflects the severity of the disease with those exhibiting all the symptoms of PCOS. Phenotype A had the highest AMH levels among the PCOS phenotypes. We could not find any correlation between AMH levels and HOMA-IR and BMI in our study.

Acknowledgements

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Conflict of interest

The authors report no potential conflict of interest.

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Does pregnancy influence eye parameters? Assessment of choroidal thickness using EDI-OCT before and after labour depending on the way of delivery method

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ABSTRACT

Objectives: The aim of this study is to assess the choroidal thickness (CT) with use of EDI-OCT in patients before and after delivery depending on the mode of delivery.

Material and methods: The study involved 146 eyes of 73 patients aged 20–34 years, after natural labour (66 eyes) and C-section (80 eyes). Main inclusion criteria: Informed consent to participate in the study, age 18–35 years, single pregnancy, spherical refraction error –4.00 to +4.00 D, no eye pathologies, no surgery and ophthalmic procedures-including refractive surgery, childbirth after 36 weeks of pregnancy, BCVA = 1.0. Patients were examined twice: in 36 WG and on 6th week after the birth. All examinations were carried out between 8:00 am and 10:00 am in order to avoid daily cycle fluctuations. CT measurements were made manually by two independent researchers at: subfoveal and 500 µm, 1000 µm, 1500 µm, 3000 µm temporally and nasally. The student's t-test was made.

Results: In C-section group CT differences before and after delivery were statistically significant in 7/9 of the analysed areas. Mean subfoveal choroidal thickness was 370.86 µm vs 388.71 µm in 36 WG and in 6th week postpartum respectively ($p = 0.0003$). In women after natural labour, differences were statistically significant in 3/9 of the analysed areas. Mean subfoveal choroidal thickness was 303.27 µm vs 308,34 µm in 36 WG and in 6th week postpartum respectively ($p = 0.4800$).

Conclusions: The thickness of the choroid was lower in women in 36 WG in comparison to 6th week after birth. Changes in the thickness of the choroid are particularly noticeable in women after caesarean section.

Key words: pregnancy; caesarean section; natural childbirth; choroid; optical coherence tomography

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INTRODUCTION

Pregnancy, being a special physiological condition of a woman's organism, is characterized by a variety of changes in many systems and organs. In recent years there has been a growing trend in research to investigate the influence of pregnancy and labour on the visual system [1].

The choroid is one of the most vascularized tissues in our body. Despite its small size, it is the structure with the highest blood flow in relation to volume. Until recently, there were no diagnostic methods available to create clear image of the choroid, therefore its physiology has not yet been fully understood. Examination of the choroid became available after Spaide's et al. [2] introduction of EDI-OCT modification, which, thanks to its high resolution and greater depth of scanning, allowed to visualize the structure of the choroid. This discovery led to an increased interest in the choroid physiology and intensified clinical trials on the influence of numerous factors and pathological conditions on the morphology of *choriocapillaris*. Among the factors that correspond to the thickness of choroid (CT, *choroidal thickness*) the most important are: vascular diseases such as diabetic retinopathy, hypertension, AMD and also AL, CCT, IOP, age and refractive error [3–5] It has been shown that CT changes during the 24-hour cycle [6, 7]. Ulaş has also shown that CT fluctuates between phases of the menstrual cycle [8], which may indicate a hormonal background for described changes. This interpretation is supported by studies of Wickham et al. [9], who showed the presence of female

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sex hormone receptors mRNA in choroid cells. For a long time, it was believed that unlike retina and anterior vascular membrane, choroid has no autoregulatory mechanism and its physiology is controlled mainly by autonomic nervous system mediators and hormonal stimulation. Recent reports indicate that small vessels of the choroid can have some degree of its own blood flow regulation, but the mechanism of this phenomenon has not yet been fully explained [10].

Results of recent studies on the influence of pregnancy and childbirth on the anterior segment of the eye showed changes, such as the increase in central corneal thickness, temporary change in spherical refraction, increase of depth of anterior chamber [11, 12], and resulting indirect drop in intraocular pressure [13–16]. Mentioned changes are interpreted as hormonally related, which is supported by in vitro studies. The presence of estrogen and progesterone receptors mRNA in the stroma of cornea has been shown. Other changes, such as the change in endothelial cell density [17], the appearance of transient astigmatism [11], or changes in corneal biomechanical parameters [14, 15, 18, 19] are controversial and require further research. In recent years, several studies have been published, assessing the impact of pregnancy and childbirth on the thickness of the choroid. However, the results obtained by the researchers are divergent and the physiological background of these changes is still unclear.

This study aims to answer the following research questions: Is the thickness of the choroid different in patients in the third trimester of pregnancy compared to post-delivery period? Does the birth method affect the thickness of the choroid?

MATERIAL AND METHODS

This prospective research was carried out in accordance with the Helsinki Declaration. Patients who agreed to participate in the study gave their written, informed consent after explanation of the nature and possible consequences of the study. The research was conducted in the period from October 2016 to September 2019.The study involved 146 eyes of 73 patients aged 20–34 years. The study involved both pregnant women giving birth vaginally (66 eyes of 33 patients) and women who gave birth by caesarean section (80 eyes of 40 patients). The preliminary qualification procedure was carried out, as part of which the following tests were performed:

— interviews: ophthalmological and obstetrical,
— BCVA to far and near vision using standardized Snellen tables,
— assessment of the anterior and posterior segment of the eye in biomicroscopy,
— auto kerato-refractometer.

Patients who met the criteria for inclusion were recruited to the research group — Table 1.

Patients qualified to participate in the project were examined twice: in 36 WG and 6 weeks after the delivery. The tests were carried out on the SPECTRALIS®OCT (Heidelberg Engineering) device. Optical coherent tomography of the choroid was performed. The measurement of the choroidal thickness was performed according to the protocol presented below. Anatomical limitations of the choroid were determined as:

— upper limit: the boundary between the end of the retinal pigment epithelium and Bruch's membrane (the lower edge of the hyperreflex line),
— lower limit: border between sclera and choroid

Measurements were made manually each time by two independent researchers. Protocol included manual measurements at 9 spots: subfoveal, 500, 1000, 1500, 3000 µm temporally and nasally from the fovea, respectively. The measurement diagram is illustrated in Figure 1.

The researchers were not informed about whether the result presents a measurement from the third trimester of pregnancy or from the period after childbirth. All examina-

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Informed consent to participate in the study	No informed consent to participate in the study
Age 18–35 years old	18 years > Age > 35 years old
Physiological delivery or cesarean section	Complicated pregnancy
Single pregnancy	Multiple pregnancy
Refraction error –4.00 to +4.00 D sph.	Operative vaginal delivery- OVD: vacuum and forceps delivery
Good cooperation during research	Pregestational diabetes melitus And gestational diabetes melitus
No eye diseases	Hypertension in pregnancy
No surgery and/or ophthalmic procedures in history	Preeclapsja
Childbirth after 36 weeks of gestation	*Intrauterine growth restriction — IUGR*
Distance BCVA: LogMAR = 0.0, (V= 20/20)	Defective spherical refraction outside of the scope of inclusion criteria
Near BCVA: Sn = 0.5/30 cm	Lack of cooperation from the patient
	Active disease of the anterior or posterior segment of the eye
	Condition after ophthalmic operations or surgeries, refractive procedure performed
	Premature delivery < 36 weeks gestation
	Distance BCVA: LogMAR > 0.0, (V < 20/20)
	Near BCVA: > 0.5 /30 cm

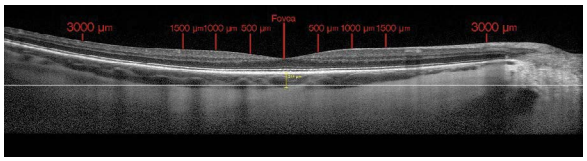

Figure 1. Measurements method

tions were carried out in the morning between 8:00 am and 10:00 am in order to avoid fluctuations related to the daily cycle.

For the analysis of the results, the values of parameters being the arithmetic mean of the measurements obtained by both researchers were used. The results were analysed statistically using StatSoft Statistica 13.1. All the results were presented as an average ± standard deviation. Before starting the analysis, the conditions for parametric tests were checked. The normality of decomposition was checked by the Shapiro-Wilk test and the homogeneity of variance by the Leaven test. Because the above assumptions were met, the analysis of significance of differences was carried out with the student's t-test for dependent samples. The $p < 0.05$ was assumed to be statistically significant.

RESULTS

Table 2 presents detailed results of CT analysis in nine areas: subfoveal, 500, 1000, 1500, 3000 µm temporal and nasal from the fovea. The results are presented as mean and standard deviation (µm). The table presents a comparison of CT between groups of women after natural labour and C-section with the level of significance p of the Student's t-test.

Regardless of the delivery method and the analysed area of the choroid, the mean and standard deviation of choroid thickness were lower in women in 36 WG in comparison to 6th week after birth. However, these differences in CT before and after delivery were not statistically significant in all areas. In addition, a significant difference was observed depending on the delivery method of pregnancy. On the basis of t-Student's analysis it was shown that in Caesarean section group CT changes before and after delivery are statistically significant ($p < 0.05$) in 7/9 of the analysed areas (subfoveal, Nasal: 500, 1000, 1500, 3000 µm, Temporal: 500, 1000 µm). In 2/9 areas (Temporal: 1500, 3000 µm), despite the observed trend, we did not observe significant differences ($p > 0.05$). Patients giving natural birth showed a similar trend as those giving birth with c-section, but the differences were not statistically significant in all regions of the choroid. In the group of women after natural labour, differences were statistically significant in 3/9 of the analysed areas (Nasal: 500, 1000, 1500 µm) and in 6/9 (Subfoveal, Nasal 3000 µm, Temporal: 500, 1000, 1500, 3000 µm) $p > 0.05$ was obtained. When measured at 3000 µm nasally, the choroid was slightly thicker before childbirth than after delivery, but the mean difference was only 1.02 µm and the difference was statistically insignificant ($p = 0.77$), so the difference is

Table 2. Results

Location (µm from fovea)	CS — group before delivery		CS — group after delivery		Mean difference (µm)	SD (µm)	p–value (t–student)	NL — group before delivery		NL — group after delivery		Mean difference (µm)	SD (µm)	p–value (t–student)
	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)				Mean (µm)	SD (µm)	Mean (µm)	SD (µm)			
SFCT	370.86	100.92	388.71	96.01	–17.86	40.95	0.0003	303.27	88.09	308.34	112.62	–5.07	47.51	0.4800
Nasal 500 µm	348.36	96.85	367.48	97.93	–19.12	32.73	0.0000	276.66	80.34	295.52	105.42	–18.86	47.62	0.0119
Nasal 1000 µm	325.25	92.73	340.6	95.27	–15.35	31.36	0.0001	256.52	78.69	275.2	100.67	–18.68	53.06	0.0243
Nasal 1500 µm	281.21	85.36	300.14	93.08	–18.94	92.07	0.0000	224.86	74.3	241.5	91.18	–16.64	35.14	0.0031
Nasal 3000 µm	156.75	56.92	171.68	79.11	–14.92	56.34	0.0228	130.61	53.09	129.59	49.14	1.02	22.96	0.7690
Temporal 500 µm	362.78	100.44	378.9	100.02	–16.12	53.87	0.0105	291.23	83.29	302.93	108.38	–11.7	47.08	0.1064
Temporal 1000 µm	352.58	96.54	363.97	99.448	–11.39	42.5	0.0213	293.32	79.95	299.07	105.83	–5.75	41.29	0.3607
Temporal 1500 µm	340.09	96.71	344.87	101.55	–4.78	39.92	0.3003	297.68	81.67	300.59	96.08	–2.91	41.26	0.6424
Temporal 3000 µm	294.04	84.61	298.73	92.32	–4.7	53.83	0.4492	268.73	51.96	277.75	75.97	–9.02	43.48	0.1758

CS: group (cesarean section group); NL: group (natural labour group)

within the limits of measurement error (SD for this measurement = 22.96 μm).

The results allow us to conclude that the decrease in the thickness of the choroid after delivery compared to the third trimester of pregnancy is significantly more noticeable in women after C-section than in women after vaginal delivery.

DISCUSSION

The lack of an unambiguous mechanism determining the character of changes in the thickness of choroid during pregnancy and after labour makes the subject controversial and worth taking an effort to conduct our own research project to clarify conflicting data [20].

Our study is the largest prospective study available in the literature (143 eyes), comparing the thickness of the choroid before and after childbirth. Our results are in opposition to the results of some researchers, but the methods of group selection and research schemes published in the literature are not identical. Choroid in pregnant patients was examined by many authors [1, 4, 11, 20, 27–33]. However, most of them [1, 11, 27–32] were clinical-control cohort examinations in which the thickness of choroid of pregnant women was compared to other, non-pregnant women. Such a study method does not allow obtaining objective results due to individual variation in the thickness of the choroid.

Dadaci et al. [28] examined 54 eyes of 27 patients in the first and third trimester of pregnancy. They showed that the choroid in the first trimester of pregnancy is thicker than in the third trimester. The authors did not perform postpartum tests, but instead used a control group of 50 eyes of 25 non-pregnant women. In this way, they found that the thickness of the choroid in the third trimester was higher than in non-pregnant patients (control group). Results [28] show the trend of CT changes during pregnancy, highlighting the differences between 1st and 3rd trimester, which is a great value of the work of these authors, but in our opinion those results do not allow to determine the direction of CT changes until the end of postpartum confinement, which was done in our work. In order to precisely trace changes in the thickness of the choroid during pregnancy, it is necessary to examine the same group of patients before and after childbirth as we have done in our study. Only such an examination scheme will allow to obtain objective results.

Goktas et al. [30] conducted a study in which they compared the thickness of the choroid in four groups of 30 eyes each, respectively in:1st, 2nd and 3rd trimester of pregnancy and control group. They found that the thickness of the choroid was highest in the 2nd trimester with no increase of this parameter in the 1st and 3rd trimester of pregnancy compared to the control group. CT is a parameter of high individual volatility and in our opinion more reliable results would bring prospective studies.

Kara et al. [27] conducted a large study of 100 pregnant women, which they compared with the control group — non pregnant women. CT was measured only in subfoveal region, and the study group consisted of pregnant women with no distinction between trimesters. Considering the results of other researchers such as Goktas et al. [30] there is a clear difference in CT measured at different stages of pregnancy. The paper provides some information about mean CT values in particular trimesters of pregnancy, but it does not allow to determine the direction and dynamics of changes of this parameter.

Takahashi et al. [4, 20] and Ulusoy et al. [33] were the only ones who have done prospective study and examine the same group of women during pregnancy and after childbirth, so their results deserve special attention in this discussion.

Takahashi et al. [20] recruited patients in the 1st trimester of pregnancy and conducted long-term observation based on four measurements: in the 1st, 3rd trimester, shortly after childbirth and one month after childbirth. By examining 62 eyes they showed that the CT was highest in the 1st trimester, decreased in the 3rd trimester and remained at a similar level to the first month after birth. This is a remarkably interesting study due to the fact that the authors have traced the changes in CT from the initial period of pregnancy to the first month after birth. It should be noted, that in comparison to our work, in which we examined 146 eyes in total, Takahashi et al. [20] have examined a relatively small group. In contrast to our study, they did not show any significant differences between CT in the 3rd trimester and the first month after childbirth ($p > 0.05$). While our team showed that the choroid is thinner in the third trimester of pregnancy compared to the period after delivery ($p < 0.05$). Takahashi et al. [20] performed the last CT measurement 4 weeks after birth, and our team-6 weeks. This may be of clinical importance as the regression of postpartum physiological changes usually occurs 6–7 weeks after birth. Takahashi et al. [20] provide valuable information on the direction of CT changes from the 1st trimester of pregnancy to the 4th week after birth, but do not determine the nature of the changes at a later stage. Our research shows that the CT may increase in relation to the values in the 3rd trimester after the end of the postpartum period, as a result of a natural regression of postpartum changes in the woman's body, leading to the return of the pre-pregnancy choroid morphology.

The study of Ulusoy et al. [33] is the second of three prospective studies so far available in the literature, in which the same patients were examined before and after labour. The same study scheme was used in our work and by Takahashi et al. [20] Like us, he also examined women in 36 WG, but carried out a follow-up 3 months after the birth. SFCT was higher in the third trimester of pregnancy than three months

after the birth ($p < 0.05$), which does not agree with the results of our studies, which show that SFCT is lower before labour in comparison to period after delivery. It should be noted, however, that statistically significant differences were obtained in women giving birth by C-section ($p < 0.05$). In case of vaginal delivery, despite the preserved tendency, the differences were not statistically significant ($p > 0.05$). Ulusoy et al. [33] did not specify in their methodology the type of birth, which, according to our research, is important for measuring CT. In the remaining regions results [33] were the same as ours — the choroid was thinner in 36 WG than after childbirth, but differences were not statistically significant ($p > 0.05$). If we do not consider the method of delivery, our research shows that the choroid is thinner in 36 WG than on 6th week after birth. However, after two separate analyses, depending on the method of delivery, these differences are most clearly seen and statistically significant, particularly in the group of women after C-sections. Perhaps the timing of the follow-up by Ulusoy et al. [33], which was carried out twice as late (12 weeks) as in the case of our follow-up (6 weeks), affects the CT value after birth.

We are the only team in the world to measure women's choroid before and after childbirth, differentiating the results according to the method of delivery. We decided that the prospective study of the same patients have greater scientific value, due to the fluctuation of individual thickness of the choroid. It is known that as soon as the 1st trimester of pregnancy, the peripheral resistance of vessels decreases and the blood flow in the choroid increases during the 1st––2nd trimester of pregnancy [34]. As pregnancy progresses, the volume of circulating blood increases steadily, increasing by 40% in the 3rd trimester in comparison to pre-pregnancy levels. As can be seen from the work of Thornburg et al. [35], blood especially at the end of pregnancy is redistributed to important organs, such as the uterus, breast glands and kidneys. Moreover, as it results from the work of Dahle et al. [36], in the third trimester of pregnancy, mainly between 37 and 39WG, the number of adrenergic alpha-1 receptors in a woman's body increases, which is supposed to prepare for the initiation of childbirth. As reported by Dadaci et al. [28] it is the increase in the activity of these receptors that may affect the redistribution of blood from the vascular system to other organs and the constriction of choriocapillaris, which may explain reduction of thickness of the choroid demonstrated in our study with use of OCT in the 3rd trimester of pregnancy. Current knowledge does not allow us to explain why in the case of a caesarean section, there is a more pronounced increase in the thickness of the choroid after birth than in the case of natural childbirth. This is the most interesting conclusion from our work -that C-section may influence the visual organ. It is possible that future research will clarify the exact mechanism of this phenomenon.

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The crown-rump length measurement — ISUOG criteria and clinical practice

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ABSTRACT

Objectives: Significance of the crown-rump length (CRL) measurement criteria in the assessments of gestational age and actual precision in daily clinical practice.

Material and methods: We recruited 806 pregnant women with singleton pregnancy and history of regular menstrual periods.We analysed retrospectively CRL measurements obtained during routine first trimester scan performed between 11 + 0 and 13 + 6 weeks gestation. Gestational age was calculated using both the last menstrual period (LMP) and the CRL. The images of the CRL measurements were assessed by the expert. The visual analysis of the images in terms of meeting the five criteria recommended by the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) was performed. Statistical analysis were used to assess how the above-mentioned criteria influenced calculation of the gestational age.

Results: The study showed 323 out of 806 of the CRL measurements (40.1%) were qualified by a specialist as accurate, 279 (34.6%) as inaccurate, and 204 (25.3%) as inaccurate, but not changing the duration of a pregnancy. With the application in the assessment of the five criteria of the ISOUG 217 (26.9%), the following results of qualification were obtained: accurate — fulfilled ≥ 4, inaccurate 341 (42.3%) — fulfilled ≤ 2, whereas inaccurate, but not changing the duration of a pregnancy 248 (30.8%) — 3 criteria fulfilled. We found that only the neutralof the fetus demonstrated a significant corellation with the assessment of the duration of a gestation.

Conclusions: a) the accurate audit of the CRL measurements is recommended; b) neutral position of the fetus is the most important criterion out of 5.

Key words: CRL, gestation, early pregnancy

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INTRODUCTION

Determining the gestation age accurately is one of the key elements influencing the provision of accurate perinatological, and obstetric care for a patient. In Poland, the assessment of gestational age (GA) is conducted with the application of two methods.

The first method is based on the date of the last menstrual period (LMP), which is easy to perform and inexpensive. However, the following conditions had to be fulfilled for this method to give accurate estimations of the gestational age: regular menstrual cycle, fertilisation in the middle of the cycle, accurate information of the first day of the LMP must be provided by the women. Determining gestational age precisely on the basis of the LMP would be possible if the exact dates of: ovulation, fertilisation, and implantation of an embryo were known, for instance in pregnancies arising using ART (Assisted Reproductive Technology) [1]. Even in cases of accurate recollection of the date of LMP, delayed ovulation can cause discrepancies between gestational age calculated using LMP and with the use of different methods.

The second method uses an assessment of crown-rump length (CRL). CRL measurement can be obtained during routine ultrasound scan in first trimester and is described as the length of the fetus from top of the head to the bottom of the rump (buttocks). The CRL measurement is seen as the most sensitive predictor of gestational age in the first trimester of pregnancy [2]. The International Society of Ultrasound in Obstetrics and Gynecology (ISOUG) recommends determining the duration of pregnancy based on CRL between 8 and 14 weeks as the most reliable method [3].

To ensure the objective assessment of crown-rump length, the ISOUG proposed five criteria to increase the

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Table 1. 10 centile comparison

Week 11 — CRL 50 mm	Week 12 — CRL 63 mm	Week 13 — CRL 76 mm	Week 13 + 6 — CRL 88 mm
–5 mm = week 10 + 4 mm	–5 mm = week 11 + 4	–5 mm = week 12 + 4	–5 mm + week 13 + 3
–10 mm = week 10 + 1	–10 mm = week 11 + 1	–10 mm = week 12 + 1	–10 mm = week 13 + 1
+5 mm = week 11 + 3	+5 mm = week 12 + 2	+5 mm = week 13 + 2	+5 mm = week 14 + 1
+10 mm = week 11 + 5	+10 mm = week 12 + 5	+10 mm = week 13 + 5	

CRL — crown-rump length

Table 2. 50 centile comparison

Week 11 — CRL 43 mm	Week 12 — CRL 55 mm	Week 13 — CRL 67 mm	Week 13 + 6 — CRL 78 mm
–5 mm = week 10 + 4 mm	–5 mm = week 11 + 4	–5 mm = week 12 + 4	–5 mm + week 13 + 3
–10 mm = week 10 + 1	–10 mm = week 11 + 1	10 mm = week 12 + 2	10 mm = week 13 + 1
+5 mm = week 11 + 3	+5 mm = week 12 + 3	+5 mm = week 13 + 2	+ 5 mm = week 14 + 1
+10 mm = week 11 + 6	+10 mm = week 12 + 6	+10 mm = week 13 + 5	+ 10 mm = week 14 + 3

CRL — crown-rump length

precision of the conducted measurements. These include accurate magnification of the image, neutrality of the fetus, horizontal position of the fetu,, accurate placement of the callipers, and the presence of pocket of amniotic fluid under the foetus chin.

Even small inaccuracies, as small as 5 mm to 10 mm, in the CRL measurement can cause significant differences in calculation of the gestational age. Examples of how inaccurate measurement of CRL can affect calculation of the gestational age can be seen in the tables (Tab. 1–3).

Objectives

The aim of the study was to evaluate the significance of the CRL measurement criteria in the assessments of gestational age and its actual precision in daily clinical practice.

MATERIAL AND METHODS

There were 806 women with singleton pregnancies and known date of LMP included into the study. All women had history of regular menstrual periods (28–35 days) and underwent a routine first trimester scan in our Prenatal Assessment Clinic between January 2017 and December 2019. Patients with irregular menstruation cycle and lactating patients were excluded from the study. CRL measurements obtained during routine ultrasound scans were assessed retrospectively by experts in fetal medicine ultrasound.. The physicians who took the measurements were obstetrics&gynecology specialists with experience in ultrasonography. Moreover, all of them were certified by PTGiP (minimum of 5 years) and FMF. The measurements were taken on GE Voluson E6 ultrasonograph in Prenatal Outpatient Clinic belonging to the Department of Obstetrics and Gynecology of Pomeranian Medical University in Szczecin. In all of the cases the duration of the pregnancy was established using two methods: based on the LMP and based on the CRL.

First, CRL pictures were assessed by an expert in fetal medicine. ISUOG criteria were applied when assessing CRL measurements. Said expert is a physician, specializing in obstetrics&gynecology and fetal medicine (with over 10 years of experience) certified by FMF (for over 12 years) and audited by FMF for over 6 years. The measurements were divided into three categories: correct, incorrect and incorrect, but with no impact on the pregnancy's duration. The difference in the duration of a pregnancy determined on the basis of the LMP, and between the duration of a pregnancy determined on the basis of the CRL, in particular groups was compared.

It was proposed that the population be divided into three separate categories depending on the number of fulfilled criteria. The measurements fulfilling four to five criteria were qualified as accurate. The measurements fulfilling three criteria were found to be inaccurate, but not changing the duration of a pregnancy, whereas the measurements fulfilling two or fewer criteria were found to be inaccurate. Afterwards, the difference in the duration of a pregnancy determined on the basis of the LMP, and between the duration of a pregnancy determined on the basis of the CRL, in particular groups was compared.

The next stage involved the assessment of the significance of the particular criteria of the ISOUG for the assessment of the duration of a pregnancy determined with the application of the CRL. For each criterion, the difference between the gestational age calculated from LMP, and use of the CRL, was assessed.

Table 3. 90 centile comparison

Week 11 — CRL 36 mm	Week 13 — CRL 47 mm	Week 13 — CRL 59 mm	Week 13+6 — CRL 70 mm
–5 mm = week 10 + 3 mm	–5 mm = week 11 + 4	–5 mm = week 12 + 4	–5 mm + week 13 + 3
–10 mm = week 10	10 mm = week 11	–10 mm = week 12 + 1	–10 mm = week 13
+5 mm = week 11 + 3	+5 mm = week 12 + 3	+5 mm = week 13 + 3	+5 mm = week 14 + 2
+10 mm = week 11 + 6	+10 mm = week 12 + 6	+10 mm = week 13 + 5	+10 mm = week 14 + 5

CRL — crown-rump length

Table 4. The assessment of the particular criteria

Criterion according to the ISOUG	Number of accurate measurements	Number of inaccurate measurements	Difference between GA on the basis of the LMP, and GA on the basis of the CRL, among accurate measurements	Difference between GA on the basis of the LMP, and GA on the basis of the CRL, among inaccurate measurements
Image enlargement	458	348	p = 0.624	p = 0.489
Neutral position	319	487	p = 0.002	p = 0.012
Horizontal position	593	213	p = 0.527	p = 0.204
Setting callipers	341	465	p = 0.127	p = 0.152
Presence of fluid under the chin	434	372	p = 0.209	p = 0.153

CRL — crown-rump length; GA — gestational age; ISUOG — the International Society of Ultrasound in Obstetrics and Gynecology; LMP — last menstrual period

Lastly, the repeated division of the images in terms of the fulfilled ISOUG criteria. As accurate measurements, those fulfilling four to five criteria were assessed; it was required that they had been positively assessed in terms of the neutral position. The measurements fulfilling three criteria were found to be inaccurate, but not changing the duration of a pregnancy, whereas these which fulfilled two or fewer criteria, and which also received a negative assessment in terms of the neutral position were found to be inaccurate. The analysis of the difference in the duration of a pregnancy determined with the application of the LMP and the CRL was repeated again.

The statistics were made using the Statistical Package for Social Sciences (SSPS TM) program. The Shapiro–Wilk test showed that the data does not distribute normally. Next, the U–Mann–Whitney test was conducted in order to compare the categories. The statistical significance level was set at $p < 0.05$.

RESULTS

Out of 806 of the CRL measurements, 323 (40.1%) were qualified as accurate, 279 (34.6%) as inaccurate, and 204 (25.3%) as inaccurate, but not changing the duration of a pregnancy. 527, i.e. 65.4% of the measurements of crown-rump length made it possible to ensure the accurate assessment of gestational age. A statistically-significant difference between the duration of a pregnancy determined with the application of the LMP and USG ($p = 0.003$) was observed in the group of inaccurate measurements, whereas in the group with accurate measurements and inaccurate, but not changing the duration of a pregnancy, no statistically significant difference was observed.

Applying in the assessment of the five criteria of the ISOUG, it was determined that 217 (26.9%) of the measurements were accurate, and 341 (42.3%) inaccurate, whereas the number of inaccurate measurements, not changing the duration of a pregnancy, was established as 248 (30.8%). Similarly, only 57.7% of the CRL measurements made it possible to ensure the proper assessment and determine the duration of a pregnancy. A statistically-significant difference between the duration of a pregnancy according to the LMP and the CRL in the group of inaccurate measurements ($p = 0.025$) was demonstrated again.

Also, 348 (43.2%) of the measurements were found to be inaccurate, and 458 (56.8%) as accurate, in terms of the criterion of image enlargement. A statistically-significant difference in the duration of a pregnancy determined on the basis of the LMP and the CRL was not demonstrated, either in the group found to be inaccurate or the one found to be accurate.

In terms of the neutrality of the fetus, 487 (60.4%) of the assessments were found to be inaccurate, and 319 (39.6%) as accurate. In the group of inaccurate measurements, and also that of the accurate ones, a statistically-significant difference between the duration of a pregnancy determined on the basis of the LMP, and sometimes the duration of a pregnancy determined on the basis of the CRL, was observed.

In 213 (26.4%) of the measurements, the horizontal position of the fetus was determined as inaccurate, whereas

it was found to be accurate in 593 (73.6%) of the measurements. A statistically-significant difference in the duration of a pregnancy determined on the basis of the LMP and the CRL in both of the groups was not demonstrated.

In the case of the criterion of accurate placement of the callipers, 465 (57.7%) of the measurements were assessed to be inaccurate, and 341 (42.3%) to be accurate. A statistically-significant difference in the duration of a pregnancy determined on the basis of the LMP and the CRL was not observed.

The presence of fluid under the chin was found to be inaccurate in 372 (46.2%) of the measurements, and it was found to be accurate in 434 (53.8%) of them. No statistically-significant difference between the duration of a pregnancy determined on the basis of the LMP and the duration of a pregnancy determined on the basis of the CRL was observed in both of the groups. The assessment of the particular criteria was presented in Table 4.

At the following stage, 189 (23.5%) of the measurements were described as accurate, i.e. fulfilling four to five criteria, and that included the positive assessment of the neutral position. The measurements of 90 (11.2%) were found to be inaccurate, but not changing the duration of a pregnancy, i.e. fulfilling three criteria, whereas 527 (63.3%) were found to be inaccurate, i.e. fulfilling two or fewer of the criteria; this group included also the measurements in which the neutral position was assessed negatively. A difference in the duration of a pregnancy determined on the basis of the LMP, and in the duration of a pregnancy determined on the basis of the CRL in the group of measurements determined to be inaccurate, and also in the group of accurate measurements and those found to be inaccurate, but not changing the duration of a pregnancy.

DISCUSSION

Presently, ultrasonography is replacing the traditional method of calculating gestational age based on the LMP. It is thought that the measurement of crown-rump length is the most accurate method of calculating gestational age in first trimester [4]. CRL can be measured very early in the first trimester and attempts were made to calculate gestational age using these measurements as early as or before 9 weeks of pregnancy [5]. However current consensus on the most accurate assessment of gestational age using CRL advises performing these measurements between the 11^{th} and 13^{th}+ 6 weeks of pregnancy.

Applying as the basis gestational age determined on the basis of the CRL parameter, results in a significant reduction in the number of premature pregnancies, postmature pregnancies [6], and also improved the effectiveness of screening of chromosomal disorders [6]. It is connected with frequently erroneous information provided by a pregnant woman on the last menstrual period, which causes a mistake in determining gestational age, and as a consequence, underestimating or overestimating it. That, in turn, leads to the misdiagnosing of premature births.

Due to the differences between gestational age determined with the application of the LMP, and with the application of USG, it is recommended to base on the EUS measurements in the case of a difference of no fewer than seven days [8].

A key element of determining gestational age accurately is the precise determination of the CRL measurement. The problem related to the subjective assessment of this parameter is raised, among others, due to imprecise criteria and the risk of different interpretation of the measurements by various specialists. According to the literature, like in our analysis, the most important criterion is the neutral position [9]. In accordance with the assessment of specialists, the assessment of the above-mentioned criterion involves a problem with determining what deviation should be interpreted as a position making it impossible to accurately assess the crown-rump length.

Another aspect is the influence of the inaccurate assessment of the CRL parameter on the diagnostics of chromosomal disorders. It is reported that a measurement error in the assessment of crown-rump length may result in reducing the detection of the trisomy of the 21^{st} chromosome, and increasing the number of distorted (underestimated) results [10]. In the literature, we can also find a correlation between a small crown-rump length and a higher risk of chromosomal anomalies [11].

Our paper proved that even in a centre specialising in prenatal diagnostics, a significant percentage of examinations include deviations from the recommended rules of measurements, which may influence determining gestational age, and, ipso facto, affect the supervision of obstetric personnel in the second half of a pregnancy (suspicion of IUGR, and necessity of inducing a delivery). A problem related to the accurate assessment of the CRL is raised in the literature, which suggests that the issue requires further studies, and a debate on the criteria proposed by the ISOUG [12]. One of the elements of the discussion should be the importance of particular criteria in the assessment of an accurate measurement or reducing their number. Our analysis brought forth the conclusion that the most important criterion is the neutral position. Yet, the issue needs commencing collaboration between various facilities, and then repeating studies on a larger group of patients. Conducting standardisation exercises for USG specialists would also increase the percentage of accurately performed examinations [13].

An assessment conducted by an expert, and on the basis of criteria of the ISOUG, is identical in terms of determining the duration of a pregnancy, which may be applied by

conducting an audit in which implementation should be sought for. One of the proposed forms of audits is introducing the assessment of the CRL measurements by specialists according to objective, such as the neutral position. It was observed that ensuring objective assessments results in a lower percentage of inaccurate measurements, and makes the measurements more repeatable [14].

CONCLUSIONS

The accurate audit of the CRL measurements are recommended in every group of researchers regardless of experience. Not all of the criteria recommended by the ISOUG influence the assessment of gestational age in the same manner; studies on a larger population are required in order to verify the significance of particular criteria. An assessment by an expert in prenatal diagnostics is identical to the detailed analysis of the criteria of the ISOUG, and may be seen as a form of audit.

Conflict of interest

None declared.

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Assessment of renal volume by 3D VOCAL Ultrasonography method in late-onset growth--restricted fetuses with normal amniotic fluid index

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ABSTRACT

Objectives: The aim of this study was to study renal volumetric alterations and renal artery doppler changes in late-onset fetal growth restricted (FGR) fetuses with normal amniotic fluid compared to healthy pregnancies.

Material and methods: This prospective study was composed of pregnant women with late-onset FGR and a control group of uncomplicated pregnancies within 32–37 weeks of gestation. Following the assessment of umbilical, bilateral uterine, middle cerebral using Doppler Ultrasonography (US), three dimensional (3D) US Virtual Organ Computer-aided Analysis (VOCAL) was executed to calculate bilateral renal volumes.

Results: A total of 76 fetuses with FGR and 51 healthy fetuses (control group) were evaluated. Umbilical artery Doppler systole/diastole and Pulsatility index values were found to be significantly different between the two groups ($p = 0.001$ and $p = 0.001$, respectively). Middle cerebral, bilateral uterine, and bilateral renal arteries' Doppler indices revealed no difference between the two groups. Right, left, and mean renal volume of the fetuses with FGR were smaller than the control group, and the differences were statistically significant ($p = 0.025$, $p = 0.004$, $p = 0.004$, respectively). Left renal volume was significantly greater than the right renal volume in the control group ($p = 0.009$).

Conclusion: Although not accompanied by oligohydramnios, and having similar renal vascular resistance as the control group, renal volumes of fetuses with late-onset FGR were still observed lower than the control group. This difference was explained by not decreased blood flow via redistribution but other mechanisms like glomeruli reduction and glomerular apoptosis.

Key words: late-onset fetal growth restriction; 3D VOCAL Ultrasonography; renal volume

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INTRODUCTION

Fetal growth restriction (FGR) affects approximately 10 percent of all pregnancies. Compared to healthy pregnancies, FGR has been associated with higher perinatal mortality and morbidity and may require more prenatal care [1].

Intrauterine hypoxemia, mostly due to placental insufficiency, causes remodeling of the fetal circulation. Because of this remodeling event, the fetal hemodynamic profile tends to distribute low oxygenated fetal blood towards to the vital organs, including the brain, myocardium, and adrenals, and a reduction in blood supplied to kidneys, fetal intestines, and lungs [2].

Interestingly, kidneys are not considered as vital organs in the intrauterine period. Contrarily, lower renal perfusion due to FGR has been related to life-time complications in adulthood, including coronary heart disease, stroke, hypertension, type 2 diabetes, and chronic kidney disease that can manifest later in adulthood [2–4].

Nephrogenesis is thought to be continued even after birth, although most of the nephrons are resolved within the intrauterine period. An adverse intrauterine environment, such as intrauterine growth restriction, causes sclerosis in multiple organ systems, including fetal kidneys [5]. Because of this sclerosis, oligonephropathy has been associated with future renal hypertension and proteinuria [2, 6, 7]. In previous studies, a decreased number of glomeruli and lower renal volume in pregnancies with FGR have been widely discussed in all aspects. However, prenatal renal changes have not thoroughly researched [2, 3, 5].

According to several doppler studies, renal arteries have been shown to have higher resistance and pulsatility indexes (PI) in fetuses with FGR. On the other hand, this difference

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can be more noticeable when FGR is accompanied by oligohydramnios [8, 9]. According to literature, hypoperfusion due to FGR tends to not affect renal artery Doppler PI values unless oligohydramnios has been detected, which can be explained by the redistribution process [9].

Late-onset FGR is defined as growth restriction that is diagnosed following 32 weeks of pregnancy. It is not associated with the reduced villous vascular area of the placenta like early-onset FGR and is not always associated with abnormal Doppler parameters and cardiovascular adaptations. Although cardiovascular changes are not always accompanying, oligohydramnios due to hypoperfusion of fetal kidney is believed to be a complication of late-onset FGR [10].

Fetal kidney volumes are expected to be smaller in fetuses with FGR, and this difference is usually explained by the lack of renal artery blood flow [6]. Oligohydramnios due to decreased renal arterial flow is the major complication of fetuses with FGR. However, we hypothesize that when oligohydramnios is not accompanying late-onset FGR, renal volumes of fetuses with FGR may not differ from healthy pregnancies.

Objectives

The aim of this study was to present the renal volumetric alterations, and renal artery Doppler changes in intrauterine growth-restricted fetuses with normal amniotic fluid indexes compared to healthy pregnancies. We hypothesize that, compared to healthy pregnancies, pregnancies with late-onset FGR have similar renal volumes and renal Doppler indexes when oligohydramnios is not present.

MATERIAL AND METHODS

Patients and data collection

This prospective study was conducted between March 2019 to December 2019 at Tepecik Training and Research Hospital, Department of Obstetrics and Gynecology, Division of Perinatology. The same institution's ethics committee approved the study. One hundred twenty-seven pregnant women between 32–37 weeks of gestation were enrolled in this study following provided written informed consent. The data on age, parity, body mass index (BMI), and smoking preference were collected, along with a detailed ultrasound scan. Women with singleton pregnancies were included, and the gestational age was calculated after confirming with the first trimester crown-rump length. All of the enrolled pregnancies were previously scanned in their second trimester and had no significant structural or chromosomal abnormalities. None of the pregnant women had a history of chronic diseases such as diabetes, hypertension, systemic lupus erythematosus, and antiphospholipid syndrome that could have led to placental vascular damage. Cases with proven FGR causes, such as preeclampsia and gestational hypertension, and pregnancies complicated by polyhydramnios were also excluded. Finally, the birth week, weight, mode of delivery, and Apgar scores were collected.

Pregnants assessed as FGR with normal amniotic fluid index after 32 weeks of gestation (late-onset FGR) were enrolled in this study. Oligohydramnios due to hypoperfusion of fetal kidney is believed to be a complication of FGR. However, FGR with oligohydramnios was not the interest of this study.

MATERIAL AND METHODS

Scanning

One sonographer with minimum of 6 years of experience made all examinations (HGP). The ultrasound machine was a Samsung Ultrasound System HS70A (Samsung Medison Company, Republic of Korea). Measurements were performed during limited fetal movement or respiration. No pressure with the ultrasound probe was applied to the fetus. All biometric measurements and amniotic fluid index calculations were performed using the 2D probe. Estimated fetal weight (EFW) was calculated by four-way biometric measurements (biparietal diameter, head circumference, abdominal circumference, and femur length) using Hadlock's formula [11]. Umbilical artery (UA), middle cerebral artery (MCA), and uterine artery Doppler assessments were made by techniques defined by the ISUOG Practice Guideline [12]. Pregnancies assessed FGR who have EFW of less than the 3rd percentile or EFW of less than the 10th percentile with an abnormal UA Doppler or an abnormal cerebroplacental ratio (CPR) according to Delphi criteria [13]. Abnormal UA is accepted as UA pulsatility index (PI) of greater than the 95th percentile or with absent or reversed UA diastolic flow. Abnormal CPR was accepted as the CPR of less than the 5th percentile Pregnancies complicated with FGR after 32 weeks of gestation was defined as late-onset FGR. Amniotic fluid index (AFI) was computed by measuring amniotic fluid in four quadrants. Amniotic pockets without cord and extremities were measured, and AFI less than 50 mm were interpreted as oligohydramnios [14]. Renal abnormalities and renal pelvis diameters more than 10 mm were excluded [15].

After presenting kidneys properly in the coronal axis, we performed 3D scanning with a 3D/4D curved array abdominal probe. The image was magnified, and kidneys were placed into the 3D volume box. In the coronal view, six consecutive images with a rotation angle of 30° were captured, two demarking arrows placed, and manual tracing was applied to all of the images. The volumetric calculation was conducted automatically by VOCAL software (Fig. 1).

Statistics

Statistical analysis was performed with IBM SPSS Statistics 25.0 package program (IBM Corp., Armonk, New York,

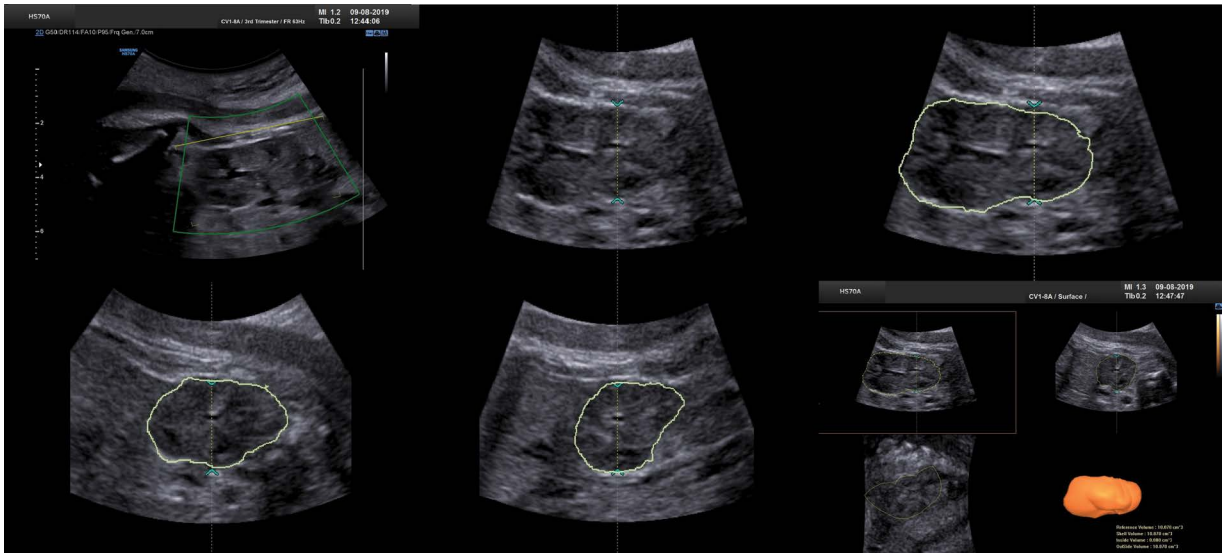

Figure 1. Volumetric calculation of fetal kidney by virtual organ computer-aided analysis (VOCAL) software

Table 1. Maternal, perinatal characteristics of the control group and FGR group (n = 127)

Characteristics	Control (n = 51)	FGR (n = 76)	p value
Maternal age	26 (10)	27.5 (11)	p > 0.05‡
Gravida	2 (2)	2 (1)	p > 0.05‡
Parity	1 (2)	1 (1)	p > 0.05‡
BMI [kg/m^2]	28 (8.4)	27.3 (6.5)	p > 0.05‡
Gestational age at exam [weeks]	34 (3)	35 (2)	p > 0.05‡
Smoking	5 (9.8%)	10 (13.2%)	p > 0.05†

FGR — Fetal growth restriction; BMI — Body mass index; Data are presented as Median and interquartile range or n (%); ‡Mann-Whitney test; † Chi-square Test

USA). Shapiro–Wilk's test, a histogram, and *Q*–*Q* plot was used to assess the normality of data of this study. After defining the normality, means and standard deviations or medians and interquartile range (IQR) were provided for continuous variables, whereas frequencies and percentages were provided for categorical variables. The Chi-Square statistic was performed for testing categorical variables. Independent sample t-test for parametric and Mann-Whitney U test for non-parametric variables was used to distinguish the differences between FGR and the control groups. The volume difference between the left and right kidney were tested by paired sample t-test. A p-value of less than 0.05 was regarded as statistically significant.

RESULTS

One hundred forty-three singleton pregnancy were enrolled in this study. Unfortunately, we could not manage to assess fetal renal volume (right, left, or both) due to inappropriate fetal position and/or movement in 16 pregnancies. A total of 76 fetuses with FGR and 51 fetuses with normal estimated fetal weight were studied. The mean gestational age was 34 weeks in the control group and 35 weeks in the FGR group. There was no statistically significant difference between groups in terms of maternal and perinatal characteristics (p > 0.05). The characteristics of the subjects are given in Table 1. There was a statistically significant difference between the right and left renal volume. The left renal volume was greater than the right one when both FGR and control groups were included. (p = 0.009). However, this difference was not prominent in the FGR group, and no statistical significance was observed within this group (p = 0.15). However, in controls, the left renal kidney was larger than the right kidney (p = 0.02) (Tab. 2).

UA Doppler studies revealed that there was a statistically significant difference between the two groups. As expected, FGR had higher S/D (S/D (Systole/Diastole) and PI values p = 0.00 and p = 0.001, respectively. MCA Doppler studies were not different between groups. Likewise, there was no significant difference between groups in uterine artery S/D and PI. Right and left renal arteries S/D values

Table 2. Difference between right and left renal volume in all pregnancies

Measurement	Mean ± SD	SEM	Mean Difference	SE Difference	t	df	p value
Control **right renal volume**	10.41 ± 2.56	0.21	0.78	0.32	2.37	50	0.021
Control **left renal volume**	11.19 ± 2.68	0.24					
FGR **Right renal volume**	9.43 ± 2.26	0.21	0.35	0.24	1.43	75	0.15
FGR **Left renal volume**	9.78 ± 2.61	0.24					

FGR — Fetal growth restriction; Data are presented as mean and standard deviation. Paired sample t-test was used

Table 3. Doppler assessment, volume, EFW and perinatal outcome differences between the two groups

	Control (n = 51)	FGR (n = 76)	p value
UA S/D	2.39 (0.65)	2.63 (0.96)	0.000‡
UA PI	0.87 (0.3)	0.98 (0.32)	0.001‡
MCA S/D	4.39 (1.75)	4.73 (1.94)	0.46‡
MCA PI	1.56 (0.49)	1.7 (0.59)	0.64‡
Right uterine artery PI	2.07 (0.53)	2.14 (0.78)	0.81‡
Left uterine artery PI	0.84 (0.33)	0.85 (0.45)	0.72‡
Right renal artery S/D	5.60 (256)	5.53 (1.91)	0.67‡
Left renal artery S/D	5.43 (1.86)	5.46 (2.47)	0.25‡
Right renal artery PI	1.99 ± 0.39	2.08 ± 0.58	0.28§
Left renal artery PI	2.04 (0.64)	2.01 (0.56)	0.76‡
Right renal volume cm^3	10.41 ± 2.56	9.43 ± 2.26	0.025§
Left renal volume cm^3	11.19 ± 2.68	9.78 ± 2.61	0.004§
Mean renal volume cm^3	10.80 ± 2.34	9.60 ± 2.20	0.004§
EFW [gr]	2611 (572)	2029 (38)	0.000‡
EFW percentile	47 (38)	3.43 (4.47)	0.000‡
Birth week	38 ± 1.80	36.91 ± 1.4	0.002§
Birth weight [gr]	3100 (730)	2282 (580)	0.000‡
Apgar score 0^{th} minute	7.35 ± 0.87	7.26 ± 1.06	0.66§
Apgar score 5^{th} minute	8.32 ± 0.90	8.31 ± 1.12	0.95§
Patients delivered in our center	31 (60.8%)	58 (76.3%)	0.094†
Patients delivered in another center	20 (39.2%)	18 (23.7%)	
Vaginal delivery	10 (32.3%)	20 (34.5%)	1.00†
C-section	21 (67.7%)	38 (65.5%)	

UA — umbilical artery; MCA — middle cerebral artery; S/D — Systole/Diastole; PI — pulsatility index; EFW — estimated fetal weight; Data are presented as median and interquartile range or mean and standard deviation or n (%); ‡Mann-Whitney test; § Independent sample test; † Chi-square Test

were not different statistically significant (p = 0.67 and p = 0.25, respectively). PI values of right and left renal arteries were also indifferent (p = 0.28 and p = 0.76, respectively)

Right, left, and mean renal volume of fetuses with FGR was less than the control group, and this difference was statistically significant (p = 0.025, p = 0.004, p = 0.004, respectively) (Tab. 3). EFW, delivery methods, Apgar scores, and perinatal outcomes were listed in Table 3.

DISCUSSION

FGR is thought to be one of the most complicated issues of perinatal medicine, and it is also considered to have effects that may manifest later in adulthood. Barker [16] hypothesized that FGR could be related to future coronary heart disease, stroke, hypertension, and diabetes. It stated that intrauterine environmental conditions could be related to altered developmental mechanisms that have long term consequences.

FGR is mostly caused by placental villous vascular damage and results in the activation of several compensatory mechanisms, including redistribution of fetal blood circulation towards the vital organs such as the fetal brain, adrenals, coronaries. In the fetal brain, middle cerebral arteries tend to have lower circulatory resistance when blood flow alteration mechanisms are active. In our study, no statistically significant difference between the two groups was observed for fetal MCA S/D or PI values. This result can be interpreted as our FGR group was not exposed to the brain sparing effect or redistribution due to FGR, and it is known that late-onset FGR does not always associate with the deprived blood supply of fetal organs [17, 18].

As compared to the brain, fetal renal arteries tend to have higher circulatory resistance to maintain the hemodynamic well-being of the fetus [19]. Vyas S et al. [19] stated that the renal artery PI is expected to be higher in fetuses with FGR; however, our data indicated otherwise. We did not find a significant difference between the FGR and uncomplicated pregnancies right and left renal arteries S/D and PI values, which is consistent with previous studies about late-onset FGR [6]. This difference might be explained by the fact that we have excluded fetuses with FGR accompanied by oligohydramnios. Oligohydramnios is proven to be secondary to decreased renal blood flow [20]. With the findings of our study, it can be commented that late-onset FGR without decreased renal flow, redistribution of the circulation is not the only mechanism in determining the fetal kidney volumes. Other mechanisms like glomeruli reduction and apoptosis may also be responsible for the decreased renal volume [7, 21, 22]. Low nephron number and small filtration area may be the leading cause of decreased renal volume than decreased renal vascular flow [23]. It is expected that MCA PI values tend to be different between the two groups, but there was no statistical difference [22].

No difference was observed between the uterine arteries of the FGR and control group on both the right and left sides. Early-onset FGR is considered to be related to higher uterine artery S/D and PI values [10, 24]. As mentioned before, our study group consisted of late-onset FGR, which uterine artery Doppler assessment typically remains normal. Therefore, a lack of a significant difference between the two groups was not suprising [25].

While most researchers in literature used 2D US in order to calculate the renal volumes of the FGR or normal pregnancies, to the best of our knowledge, this is the first study that concentrated on volume computing with the 3D VOCAL method [6]. Although there are controversial data about the volumetric organ calculations with the 2D US compared to the 3D VOCAL technique [4, 20, 26], 2D US has more commonly been used in organ volume calculations. However, the 3D VOCAL method is thought to be more accurate in organs with non-geometric shapes such as kidneys. Additionally, calculations can be done simultaneously in about three minutes with the VOCAL method, while the 2D US calculations should be made using a formula for ellipsoid shapes, even though kidneys are not ellipsoid [27]. Our control group renal volumes were consistent with calculations of 2D US; nonetheless, the data were limited [28]. Further studies are needed on the compatibility of two methods. We have conducted research with healthy pregnancies because of insufficient data with 3D VOCAL method.

In literature, some studies suggest that there was no significant difference between the right and left renal volume [26, 28, 29]. However, according to our data, left renal volume was significantly greater than the right renal volume in the control group), but there were no differences between the two sides of renal volumes in fetuses with FGR. The number of our control group was 51, and this difference can be explained by a lack of subjects. Only, Shi et al. [30] mentioned to have found the left renal volume greater than the right one but in pediatric patients.

We should mention the limitations of this study. We conducted the study with a single operator, ignoring the interobserver reliability. Authors are now carrying out another study about the interobserver variability of organ volumes in the 3D US VOCAL method. Secondly, we have only concentrated on fetuses between 32–37 weeks and predominantly late-onset FGR. Fetuses with early-onset FGR is known to be generally exposed to intrauterine hypoxia longer; therefore, struggling more for supplying organ systems [31]. Lastly, our mean EFW percentile was 3.43 (IQR 4.47), and we accepted fetuses with FGR when EFW was less than ten percentile. Although it is obvious that our FGR group seemed to be formed by growth-restricted fetuses, they can be small for gestational age fetuses inside the FGR group. However, all of them had an abnormal UA Doppler.

CONCLUSIONS

Our findings suggest that not only renal vascular adaptations, as well as glomerulosclerosis or apoptosis, may be responsible for the decreased renal volume in fetuses with FGR.

Ethical statements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Financial Disclosure

There are no *financial* conflicts of interest to *disclose*.

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Morphological estimation of incomplete uterine scar rupture (dehiscence) in post-cesarean deliveries. Immunohistochemical studies

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ABSTRACT

Objectives: No studies were found that analysed the properties of the caesarean scar, therefore the new study analysed the myometrial immunohistochemical expression of elastin, collagen type VI, alpha smooth muscle actin, smooth muscle myosin heavy chain, and endothelial cell marker CD31.
The aim of the study was to determine the risk of uterine rupture in future pregnancies.

Material and methods: A total of 89 women of Caucasian ethnicity were eligible: 20 healthy pregnant women, who underwent repeat caesarean section complicated by incomplete uterine scar rupture before labour, and 69 healthy pregnant women, who underwent repeat caesarean section without subsequent uterine scar rupture as the control group. In all cases, uterine tissue sample from the scarred region was collected during the caesarean section operation.

Results: The lack of observed significant changes of elastin, collagen type VI, alpha smooth muscle actin, smooth muscle myosin heavy chain and endothelial cell marker CD31 concentrations in ruptured and unruptured uteri indicates that these components cannot be found to be a marker of risk of uterine rupture in future pregnancies.

Conclusions: It could be suggested that the examined components do not contribute to the mechanism of maintaining integrity and are not responsible for the biomechanical properties of the uterine scar.

Key words: uterine scar; elastin; collagen; actin; myosin; endothelial cell marker CD3

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INTRODUCTION

The latest data show an increased global trend in caesarean section (CS) rates, consistently increasing over the past five years [1]. The uterine scar seriously affects the integrity of the uterus [2, 3], and recently, many more cesarean scar defects have been found to lead to unexpected complications, such as: abnormal uterine bleeding, painful menstruation, pelvic pain, dyspareunia and infertility in non-pregnant women [4]. Additionally, primary CS delivery carries potential risks in subsequent pregnancies: cesarean scar pregnancy, placenta previa, accreta, increta or percreta, scar dehiscence or rupture of the uterus in pregnant women [3–5].

Uterine ruptures are usually divided into complete and incomplete (dehiscence) ruptures. Incomplete uterine rupture defines a process of gradual or full myometrial rupture where the serosa and amniotic sac are intact, and the patient is virtually always asymptomatic. Complete uterine rupture is used to refer to a situation in which a patient has a uterine rupture coexisting with strong clinical manifestations (intraabdominal haemorrhage, tachycardia, rebound abdominal tenderness) [6]. Compared to complete uterine rupture, uterine dehiscence relates to much lower maternal and neonatal mortality and morbidity. There is a little known about complex process of the uterine wound repair and healing after cesarean delivery [7]. Improper healing may lead to thinning of the anterior uterine wall. In most of investigations, anatomical defects resulting from previous cesarean sections, have been reported to be associated with a higher probability of complete uterine rupture during labor [5, 6]. The uterine closure technique during CS may influence on further biomechanical uterine wound proprieties during subsequent pregnancies and thereby determine the perioperative or long-term maternal outcomes. Based on a meta-analysis including twenty studies (15,053 women),

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it has been shown that double-layer unlocked sutures are more effective than single-layer locked sutures. In terms of wound healing and residual myometrium thickness has been found to be decreased by 1.26mm after single-layer closure when compared to double-layer closure technique [8]. Dysmenorrhea symptoms have been observed more often in the single-layer group, whereas incidence of uterine rupture was similar in both groups: with single- and double-layer sutures. [8].

In turn, in the CORONIS trial conducted in 13,153 women during the 3.8 years period, there was no evidence of any difference in the incidence of pelvic pain symptoms, dyspareunia and subsequent pregnancy outcomes, depending on single- or double-layer closure of the uterus [9]. Finally, it is suggested that double-layer uterine closure with unlocked first-layer suture during caesarean delivery appears to be the most accurate method in terms of postpartum uterine scar thickness [10, 11]. There is also evidence suggesting that locking a single-layer suture in primary CS may increase the risk of uterine rupture at a subsequent delivery. Regardless of whether the uterine incision is closed using one or two layers, thickness of uterine myometrium in the site of previous incision is reduced by about 50%. [12]. The current randomized controlled trial (2Close Study) results publication will surely help to choose the preferable technique of uterus closing during CS in relation to postmenstrual bleeding, fertility and the development of a niche, measured by ultrasound [13]. Though sonographic lower uterine segment (LUS) thickness seems to be a strong predictor for uterine scar defect and full LUS thickness of less than 2.3 mm is associated with severe complications during labor (uterine dehiscence, rupture, hemorrhage), no ideal diagnostic method can yet be recommended [14, 15]. The possible pitfalls in ultrasound diagnostics may lead to LUS diagnosis difficulties, as well as incorrect finding of valuable reference values for LUS thickness [16]. In cases of altered anatomy and impaired ultrasound conditions, the use of 3T magnetic resonance imaging (MRI) as an additional LUS diagnostic tool may be useful [16]. In studies focused on suturing operative techniques, it is suggested that full thickness uterine suturing technique plays a role by lowering the incidence of incomplete healing of the uterine incision after CS [17, 18]. Labour before previous CS and the use of synthetic sutures for the uterine closure may be associated with a thicker myometrial LUS [14]. It has been also proposed that prostaglandins used for uterine contractions induction may act locally by leading to biochemical modifications that weaken the scar and subsequently predisposed to rupture [19]. It is possible that an increased risk of incomplete healing after the uterine incision is related with cesarean operation in advanced labor (second stage of labor) [20, 21]. The occurrence of post caesarean scar defect may be also influenced by risk factors such as age > 30years, BMI > 27.3, premature rupture of membranes, elective caesarean section, postoperative anaemia and retroposition of the uterus [2]. Delivery may alter the viscoelastic proprieties of myometrium and the pattern of collagen organization. The regenerative ability of a uterus can be result of histological, mitotic and functional differences in biomechanical proprieties of the scarred myometrium after cesarean section. Tensile properties of the LUS can be also connected with its biochemical structure and sulfated glycosaminoglycans, hydroxyproline, pyridinoline — deoxypyridinoline concentrations [17, 22, 23]. Extracellular matrix (ECM) remodeling during healing process lead to new ECM forms creation that never achieve biomechanical proprieties (flexibility and strength) of the original unscarred tissue [24]. The tissue scarring process is proceeding in various ways, leading in some cases to abnormal ECM reconstruction with excessive scars formation (keloid or hypertrophic scar) [24]. The uterine scar alpha smooth muscle actin concentrations differences detected with use of IHC assay may facilitate understanding their role in the pathogenesis of reparative process [25], due to regenerative endothelial cells activity that is enhanced by smooth muscle cells [26]. In the ischemic organs the reparation process proceeds with new blood vessels formation, where the vascular network creation is stimulated by endothelial cells and smooth muscle cells [26].

Essential for wound repair, angiogenesis is regulating by signals coming from serum and ECM, providing scaffold support with non-collagenous laminins 8 and 10. This dynamic healing process is moderating with cooperative angiogenic cytokines regulation. Vascular endothelial growth factor, angiopoietin, fibroblast growth factor, transforming growth factor beta are the most important and well recognized angiogenic cytokines. Uterine wound healing process involves many other cells, such as connective tissue growth factor, basic fibroblast growth factor, platelet-derived growth factor, tumor necrosis factor alpha expression of some of these factors in the myometrial smooth muscle is suspected to be altered in cases of uterine dehiscence [7]. Therefore, the investigation of uterine scar proprieties with determination of elastin, collagen type VI, alpha smooth muscle actin, smooth muscle myosin heavy chain, endothelial cell marker CD31 concentrations may be helpful to recognize possible determinants of uterine rupture in scared uteri.

Collagens and elastic fibres are *ECM fibrous proteins* constituting networks, present in myometrium tissue. Type VI collagens have many functions, including clinical evidence of involvement of connective tissue [27–29]. Its deficiency is associated with morphological abnormalities of the tendons and large spectrum of collagen VI-related myopathies. It also acts throughout interaction with collagen IV of basement membrane. Elastin is a connective tissue polymeric protein,

Table 1. Characteristics of analyzed groups of patients

	Unruptured uterus	Ruptured uterus	p
The average age of women [years]	33.36 ± 4.6	32.95 ± 5.3	NS*
Mean gestational age [weeks]	39.22 ± 1.5	37.60 ± 1.2	NS*
Number of cesarean sections (n)	2.42 ± 0.8	3.10 ± 0.9	0.005
Period after previous cc [months] cesarean section [months]	70.40	59.2	NS*
Pregnancy complications	None	None	
Previous uterine incision closure technique	single-layer closure	single-layer closure	
Newborns' birthweight [grams]	3445.94	3045.50	0.002

NS — not significant

synthesized as a single chain protein, which undergoes organization into an elastic fiber in the extracellular space. It is likely elastin tissue distribution may help to explain the normal contractile function of myometrium during labor. We speculate that there is a correlation between the occurrence of uterine dehiscence or rupture incidence in term pregnant scarred uteri and biochemical changes in LUS structure, ascertained by myometrial immunohistochemical expression of elastin, collagen type VI, alpha smooth muscle actin, smooth muscle myosin heavy chain, endothelial cell marker CD31 (Platelet endothelial cell adhesion molecule - PECAM-1). Differences may occur in incompletely ruptured, fully ruptured or unruptured scarred uteri in term pregnancies.

MATERIAL AND METHODS

Test group

The study was conducted in Department of Perinatology, Obstetrics and Gynecology, Pomeranian Medical University in Szczecin, Poland in years 2016–2018. Institutional ethics approval from Pomeranian Medical University in Szczecin was received for all experiments and patients gave written consent for the investigation. During the three year prospective observation, total number of deliveries in our department was 4668 and the cesarean sections were performed in 2395 (51.3%) cases. From a total of 2395 cesarean sections, 20 (0.83%) were complicated by incomplete uterine scarred rupture. In all cases, the rupture of previously scarred uteri has occurred occasionally in the antepartum period. All analysed pregnant women were at term, without previous signs and symptoms of labor or regular uterine contractions. No pro-contractile agents have been administrated. All women who previously had one or more cesarean sections and did not accept vaginal route delivery after previous cesarean section, were qualified for elective cesarean section. Eighty-nine Caucasian ethnicity women took part in the study: 20 healthy pregnant women, who underwent repeat cesarean section complicated by incomplete uterine scar rupture before onset of labour and 69 healthy pregnant patients, who underwent repeat cesarean section without uterine scar rupture were analysed. The mean *age of* pregnant women in our total sample was 33.30 (SD ± 5.34) years with a range of 18 to 39 years. In all analysed women, a pre-pregnancy body mass index (BMI) had been calculated by dividing weight (kg) by height (m) squared. The BMI ranged between 19.8–29.0. Seventy-nine percent of the sample was classified as normal weight. There were no significant differences among analysed groups of patients in terms of age, gestational age and period after previous cesarean section. The time that had elapsed since the last caesarean section was generally longer than six years (Mean 6.1 SD ± 1.87) and did not statistically differ between either group. The mean number of cesarean sections in the group of women with unruptured and ruptured uterus was statistically significantly different, 2.42 (SD ± 0.61) and 3.10 (SD ± 0.72) respectively (Tab. 1).

Surgical procedures

All patients were operated under epidural anesthesia. Cesarean section was performed in sterile conditions. A transverse skin incision was made and carried through to the underlying layer of fascia. The fascia was incised in the midline and extended laterally. Once the abdomen was opened, the lower uterine segment in place of previous cesarean section was incised in transverse fashion. The infant was delivered atraumatically. After fetus removal, a uterine scar had been identified and a 2 × 2 cm sample of uterine lower segment was cut out. In all cases of incomplete uterine ruptures or unruptured uteri, an analogical procedure for collecting samples was performed. The uterine incision was closed by using one-layer closure technique with continuous lock stitches. No hysterectomy was required and there were neither maternal nor neonatal deaths.

Morphological study

Obtained tissues were fixed in 4% buffered paraformaldehyde and subsequently embedded in paraffin. The ovaries

were sectioned into slices of thickness of 3–5 µm with a Microtome HM 325. These sections were then mounted onto *poly-l-lysine coated slides*. The slides were stained with H-E (hematoxylin and eosin) for morphological study, and immunohistochemistry (IHC) was used to detect the presence of specific protein markers in uterine scars: CD31 (PECAM-1) endothelial cell marker; a-actin and myosin heavy chain — elements of myofilaments in smooth muscle cells; elastin and collagen type VI — elements of extracellular matrix. To visualize the proteins in myometrium scar, following mouse anti-human antibodies (Novocastra distributed by Leica Biosystems, Zalesie Gorne, Poland) were used: anti-CD31 (clone 1A10; optimally diluted); anti-smooth muscle actin, alpha (clone ASM-1; optimally diluted); anti-myosin heavy chain (smooth muscle) (clone S131; optimally diluted); anti-elastin (clone BA-4; diluted 1:100); anti-collagen type VI (clone 64C11; diluted 1:500). The deparaffinized sections were microwaved in citrate buffer (pH 6.0) for heat-induced epitope retrieval. After slow cooling to room temperature, the slides were washed in PBS twice for five minutes and then incubated for 60 minutes with primary antibodies. Next, the slides were incubated with Invitrogen Alexa Fluor Plus 488 goat anti-mouse IgG secondary antibody (Product # A32723) at a dilution of 1:1000 for 1 hr at room temperature (Invitrogen Thermo Fisher Scientific, USA).

The samples were viewed by fluorescent microscopy Olympus BX 46 and Olympus DP 25 camera. Additionally, samples were analyzed by high content screening for rapid quantitation and comparison of data from multiple samples. A digital computer-assisted analysis technique was based on the use of an image processing program (cell Sense Dimension 1.5), where three parameters were obtained: percentage of labeled cells, digital immunostaining intensity, and digital expression index. Sample images of staining analysed all the components shown in the picture below (Fig. 1 A–E).

Statistical analysis

To choose the right statistical analysis, we checked if the dependent variables were normally distributed using Shapiro-Wilk normality test. Because of non-normal data distribution, a nonparametric Mann-Whitney U test was used for determination of differences between analyzed groups. We compared mean, median scores of samples and performed one-way analysis of variance with the aid of Statistica10 statistical software (Oklahoma, Tulsa, USA). A $p \leq 0.05$ was considered to indicate statistical significance.

RESULTS

In our study, the majority of ruptures occurred in para 3–4, before trial of labour and uterine rupture was significantly more frequent, when the number of previous cesarean sections exceeded three. In turn, the period that had passed since the previous cesarean section and uterine incision closure technique previously used did not play a significant role. *Significant differences* were found between the birth *weight* of *newborns. In the group of unruptured uteri, the newborns* where significantly heavier when compared to those coming from the *ruptured uteri group* (Tab. 1).

Our study has demonstrated that collagen type VI, elastin, alpha smooth muscle actin, smooth muscle myosin heavy chain, endothelial cell marker CD31 are active and regular constituents of uterine scarred myometrium, which surround and associate smooth muscle cells (Tab. 2). Their concentrations, however, did not differ in the scarred unruptured and ruptured uterine tissue. The analysis of significance of the sample's correlation coefficient in two groups did show significant negative correlation between alpha smooth muscle actin and smooth muscle myosin heavy chain concentrations and elastin and CD31 concentrations in the unruptured uteri group as well (Tab. 3). Analysis of products of IHC reaction of tested components in myometrial scar did not show any significant differences in both groups of women delivered by cesarean section.

DISCUSSION

Cesarean section is the most frequent obstetrical procedure which the rate has dramatically risen in few last years. The presence of cesarean scar defect (CSD) in the lower uterine segment became a life-threatening problem mainly in cases when women wish to be pregnant more than once [18]. Previous caesarean section is known to be the main risk factor for incomplete and complete uterine rupture. Therefore, uterine scar rupture remains one of the most frightening late complications in obstetric care [5, 6]. Absence of peritoneal signs in incomplete uterine rupture in non-labouring women may delay its diagnosis, especially when connected with little or lack of bleeding into the abdominal cavity.

There is no consensus about the role of uterine closure technique for the risk of uterine rupture [8]. It is suggested that the risk of uterine rupture during labor after a single-layer closure is not significantly different from that after a double-layer closure [30]. In other studies, is postulated that a double-layer closure of the uterus during previous cesarean section is related to a thicker LUS, which may subsequently reduce the risk of LUS thickness lowering for less than 2mm and uterine dehiscence in the next pregnancies [11]. Contrarily, the type of used thread for uterine closure does not significantly influence on LUS thickness in next pregnancies [11]. There are also other factors that may have an impact on LUS integrity, such as: inter-cesarean interval longer than 54 months, maternal age beyond 35 years, cesarean section performed in labor, baby weighting more than 3000g, period longer

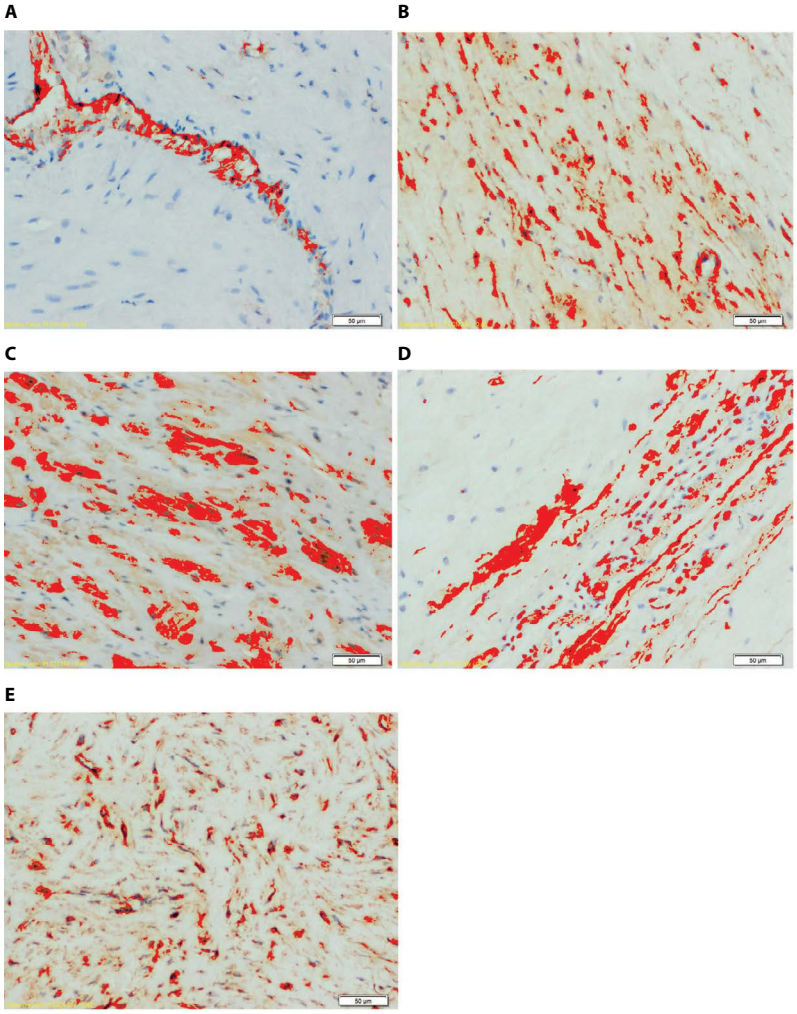

Figure 1. Representative IHC staining of **A.** CD31 (PECAM-1); **B.** Alpha smooth muscle actin; **C.** Smooth muscle myosin heavy chain; **D.** Elastin; **E.** Collagen type VI in ruptured uteri. Images were obtained under an ×20 magnification. Scale bar, 50 µm

Table 2. Relationship between immunohistochemical morphological parameters of unruptured and ruptured uterine cesarean scar

	alpha smooth muscle actin						smooth muscle myosin heavy chain					
	area [%]	**z**	**p**	**area fraction [µm²]**	**z**	**p**	**area [%]**	**z**	**p**	**area fraction [µm²]**	**z**	**p**
Unruptured uterus (n = 69)	23.14	–0.29	NS*	40755.87	–0.49	NS*	2.99	1.16	NS*	4 235.89	0.60	NS*
Ruptured uterus (n = 20)	29.86			46194.99			2.08			2 948.88		
	elastin						collagen type VI					
	area [%]	z	p	area fraction [µm²]	z	p	area [%]	z	p	area fraction [µm²]	z	p
Unruptured uterus (n = 69)	2.54	1.28	NS*	3 496.22	0.89	NS*	11.72	–0.47	NS*	4 235.89	–0.24	NS*
Ruptured uterus (n = 20)	2.06			2 936.05			12.15			2 948.88		
	CD31											
	area [%]	z	p	area fraction [µm²]	z	p						
Unruptured uterus (n = 69)	1.16	–1.05	NS*	1 857.15	–0.82	NS*						
Ruptured uterus (n = 20)	1.37			2 088.51								

NS — not significant

Table 3. Significance of the samples correlation coefficient in analyzed groups

Variables		Unruptured uterus n = 69 p	Ruptured uterus n = 20 p
Actin	Myosin	0.025	NS*
Actin	Elastin	NS*	NS*
Actin	Collagen	NS*	NS*
Actin	CD31	NS*	NS*
Myosin	Elastin	NS*	NS*
Myosin	Collagen	NS*	NS*
Myosin	CD31	NS*	NS*
Elastin	Collagen	NS*	NS*
Elastin	CD31	0.028	NS*
Collagen	CD31	NS*	NS*

NS — not significant

than 18 hours after rupture of membranes [21]. Myometrial discontinuity at the site of a previous cesarean section in nonpregnant women may be responsible for postmenstrual spotting, dysmenorrhea, dyspareunia and chronic pelvic pain. Moreover, patients after multiple cesarean sections have larger CSD followed by more severe clinical symptoms. It is reported that the CSD rate varies widely in range 0.3–19.4%, probably due to asymptomatic group of patients with CSD, who are not under control or at analysis [5, 19, 20].

Uterine wound repair has been analysed in just a few studies [17, 22]. It is likely that individual biochemical and biomechanical tissues' proprieties play a certain role in myometrium healing process [3].

Many investigations were focused on risk factors for uterine rupture and its prediction by LUS sonographic evaluation [2, 8, 15]. Until now, there is no evidence which factors have most significant and important impact on uterine healing process. There are a few data for the field of morphological and histological uterine wall repair process and there is little known about human uterine scar protein contents as well [17, 22]. The wound healing as a biological response for tissue injury can proceed as a repair and regeneration [23]. The wound repairing usually undergoes by patching, rather by restoring to its original structure. In normal conditions, wound repairing is processing through three phases: inflammation (onset of injury to days 4–6), tissue formation (days 4–14), tissue maturation and remodeling (week 1–year 1). A fibro-proliferative response involves mediators, blood cells and ECM parenchymal cells. The human myometrium is mainly composed of smooth muscle cells that have the ability to undergo hyperplasia and hypertrophy during pregnancy and can also regenerate as a repair response of injured tissue. The cells are interspersed with elements of ECM, a reservoir for matricellular proteins, growth factors, and cytokines [29]. Parallel to presence of smooth muscle cells, interstitial collagen fibrils are also detected. Collagens and elastic fibres are *ECM fibrous proteins* constituting networks, present in myometrium tissue. Type I, type III and type V are the predominant in human myometrium, additionally to type IV (basement membrane) and type VI that are present. The collagen VI plays a structural role as well as influences the migration of cells probably through fibronectin-dependent agents. [31]. Type VI collagens have many functions, including clinical evidence of involvement of connective tissue [27–29]. Its deficiency is associated with morphological abnormalities of the tendons and large spectrum of collagen VI-related myopathies. It acts also throughout interaction with collagen IV of basement membrane. The collagen VI homeostasis is regulating by capillary morphogenesis gene 2, also known as anthrax toxin receptor 2 (CMG2/ANTXR2). In cases of loss of CMG2 function, an accumulation of collagen VI lead to nodule formation in patients suffering from hyaline fibromatosis syndrome. In animal studies, a massive mice uterine collagen type VI accumulation induces progressive fibrosis and sterility. It is proposed, that CMG2 may mediate collagen VI intracellular degradation and plays a role of signalling receptor [32]. We suggest that over-accumulation of collagen VI may affect the uterine integrity by abnormal healing process, leading to changing the biomechanical wound proprieties. Another collagen VI function is an interaction with basement membrane collagen IV [28, 29]. In human wound collagen type VI is reported to be present after a post injury period of at least three days in a network connected with fibroblasts in the wound area. It can be also found in scar tissues and may play a role in modulation of haemostatic response to vascular injuries. Though the uterine scar collagen deposition after cesarean section is not the primary healing mechanism, collagen seems to be the most critical element, responsible for maintenance of tissue structural integrity. Pollio et al. demonstrated a higher collagen content in scarred lower uterine segment in cases of uterine dehiscence [17]. Our histological analysis of the uterine scar did not show any difference in scar integration and collagen type VI remodeling at the site of myometrial injury between ruptured and nonruptured uteri.

Elastin is a connective tissue polymeric protein, synthesized as a single chain protein, which undergoes organization into an elastic fiber in the extracellular space. The elastin is a stable element of ECM, and its myometrial tissue concentration remains unchanged even at pregnancy. In our investigation the myometrial elastin concentrations did not vary in groups of patients with ruptured and unruptured uterine scars. Our study provides tendency that there is a gradient of elastin uterine scar distribution and the scar seems to be more elastic in pregnancies uncomplicated by uterine rupture when compared to pregnancies complicated by uterine rupture.

It has been proven, that eNDOTHELIAL CELLS have many functions and play a role in the control of coagulation, thrombolysis, vascular tone, permeability, inflammation, tissue repair and angiogenesis [33]. The expression of anti-platelet-endothelial cell adhesion molecule-1 (endothelial cell marker CD31, 130-kDa transmembrane glycoprotein) has been recently demonstrated on surface of platelets, monocytes, macrophages [34]. Neoangiogenesis understood as a formation of new blood vessels, seems to be an essential process during wound healing. In our study we demonstrated the presence of endothelial cell marker CD31 in human uterine scar of ruptured and unruptured uteri as well. The CD31 expression and angiogenesis in the uterine scar may be associated with the inflammation phase of wound repairing after cesarean section, oxygen deliverance, nutrients, and inflammatory cells as well. Its angiogenic and facilitating leukocyte migration role, may be important in myometrial continuity repair process. We did not identify any significant differences in CD31 expression in scarred ruptured and non-ruptured uteri. In animal model studies, the formation of capillaries, reflected by expression of CD31 haven't been increased in uterine wound tissue [23]. The scarred and unscarred tissues are composed of the same molecules of extracellular matrix, but the ratios in scarred tissue are different when compared to normal tissue [24, 35], which was also partially confirmed in our studies in the analysis of actin and myosin in the unruptured uteri group (Tab. 3). The contractile smooth muscle activity is based on cytoplasmic structural proteins' microfilament system, where actin and myosin play a basic role and constitute about 55% of all the proteins of the smooth muscle cells. Immunoexpression of alpha smooth muscle actin (SMA) is found in vascular walls and muscularis mucosae of many organs, including uterus, therefore is reported to be useful in the identification of leiomyomas and leiomyosacomas pleomorphic adenomas. In our investigation we identified presence of SMA in scarred uteri and its concentration did not differ in ruptured and non-ruptured uteri. The smooth muscle myosin heavy chain (SM-MHC) that is major component of the contractile system also did not vary in analysed groups of patients. Our study can suggest indirectly that in unruptured scarred uteri the contractile uterus activity is less expressed than in ruptured uteri. Myometrial contraction is mediated *via* interaction of actin and myosin and regulated by enzymatic phosphorylation.

CONCLUSIONS

Our study demonstrated that collagen type VI, elastin, endothelial cell marker CD31, alpha smooth muscle actin, and smooth muscle myosin heavy chain, are active and regular constituents of ruptured and unruptured uterine scarred myometrium.

The obtained results indicated correlated distribution of actin and myosin as well as elastin and CD31 in unruptured uteri while this fact hasn't been observed in ruptured uteri.

There is no statistically significant difference between myometrial immunoexpression of studied fibrous proteins of extracellular matrix, endothelial cell marker and markers of smooth muscle cells in ruptured and unruptured scarred uteri. It suggests that myometrial wound healing is related to multicomplex cell interactions, where the direct mechanism of abnormal uterine healing and myometrial rupture remains unclear.

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Conflict of interest

The authors have not reported any conflict of interest.

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Evaluation of indications for amniocentesis in cases of normal fetal ultrasound results

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ABSTRACT

Objectives: The objective of this study was to analyze indications for amniocentesis in cases of patients with normal fetal ultrasound results between 11+0 and 13+6 weeks of gestation.

Material and methods: The results of first-trimester screening tests performed between 2014 and 2018 on 6,863 patients of the Prenatal Testing Outpatient Clinic at the Clinical Department of Obstetrics and Gynecology, Pomeranian Medical University, Szczecin, Poland, were analyzed. The inclusion criteria were a singleton pregnancy and normal results of fetal ultrasound between 11+0- and 13+6-weeks' gestation. Depending on the calculated risk of fetal trisomy 21, the patients were divided into three groups (group A = RS > 1:300, group B = RS 1:300 – 1:999, group C = RS ≤ 1:1000). Subsequently, values such as PAPP-A and fβ-hCG protein levels and maternal age were analyzed for each of the groups.

Results: The patients, 6,310 (91.94%) met the inclusion criteria. A high risk of fetal trisomy 21 was identified for 514 women (8.15%). Group B had 733 (11.62%) and group C 5,063 (80.23%) patients. In group A, an fβ-hCG level of ≥ 2.000 MoM was shown for 50.97% of the women. A PAPP-A level ranging from 0.001 to 0.499 MoM was observed for 38.72% of group A patients. The mean maternal age in groups A, B and C was 36.45, 36.08 and 31.64 years, respectively.

Conclusions: In the first-trimester, patients with normal ultrasound results obtained during prenatal screening tests, the main cause of an increased risk of trisomy 21 was elevated PAPP-A and fβ-hCG concentrations. According to this paper's authors, in these cases extension of diagnosis to include other gestational complications, *e.g.* preeclampsia, should be considered.

Key words: amniocentesis; ultrasonography; prenatal; PAPP-A; HCG-beta

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INTRODUCTION

Although first-trimester screening tests are recommended to every pregnant patient, the percentage of such tests performed in Poland continues to be low. As an example, 389,455 children were born in 2018, while only 116,079 pregnant women, including 74,543 (64.2%) below the age of 35, took advantage of the screening project funded by the National Health Fund (NFZ). Biochemical tests, together with maternal age and ultrasound results, obtained between 11+0- and 13–6-weeks' gestation allow for detecting almost 95% of all chromosomal aberration cases, with 5% of false positive results [1]. Every result suggesting an increased risk of fetal aneuploidy is an indication for a karyotype test, with the most common invasive method allowing for the test to be carried out being amniocentesis. In 2018, 6,926 such procedures were performed under NFZ financing. The NFZ program is designed to support chromosomal aberration diagnosis, but its enormous potential allowing for the screening of distant obstetric complications is not utilized. World literature reports that false positive results of prenatal tests can be useful in indicating pregnancy complications that are not related to an abnormal number of chromosomes. Trisomy 21 is associated with characteristic values of the proteinsare determined in these tests. For human chorionic gonadotropin (β-hCG), that value is most deemed to be over 2 MoM, and for pregnancy-associated plasma protein A (PAPP-A) — below 0.5 MoM [1]. These values, with a normal ultrasound picture and fetal euploidy indicated by the karyotype test, may be linked to numerous second trimester complications, such as preeclampsia, PIH,

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gestational diabetes mellitus, IUGR, premature rupture of membranes and preterm labor [2, 3].

The aim of this paper was to assess the indications for amniocentesis, accompanied by a detailed analysis of cases with normal ultrasound results between 11 + 0- and 13 + 6-weeks' gestation.

MATERIAL AND METHODS

The results of first-trimester screening tests performed between 2014 and 2018 on 6,863 patients of the Prenatal Testing Outpatient Clinic at the Clinical Department of Obstetrics and Gynecology, Pomeranian Medical University, Szczecin, Poland, were analyzed retrospectively. The patients were aged between 14 and 46 and had singleton pregnancies. The analysis included patients whose ultrasound scans made between 11 and 13+6 weeks' gestation showed normal fetal anatomy and for whom all ultrasound markers for chromosomal abnormalities were within normal ranges, according to the Recommendations of the Ultrasound Section of the Polish Society of Gynecologists and Obstetricians. Subsequently, the patients were divided into three groups according to their test results:

- group A — an increased risk of fetal trisomy 21 (RS > 1:300);
- group B — a moderate risk of fetal trisomy 21 (RS 1:300–1:999);
- group C — a low risk of fetal trisomy 21 (RS ≤ 1:1000).
- Additionally, group A was divided into two subgroups:
- subgroup A1 — an extremely high risk (RS > 1:100);
- subgroup A2 — a high risk (RS 1:100–1:299).

Within these groups, PAPP-A MoM and free β-hCG MoM were analyzed to establish their correlations with the risk of trisomy 21. The ranges determined for PAPP-A MoM were 0.001–0.499 and ≥ 0.500, while those for free β-hCG MoM were of 0.001–1.999 and ≥ 2.000.

The results were then analyzed using the Statistica software (ver. 13.1). The statistical analysis was performed using the Mann-Whitney U test and Pearson's χ^2 test, assuming the significance level of $p < 0.05$. The obtained results are shown in the tables and figures below.

RESULTS

Out of the whole study population, 6,310 (91.94%) patients satisfied the inclusion criteria. A result indicating an increased risk of fetal trisomy 21 was obtained in 514 cases (8.15%). Out of those, 222 (3.52%) showed a value of > 1:100, while for 292 (4.63%) the risk ranged between 1:100 and 1:299.

The mean PAPP-A MoM values for groups A, B and C differed in a statistically significant manner, and were 0.65 ± 0.36, 0.80 ± 0.43 and 1.08 ± 0.60 (p_{AB}, p_{AC}, $p_{BC} < 0.001$), respectively.

The mean fβ-hCG MoM values for groups A1 and A2 differed in a statistically significant manner and were 2.70 ± 1.81 and 2.14 ± 1.32 ($p < 0.001$), respectively.

The mean PAPP-A MoM values for groups A1 and A2 differed in a statistically significant manner and were 0.55 ± 0.28 and 0.73 ± 0.39 ($p < 0.001$), respectively.

An fβ-hCG level of ≥ 2,000 MoM was shown in 50.97% of group A patients. This figure was 56.31% for subgroup A1 and 46.92% for A2. For group B, such levels were determined for 26.06%, and for group C for 10.21% of the patients.

A PAPP-A level ranging from 0.001 to 0.499 MoM was determined for 38.72% of group A patients. This figure was 49.55% for subgroup A1 and 30.48% for subgroup A2. For group B, such levels were determined for 24.56%, and for group C for 9.54% of the patients.

Table 1. Descriptive statistics of the variables analyzed for groups A, B and C.

		n	M	Me	SD	Min.	Max.
Group A	Age	514	36.45	37.00	4.75	16.00	46.00
	CRL [mm]	514	64.67	64.40	7.63	45.00	84.00
	β-hCG MoM	514	2.38	2.05	1.57	0.22	13.14
	PAPP-A MoM	514	0.65	0.58	0.36	0.12	3.65
Group B	Age	733	36.08	36.00	4.85	20.00	45.00
	CRL [mm]	733	64.92	64.50	7.66	45.20	84.00
	β-hCG MoM	733	1.64	1.40	1.02	0.06	6.89
	PAPP-A MoM	733	0.80	0.69	0.43	0.07	3.07
Group C	Age	5,063	31.64	33.00	5.41	14.00	41.00
	CRL [mm]	5,063	63.78	64.40	7.83	43.00	84.00
	β-hCG MoM	5,063	1.15	0.96	0.74	0.005	7.94
	PAPP-A MoM	5,063	1.08	0.95	0.60	0.002	16.73

Table 2. Descriptive statistics of the variables analyzed for subgroups A1 and A2.

		n	M	Me	SD	Min.	Max.
Group A1	Age	222	36.83	38.00	4.64	19.00	45.00
	CRL [mm]	222	64.65	65.15	7.28	48.30	80.70
	β-hCG MoM	222	2.70	2.31	1.81	0.26	13.14
	PAPP-A MoM	222	0.55	0.50	0.28	0.12	1.65
Group A2	Age	292	36.16	37.00	4.82	16.00	46.00
	CRL [mm]	292	64.68	64.20	7.90	45.00	84.00
	β-hCG MoM	292	2.14	1.87	1.32	0.22	8.64
	PAPP-A MoM	292	0.73	0.65	0.39	0.16	3.65

The mean fβ-hCG MoM values for groups A, B and C differed in a statistically significant manner, and were 2.38 ± 1.57, 1.64 ± 1.02 and 1.15 ± 0.74 (p_{AB}, p_{AC}, p_{BC} < 0.001), respectively

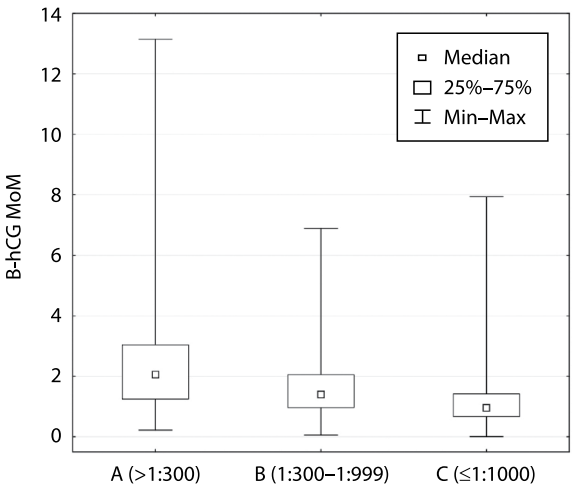

Figure 1. A comparison of fβ-hCG MoM distributions for groups A, B and C

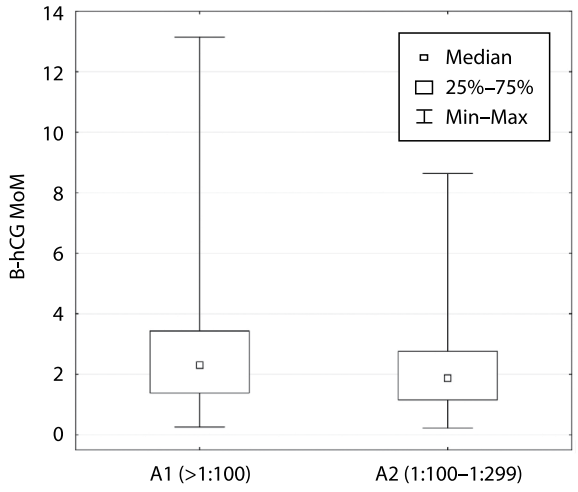

Figure 3. A comparison of fβ-hCG MoM distributions for groups A1 and A2

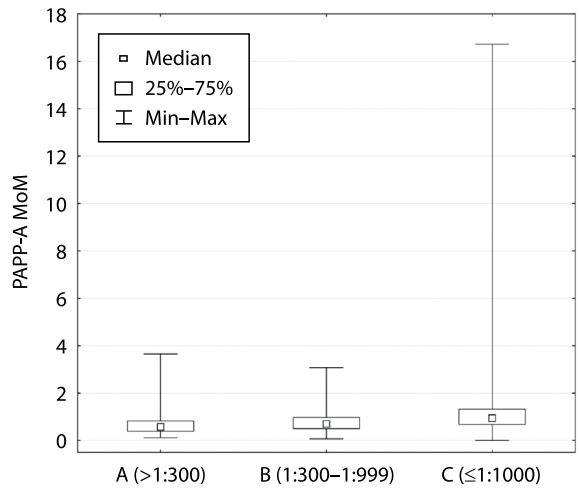

Figure 2. A comparison of PAPP-A MoM distributions for groups A, B and C

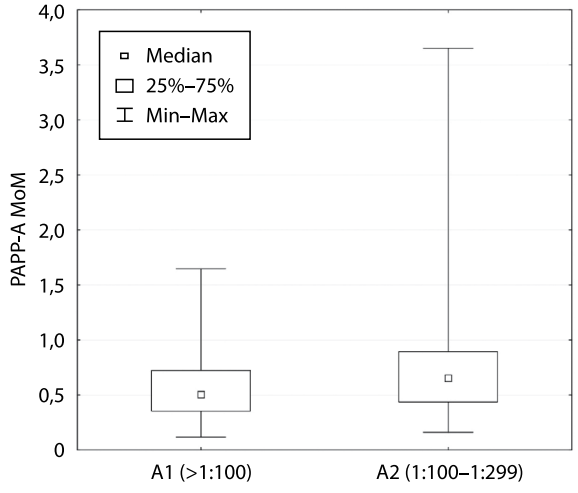

Figure 4. A comparison of PAPP-A MoM distributions for groups A1 and A2

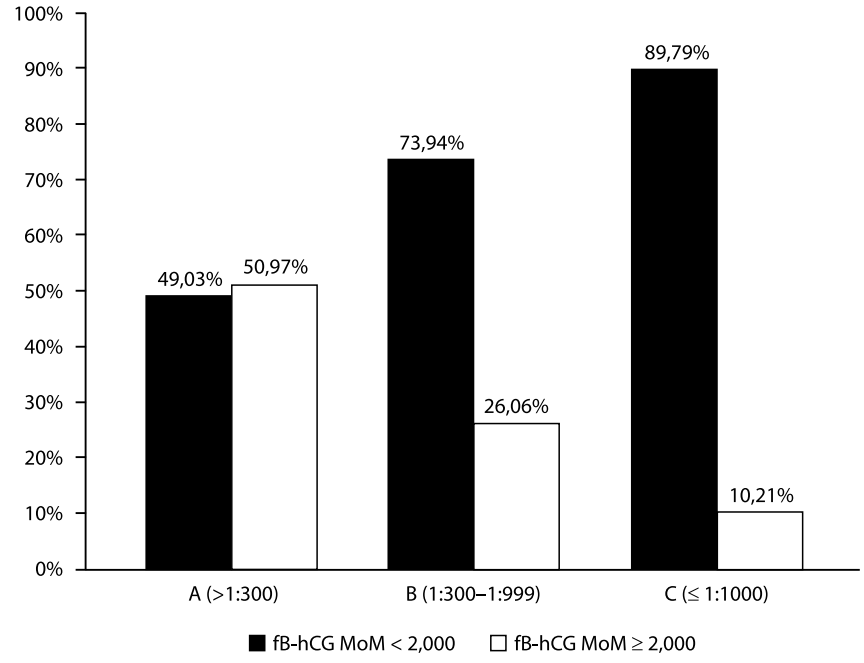

Figure 5. Correlations between fβ-hCG levels and the adjusted risk of fetal trisomy 21 for groups A, B and C

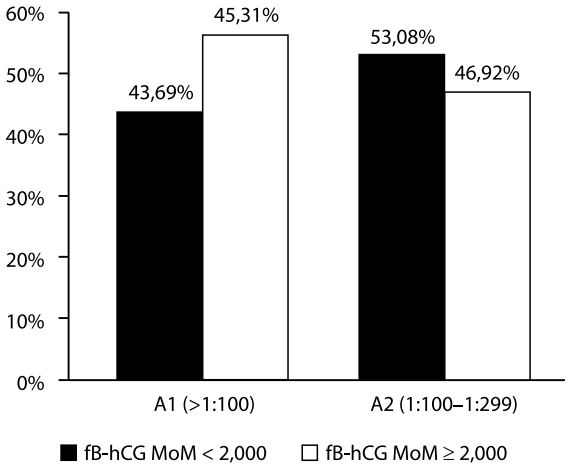

Figure 6. Correlations between fβ-hCG levels and the adjusted risk of fetal trisomy 21 for subgroupsA1 and A2

DISCUSSION

The Polish National Health Fund introduced a first-trimester screening program in 2004. Since then, the percentage distribution of the most common indications for genetic amniocentesis has changed [4, 5]. Similarly, to any other invasive procedure, amniocentesis carries the risk of such complications as pregnancy loss (0.1–1%), rupture of membranes after the procedure (1–2%) and chorioamnionitis (0.01%) [6]. According to research, the prevalence of detected karyotype irregularities ranges between 11.7% and 33.9%, while an abnormal ultrasound result is the most frequent indication for amniocentesis in such cases [4, 7]. The calculated risk of trisomy 21 may be affected by three main groups of parameters, namely maternal age, maternal serum concentrations of free β-hCG and PAPP-A, and fetal ultrasound results. The woman's age, if considered alone, renders a detection rate of 30%. If biochemical markers (PAPP-A and β-hCG) are added to the test, the rate rises to almost 65%. Only after the third element, namely ultrasound parameters, is included does the detection rate reach its highest value of 90–95% of chromosomal aberration cases [8].

The risk of trisomy 21 increases with age. At the same time, women in developed countries are choosing to have offspring increasingly later. Nevertheless, there is credible evidence that the age of over 35 is a poor factor determining the development of fetal chromosomal abnormalities. In our study, the mean age in the increased risk group was 36.4 years with an SD ± 4.75, whereas women aged over 35 accounted for 66.73% of the group. By way of comparison, the mean age in the low risk group was 32.2 years with an SD ± 5.54, and the share of women aged over 35 was 32.13%.

Abnormal fetal nuchal translucency (NT) values are claimed to be especially useful in diagnosing chromosomal abnormalities, although not all reports seem to confirm that. For instance, Maket et al. studied women bearing an increased risk of trisomy 21 for correlations between PAPP-A and β-hCG on the one hand and the NT measurements on the other. They established that although the mean NT was significantly higher in fetuses with confirmed trisomy 21 than in euploid fetuses, a considerable proportion of the former group showed normal NT values. For

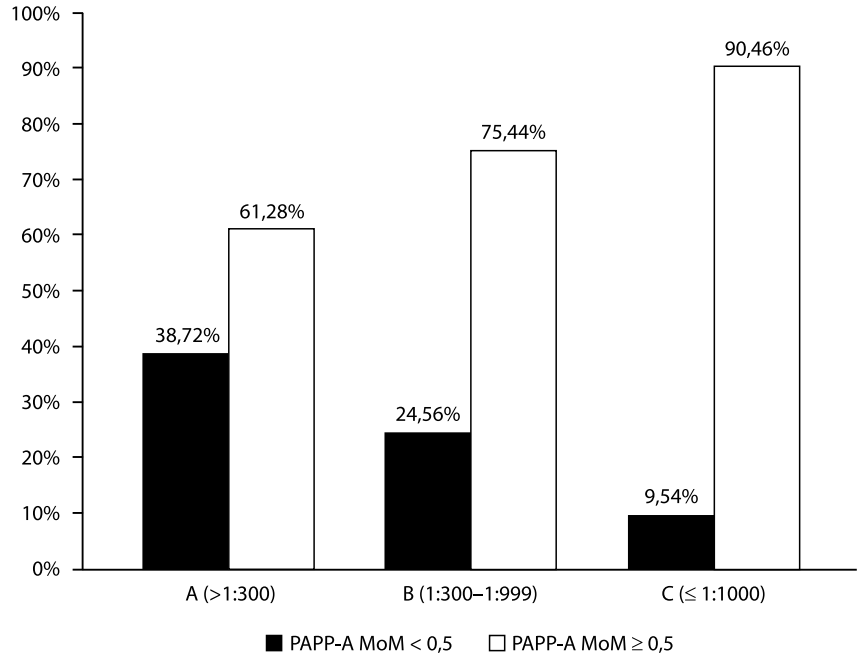

Figure 7. Correlations between PAPP-A levels and the adjusted risk of fetal trisomy 21 for groups A, B and C

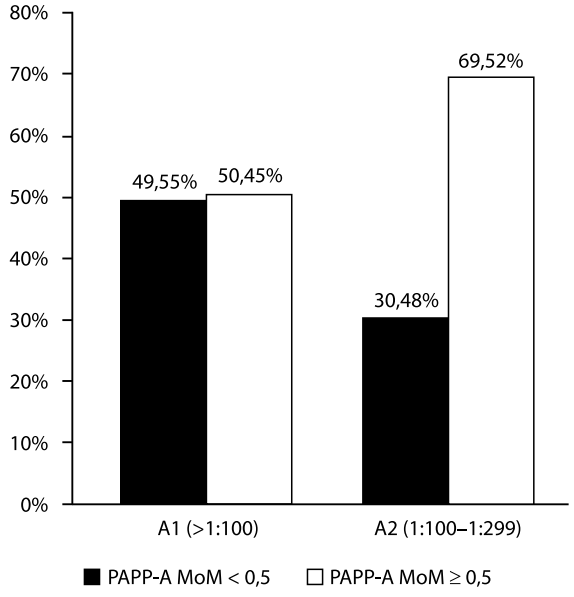

Figure 8. Correlations between PAPP-A levels and the adjusted risk of fetal trisomy 21 for subgroups A1 and A2

cases with PAPP-A concentrations of less than 0.5 and β-hCG levels of over 1.5 MoM, the figure was 44.15% and 26.5%, respectively [9].

Another element taken into account is nasal bone imaging. Wojda et al. [10] examined 941 fetuses, with trisomy 21 diagnosed in 45 of them. In their study, absent nasal bone demonstrated a mere 27% sensitivity and a 97% specificity as a marker for trisomy 21. As for its positive predictive value, it was estimated to be 35%. In their conclusions they found that nasal bone imaging was such a poor marker for aneuploidy that it should not be accounted for in risk algorithms [10].

In our study, although 6,310 patients satisfied the condition that all the ultrasound parameters should be normal, yet 514 (8.15%) of them were found to carry an increased risk of trisomy 21.

PAPP-A is a macromolecular protein, produced mainly by the syncytiotrophoblast. Due to the increase in its levels from as early as 6 weeks' gestation, the woman's system does not recognize the trophoblast as foreign tissue and, thus, allows the pregnancy to develop. Another role of PAPP-A is that of a growth-stimulating enzyme, as it releases bioactive insulin-like growth factors IGF-I and IGF-II of the insulin-like growth factor-binding protein (IGFBP) subgroup. Both these factors stimulate normal development of the placenta and, therefore, the embryo. Low PAPP-A concentrations are responsible for low IGF-1 and IGF-II expression, which may lead to impaired trophoblast invasion and, consequently, placental insufficiency. This mechanism is associated with numerous gestational complications, such as preeclampsia, PIH, gestational diabetes mellitus, IUGR, premature rupture of membranes and preterm labor [2, 3].

In our study, the measured mean PAPP-A concentration differed significantly between all the compared groups. Its value grew as the risk of trisomy 21 fell, and was 0.65 ± 0.36 MoM for group A, 0.80 ± 0.43 MoM for group B and 1.08 ± 0.60 MoM for group C.

Staboulidou et al. [11] compared PAPP-A concentrations at 11 to 13 weeks' gestation in 165 patients with preeclamp-

sia and 301 patients with parameters indicating fetal aneuploidy, including 200 cases of fetal trisomy 21. The levels of PAPP-A were similar for the trisomy 21 group (0.54 MoM) and the early-onset preeclampsia group (0.58 MoM). Significantly higher values were observed in women with the late-onset variant of preeclampsia (0.9 MoM) [11].

Spencer et al. [12] carried out a retrospective study of PAPP-A levels in 5,867 pregnant patients at 11 to 13 weeks' gestation diagnosed with preeclampsia. They established that low PAPP-A values were associated with a more severe course of the condition.

Similar conclusions were drawn by Odibo AO et al. [13] They found that PAPP-A concentrations, in conjunction with uterine artery PI and PP-13 protein (placental protein 13), may be reasonable individual predictors in women at risk of developing preeclampsia.

The second component of the so-called double marker test is human chorionic gonadotropin (β-hCG). Its production commences as early as approx. 7 days after conception, during the blastocyst stage [14]. In a physiological pregnancy, its concentration increases until approx. 10 weeks' of gestation, and subsequently falls to reach 10–20% of its peak value at 13 to 15 weeks' gestation. In trisomy 21, on the contrary, its concentration remains increased throughout the whole period (> 2.0 MoM) [2]. β-hCG is a hormone influencing a number of processes related to implantation, being also a key factor regulating angiogenesis in the chorion and the placenta by affecting angiogenic factors, e.g. vascular endothelial growth factor (VEGF) and angiopoietin (Ang-1) [14]. A high level of human chorionic gonadotropin in the second trimester of pregnancy is associated with impaired trophoblast invasion of the uterine spiral arteries, which is already observed in the first weeks after fertilization. This mechanism leads to chronic hypoxia and may be related to numerous gestational complications, such as preeclampsia and gestational hypertension [2, 15].

In our study, the mean β-hCG values for groups A, B and C were 2.38 ± 1.57 MoM, 1.64 ± 1.02 MoM and 1.15 ± 0.74 MoM, respectively.

According to the Fetal Medicine Foundation, β-hCG values exceeding 2.0 MoM may be indicative of, *inter alia*, trisomy 21, which is concordant with results reported by numerous researchers [16]. Its high concentrations are also associated with many other gestational complications. Revankar et al. [17] came to the conclusion that a high serum β-hCG level may be a predictor of gestational hypertension. When analyzing the results of tests on 7,754 women, Norwegian researchers observed a positive correlation between the total hCG content in early pregnancy and the risk of preeclampsia [18].

In most cases, positive biochemical parameters entail the application of invasive procedures. All of them, however, carry the risk of pregnancy loss. Therefore, it is essential that the results be correctly interpreted and the patients appropriately qualified for further diagnosis. On the one hand, it must be remembered that a normal ultrasound picture alone provides insufficient evidence of absent chromosomal abnormalities, on the other hand, however, it must be stressed that biochemical tests can be very useful in detecting not only genetic defects, but also other conditions of the fetus.

CONCLUSIONS

In patients with normal fetal ultrasound parameters obtained in first-trimester screening tests and an increased risk of trisomy 21, the main causes of the increased risk are abnormal PAPP-A and β-hCG concentrations. In such cases, extension of diagnosis to include other gestational complications, e.g. preeclampsia, should be considered.

It is particularly important that patients who receive their first-trimester screening results should be made aware that normal ultrasound scan parameters do not guarantee they will deliver a healthy child, but also that a positive result does not necessarily imply aneuploidy.

In a woman with a singleton physiological pregnancy, β-hCG MoM shows a positive correlation, and PAPP-A MoMa negative correlation with the adjusted risk of fetal trisomy 21.

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Misoprostol vaginal insert and Foley catheter in labour induction — single center retrospective observational study of obstetrical outcome

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ABSTRACT

Objectives: Induction of labour is one of the most common procedures used in obstetrics and its prevalence tends to increase. In patients with an unripe cervix (Bishop score < 7) pre-induction procedures are used before the start of oxytocin induction. Currently there is no consensus among scientific societies on the optimal way of pre-induction. We have conducted a single-centre retrospective observational study comparing obstetric induction results of patients after 37 weeks of gestation who were pre-induced with misoprostol vaginal insert (MVI) with 200 µg of misoprostol (Misodel — Ferring Pharmaceuticals Poland) or Foley catheter (20 F, 60 mL baloon).

Material and methods: We have reviewed the medical records of 503 patients (group A pre-induced MVI — 135 patients, group B pre-induced Foley catheter — 368 patients) who were in a single, full-term pregnancy, pre-induced due to unripe cervixes (Bishop score < 7) with a Foley catheter or Misodel (MVI 200 µg). We compared obstetric results between groups.

Results: Group A patients had a lower chance of using oxytocin in labour induction/augmentation (OR = 0.21 95% CI = 0.13–0.32), and a greater chance of surgical delivery by caesarean section (OR = 2.14 95% CI = 1.42–3.23) and vacuum extraction (OR = 3.29 95% CI = 1.08–10.00). Group A patients also had a greater chance of abnormal CTG (OR = 2.66 95% CI = 1.5–4.7) compared to group B. The groups did not differ in terms of meconium stained amniotic fluid and postpartum haemorrhage. The percentage of children born with a pH from umbilical cord blood < 7.2 and < 7.1 and newborns of medium general condition (Apgar 4–7) did not differ between the groups.

Conclusions: Neonatological results of children from Foley catheters and MVI induced delivery do not differ. Patients pre-induced with MVI rarely require labour augmentation with oxytocin. MVI-preinduced patients have a better chance of having a delivery by CS or VE compared to the Foley catheter.

Key words: misoprostol; induction of labour; dinoproston; cervical ripening; Foley catheter

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INTRODUCTION

Induction of labour (IOL) is a procedure whose prevalence varies dramatically from country to country and may range from 1.4% to 35.5% [1]. In countries with high levels of economic development, it is used in about 1 in 5 pregnant women after the 37th week of pregnancy [2]. It is undoubtedly a procedure reducing the mortality and morbidity of newborns and pregnant women in the case of specific complications in pregnancy and should be used when, in the opinion of the clinician, the risk associated with waiting for spontaneus onset of labour is greater than the risks associated with shortening the duration of pregnancy by IOL. It seems to be generally accepted that for unripe cervix (usually defined by Bishop score < 7) cervical ripening (pre-induction of labour) is necessary. At present, there is no consensus among scientific societies in the world on the optimal method of IOL, and the differences in local recommendations mainly concern cervical ripening methods due to their diversity.

Objectives

Comparison of obstetric results of patients pre-induced with misoprostol vaginal insert which contains 200 µg of misoprostol slowly released 7 µg/hour for 24 hours (Misodel — Ferring Pharmaceuticals Poland) with patients in whom a Foley catheter was used to pre-induce labour. (20 F, 60 mL).

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MATERIAL AND METHODS

We reviewed the medical records of 503 patients who delivered in the Department of Gynecology and Obstetrics of the Provincial Hospital Complex in Kielce between 4.03.2017 and 21.07.2018. All labours were induced. Indications for labour induction were in accordance with the recommendations of the Polish Society of Gynaecologists and Obstetricians [3]. The study included patients with single, full-term pregnancy (completed 37 weeks of pregnancy) who had unripe cervixes (Bishop score < 7) at the time of the decision on labour induction. MVI 200 (misoprostol vaginal insert with 200 micrograms of misoprostol) — (group A — 135 patients) or Foley catheter (group B — 368 patients) 20 F thick with 60 mL of saline balloon filling were used for labour pre-induction. Patients were sent to the delivery room at the start of regular contraction and cervical dilation of 3–4 cm or 20–24 hours after pre-induction in the absence of the start of labour. Oxytocin was used for induction and labour stimulation in a low-dose protocol, at 4–6 cm dilation amniotomy was performed. The groups were compared for obstetric results: — Apgar scores in one minute, pH from venous cord blood — in quantitative terms, as well as the percentage of children born with pH < 7.2 and < 7.1.

Table 1. Characteristics of groups

	A (n = 135)	B (n = 368)	
age [years] (mean ± SD)	27.5 ± 4.15	28.37 ± 4.72	p = 0.43
pluripara	17.78%	20.92%	p = 0.48
gestational age (median, IQR)	40 (0.8)	40 (0.8)	p = 0.93
Membranes rapture before pre-induction	17.05%	0%	p < 0.01
epidural analgesia	15%	21%	p = 0.06

SD — standard deviation; IQR — interquartile range

We compared the percentage of meconium stained amniotic fluid (MSAF), surgical deliveries [vacuum extraction (VE) and cesarean section (CS)], postpartum haemorrhage (PPH), and the duration of stay in the delivery room. We performed the statistical analysis using Statistica 13.1 (StatSoft Poland). For continuous variables we presented the arithmetic mean when the distribution was close to normal and as a median for skewed distributions. Standard deviation and interquartile range were used as measures of scatter, respectively. We compared the groups when the assumptions of near-normal distribution and equal variance were met with the Student's t-test, and when the above-mentioned criteria were not met with the Kruskallis-Wallis U test. In case of qualitative variables, we presented the data as a percentage of events in a given group and the quotient of chances of group A vs. B (OR), and compared the groups using Pearson's $\chi 2$ test, and in case of small expected numbers we used Yates correction. The differences were considered statistically significant in case of $p < 0.05$.

RESULTS

The demographic characteristics of the groups were presented in Table 1.

The groups did not differ in terms of age, fertility, median gestational age and the percentage of patients who received epidural analgesia. The Foley catheter was not used for patients with pre-labour rapture of membranes (PROM). In group A, the percentage of patients with amniotic fluid drainage prior to MVI insertion was 17.05% (23 patients). The total percentage of CS was 32% and VE 2.5%.

The results of the comparison of groups A and B are presented in Table 2.

Among patients in group A compared to group B, oxytocin was used significantly less frequently in the stimulation or induction of labour [26% vs 62% (p < 0.001, OR = 0.21 95%

Table 2. Comparison of groups

	A (n = 135)	B (n = 368)	p	OR (95% CI)
oxitocin stimulation/induction	26%	62%	p < 0.001	0.21 (0.13–0.32)
time at delivery room [h] (median, IQR)	8.75 (7.83)	8.16 (5.58)	p = 0.13	N/A
cesarean section	45.19%	27.72%	p < 0.001	2.14 (1.42–3.23)
unreassuring fetal heart rate pattern	24.55%	10.88%	p < 0.001	2.66 (1.5–4.7)
arrested labour and failed induction	19.26%	13.59%	p = 0.11	1.51 (0.90–2.55)
vacuum extraction	5.19%	1.63%	p = 0.02	3.29 (1.08–10.00)
postpartum haemorrhage	2.22%	2.17%	p = 0.97	1.02 (0.26–3.19)
meconium stained amniotic fluid	12.59%	12.50%	p = 0.97	1.00(0.55–1.82)
pH (median, IQR)	7.358 (0.085)	7.374 (0.068)	p < 0.01	N/A
pH < 7.2	3.70%	2.17%	p = 0.33	1.73 (0.55–5.38)
pH < 7.1	0%	0%	N/A	N/A
Apgar 4–7 points	2.96%	1.63%	p = 0.34	1.83 (0.51–6.61)

CI = 0.13–0.32)]. Considering only the patients and groups A and B, whose labour ended in natural ways, the difference is similar (28.3% vs. 67.67%, p < 0.001, OR = 0.18 95% CI = 0.10–0.33). Patients pre-induced with MVI compared to the group pre-induced with Foley catheter had a significantly higher chance of completion of labour through CS (OR = 2.14 95% CI 1.42–3.23) as well as VE (OR = 3, 29.95% CI = 1.08–10), the most common indication for operative labour in group A was nonreassuring fetal heart rate tracing, which was statistically more frequent in comparison to group B (OR = 2.66 95% CI = 1.5–4.7). Among patients in group A and B who gave birth by nature the chance for VE was more than 5 times higher (9.46% vs 1.88%, p = 0.001, OR = 5.45 95% CI = 1.62–17.72). Groups A and B did not differ in terms of the most frequent indication for operative delivery, *i.e.* incorrect fetal CTG recording as well as frequency of MSAF and PPH. The median pH in group A was significantly lower, although both values were within the norm range (7.35 vs 7.37 p = 0.008), groups A and B did not differ in terms of the percentage of newborns born with pH < 7.2 and < 7.1. Patients in groups A and B did not differ in terms of the time they spent in the delivery room. In the whole study group, there were no newborns born in severe condition (defined as Apgar scores in 1 minute of life ≤ 3). Therefore, we compared groups in terms of the percentage of newborns born in general medium condition (Apgar 4–7 points in 1 minute), the groups did not differ significantly.

DISCUSSION

Although induction of labor (IOL) is one of the most common interventions in obstetrics, the proportion of patients who will undergo this procedure may increase significantly in the coming years. Post-term pregnancy is one of the most common indications for induction of labour. The practice of post-term induction differs between countries [4], but usually in low-risk pregnancies this procedure is not used until the 41st week of pregnancy. A multi-centre randomized study published in 2018 indicates that induction of a low-risk pregnancy at 39th week of pregnancy may reduce the percentage of caesarean sections, hypertension-related pregnancy complications, improve patient satisfaction and through the reduction of pain without compromising neonatal outcomes [5]. Following the results of the this ARRIVE study [5]. The Society for Maternal-Fetal Medicine concluded that it is reasonable to offer elective induction to low-risk nulliparous women who are ≥ 39 + 0 weeks of gestation [6]. In the year preceding the publication in the United States, delivery after the 41st week of gestation concerned about 7% of pregnancies, and between 40 and 41 weeks of gestation about 25% of pregnancies [7]. The implementation of the results of the ARRIVE study into clinical practice may result in a significant increase in the IOL percentage in the future, through additional qualification for induction of labour of approximately 30% of pregnant women. Additional factors like increasing population rate of obesity and age of procreation will also take their role in this process [8]. Due to the large scale of the problem, research is needed to optimize the entire process of IOL in terms of woman and child safety as well as cost-effectiveness. One of the pre-induction methods used in patients with an unripe cervix is MVI. It is a therapeutic system applied to the posterior vaginal vault releasing misoprostol (prostaglandin E1 analogue — PGE1) at a dose of about 7 µg per hour for a period of 24 hours (the total dose of misoprostol is 200 µg). The unquestionable advantage of the preparation is the ease of removal in the case of complications occurring during the application and the possibility of use at the outflow of amniotic fluid, moreover, the preparation is registered from the end of 36 weeks of pregnancy. MVI is removed from the vagina at the beginning of labour or at 4 cm cervical dilation, 30 minutes after the removal of the preparation, an infusion of oxytocin can be started. In the Phase 3 key study for product registration, the product was used in patients [9] in whom cervical maturity was assessed to be 4 or less on the Bishop scale. There are no studies in the literature that directly compare the efficacy and obstetric performance of Foley catheter-induced patients to MVI, but there are studies to evaluate other forms of misoprostol in IOL. The 2016 meta-analysis showed that vaginal use of misoprostol tablets (compared to Foley catheter, vaginal dinoprostone and oral misoprostol) is associated with the highest chance of vaginal delivery within 24 hours and the highest risk of uterine hyperstimulation and abnormal CTG recording [10]. The main advantage of MVI is its ease of removal in case of the above-mentioned complications. The risk of developing hyperstimulation in a patient pre-induced with MVI is about 13% and is one of the most common complications [9]. One of the studies [11] to analyze the use of MVI for pharmacoeconomic purposes used an indirect comparison of MVI with the Foley catheter for prenatal pre-induction in accordance with Bucher's method [12]. In the study cited, dinoprostone vaginal insert (DVI — 10 mg of dinoprostone in insert releasing 0.3 mg/h —Propess, Cervidil) was the common comparator. The median time needed to achieve active phase of labour with MVI was 44% lower than with Foley catheter (95% CI 33.5–54.3%), there were no differences in the percentage of patients who gave birth vaginally, percentage of CS , frequency of PPH, MSAF and chorioamnionitis. In the group of MVI pre-induced patients, less frequent prenatal oxytocin before delivery was used (RR = 0.5 95% CI 0.39–0.62). The risk of tachysystole was almost 40 times higher compared to the Foley catheter (RR = 39.91 95% CI = 5.02–317.5) [11]. In our cohort of patients, these correlations were similar, the chance of using oxytocin was about 5 times lower in the group of

patients who were pre-induced with MVI compared to those pre-induced with Foley catheter, this regularity applied both to patients who gave birth vaginally and to those who had a CS. The limitation of our study involves the lack of data about induction to delivery time (ID — time), it only includes the time the patient spent in the delivery room, which did not differ significantly. This value is less useful especially in the context of cost-effectiveness studies. The Expadite randomized study [9] comparing MVI with DVI revealed no differences in the percentage of caesarean sections between groups and the percentage of CS in the MVI group was 26%. However, observational studies in the Polish population indicate a higher percentage of CS. In one of the works in the Polish cohort of patients published by Jagielska et al. [13] the percentage of caesarean sections was 40.58% in the primagravida and 16.13% in the plurigravida group, and the overall percentage of caesarean sections in the group of patients induced by MVI was 33%, which is lower than in our cohort. However, in the study cited above [12], the proportion of plurigravidas to the primagravidas in the studied group (31/69 = 0.45) was higher, and in our studies it was lower (24/111 = 0.21), (p = 0.018). Numerous studies show that vaginal childbirth in the history is one of the strongest predictive factors of induction efficiency and occurs in most of the predictive models published in the literature [14, 15]. In our opinion, this proportion is crucial if we want to compare the percentage of caesarean sections between individual studies. This study demonstrates the high effectiveness of misoprostol in the form of MVI as the only method without the need to augment the delivery, however, the higher risk of surgical delivery compared to Foley catheter brings some concern. Clinical and biochemical condition of newborns did not differ significantly between groups. The question remains open whether this situation was influenced by the increased number of obstetric interventions (CS and VE) undertaken by supervising obstetricians. The limitation of the study is also related to the lack of division of patients according to the indications for induction of labour.

CONCLUSIONS

Neonatological results of children from births induced with Foley catheter and MVI 200 do not differ.

Patients pre-induced with MVI 200 less frequently require oxitocin augumentation of labor.

MVI 200 pre-induced patients have a greater chance of delivery by CS and VE compering to patients pre-induced with Foley catheter.

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Pelvic venous insufficiency — an often-forgotten cause of chronic pelvic pain

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ABSTRACT

Chronic pelvic pain is a common health problem that afflicts 39% of women at some time in their life. It is a common challenge for gynecologists, internists, surgeons, gastroenterologists, and pain management physicians. Pelvic venous insufficiency (PVI) accounts for 16–31% of cases of chronic pain but it seems to be often overlooked in differential diagnosis. The aim of this article was to summarize current data concerning PVI. The embolization of insufficient ovarian veins remains the gold standard of therapy but the optimal procedure is yet to be determined. Well-designed randomized trials are required to establish the best treatment modalities.

Key words: chronic pelvic pain; pelvic venous insufficiency; pelvic congestion syndrome; embolization

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The term chronic pelvic pain (CPP) refers to a pain syndrome experienced by women that lasts more than six months and negatively impacts their everyday activities to a high degree, decreasing their quality of life. The pain is hardly associated with the menstrual cycle and pregnancy but can be exacerbated by some hormonal or behavioral conditions (sexual intercourse). The origin of this bothersome syndrome is related to a variety of pathologies. CPP afflicts millions of women worldwide and often requires a multi-specialization medical approach in the diagnostic and therapeutic process. The incidence of CPP in the UK is estimated to be 38/1,0000 patients per year [1]. Since the diagnosis of chronic pelvic pain still remains a challenge, one third of the patients who are evaluated yield no clear etiology. Subsequently, one third of those with no apparent cause of pelvic pain have pelvic venous insufficiency (PVI). Two definitions related to this condition are often mixed and covered by the term CPP. Pelvic (venous) congestion syndrome (PCS) refers to chronic pelvic pain resulting from pelvic venous distention. However, due to the lack of detailed diagnostic criteria, PCS should not be a recognized entity. Some data suggest that CPP could be secondary to PVI, with clinical manifestations of pelvic and vulvar discomfort, dyspareunia as well as lower back pain. The symptoms usually potentiate in the evening and often release in a supine position [2]. The pathophysiology of PVI is related to retrograde flow through incompetent gonadal and pelvic veins. This pathology can result from primary vulvar insufficiency, venous outflow obstruction, and hormonally mediated vasomotor dysfunction. The term PVI should be preferred as it seems to be the closest to the pathological background of this condition [3].

The problem affects women of childbearing age, suggesting its relation to hormonal status. Animal studies have shown ovarian vain distention when exposed to increased doses of estrogens. Furthermore, the exacerbation of signs and symptoms is demonstrated during pregnancy and the menstrual cycle with relief from pain occurring after the menopause [4]. Multiparity seems to be a risk factor for PVI. Vein capacity during pregnancy can increase by as much as 60%. Additionally, the anatomical changes in the pelvis and increase in weight during pregnancy may result in temporary venous obstruction [5]. From a mechanical point of view, venous dilatation could result from incompetent valves and retrograde venous flow. The venous system of the pelvis builds an anastomotic plexus with the main direction of the blood flow going from the uterus, parametrium, and mesosalpinx to the internal iliac and ovarian veins [6]. In typical anatomic conditions, the left ovarian vein drains to the left renal vein and the vena cava receives the right ovarian vein. Anatomical variabilities may cause an obstruction of the blood flow leading to venal dilatation and insufficiency. Additionally, compression of the left ovarian vein by the superior mesenteric artery may occur (nutcracker phenom-

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enon) and the left iliac vein may be compressed against the lumbar vertebral bodies by the right common iliac artery, which could result in endothelial damage, thickening, and venous congestion (May-Thurner syndrome) [5, 7]. Imaging studies have shown that an ovarian vein diameter greater than 8 mm is associated with significantly wider peri-uterine veins compared to a diameter less than 8 mm [3]. In general, two physiopathological mechanisms underlie this condition:

1. Venous outflow obstruction impeding the centripetal direction of flow.
2. Venous vulvar dysfunction and venous leakage through collateral routes, permitting flow reentry in a centrifugal (proximal-to-distal) direction [8].

One of the classifications of pelvic varicose veins distinguishes three types of vein damage:

1. Vascular wall pathology (valvular incompetence, malformations or agenesis);
2. Vascular compression effects such as the nutcracker syndrome or the May-Thurner syndrome; and
3. Pathology-induced (e.g., endometriosis or pelvic tumor) local extrinsic compression [6].

Pelvic congestion syndrome is usually related to incompetent internal pudendal and broad ligament parametrial branches, whilst vulvar and lower limb varicosities refer to incompetent branches of the circumflex femoral and obturator veins [9].

CLINICAL PRESENTATION

Most women suffer from prolonged noncyclical pelvic pain accompanied by dyspareunia and post-coital discomfort, bladder irritation, and dysmenorrhea. The pain may increase while sitting, standing, at the end of the day, or just before the onset of the menses. Additionally, vaginal discharge, vulvar edema, and tenderness of the pelvis that may contribute to depression could also be present. Vulvar varicosities and varicose veins on the posterior surfaces of the thighs may be found in physical examination. A gynecological examination often reveals uterine dyskinesia and palpatory tenderness over the ovaries. Moreover, varices of the vulva, vagina, and in perianal area are often identified. One in five women presenting with varicose veins have reflux of non-saphenous origin, with the associated pelvic vein reflux present in one in six [10]. The differential diagnosis should include endometriosis, pelvic inflammatory disease (PID), ovarian tumors, bowel pathology, fibroids, pelvic organ prolapse, urologic pathology, and porphyria [5, 6]. A case of PCS initially misdiagnosed as a hydrosalpinx was also reported [11].

DIAGNOSIS

Pelvic ultrasound (US) is typically the first-line imaging modality in the diagnostic process of patients with chronic pelvic pain. The advantage of a Doppler ultrasound examination is the ability to provide information about venous blood flow. Beard et al. have established criteria for the sonographic diagnosis of pelvic varices:

1. Visualization of the ovarian veins dilated above 4 mm in diameter,
2. Communication of the dilated tortuous arcuate veins in the myometrium with bilateral pelvic varicose veins,
3. Blood flow less than 3 cm/s, and
4. Detection of retrograde venous blood flow in the left ovarian vein [12].

The dilatation of pelvic veins > 8 mm is associated with reflux and symptoms, while the diameter 4–8 mm is usually related to asymptomatic reflux [13]. In contrary, Dos Santos et al. showed no significant difference between the diameters of competent and refluxing ovarian veins, concluding that it is not acceptable to use vein diameter as an indicator of ovarian venous reflux [14]. Park et al. [15], with a combination of transabdominal and transvaginal Doppler ultrasound in the evaluation of women with CPP and PVI, found that the mean left ovarian vein diameter was greater in symptomatic patients as compared to healthy controls (0.79 vs 0.49 cm, $p < 0.001$). Furthermore, the authors reported a sensitivity of 100% and specificity of 75% when evaluating retrograde flow in gonadal veins with the use of transabdominal ultrasound. In another study, the sensitivity and specificity of duplex ultrasound for the detection of a dilated left ovarian vein (LOV) were 100% and 57%, and for the right ovarian vein (ROV) they were 67% and 90% [16]. The high efficiency of US in detecting venal reflux was confirmed in the study by Hansrani et al. [17]. The authors reported better visibility of pelvic veins in the supine position against the semi-standing position (76% vs 64%), but better identification of pelvic vein incompetence in the semi-standing position as compared to the supine position (76% vs 68%), concluding that a complete US evaluation should be performed in both cases, including the Valsalva maneuver.

Magnetic resonance imaging (MRI) provides excellent pelvic organ imaging and can be utilized for PVI detection. Although, it carries the risk of underestimation of the extent of the pelvic venous plexus and dilatation of the gonadal vein, when performed in dorsal decubitus, several studies showed its high sensitivity and specificity reaching 88–100% and 38–75%, respectively [2, 18]. A comparable efficacy in the assessment of flow velocity and reflux in ovarian veins is observed in phase-contrast MRI. However, the time-resolved MRI achieved the best efficacy in the imaging of retrograde flow in ovarian veins, with an accuracy of 79–85% as compared to conventional venography. Time-resolved imaging (TRI) is a specialized 3D contrast-enhanced MR angiographic sequence particularly useful for venous imaging and indi-

cated in cases when a proper diagnosis is dependent on the presence and direction of the flow. TRI is effective in the detection of non-dilated incompetent ovarian veins and dilated but competent ovarian veins and, contrary to conventional venography, reveals possible arteriovenous (AV) malformations [19]. Conventional venography is an invasive procedure but still constitutes the criterion standard for the evaluation of PVI. The diagnostic criteria for PVI include:

1. Dilatation of the uterine and ovarian veins above 5 mm,
2. Presence of retrograde flow in gonadal veins,
3. Stasis of contrast material in pelvic venous plexus, and
4. Opacification of vulvar and thigh varices.
5. Retrograde flow of contrast material through the utero-ovarian arcade to the opposite side [3].

In 2019, a consensus was achieved among UK-based interventional radiologists on the optimal imaging strategy and definition of important imaging diagnostic features in women with PVI. Three consensus statements were defined:

1. Catheter venography is the gold standard investigation for the diagnosis of pelvic vein incompetence;
2. PVI should be defined as "retrograde flow along the ovarian or internal iliac veins"
3. Pelvic varices should be defined as "tortuous, often dilated, vulval, adnexal, para-uterine veins arising from incompetent internal iliac or ovarian veins" [20]. Anyhow, it seems that noninvasive imaging modalities are efficient enough to make a proper diagnosis of PVI. In summary, the most important imaging feature for the diagnosis of PVI is the demonstration of a reflux in one or both gonadal veins. Furthermore, the presence of a dilated vain which crosses the midline of the uterus seems to be the most specific sign of PVI [18].

The differential diagnosis of CPP could be long and trying as chronic pain is often the result of a multiple, overlapping pain condition, with each contributing to the generation of pain. Other gynecologic causes of pain in the pelvis should include endometriosis with adenomyosis, intra-abdominal adhesions, leyomyomas, ovarian remnant and residual ovary syndrome, gynecologic malignancies, vulvodynia, and dyspareunia. Endometriosis is the most common gynecologic cause of CPP and the coexistence of other pain syndromes in women with endometriosis is higher than in the general population [21]. However, the presence and severity of endometriosis does not often correlate with symptom severity. Adenomyosis coexists in approximately 20% of women with deeply infiltrative endometriosis, however, the relationship between adenomyosis and CPP is not fully understood [22]. As it had been shown in the Swann study, adenomyosis is frequently identified in asymptomatic women with no correlation to pelvic pain or abnormal bleeding [23]. The relationship between CPP and abdominal adhesions is poorly defined, however, there is some evidence that dense adhesions limiting organ mobility may cause pelvic pain [24]. Uterine leiomyomas are hardly related to chronic pain, but in one survey CPP was reported by 15% of women with fibroids as compared to 3% by women without fibroids [25]. Ovarian remnant syndrome refers to patients who have undergone bilateral oophorectomy with ovarian tissue inadvertently left behind, while residual ovarian syndrome is related to patients who had ovarian conservation and subsequently developed pathology. These patients may present cyclic or chronic pain with acute flare-ups [26]. Chronic pelvic pain is usually a complex condition, thus, the differential diagnosis should consider the signs and symptoms of gastrointestinal tract dysfunction such as: irritable bowel syndrome, inflammatory bowel disease, diverticular colitis, celiac disease; urinary tract dysfunction like painful bladder syndrome and recurrent urinary tract infections; neurologic causes as nerve injury or central sensitization, and musculoskeletal causes, for instance, myofascial pelvic pain syndromes or fibromyalgia. Depression and other psychiatric comorbidities, opioid dependency, and sexual abuse in the history should also be included into clinical investigation.

TREATMENT

A conservative medical management is suggested by several authors as a first-line therapy. The data are limited as they come from small randomized trials. Women treated with goserelin (3.6 mg per month), medroxyprogesterone acetate (30–50 mg per day) or an etonogestrel implant reported improved pain and venography scores [27–29].

Patients who do not respond to medical therapy can pursue invasive treatment. Embolization is the gold standard in the treatment of PVI. Insufficient ovarian veins generate a hydrostatic pressure that by embolization could be eliminated. Embolization is usually performed on an outpatient basis. The procedure of embolization of bilateral ovarian veins in PCS treatment was introduced by Edwards in 1993 [30]. Usually the transfemoral or transjugular approach is used to achieve gonadal and internal iliac pelvic vein access. Transcatheter embolization is performed after gonadal venous insufficiency is confirmed by venography. For clinical success, scrupulous and complete embolization is crucial as there are multiple tributaries of the ovarian veins and many collateral veins supporting the flow between the contralateral sides of the pelvis. For this purpose, embolization should cover the main ovarian veins with their tributaries to the level of the inferior vena cava (IVC) on the right side and a level of 3 cm from the renal vein on the left. Foamed sclerosants, such as 3% sodium tetradecyl sulfate or 5% sodium morrhuate, are often used as adjuncts to coils to reduce recanalization and treatment failure. Platinum coils are MRI compatible up to 1.5T and go undetected by

airport scanners. Usually six coils are sufficient for complete embolization but the number can vary from two to ten depending on the clinical situation [6]. Complete occlusion is obtained by gradually untwisting the fibered platinum coil (FPC) along the vessel [9]. One of the studies showed the high effectiveness of the ethylene vinyl alcohol copolymer as a sclerosant in the embolization of the ovarian vein [31]. Gonadal, vena cava, and internal iliac vein venography should be finally performed to confirm the gonadal vein occlusion with subsequent selective embolization if residual reflux into ovarian, vulvar, or thigh region varices is present [3].

The initial venous puncture and embolization process should be performed with reasonable care to avoid serious complications. Intraoperative complications include: hematoma, pneumothorax due to venous puncture, and embolization of non-target vessels (coil misplacement). Stroke caused by an emboli from coil migration or uncontrolled foam has been also reported. Delayed complications include an enlarging pneumothorax and coil migration [9].

PVI coexists with leg varicosities in approximately 76%. Embolization of incompetent pelvic veins seems to be mandatory in cases of lower limb varicose veins. In one study, embolization led to an improvement of PCS in 91%, and of lower limb varicose veins in 51% on its own [32]. Furthermore, there is high incidence of PVI in patients with recurrent varices after surgery (REVAS). Monedero et al. [8] reported a significant relief of clinical signs and symptoms of pelvic and lower extremity venous stasis after the embolization of gonadal and hypogastric mainstem and collateral vessels in patients with REVAS and PVI. In contrary, Rabe and Panier, based on a literature review, did not find satisfactory evidence for the efficiency of ovarian and pelvic vein embolization in the treatment of varicose veins of pelvic origin in patients without PCS. The Authors suggest performing foam sclerotherapy or phlebectomy in these patients [33].

Abdelsalam reported a 3-year follow-up of 11 patients with lower abdominal pain and vulvar varicosities treated with unilateral ovarian vein (OV) embolization (6 cases) or bilateral OV embolization (5 cases) with the use of spiral coils. The procedural success rate was 100%. Post-embolization pain relief and relief of vulvar varices were encountered in 70% of patients within 3 months of the procedure; however, in 1 patient (10%) the symptoms returned after 6 months. No significant complications were reported [34]. The effectiveness of ovarian vein embolization was confirmed in the study by Pyra, in which the clinical success was reflected by a decrease in the visual analogue scale (VAS) from 8 points at the baseline to 1 point at the 3-month follow-up ($p < 0.001$) [35]. Guirola et al. [36] compared the efficacy of vascular plugs (VPs) and FPCs for embolization in PVI. They found no statistically significant differences in clinical success and subjective improvement concerning dyspareunia, dysmenorrhea, urinary urgency, and pelvic pain at 1-year follow-up. VPs were associated with decreased fluoroscopy time and radiation dose but also with a significantly higher cost of therapy.

As revealed by Riding et al. [2], a substantial number of studies reporting significantly improved patient outcomes following endovascular embolization are of a relatively low quality, varying in terms of patient demographics, inclusion criteria, various embolization procedures performed, and different outcome measures used. There is also heterogeneity among the diagnostic criteria for PVI and CPP. The approach to the therapy and treatment modalities varies among vascular specialists. A survey conducted in the UK revealed that 9% of them do not regard pelvic vein reflux as a pathological entity and 11% never investigated or treated it. Indications for investigation include labial (94%) and buttock/upper thigh (70%) varicose veins where 46% used MR venography and 16% transvaginal duplex. The treatment modalities include transcatheter coil embolization (89%), sclerotherapy via the thigh varicose vein (47%), and transcatheter sclerotherapy (26%). Both ovarian veins and internal iliac tributaries were treated by 61% of responders, while 34% treated only ovarian vein reflux. Such substantial variation in the management of pelvic vein reflux requires well-designed clinical trials to establish recommendations for good clinical practice [37].

Chronic pelvic pain still remains a diagnostic and therapeutic challenge. Due to its complexity, it often requires a multidisciplinary approach involving gynecologists, vascular surgeons, and interventional radiologists. PVI, as a cause of CPP, seems to be underestimated, although it can be successfully treated when properly diagnosed. The therapeutic uncertainty raises questions about the number of veins that should be closed, the kind of embolization material used, and the treatment of vulval varicosities in patients with asymptomatic PVI. Well-designed, prospective long-term trials are needed to clarify these issues and to establish recommended guidelines for clinicians.

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Perinatal factors affecting the gut microbiota — are they preventable?

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ABSTRACT

Intestinal microbiota affects many aspects of physiological processes. The type of microbiota in the early stages of life is a critical element conditioning the development of the immune response and food tolerance. Disturbed colonization of the digestive tract resulting from the amount or diversity of bacteria colonies stimulates an inflammatory response that is associated in later life with inflammatory and autoimmune diseases. One of the elements disturbing normal colonization in the perinatal period is the operative way of delivery by caesarean section and the administration of antibiotics, used as a prophylactic measure as well as for therapeutic reasons. Based on the current state of knowledge, there is a lot of evidence demonstrating the long-term adverse effects of these modifying agents for gut microbiota, which should be kept to a minimum as far as possible.

Key words: gut microbiota; neonate; antibiotics; cesarean section

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INTRODUCTION

In recent years, increasing attention has been focused on disorders of newborn gut colonization and their impact on its further development and functioning in adulthood, however, these data are largely not systematized. Initial microbial colonization is one of the most important factors affecting a child's development having a significant impact on triggering of diseases in his adult life. Such disorders as diabetes, obesity, allergies, inflammatory bowel diseases and other autoimmune disturbances are found to be linked with abnormal colonization during the first two years of life, that commence since the perinatal period. Epigenetic changes may induce transgenerational issues with fixation of mutant genes, which functional products have effect in subsequent generations. The perinatal factors that impair proper newborn's gut colonization include caesarean section, use of antibiotics during pregnancy and delivery, among others, as a prevention of GBS infection, as well as early abandonment of breastfeeding replaced by formula feeding. A recent significant increase in both the incidence of surgical deliveries as well as use of antibiotic therapy have become the reason for more and more frequent researches on the negative impact of these procedures.

THE COLONIZATION OF THE FETUS IN UTERO

The degree of sterility of the fetal environment and the possibility of transfer of the microbiome to the uterine cavity have been the subject of scientific discussion for a long time. For many years, it was believed that the fetal amniotic environment would remain sterile until the premature rupture of membranes had place during the delivery and that the intestinal tract of the newborn remain sterile until birth. According to the above paradigm, the first contact of a child with bacteria occurs through the vertical (from the mother) and horizontal (from the environment) way, and the first signs of bacterial colonization can be recognized only a few hours after delivery [1]. The implementing and use of highly specialized methods for microbial identification, including polymerase chain reaction (PCR), undermined the theory mentioned above and allowed for precise determination of the microbiota of the fetal environment [2–4]. A modified PCR technique, *i.e.* PCR-DGGE (polymerase chain reaction — denaturing gradient gel electrophoresis) is used

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to assess bacterial DNA and the 16S rRNA sequence in the material being examined (*e.g.* stool, placenta, amniotic fluid, meconium). The presence of bacteria in placental tissue, umbilical cord and meconium was confirmed, proving the physiological contact of the fetus with microbiota [2]. In several investigations it is reported that intrauterine colonization of the fetus plays a pivotal role in the maturation of the immune system and has a significant impact on its development in the neonatal, childhood and ultimately in adulthood [5]. Sodeborg et al. [6] showed a relationship between disturbed bacterial colonization in pregnant women complicated by obesity and diabetes, with excessive birth weight of their newborns, abnormal fat distribution, overweight, immune-related dysfunction and the presence of non-alcoholic fatty liver in their adult life. Studies confirming the association between abnormal intestinal colonization and autism in children have been also published [7]. If the "hypothesis of in utero colonization" would prove to be correct, the role of the primary microbiome, environment, lifestyle and clinical factors determining human health as well as regulatory epigenetic aspects should be redefined [8]. As consequence of this assumption the clinical activities promoting proper colonization with correct transfer of microbiota in pregnant women with among others reducing the incidence of cesarean sections should be implemented [1]. Human microbiota shows an individual variability, determined by the number and diversity of bacterial strains. During pregnancy, microbes and metabolites of the microbiome are transferred to the fetus and then to the newborn during labor and lactation [9]. The composition of the microbiome is moderated by the environment, including diet, pregnancy health, metabolic diseases, gestational weight gain, termination of pregnancy, genetic factors and antibiotics used in the perinatal period [10–12].

The native intestinal microflora differs in women with normal body weight and obesity. Pregnant women with excessive weight gain have shown an increase in Firmicutes (Staphylococcus) and some Proteobacteria (Escherichia coli), especially in the second half of pregnancy [12, 13]. It has been shown that differences in microbiota species in pregnancy determine the increased birth weight of newborns [10, 12]. One of the factors affecting the developing fetus in obese women is the excessive amount of plasma endotoxins, the complex lipopolysaccharides (LPS) forming an inherent fraction of the outer cell wall of bacteria. Endotoxins may increase the intestinal translocation of bacterial products across the intestinal mucosa, contributing to the formation of an inflammatory response within the placenta and insulin resistance. Collado et al. [2], using the 16S rRNA sequencing method to identify bacterial species in amniotic fluid and placental tissue samples, found that the most common strain was the bacteria belonging to the Proteobacteria group, especially from the Enterobacteriaceae family (genera Escherichia and Shigella). The presence of the same microorganisms has been also identified in fetal vernix caseosa, meconium and faeces of newborns, although their growth was less abundant. Propionibacterium, in turn, was the second most common strain that has been found in placental tissue, amniotic fluid and meconium as well. Other bacterial strains have been also shown to be present, although in a much smaller quantity (Streptococcus, Staphylococcus, Lactobacillus) [3, 11].

EARLY BACTERIAL COLONIZATION OF THE NEWBORN AFTER BIRTH

According to Langherdries et al. [11], the postnatal contact of a newborn baby with an invasive microbiological environment and exposure to the new antigens, intestinal bacteria and their products, triggers immune mechanism within the intestinal tissues. It is likely that the modification of the quality of the host bacteria and their interaction leads to disturbed colonization, favoring the acquisition of abnormal immunity of neonates and an appearance of autoimmune and allergic diseases in later life. Various environmental factors occurring in the early neonatal period may affect the intestinal bacterial composition, potentially affecting the subsequent risk of diseases such as asthma, metabolic disorders and inflammatory bowel diseases [14]. The intestinal microbiome and adaptive immunity of infant is controlled by innate lymphoid cells (ILCs), which play an important role in coordination the inflammation, immunity, wound healing, and tissue homeostasis [15]. The above observations particularly apply to developing countries. Therefore, it should be recognized that the composition of the intestinal flora determines the type of immune response and the change in the profile of the intestinal flora, the so-called dysbiosis precedes the development of allergies in later life of infants. The mechanism responsible for the regulatory effect of a microbiota on the body is the ability to change gene expression in the host gut via the gut flora. Bacteria mainly colonize the large bowel, forming colonies containing an average of 1011 CFU (Colony Forming Unit) in 1 gram of tissue tested. Their content during life does not change much in healthy individuals, provided that there are no infectious diseases and antibiotic therapy. The anaerobic microflora as the most important is represented in 80% by strains of the genera: Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus. The factor initiating colonization of the intestine is a direct contact with maternal rectovaginal flora during delivery, followed by breastfeeding [1, 4, 16]. Usually this process lasts several weeks. During the first 48 hours, the number of bacterial cells is already very high, 104–106 CFU/mL of intestinal content, and is independent of how the neonate is fed. During this period, antibiotic therapy is a factor that

significantly disturbs the quality and quantity of colonizing bacteria. Over the next 10 days, an increase in Bifidobacteria and Lactobacillus bacteria number is observed, achieving a stable concentration of approximately 109 CFU/mL of intestinal content. In the next phase of colonization, an increase in Escherichia coli, Bacteroides spp and a much less significant increase in Clostridiae are detected. In nonphysiological conditions during the perinatal period, it is the Clostridia strains that show the strongest positive association with increased neonatal morbidity [11, 12, 17]. At the end of the first month of life, there are differences in the type of bacterial colony, depending on how the newborn is fed. In the breast-fed babies' group, the intensive intestinal growth relates to the Bifidobacteria colony, compared with only 30–40% bacterial content in formula-fed babies. By the end of the first and up to the second year of life, the content of intestinal microflora is approaching that in adulthood.

THE GUT MUCOSAL IMMUNE SYSTEM

A particularly important element of acquired immunity is the intestinal mucosa barrier. Commensal intestinal microflora determines the functions of lymph tissue, occurring within the gastrointestinal tract, the so-called GALT (gut-associated lymphoid tissue) by transmitting information to enterocytes and M cells of the intestinal lymphoid epithelium. The GALT system fulfills a dual role: it induces an immune system against bacterial pathogens and viruses through an enhanced, well-controlled pro-inflammatory response and increases complex immune mechanisms that produce antigen tolerance [12]. The initial colonization of gut microorganisms depends on the GALT maturation and is regulated by the Gastrointestinal-Blood Barrier (GIB) throughout selective absorption to the blood as well. The establishment of a balance between immunity and infections is determined by GALT and critical closure of GIB. Enterocytes, belonging to the major histocompatibility complex (MHC), can partially transfer information about the type of antigen, thus fulfilling the role of the first mucosal immunity mechanism. The interaction of enterocytes with endothelial T lymphocytes, mainly CD8 + (cytotoxic T lymphocytes) is of fundamental importance here. The degree of neonatal intestinal maturity varies individually and shows differences depending on the week of gestation [18]. Although the human intestine reaches structural maturity around 19 weeks of pregnancy and all cellular elements of the intestinal immune system are already present, its functional full maturity occurs after the onset of antigenic stimulation with colonizing microflora [18]. Studies have shown that premature exposure of the fetal intestine to microorganisms contained in the amniotic fluid may be associated with premature birth [19]. Also, the exposure of a pregnant woman's body to bacteria may affect the development and maturation of the fetal epithelium of the intestine, which in turn promotes its hypersensitivity to pro-inflammatory factors, leading, among others to necrotic enterocolitis in a newborn [20]. Newborns during labor are exposed to maternal bacteria of the vagina and anus mucosa, which are mainly facultative anaerobes, Streptococci [18]. Within a few days, Enterococci and enterobactriaceaea appear, whose activity is associated with a decrease in oxygen concentration, thus facilitating further colonization with Bifidobacteria and Bacteroides spp. and Clostridium spp. Exposure of the nascent baby to ligands of colonizing bacteria seems to be a key factor developing an intestinal tolerance . Bacterial ligands are recognized by innate immune receptors, Toll like Receptors (TLRs), whose expression is present in fetal, neonatal and adult life. TLR2 and TLR4 are present in the fetal intestinal epithelium since the 18th week of gestation [21]. As a result of receptors activation that occurs throughout the contact of newborn's intestinal epithelial cells with LPS, an endotoxin present in the outer membrane of Gram-negative bacteria, the immunotolerance process develops [22]. Perinatal contact of bacterial LPS with fetal epithelial TLR induces the production of microRNA (miR) -146a, which in turn inhibits the translation of interleukin-1 receptor-associated kinase (IRAK-1), a key enzyme in the TLR4 metabolic pathway. Low IRAK-1 concentration protects the intestine during the first contact with bacteria. After recognition of microorganisms by a newborn's intestinal epithelium, it also begins to produce cytokines such as interleukin-10 (IL-10) and transforming growth factor beta (TGF-β), which have immunoregulatory properties. Among the secreted molecules responsible for determining microbiological tolerance homeostasis, the peptides defensin (alpha and beta) and cathelicidin play an important role. In other words, there are antimicrobial peptides that regulate commensal flora and protect against pathogens. Located in the small intestinal crypts, Paneth cells produce large amounts of alpha defensin, an antimicrobial peptide which accounts for over 70% of their secretory bactericidal activity. In turn, beta defensins are mainly present in the large intestine. In contrast, cathelicidins are peptides expressed in the intestinal epithelium and produced by various intestinal cells, including neutrophils, mast cells, and epithelial cells. The bactericidal activity of these cationic peptides is associated with their amphipathic properties. Endogenous cathelicidin affects the intestinal barrier integrity and modulates the infiltration of neutrophils and macrophages during infection and bacterial sepsis [23].

MODE OF DELIVERY

One of the factors disturbing the proper colonization of the newborn's intestine is an operative delivery by caesarean section [17, 24]. Differences in newborns' gut microbiome content and diversity on the 4th and 120th day after delivery

have been shown, suggesting that the composition of the so-called early postpartum microbiota determines the microbiota during the subsequent years. The changes in gut bacteria populations diversity, however, largely disappear after six months of life of the infant. [24]. In case of caesarean section delivery, colonization of the newborn's intestine seems to occur later in relation to infants born by vaginal route. Many studies have also shown that microbiota of newborns after caesarean section differs from that of delivered by vaginal route [25, 26]. In an animal model, it was demonstrated that the initial bacterial flora of newborns is mainly composed of microorganisms of the rectovaginal origin of mothers [18]. In the case of neonates after caesarean section, the dominant colonizing microorganisms are Staphylococcus and Propionibacterium, derived from the mother's skin. On the other hand, vaginal birth is associated with colonization mainly by Lactobacillus and Prevotella strains occurring in the female urogenital area [3]. The most efficient colonization of a baby, therefore, occurs along with its inoculation with vaginal discharge microbiota during childbirth. During childbirth, the baby is also colonized by maternal digestive tract bacteria [27]. It is likely, that operative way delivery seems to be associated with a higher incidence of asthma, inflammatory bowel disease and obesity in adulthood through disturbed colonization of the neonate leading to dysbiosis [2]. Other factors affecting the development of a newborn microbiota are also the duration of pregnancy, diet during pregnancy, breastfeeding, genetic and environmental conditions.

PERINATAL ANTIBIOTIC THERAPY

Exposure to antibiotics is associated with their destructive effects on the intestinal bacterial flora. The implementation of both early short-term and prolonged antibiotic therapy may be associated with an increased risk of being overweight or obese in late childhood as well as other fat accumulation related disturbances [28]. The use of antibiotic therapy during labor in women GBS positive pregnant women as a prevention of infectious complications in newborns, including sepsis, significantly disturbs the microbial balance of the rectovaginal region, impairing the proper colonization of the newborn during delivery [29]. Also, pregnancy exposure to antibiotic therapy used in both premature babies and full-term children significantly affects the composition of the microbiota of newborns, increasing the risk of early neonatal sepsis [29]. The effect of perinatal antibiotic therapy inducing maternal vaginal and infant gut microbiota dysbiosis, is a significant decrease in the number of Lactobacillus spp. In Fouhy F. et al. [30] study, carried out in nine full-term newborns treated with ampicillin and gentamicin, the exposure to antibiotics was associated with an increase in growth of Proteobacteria colony and decrease of Actinobacteria in faeces, especially of Bifidobacterium species within four weeks, compared to unexposed for antibiotics newborns. According to Zhou et al. [29], the neonates born at term without antibiotic exposure showed no early sepsis, while in the group of neonates born at term or prematurely exposed to antibiotic therapy before labor, early-onset sepsis has been diagnosed in at least one case. Therefore, antibiotics routinely used in the perinatal period may cause gut dysbiosis, resulting in both short- and long-term diseases. Early empirical antibiotics in newborns increase the risk of necrotizing enterocolitis (NEC) and late-onset sepsis (LOS) in premature babies, while maternal intrapartum antibiotic prophylaxis (IAP) is associated with inflammatory bowel diseases, obesity and atopic diseases in infants [31]. Antibiotics have a negative impact on the homeostasis of the pregnant woman's microbiota, while the use of properly selected probiotics has a beneficial effect on both pregnant women and their offspring. In order to prevent metabolic complications and in the case of therapeutic intervention, it is recommended to use good-quality pre- and probiotics in combination with an individually selected, high-nutritious diet [32]. The use of antibiotics during the first half of a child's life is associated with systematic excessive weight gain at a later time, while this effect is noticeable when women took medicine in the second and third trimester of pregnancy, as this correlation has not been seen in the first trimester of pregnancy. The consequences of taking antibiotics were still visible in children in the age of two years [33, 34]. The type and duration of antibiotic administration is important in the context of the effects on the pregnant women microbiota and the development of the child's immune tolerance. For this reason, according to the researchers, the use of antibiotics should remain under strict control, not only because of the possibility of the evolution of antibiotic-resistant microorganisms, but also because of the long-term metabolic consequences [28].

SUMMARY

According to the current state of knowledge, preliminary microbiotic colonization begins before the onset of labor and the microbiota derived from the amniotic fluid, placenta and maternal intestine may support its development in the infant. There are perinatal factors connected with a higher risk of impairing infants and children health, such as exposure to antibiotics and cesarean section. These two factors seem to be extremely important and may have a destructive impact on the infants' microbiota, leading to dysbiosios and subsequent health disorders. The presence of Lactobacillus and Bifidobacteria species reduces the risk of intestinal inflammation, while the Firmicutes/Bacteroides strains growth increases the risk of type 2 diabetes, overweight, obesity and lipid disorders, which is particularly visible in premature babies. The gut of infants born by cesarean section are mostly

colonized by environmental skin bacteria with decreased diversity. Also, cesarean section results in delayed colonization, leading to weaken beneficial immunomodulatory signals.

It is extremely important for both women and physicians to be aware of the harmful use of antibiotic therapy in unjustified cases, as well as the use of cesarean section in conditions where there are no obstetric contraindications to terminate pregnancy by nature. The use of antibiotics should remain under strict control, not only because of the possibility of the evolution of antibiotic-resistant microorganisms, but also because of the long-term metabolic consequences. Considering the above facts may contribute to reducing the incidence of lifestyle diseases.

Conflict of interest

The authors declare no conflict of interest.

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RECOMMENDATIONS

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Recommendations of the Group of Experts of the Polish Society of Gynecologists and Obstetricians regarding gynecological examination and treatment of a minor person (01.01.2020)

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The recommendations present the current knowledge and procedures, which can be modified and changed in some cases, after careful analysis of a given clinical situation, which in the future may become the basis for their modification and updating.

According to the regulations of the Civil Code, a minor is a person under the age of 18. Exceptionally, a woman who turns 16 and gets married with the consent of the family court becomes an adult.

The gynecological examination is one of the most intimate procedures that can cause embarrassment in many women. Before visiting a gynecological clinic, a minor should be prepared for the examination by the mother, which will allow them to feel confident, reduce anxiety, and improve the minor's well-being before the examination.

Gynecological examination of a minor should be carried out by an experienced pediatric gynecologist. However, in emergency cases, injuries of the genital organs (*i.e.* emergent conditions), this examination can be performed by an experienced obstetrician-gynecologist or a doctor of other specialization who will provide proper care to the examination and medical documentation.

The indications for a gynecological examination of a minor are:

- abnormal vaginal bleeding;
- abdominal/lower abdominal pain; injuries of the genital organs, pelvis;
- suspected malformation of the genital organs;
- pubertal disorders;
- menstrual disorders;
- genital organ infections;
- suspected sexual abuse
- preventive examinations (not only in sexually active minors).

MEDICAL HISTORY

Taking medical history should be conducted with a minor patient and her representative present during the gynecological visit. According to the regulations of the Family and Guardianship Code, the legal representative is, in principle, the parent or legal guardian.

The interview should include:

- the minor's reason for visiting a gynecologist — symptoms;
- gynecological history: the course of the somatic and sexual development, the date of the first and last menstruation, characteristics of the menstrual cycle, sexual initiation, contraception, history of sexually transmitted infections;
- systemic diseases or other current diseases;
- illnesses and surgical procedures, treatment used;
- the course of pregnancy and childbirth, the neonatal period;
- family history of diseases: cardiovascular diseases, obesity, metabolic diseases, endocrinopathies, cancer;
- socioeconomic conditions of the family

GENERAL PHYSICAL EXAMINATION

The physical examination of a minor should be carried out in an atmosphere ensuring full privacy and intimacy, and with respect for the dignity of the examined person. At the outset, the purpose and nature of the study should be clarified. The general physical examination should evaluate:

- general and emotional condition;

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- physical development (body weight, height, body mass index (BMI), body type, proportions, presence of dysmorphic features, malformations);
- the skin of the whole body (excluding the presence of symptoms related to physical violence);
- stage of sexual development (according to the Tanner scale);
- type of hair (female type, hypertrichosis, hirsutism — degree of intensity according to the Ferriman-Gallwey scale).

GYNECOLOGICAL EXAMINATION

Gynecological examination of a minor patient can be performed in the lithotomy position, "frog position" or knee-thoracic position on the gynecological chair or the mother's lap - in the most comfortable way for the minor patient. The examination should begin with a thorough assessment of the external genitalia (pubic hair, skin and mucosa of the external genitalia - labia majora and minor, clitoris, hymen, vaginal vestibule, external urethra, medial surfaces of thighs, buttocks, perineum, anus). Then, the presence and type of vaginal discharge should be assessed and, in selected clinical cases, material should be collected for bacteriological examination.

In young children and girls who are sexually inactive, a two-handed transrectal examination should be performed to assess the reproductive organ, and in some clinical cases (*e.g.* vaginal bleeding, the presence of a foreign body in the vagina, suspected malformations), child specula (heated, moistened 0.9% NaCl solution) or a vaginoscope can be used. In sexually active girls, a gynecological examination should be performed through a vaginal speculum (appropriately selected vaginal speculum) and a two-handed examination. It is also recommended in this group of patients to collect a pap smear up to three years after sexual initiation.

In case of exceptional situations (urgent examination, genital or pelvic trauma, suspected presence of a foreign body in the vagina), a gynecological examination should be performed after administration of sedatives or under general anesthesia.

The last part of the gynecological examination is the ultrasound examination of the pelvic organs, which allows to assess: the degree of development and regularity of the genital organs, the thickness of the endometrium, the number and size of the ovarian follicles, and the detection of ovarian cysts and tumors. Ultrasound examination can be performed with a transabdominal probe with a full bladder or rectal probe (young children and girls sexually inactive) and a vaginal probe (sexually active girls).

For precise gynecological diagnostics in selected clinical situations, additional laboratory tests (*e.g.* hormonal tests, tumor markers) or imaging tests (computed tomography, magnetic resonance imaging) may also be recommended.

MEDICAL INFORMATION

After the gynecological examination, the minor should be informed about the result of the examination and the correct structure of her genital organs in a manner that is most understandable to her. A statutory representative and a minor over 16 years of age should be clearly informed about the health condition, diagnosis, proposed and possible diagnostic and treatment methods, treatment results and prognosis.

A pediatric gynecologist should also present to the statutory representative and a minor over 16 years of age the need for selected preventive examinations (including pap smear tests, with a recommendation every 12 months in sexually active minors) and the possibility of preventing HPV infections (protective vaccinations).

LEGAL ASPECTS OF TREATMENT OF MINORS

Legal issues related to the treatment of minors are regulated by: the Convention on the Rights of the Child adopted by the UN General Assembly on November 20, 1989; the Act of November 6, 2008 on the rights of the patient and the Patient's Rights Ombudsman, the Act of December 5, 1996 on the Professions of a Doctor and Dentist (Chapter 5. Principles of practicing the medical profession), Penal Code (Chapter XXV Offenses against sexual freedom and decency), the Code of Criminal Procedure (art. 304), the Family and Guardianship Code (title II, Section 1a, Chapter II, Relationships between parents and children).

The Code of Medical Ethics also plays an important role as a set of deontological principles addressed to the medical community.

A minor's visit to a gynecologist should take place in the presence of a statutory representative or actual guardian. According to Art. 3 sec. 1 point 1 of the Act on Patient Rights and the Ombudsman for Patients' Rights, actual guardian means a person who, without statutory obligation, takes permanent care of a patient who requires such care due to age, health or mental state.

A minor may request a gynecological examination in intimate conditions — without the presence of a statutory representative/actual guardian, which should be recorded in the medical documentation. If the statutory representative/actual guardian does not consent to the examination without his presence, this fact should be noted in the medical documentation and the examination should be carried out in his presence.

In minors before the age of 16, gynecological examination, diagnostic and treatment procedures (including pre-

scribing contraceptives) require the consent of the statutory representative (an appropriate annotation should be made in the medical documentation). The fact of confirming the beginning of sexual intercourse should not be concealed from the legal representative of the minor. Additionally, about the fact of sexual intercourse, the doctor should notify the relevant authorities - the prosecutor's office, the police (pursuant to Art. 200 of the Penal Code and Art. 304 §2 of the Code of Criminal Procedure) in the case of minors under the age of 15.

In minors before the age of 16, gynecological examination, diagnostic and treatment procedures (including prescribing contraceptives) require the consent of the statutory representative (an appropriate annotation should be made in the medical documentation). The fact of confirming the beginning of sexual intercourse should not be concealed from the legal representative of the minor. Additionally, about the fact of sexual intercourse, the doctor should notify the relevant authorities - the prosecutor's office, the police (pursuant to Art. 200 of the Penal Code and Art. 304 §2 of the Code of Criminal Procedure) in the case of minors under the age of 15.

It is possible to carry out a gynecological examination with the consent of the "actual guardian". However, undertaking other medical activities (than examination) requires the consent of the statutory representative each time. If it is not possible to reach an agreement with the legal representative, the appropriate permission is issued by the guardianship court. The guardianship court is also authorized to issue a substitute consent in the event of an objection by the statutory representative, when medical activities towards a minor under the age of 16 are — in the doctor's opinion — are necessary to eliminate the risk of loss of life or serious injury or serious health disorders.

In minors who are 16 years of age and under 18 years of age, a gynecological examination, diagnosis and treatment (including prescription of contraceptives) requires a parallel (double) consent — both the minor and his/her legal representative (please put an appropriate note in the medical records). Lack of consent of any of the above persons causes the illegality of the health service.

It is also possible to conduct examination on the basis of the parallel consent of a minor and his de facto (actual) guardian in the absence of a statutory representative.

If one of the persons authorized to give parallel consent (a minor over 16 years of age or their statutory representative/actual guardian) objects to the provision of a health service, including an examination, a consent of the guardianship court is required.

The consent to diagnostic and therapeutic procedures involving increased risk should be given in writing.

An emergency situation (requiring immediate medical assistance), when it is not possible to communicate with the legal representative/actual guardian of the minor, allows the doctor to conduct an examination or provide another health service with no increased risk. In this situation, the doctor, if possible, should consult another doctor and note this fact in the medical records.

An emergent situation which is connected with the risk of loss of life, serious injury or serious health impairment allows for high-risk medical activities to be carried out without appropriate consent, if obtaining the consent of the guardianship court is impossible in a short time. In such cases, the physician is obliged to consult another physician, possibly of the same specialty, and notify the statutory representative, actual guardian or the guardianship court about the performed activities.

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CLINICAL VIGNETTE

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Laparoscopic treatment of a mature teratoma with a fistula into the rectum — a NOTES technique

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INTRODUCTION

Teratomas are common, mostly asymptomatic benign tumors with an incidence rate of 10%–30% [1, 2]. Perforated teratomas with subsequent formation of a fistula into adjacent organs are extremely rare [3]. Fistulas most often connect to the bladder, ileum, sigmoid, transverse colon, and rectum. Gastrointestinal symptoms such as diarrhea/hemodiarrhea, fatty stools with or without hairs, and the massive expulsion of gases suggest perforation to the large bowel. So far, 45 perforated teratomas have been described (Tab. 1) [4]. Pre-operative diagnosis of malignant transformation is supported by the presence of risk factors (age > 45 years, tumor size of > 10 cm, elevated tumor markers) [5]. Computer tomography (CT) and magnetic resonance imaging (MRI) are recommended to detect teratomas and estimate possible complications (torsion, rupture, malignant transformation) [2].

CASE

In a 49-year-old woman experiencing abdominal pain, hemodiarrhea and the discharge of hairs in her stool, a CT scan detected a thick-wall cyst (7.8 × 7.4 × 7.3 cm), with fluid and air bubbles inside, located in the left adnexa. The cyst was adherent to the rectum on a length of 6 cm. Typical criteria and the presence of calcified tooth-like formations suggested teratoma. Inflammation of the surrounding fatty tissue was seen, but no enlarged lymph nodes in the pelvis were found. During a colonoscopy, an 11 mm fistula to the anus with passage of hairs through the fistula was detected. CA 125 and other blood parameters were normal, but CRP was elevated. Preoperatively, mature teratoma perforated to the rectum was suspected. Surgery plan included a total laparoscopic hysterectomy (TLH); tumor resection; resection of the rectum wall damaged by perforation; transvaginal extraction of the specimen and transanal laparoscopic anastomosis.

During surgery (Supplementary Video), we found a 10 cm tumor, strictly adherent to the pelvic sidewall laterally, the uterus and broad ligament of the uterus posteriorly, and the sigmoid and rectum inferiorly. First, the tumor was dissected from the sidewall (Fig. 1A) and separated from the uterus. The left broad ligament of the uterus and left proper ovarian ligament was coagulated and cut. The same was done with the left round ligament of the uterus and salpinx. TLH with the bilateral adnexectomy was performed. The uterus and the tumor were removed through the vagina (Fig. 1 B). The superior rectal vessels were separated from the mesosigmoid, and then clipped and cut. Partial resection of the sigmoid and upper part of the rectum with the fistula was carried out using a linear stapler (Fig. 1C). The fistula opening was found 15 mm from the distal margin of the resected rectum. The resection of the sigmoid colon was done extracorporeally. The part of the bowel with fistula planned to resection was removed from the abdominal cavity through the vagina, and the proximal margin of the resected bowel was established due to the limit of the proper vascularization of the bowel. Then a segment of the bowel with fistula was cut out of the vagina, and the anvil of the circular stapler was fixed in the saved part of the sigmoid colon. The anvil was introduced into the abdomen through the vagina. The transanal anastomosis of the distal (rectal) and proximal (sigmoid) part of the bowel with the fixed anvil was done intracorporeally (Fig. 1D). Finally, the vagina was sutured. No bowel leakage was detected post-surgery. A histopathological examination confirmed a mature teratoma of the left ovary; in the right ovary — an adenomatoid tumor of 5 mm in diameter; in the resected bowel of the length of 10 cm — inflammation, hemorrhage, fibromatosis in the para-bowel tissue and the wall of the bowel. Two lymph nodes with signs of chronic reactive inflammatory changes were harvested. No complications occurred in the short- and long-term after surgery. The patient returned to full health and social life.

The presentation of this case shows that laparoscopy is equally useful as laparotomy for the treatment of pathologies of the genital and intestinal tract. It allows for perfect visualization and good access to all spaces in the abdomen and pelvis. Laparos-

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copy minimizes trauma but ensures a similar level of radicality as laparotomy. Furthermore, cooperation between surgeons and gynecologists in complicated pathologies, such as the case presented, here is of high importance.

Table 1. Reported cases of ovarian teratoma complicated with fistula formation (updated and adapted from Kizaki et al. [4])

Author	Age	Symptoms	Organs	Urgent	Approach	Method	Cause of fistula
Park; Endoscopy 2006: 38 Suppl 2: E36	41	Intermittent abdominal pain during defecation	Sigmoid colon	no	LT	Right adnexectomy, anterior resection of rectum	IF
Wickremasinghe; Ceylon Med J 2010; 55: 133	39	Abdominal pain, hematochezia	rectum	no	LT?	Resection of left ovary, anterior resection of rectum	IF
Singh; Endoscopy 2012:44 Suppl 2 UCTN: E260.	23	Abdominal pain, hairs in urine and stools	Rectum, bladder	no	LT	Excision of cyst, closing of bladder and colon defect	IF
Chong; World J Gastroenterol 2011; 17: 3659	85	Severe pain, fever Constipation, weight loss in past 6 months	Sigmoid, colon	yes	LT	Explorative	MAL
Atalay; Gynecol Obstet Invest 2015; 80: 64	20	Foul-odor groin and flank pain	Cutaneus in low abdomen	no	LAP Conv LT	Excision of cyst, closing fistula	IF
Kim; Obst Gynecol Sci 2017; 60: 383	17	Afebrile, abdominal tenderness	Middle rectum	no	LAP	Enucleation of cyst, closing fistula	IF
The present case; 2018	47	Hairs in fatty stools	Rectum	no	LAP	TLH, anterior resection of rectum	IF

IF — inflammation; MAL — malignant; Conv — conversion to; LT — laparotomy; LAP — laparoscopy; TLH — total laparoscopic hysterectomy

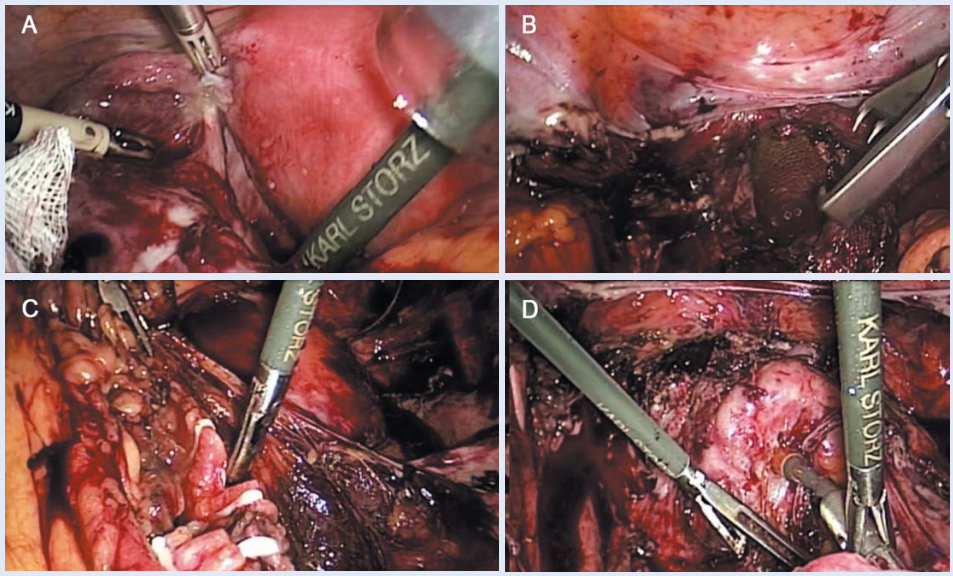

Figure 1. Laparoscopic procedure. **A** — dissection with resection of dermoid cyst; **B** — extraction of the uterus through the vagina; **C** — bowel resection; **D** — end-to-end bowel reconstruction

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