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In memoriam: Professor **Ronit Haimov-Kochman**

Born on October 22, 1965, in Tel Aviv, Israel, Professor Ronit Haimov-Kochman was the daughter of Rafael and Denise Haimov. She married Tuvia Kochman on September 15, 1986. They had three children: Yoav, Naama and Amit.

Ronit studied at the Hebrew University Hadassah Medical School in Jerusalem (1986–1989), graduating as a medical doctor in 1989. After completing her military service as a captain in the Israeli Defense Forces (1991–1994), she became a resident at the Hebrew University Hadassah Medical Center, Jerusalem, and then senior physician (1995–2000) at the Division of Reproductive Endocrinology and Infertility. She was appointed as fellow of the university Department of Cell and Tissue Biology in San Francisco in 2003–2004. From 2004, she was a member of the Hebrew University Medical School (as Administrator teaching program 2004). She supervised the Fertility Clinic at Hadassah Mount Scopus Hospital, Jerusalem.

She published 69 manuscripts in peer-reviewed international journals. Her achievements include research into the characterization of heparanase in human placenta and in the corpus luteum, as well as immunological studies on embryo tolerance and immunity to human viruses. She was a member of the Jerusalem Obstetrics and Gynecology Society (research coordinator 2005), Israel Menopause Society, Israeli Society of Fertility and Reproduction, and the Society of Gynecologic Investigation. She was a Vice-President of the European Society of Gynecology and an active member of the board. Her medical, social and societal interests reflected her personality. For example, she conducted studies to understand the religious, social and cultural background of patients, particularly religious Orthodox women, so as to provide appropriate fertility counseling and treatment. She also documented the impact of regulatory restrictions on agricultural organophosphate pesticide use in Israel. Beyond these professional achievements, I wish to add that Ronit was very special to me, and became a close friend. We first met in 2012, when Dr Nyssen and I gave a scientific presentation at the Hadassah Mount Scopus. We were deeply involved in the supervision of the Holy Family Hospital in Bethlehem (the Palestinian maternity hospital run by the Order of Malta). We suggested that the Hadassah maternity staff might supervise the Palestinian Holy Family Hospital in Bethlehem. Ronit immediately accepted this humanitarian mission with enthusiasm and came to Bethlehem on several occasions. It was the start of a true friendship. We met again many times, in Jerusalem, Tel-Aviv, Brussels and Bethlehem, and in the various locations of the European Society of Gynecology congresses. With her kindness, combined with her dedication to her patients and her professional commitments, Ronit was a particularly impressive lady. I also met her husband, daughter and sons at their home. They were all charming and made a lovely, happy family that embraced the joy of life. Her daughter, a marine biologist, came with her mother to Brussels in September 2013.

On that occasion, during the official dinner of the ESG Congress in the Egyptian Museum of Brussels, Ronit took the initiative of modifying the speakers' seating plan, so that the Israeli and Palestinian gynecologists ended up sitting together at the same table, united in friendship by their shared interest in women's health care and humanitarian concerns, in true ESG spirit!

On behalf of the ESG, I send her husband, daughter and sons our sincere condolences, and wish to assure them that, beyond borders, and cultural differences, their wife and mother lives on in our hearts and will remain forever a great and unforgettable lady! *Rest in peace, Ronit*

Professor Jean-Michel Foidart

EDITORIAL

Dear Colleagues and Friends,

It is my great pleasure and honor to introduce this, the journal of the European Society of Gynecology, named European Gynecology and Obstetrics, or EGO.

As we embark on this new project, we must first remember, and thank, Professor Albert Netter, who founded the European Society of Gynecology more than 20 years ago. Today, the ESG is a huge society that embraces numerous experts of obstetrics and gynecology, whose contributions are central to the success of our Annual Meetings and the "Alice and Albert Netter days".

Thanks must also go to the current President, Professor Andrea Genazzani, who urged the executive board to create a new journal in order to spread new discoveries and disseminate reviews. In December 2018, I was nominated Editor in Chief of EGO. I was deeply honored and pleased to accept this crucial role, and look forward to contributing to the development of our journal. But this new challenge is possible only thanks to all of you: colleagues, reviewers and section editors, who are all actively involved in the editorial process.
Different types of manuscript may be submitted for publication, such as original articles, systematic reviews, meta-analysis studies, short reviews, case reports, editorials, statement papers, position papers and also short videos. For full information, visit our website: www.egojournal.eu

The possible topics of interest are numerous, and among them we will welcome contributions relating to: gynecological endocrinology, menopause and osteoporosis, assisted reproductive technology, obstetrics, ultrasonography and imaging, breast pathology and gynecological surgery.

The goal of our journal is, of course, to reach a high scientific level by including a variety of manuscripts covering all the gynecological and obstetrical pathologies that we encounter in our practice. This is certainly feasible, as the ESG is a multidisciplinary society and covers all the topics already dealt with by Professor Albert Netter during his career. The idea is to promote EGO as much as possible through young gynecologists at the beginning of their professional lives, and we hope that they will contribute enthusiastically with papers of their own.

I would like to warmly thank the present contributors for sending their manuscripts in good time for this first issue. I hope that the members of ESG will enjoy reading the selected articles, and will feel motivated submit their own papers in the near future.
I naturally hope that EGO will be a great success and I look forward to receiving many scientific manuscripts describing our amazing specialty of obstetrics and gynecology.

I would like to take this, our first editorial, as an opportunity to inform you, with great sadness, that in December 2018 we learned, from Professor Jean-Michel Foidart, of the death of our colleague and friend Ronit Haimov-Kochman, who was still with us as recently as last November at the Alice and Albert Netter days held in Riga. She will be deeply missed.

I hope you all enjoy reading this first issue of EGO.

Professor Michelle Nisolle
Editor in Chief, EGO
ESG Vice-President

The Alice and Albert Netter Award

Dear colleagues,

As you know the European Society of Gynecology founded a prize called Alice and Albert NETTER. It is a real opportunity to promote it in the first issue of EGO and I hope we will receive many candidatures.

Professor Michelle Nisolle

Preamble	Professor Albert NETTER took part in defining the concept of Medical Gynecology and fostered the creation and the organization of the European Society of Gynecology (ESG). A great clinician and researcher, he always taught clinical excellence and aroused the scientific curiosity of his pupils. Concerned about the perpetuation of this spirit of creativity and competence, the Professor and Mrs NETTER, through the ESG, started and financed an award aimed at encouraging original research in Gynecology.
Object of the Prize	The prize is intended to reward university research work of original level in the field of human gynecology. The purpose is to stimulate research and creativity in young and talented researchers. The work can include clinical studies, epidemiological studies or basic research in the various fields of medical gynecology. A jury will assess the eligibility of the submitted works. For the year 2021, 2 prizes (5.000 Euros) will be awarded, one for clinical research work and another for basic research work.
Researchers	The Alice and Albert NETTER Prize is intended to reward young individual researchers under 40 years of age. It does not aim at ensuring the consecration of experienced researchers or established and already scientifically recognized teams. However, the joint candidature of two or several young researchers belonging to distinct scientific units but working in collaboration on the same topic is authorized. In these cases, their individual age, the importance of their total scientific contributions as well as the proof of their scientific interaction will be evaluated by the jury. The Alice and Albert NETTER Prize will be then assigned to the group as a whole.
Work	The candidatures must be addressed - by e-mail in PDF format - to the President of the ESG (President@esgynecology.org), by March 21st 2021. They will consist of a short scientific presentation (maximum 1000 words), written in French and/or in English, which will reveal the nature and the interest of the scientific research submitted for the Prize. The candidate(s) will include: a letter of motivation describing the continuation of work and the future prospects of the candidate(s); a copy of their curriculum vitae; a list of publications, followed by a list of other works, where available. The impact factor and the scientific index of quotation must accompany the list of original works. A list of stays abroad, national and international collaborations of each candidate (not of the team), as well as national and international funds personally obtained will also have to be attached. Only the original articles published in international reviews will be taken into account. Their acceptance for publication must be certified by a letter of the editors. Literature reviews, compilations of works, etc... will not be accepted. The works submitted for publication will not be taken into account. The works completed collectively by important teams is authorized, but the essential contribution of one or more candidate(s) must be documented by original publications, where they must appear as first author, at least on certain major publications. The work submitted for the contest must not have already received a National or International prize.
The jury	It is composed of the members of the ESG Board, plus the Honorary President Prof. Clara Pellissier. The President of the ESG will be automatically appointed as the President of the jury. In case of unavailability, the President will be replaced by the Honorary President Prof. Clara Pellissier. The decisions of the jury are sovereign and the simple majority rule will apply. The President will rule in case of ex æquo. The evaluation will be carried out in two phases.
	No later than 15 days prior to the jury meeting, each member of the jury will submit their classification, in writing, to the President. Should a member fail in meeting the deadline, he or she will not be able to take part in the final vote. The evaluation, calculated on a 100 point rating scale, will be based on the following criteria:

1. Scientific excellence of the work (many publications related to the work, factor of total impact, index of quotations): 50 points maximum.
2. Excellence of the curriculum vitae (lessons, stays abroad, national and international collaborations, national and international contracts in connection with work...) 20 points maximum.
3. Importance of the research topic: 30 points maximum.
4. The individual score will be communicated during the voting meeting. The final classification and the winner nomination will be decided during the jury meeting.
5. The members of the jury whose one or more collaborators wish to apply for the Alice and Albert NETTER Prize, cannot take part in the pre-classification nor in the vote.
6. The prize will be announced during the General Assembly of the 2021 ESG Congress, and the Winner will also be granted the Congress free registration.

Periodicity	The Alice and Albert NETTER Prize is allotted every two years. The official announcement of the opening of the candidatures is carried out at the time of the Albert NETTER Days.

Anorexia nervosa and cardiovascular disease: a narrative review

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ABSTRACT

Anorexia nervosa (AN) is an eating disorder with one of the highest mortality rates among mental illnesses. Cardiovascular complications seem to account for more than half of all deaths. The aim of this review is to summarize the cardiac manifestations of AN and provide guidance for their diagnosis and management. Structural alterations, electrocardiogram abnormalities and hemodynamic changes are the most common cardiovascular manifestations in AN. High clinical suspicion and vigilance are of great importance, as cardiovascular complications in AN are potentially fatal. Avoidance of drugs with cardiovascular side effects is also crucial, while nutritional rehabilitation can stabilize and even reverse overt cardiovascular alterations.

KEYWORDS

Anorexia nervosa, cardiovascular disease, structural alterations, bradycardia, repolarization, hemodynamic.

Introduction

Anorexia nervosa (AN) is a psychological and potentially life-threatening eating disorder with a poorly understood etiology. It is mainly characterized by an abnormally low body mass index (BMI), severe self-induced malnutrition, an excessive fear of gaining body weight, and a distorted body image [1]. Increased physical activity, depression, stress thoughts, emotional disorders and amenorrhea often coexist. On the basis of their dietary behavior, patients are classically divided in two types of AN: (i) the "binge-eating/purging type", when food intake is followed by purging behaviors, such as self-induced vomiting, and (ii) the "restricting type", when food intake is kept strictly low [2,3].

The etiology of AN is believed to be multifactorial, involving genetic, environmental, psychological, sociological and cultural factors [4,5]. The average prevalence of AN has been reported to be 0.3% [6], with an increased number of diagnosed cases since 2013, when the diagnostic criteria for AN in the fifth edition of the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-V) were revised. Among psychiatric disorders, AN has the highest mortality rate, which reaches 0.3-0.5% [7-9]. It mainly affects young women aged 15-19 years in Western societies [10]. The mortality rate is 5.1 deaths per 1000 person-years [11]. At least one third of these deaths are estimated to be of cardiovascular etiology, mainly sudden cardiac death [12,13]. The literature describes a large number of cardiac abnormalities associated with AN, including pathological changes in the myocardium, valvular disorders, conduction diseases, hemodynamic alterations, abnormal lipid profile and pericardial effusion. Cardiovascular complications worsen the prognosis of AN, and thus their management constitutes a major challenge for physicians. The aim of this paper is to review the cardiac manifestations of AN and provide suggestions for their diagnosis and management.

Methods

This review is based on an extensive search in the "PubMed" database utilizing MEDLINE and the Cochrane Library. During the search, the term "anorexia nervosa" was used in combination with the terms "structural abnormalities", "cardiac mass", "cardiac chamber diameters", "ejection fraction", "valvular abnormalities", "myocardial fibrosis", "pericardial effusion", "bradycardia", "ventricular repolarization", "long QT", "QT prolongation", "QT dispersion", "heart rate", "blood pressure" and "ventricular function". We also assessed references of review articles for other relevant literature. Non-English articles were excluded. This review discusses a total of 67 references.

Structural alterations

A large number of cardiac structural abnormalities associated with AN have been described in the literature over the last two decades, including low cardiac mass, decreased cardiac chamber diameters, reduced left ventricular ejection fraction (LVEF), valvular abnormalities (such as mitral valve prolapse), myocardial fibrosis and pericardial effusion.

Several studies using Doppler echocardiography provide essential clinical information about left ventricular (LV) mass and cardiac chambers. A cross-sectional study, which assessed

cardiac function in 13 patients with AN, showed that lower BMI is correlated with decreased LV mass index (LVMI; r = 0.74, p = 0.040) as well as with smaller LVMI (r = 0.74, p = 0.004) [14]. Similar results were obtained by Escudero et al. in a large case-control study of 95 female adolescents with AN and 58 healthy controls. They showed that LV dimensions, LV wall thickness, LVMI and left atrium dimensions were decreased by 5%, 10%, 20% and 8%, respectively, in females with AN (p<0.05). They also found that the most malnourished AN patients (with a BMI≤10th percentile) were at higher risk than those with a BMI>10th percentile [15]. Another case-control study, which investigated ultrasound findings in females with eating disorders, confirmed the relationship between AN and reduced LVMI and dimensions (p<0.01) [16]. Older studies evaluating cardiovascular alterations also revealed significantly lower LVMI in patients with AN compared with healthy individuals [17-20]. Indeed, lower LVMI values are associated with LV remodeling in patients with purging-type AN. These results were derived from "two-dimensional speckle tracking echocardiographic-derived strain imaging", an emerging method for assessing LV structure and function in newly diagnosed patients before the onset of clinical symptoms of cardiac dysfunction [21].

On the contrary, data on the association between AN and LV function are less conclusive. Most studies have failed to demonstrate a significant correlation between AN and LV function despite the decreased LVMI measured in individuals with AN [14,17-19,21,22]. Yet, Lelli et al. [16] and Romano et al. [20] showed that AN is significantly associated with lower ejection fraction (p<0.01 and p<0.007, respectively).

With regard to the valvular abnormalities occurring in AN, the most frequent is mitral valve prolapse. Echocardiographic studies in 95 and 40 young females with AN concluded that the incidence of mitral prolapse in these patients was 10% and 20%, respectively, [15,23] in comparison with the 2.4% in the general population calculated by the Framingham Heart Study [24]. Similar results were obtained in a cross-sectional study, where 9 out of 40 patients with AN and 1 of 28 members of the control group had mitral valve prolapse (RR=7.8, 95% CI: 1.03–65, p=0.03) [25]. Although this association has been known for a long time, it is still unclear whether it is secondary to decreased heart muscle dimensions or whether there is another underlying mechanism [26].

The widespread use of transthoracic echocardiography has led to an increase in the number of reports of pericardial effusion (PE) cases in AN. In general, the prevalence of PE in AN fluctuates within the range 8.5%-71.4% [19,22,27]. Kastner et al. showed that 34.7% of patients with AN presented with PE, in contrast to none of the control group (p<0.001) [28]. Another cross-sectional study showed that the risk of PE among females with AN was 11.5 times higher than in control individuals (p=0.005) [25]. Risk factors for the development of PE among AN patients seem to be a lower BMI and low T3 syndrome [27-29]. PE is generally mild, asymptomatic and regresses after weight restoration [27,28]. However, a few cases which required pericardiocentesis to prevent tamponade have been reported [30,31] and in two case-report studies PE progressed to tamponade requiring surgical intervention [32,33].

Myocardial fibrosis is another structural alteration affecting patients with AN. A recent case-report study, in which the postmortem heart of a patient with AN was examined histologically, revealed diffuse endocardial and interstitial fibrosis, as well as areas with myxoid material deposition and mast cells in the background [34]. An older case-report study confirms these histological findings, reporting myocyte attenuation and foci of fibrosis [35]. Nowadays, with the widespread use of cardiac MRI, subclinical cardiac involvement can be assessed. A cross-sectional study, among 40 AN females and 28 age-matched healthy controls, found signs of myocardial fibrosis, detected as late gadolinium enhancement, in 23% of patients with AN (p<0.007). The fibrosis involved the subendocardial region in three patients and the transmural one in six [25].

Electrocardiogram abnormalities

The most frequently described electrocardiogram (ECG) abnormalities are sinus bradycardia and impaired ventricular repolarization, which is most commonly manifested on the ECG as prolongation and increased dispersion of the heart rate-corrected QT (QTc) interval.

Sinus bradycardia is conventionally defined as a heart rate of less than 60 beats per minute with a normal P wave vector on the ECG, and it is the most common ECG abnormality among AN patients, with almost 40% found to be bradycardic [36]. Also, it has recently been found that sinus bradycardia affects more patients with the restricting, as opposed to the binge-eating/purging, type of AN: 48.4% vs 38.6%, respectively (p=0.03) [37]. Yahalom et al. found sinus bradycardia, assessed by 24-hour Holter monitoring, to be the most common clinical finding in AN and reported long periods (more than 180 minutes) of bradycardia with a mean lowest heart rate of 44 ± 6 bpm. Therefore, it is suggested that bradycardia can be used as a screening diagnostic tool in young adults, especially females with weight loss [38]. Bradycardia can be attributed to autonomic dysfunction and augmented vagal tone in patients with AN; these patients, though, seem to have enhanced baroreflex sensitivity and preserved sympathetic tone [39,40].

The heart rate for hospitalization of patients with AN ranges between <40 bpm 2 and <50 bpm.3 However, high clinical suspicion and vigilance are required for AN patients with resting tachycardia, too, as this may suggest underlying acute medical illness which does not manifest with obvious clinical features [41,42].

A prolonged QT interval has long been considered a potential cause of increased risk of sudden death in AN patients due to its known association with polymorphic ventricular tachycardia, also known as torsade de pointes [43]. Older studies highlighted the relationship between AN and QTc prolongation [44-46]. In a recent study, the prevalence of QTc prolongation among patients with restrictive eating patterns during hospitalization was found to be 9.7%, and no association between prolonged QTc interval and electrolyte abnormalities was shown [47]. However, a meta-analysis, including 10 studies, failed to demonstrate prolonged QTc in patients with AN, although it showed longer, but within-normal-range, QTc intervals in

AN individuals compared with controls [48]. In line with this, a recent study by Padfield et al., in which exercise testing was used in order to unmask QT prolongation, demonstrated impaired repolarization reserve in AN patients in comparison with controls, but failed to reveal QT prolongation.49 Similar results were obtained by Frederiksen et al., namely no difference in mean QTc interval or risk of prolonged QTc between AN females and healthy controls [50]. Yet, it has to be stressed that prolonged QTc, reported in many cases, can be due to other confounding factors, such as antipsychotic drugs [51], commonly used in AN, or other concomitant provocative conditions, such as electrolyte abnormalities [52] and bradycardia [53].

QT dispersion, defined as the difference between the maximum and minimum QT interval in the 12-lead ECG, reflects inhomogeneity of myocardial repolarization and a risk of arrhythmia [54]. QT dispersion seems to be greater in patients with AN ($p<0.001$) compared with controls, but no correlation with BMI could be identified [46]. However, Krantz et al. reported that QT dispersion was markedly raised in patients with AN (66.67 ± 6.15 vs 26 ± 2.67, $p = 0.01$) especially in older patients with more chronic AN [55]. However, these changes in QT dispersion are likely to be reversed after weight restoration [56].

Other ECG findings reported in AN patients are QRS wave prolongation, PR interval prolongation, low voltage R wave in the V6 lead, sinus node dysfunction, conduction disorders and arrhythmia, and QRS wave right axis deviation [57,58].

Hemodynamic changes

Hemodynamic changes are commonly encountered in patients with AN. In a study of 214 patients with AN, heart rate was found to be less than 60 bpm in 43%, whereas, 17% had values less than 50 bpm. In addition, systolic blood pressure was less than 90 mmHg in 15% of the study population [36]. Orthostasis seems to be the commonest hemodynamic alteration encountered in AN patients. A study in 36 AN patients found a mean pulse rate of 54.4 bpm on hospital admission. Importantly, 60% and 15% of these patients had orthostatic pulse and blood pressure changes, respectively, while resolution of orthostasis was achieved after 21 days of re-feeding [59]. Both hypovolemia-induced orthostasis and starvation-related muscular atrophy significantly reduce venous return to the heart and increase the risk of dizziness and syncope; when these conditions are combined with bradycardia, the risk of such episodes is even greater.

Reduced ventricular mass is also a common finding in patients with AN, which, it has been suggested, might result in reduced ventricular function. Older studies, however, failed to provide such findings in patients with AN. On the contrary, more recent trials showed reduced cardiac function in patients with AN compared with patients with normal body weight. Specifically, a study assessing LVEF in AN patients before and after exercise noted that resting LVEF was normal before and after weight restoration. However, LV response to exercise was abnormal in all AN patients, and was normalized in all of them after 25-75% of weight restoration [60]. The reduction in muscle mass and wall thickness, along with a reduction in cardiac ejec-

tion fraction, seem to the main contributing factors resulting in cardiac dysfunction in the setting of AN [61]. Other potential factors involved in cardiac dysfunction include electrolyte abnormalities and hypoglycemia [62-64].

Conclusions

Prevention and management of cardiovascular complications should be part of the initial treatment of patients with AN. Potentially life-threatening clinical symptoms and signs, such as bradycardia, conduction abnormalities, prolonged QT interval, T-wave inversion, hypotension and dyspnea should be verified at each medical evaluation. Hospitalization for continuous monitoring and observation may be required [65]. During the re-feeding phase, frequent evaluation of plasma levels of electrolytes and minerals is highly recommended, so that potential arrhythmias can be prevented [66]. In patients considered to be at high risk of major adverse cardiac events, administration of drugs with major cardiovascular side effects should be avoided. Many commonly used drugs in psychiatry, such as common antidepressants and antipsychotics, can prolong the QTc interval and may increase the risk of sudden death [67].

Clinicians should be aware of the cardiovascular complications which often occur in patients with AN. Therefore, a meticulous evaluation and a close follow-up are recommended in all AN patients to avoid the development of overt cardiovascular disease. Importantly, correct re-feeding seems to stabilize and even reverse such complications. Therefore, timely management of reduced energy intake is of paramount importance in order to reduce the risk of AN-related morbidity and mortality.

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Re-conceptualizing fetal monitoring

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ABSTRACT

Electronic fetal monitoring (EFM) has been used extensively in labor for over 40 years despite its appreciated failure to identify, in a timely fashion, and help prevent a large proportion of cases of neonatal encephalopathy and cerebral palsy. Our analysis suggests that the poor performance of EFM derives from a fundamental misunderstanding of the differences between screening and diagnostic tests, large inter-observer variability in its interpretation as a result of very subjective classifications, failure to follow the physiology of fetal compromise, and poor statistical modeling for its use as a screening test. We have recently developed a new methodology, the fetal reserve index (FRI) which contextualizes the interpretation of EFM by 1. breaking EFM down into four components: heart rate, variability, accelerations, and decelerations; and then 2. adding increased uterine activity, and 3. risk factors (maternal, fetal, and obstetrical) to create an 8-point algorithm. In a direct comparison of the ACOG monograph criteria, ACOG Category system and the FRI, the FRI performed much better in identifying cases at risk before damage had occurred, and reduced both the need for emergency deliveries and overall Cesarean delivery rates.

KEYWORDS

Fetal reserve index, electronic fetal monitoring, cerebral palsy, neonatal encephalopathy, technology assessment.

Basic principles

Most clinicians are not aware that there exists a distinct field of technology innovation and assessment which is generalizable across disciplines and individual situations. It has its own literature, societies and meetings, [1, 2] as well as norms, models and expectations. Understanding this can avoid pitfalls commonly seen in the introduction of new techniques — pitfalls that, with a grasp of the history of previous approaches, could have been completely predicted and often prevented. First there is the phase of "development". As a generalization, new ideas have often originated from academic settings, where ideas are conceptualized, tested, possibly patented and published [1, 2]. Demand is created, and then it starts to move out into practice. As demand for a new concept expands, the originators cannot handle the demand, others want "in" on the game, and new utilizers emerge. This is the "diffusion" phase. It is well understood that, during diffusion, utilization rapidly expands, but complications often skyrocket. This is where misinterpretation of electronic fetal monitoring (EFM) fits in. These same concepts equally apply to medical therapies, surgical procedures and laboratory tests [1, 2].

Overall, the incorporation of new technologies in medicine has proceeded at a slower pace than seen in many industries. The culture of medicine, while desperately seeking new approaches to critical problems, has also been, simultaneously, notoriously resistant to radical changes. The timing of adoption of new techniques is often very variable in practice with physicians/institutions/countries ranging, across a spectrum, from being "early adopters" to "late adopters". There are many components underlying how such variability comes into play, including technological capabilities, the resources available for

bringing in new technologies, the cost/benefits of such developments, return on investment, and perceived liability reductions and exposures deriving from such moves [1, 2].

Overall, a move from one technology to its replacement requires two constructs: 1. that the new technology seems ready and is an improvement over the existing one, and 2. that providers become uncomfortable with staying with the old approach. With minimal exceptions, there is never universal agreement that the new technology should immediately replace the old one — just as there is not usually universal acceptance that a new paradigm should replace an older one. It is not a simple, mechanistic process [3]. Occasionally, the abandonment of an old practice (e.g. the use of diethylstilbestrol for prevention of miscarriage) comes rapidly because of overwhelming evidence [4]. External and political forces can also come into play. In the 1980s, testing blood samples for HIV was developed in France and gained acceptance there. Even after its implementation in France and in other countries, in the United States, such testing remained prohibited by the Food and Drug Administration. Then, all of a sudden, they announced that this testing was to go from prohibited to mandatory, essentially overnight. Needless to say, there was considerable chaos as laboratories and blood banks had to reverse course with little notice [5].

On the positive side, in the case of myocardial infarction,

the identification of the role of CPK isoenzymes changed the paradigm of diagnosis of that condition from a clinical gestalt to a "lab test" fairly rapidly [6]. Acceptance, then, rests on a combination of factors that must be in place for the process to move forward. It may depend as much on the ability to resolve practical problems and hold promise for the future as on technical assessment of evidence. To put all this in context, currently debated evolutions include the use of cell-free fetal DNA (cffDNA) versus procedures with microarrays, and panethnic Mendelian screening [6-9]; cffDNA clearly identifies an increased percentage of Down syndrome, but it comes at the cost of abandonment of diagnostic procedures in which microarray analysis could detect a far higher number of serious disorders [7,8]. Conversely, pan-ethnic Mendelian screening is still underutilized. Even in well identified risk groups such as the Ashkenazi Jewish population, such screening finds more abnormalities that are not within the typical Ashkenazi panel than are within it [9]. One possible influence on the divergence of utilization of cffDNA versus microarrays and expanded Mendelian screening has been that cffDNA was rushed into practice with high marketing budget pressures by companies with only minimal refereed publications, whereas microarrays followed a much longer, traditionally rigorous process of multiple studies, including an NIH sponsored multicenter randomized trial, before entering practice [7-10]. Similarly, EFM was adopted quickly and before many basic principles had been established and properly understood [11]. There are 7 criteria that it is generally felt necessary to consider before deciding to screen for a condition (Table 1) [12]. Not all tests currently in use, however, actually follow these guidelines, which can lead to disproportionate expectations, expenditures and complications from follow-up testing that was likely unnecessary. Furthermore, the goal of a screening program is to detect the maximum number of "affected" individuals for the least number found to be screen positive. Where to put the cutoff point is, by definition, arbitrary, but it must be maintained to ensure maximum efficiency. Specifically, a program is not judged by whether any particular patient's problem can be identified [12].

Medicine, and particularly obstetrics, involves repetitive use of both diagnostic and screening tests. Most patients and many physicians do not understand the difference. Diagnostic tests are meant to give a definitive answer, may carry risks, may be expensive, and are only meant for patients at a risk high enough to warrant their use. Conversely, screening tests are meant for "everyone", and all they are asked to do is divide individuals with high enough risk to warrant diagnostic testing from those

Table 1 Criteria for screening programs.

CRITERIA FOR SCREENING PROGRAMS
Relatively frequent disorder
Impairing or fatal
Beneficial intervention possible
Good performance metrics (high sensitivity, specificity and predictive values)
Prompt testing and follow up
Benefits outweigh costs
Voluntary and Educational

without such risk. They do not give definitive answers [12]. How well they do their job is defined by the metrics of sensitivity, specificity and positive and negative predictive value. These principles of evaluation, or performance characteristics, were introduced into practice in the 1970s by Galen and Gambino [13], and they establish the boundaries of a playing field and a scoring system within which competitors, which may possibly offer better ways of doing things, can be evaluated. Sensitivity and specificity are relatively well-known test properties. Less often used are the criteria that summarize some of the critical tradeoffs that clinicians face. One such tradeoff is the relative number of true positive cases and false positive cases, which is reflected in the ratio of the two, i.e. true positives/false positives (TP/FP), and expressed as the positive likelihood ratio (PLR). A second tradeoff involves the relative number of false negatives and true negatives, reflected in the ratio of the two, i.e. false negatives/true negatives (FN/TN), and expressed as the negative likelihood ratio (NLR). Competitive approaches should be characterized by high PLRs and fractional NLRs.

Applications to electronic fetal monitoring

Physiological basis of EFM

In 2008, the American College of Obstetricians and Gynecologists (ACOG) introduced a three-tiered "category system" (CAT system) based on the presumed presence of fetal acidemia [14]. Category I (CAT I) represents a completely reassuring tracing (i.e. absent acidemia). Category III (CAT III) suggests imminent danger (or presence of injury) and the need for immediate delivery from presumed acidemia to prevent or decrease worsening of the fetal injury. Category II (CAT II) shows "elements of concern", but it is "intermediate" (meaning non-diagnostic). There is no specific understanding of or agreement on how hypoxia or acidosis came to be present, or how much time the fetus has left before irreversible neurological injury occurs. Furthermore, and more concerning, is that there is no recommended course of action other than "continued observation". Implicit is the assumption that, without acidemia sufficient to cause neurological injury (an "essential" parameter of intrapartum injury), the fetus is otherwise "normal". The CAT II tracing has received considerable criticism and been redefined by others, but such reformulations have not successfully improved outcomes [15-17]. As per the principles articulated above, the goal of a screening program is to identify cases at high risk with enough discriminatory power to signal concern but to do so before irreversible sequelae occur [12]. Only then can EFM be a true screening test for neurological injury accompanied by the opportunity to correct the pathophysiology before irreversible fetal neurological injury occurs.

There is no obvious pathophysiological basis for the ACOG's three-tiered system in fetal heart rate (FHR) pattern surveillance. Based on the retrospective analysis of the ACOG monographs on neonatal encephalopathy and cerebral palsy (CP), the CAT system can actually only serve as a diagnostic screening test for injury that has already occurred or is in the process of occurring [18, 19]. By the time the CAT III stage is reached, it is often already too late to effectively alter the process of fetal injury, even with emergency operative delivery

(EOD). The EFM characteristics of CAT III tracings (which coincide with the acid-base values of the ACOG Monograph criteria) are: absent or sinusoidal variability of the FHR baseline, absence of FHR accelerations, FHR decelerations with late recovery, absent variability during the recovery and tachycardia (often >180 bpm), or an agonal baseline. Unfortunately, logic requires that in order to prevent neurological injury from occurring, the CAT III diagnostic criteria must be replaced by criteria that, being applicable earlier in the pathophysiology, serve to screen for the risk of neurological injury if labor were to continue without resuscitation or intervention. Therefore, it is imperative to understand the relationships among the EFM monitoring parameters since the presence of one abnormality affects the others in an associative relationship. The parameters do not exist in a vacuum as the onset of fetal hypoxia/acidosis triggers a cascade of physiological neurological changes which do not occur simultaneously. We have analyzed patients who entered labor with CAT I tracings and delivered a baby with CP without another apparent cause other than labor [20, 21]. We analyzed the degree of abnormality of the individual EFM parameters, and the timing and duration of abnormalities during the course of labor and delivery. Fetuses normal at the onset of labor that went on to develop CP demonstrate a characteristic pattern: hypoxia/ischemia, and predictable deterioration to the point of injury in association with excessive uterine activity (≥8 uterine contractions/20 minutes) (Table 2). The apparent ontogeny of hypoxia/asphyxia in

Table 2 Electronic fetal monitoring variables.

	Reassuring	**Non-Reassuring (Point A)**	**Abnormal (Point B)**
Uterine contractions	≤ 8/20 Minutes	>8/20 Mins	>12/20 MINS
FHR baseline variability	5-25 BPM	<5 or ≥15 BPM	0 BPM or >25 BPM, Sinusoidal
FHR accelerations	>15 X 15 BPM/15 Secs	<15 BPM/15 Secs	<10 BPM/15 SECS
FHR decelerations	No late return to baseline	Late return to baseline (i.e. +OCT)	Late/Prolonged decelerations
Baseline FHR (BPM)	110-160 BPM	>15 BPM Rise since admission (<160)	<110/>160 BPM, Agonal

FHR: Fetal heart rate; BPM: beats per minute

Table 3 Deterioration of electronic fetal monitoring variables during labor in cerebral palsy cases (hours to reach).

	Non-Reassuring (Point A)	**Abnormal (Point B)**	**Delivery**
Order of appearance			
Increased uterine activity	7.7 Hours	10.4 Hours	11.8 Hours
Abnormal FHR Variability	6.1 Hours	8.6 Hours	10.1 Hours
Late return to baseline	4.8 Hours	7.6 Hours	8.7 Hours
Non reactivity	3.7 Hours	6.2 Hours	7.7 Hours
Abnormal FHR baseline	0.30 Hours	3.1 Hours	4.5 Hours

FHR: Fetal Heart rate

pregnancies where fetuses are "normal/uninjured" at the onset of labor starts with the occurrence of contractions. For control patients (good outcomes), the average length of labor was 11.3 hours. For those who developed CP, it was 17.7 hours. There were several other differences in the average time to initial appearance of EFM abnormalities and the order of deterioration of EFM variables (Table 3), as there was progressive and relatively orderly loss of reassuring characteristics of EFM parameters. With traditional overall assessment of the FHR tracing, we noted, as internal benchmarks for our studies, both the point when the fetus became "no longer reassuring (which we define as Point A)" and then the point at which it became "injured (which we define as Point B)." While almost all the CP cases reached both Points A and B, only 30% of CP cases reached CAT III, and when this did occur it occurred later than Point B in every case, and most often in the 2nd stage of labor, within 20 minutes of delivery. These aspects have been discussed more extensively in our previous publications [16, 22-24]. Only by correlating the pathophysiological relationships between the onset of hypoxia/asphyxia and the pattern of deterioration of the EFM parameters can an effective EFM "screening" protocol be created. In its simplest terms, the analysis of decelerations is based on an assessment of their impact on baseline rate and variability. This was first revealed in the 1970s in the normal outcomes of fetuses with reactive positive oxytocin challenge test (OCT) results (i.e., late decelerations associated with accelerations and normal variability) and the frequent adverse outcomes in patients with nonreactive positive OCT results (i.e., late decelerations and diminished variability, tachycardia, etc.) [25]. The decelerations seen under these circumstances frequently represented fetal breathing movements and not significant asphyxia [26]. These same principles exist today. The deterioration of reassuring EFM parameters should be used to determine caution, and prompt intrauterine resuscitation (IR), and intervention when necessary, rather than waiting for the presence of a CAT III tracing and irreversible fetal neurological injury. As shown by our previous data, in the normal fetus, in the presence of contractions, reduction of oxygen availability due to impaired uterine, umbilical or cerebral blood flow begins with decelerations well before any alteration in the baseline features in response to uterine contractions. Thus, by the time variability disappears, the fetus has already spent considerable time and effort compensating for impaired oxygen availability / blood flow. To require, as the CAT system does, complete absence of variability before the pattern can be called CAT III ignores the general ontogeny of these changes which is: 1. FHR decelerations with decreasing, but not yet absent, FHR variability, 2. mild elevation of baseline rate with slow return to baseline following contractions, 3. Loss of FHR accelerations, and finally 4. fetal tachycardia (>160 bpm) or bradycardia (<110 bpm). It is a well understood axiom in medicine that one cannot treat something until it has been diagnosed. Unless the emerging changes in fetal tracings are recognized before neurological injury occurs, there are no options for earlier intervention and prevention of neurological injury. Thus, we believe that a screening method must ask how the fetus is able to tolerate essentially each contraction, from the point of view of how much "reserve" it has to withstand the next one, etc. And these two aspects must be assessed in a contemporaneous fashion.

## Statistical failures of the cat system

EFM parameters must be re-set to clearly identify (yes/no) fetuses at the precise point in time that they first suggest that the fetus is having to compensate (successfully) for diminished oxygen availability and/or impaired blood flow, instead of waiting until signs of decompensation and acidemia are present (or "diagnostic" as in the CAT III criteria). To serve as a successful screening test, the parameters must be identified as being abnormal when the characteristics have only reached "no longer reassuring" as opposed to already identified as "injured."

The CAT system is a clear screening system failure because almost 80% of the patients reach CAT II, and CAT III comes far too late in the pathophysiology. By contrast, maternal serum alpha fetoprotein screening for neural tube defects in the 1970s had a cutoff point at 2.5 multiples of the mean (MoM) that identified about 90% of affected cases for a false positive rate of 5%.27 CAT III would be equivalent to moving the aforementioned cutoff to 4.00 MoM (Figure 1). At that far right point on the distribution curve, the positive predictive value of screen-positive cases would be very high, but the false negatives would be pervasive. As mentioned, CAT III is reached too late in the pathophysiology, such that interventions would be unlikely to prevent neurological injury. The positive predictive value is very high only because many such babies have already suffered damage. The very high proportion of false negatives suggests that CAT III is incapable of preventing neurological injury. Alternatively, the CAT II cutoff would be about 0.7 MoM, whose sensitivity would be very high only because it includes so much of the population. However, with a 70-80% false positive rate, it violates the fundamental principles of screening tests mentioned earlier, rendering it clinically useless

Figure 1 CAT II and CAT III superimposed upon maternal serum alpha fetoprotein distributions for controls and neural tube defect (NTD) pregnancies. In the 1970s, MSAFP cutoff of 2.5 multiples of the median (MoM) detected 90% of NTDs for 5% false positives. CAT III would be equivalent of having cutoff of 4.0 MoM. Achieves very high positive predictive value but poor sensitivity. CAT II with cutoff about 0.7 MoM would have high sensitivity because false positive rate would approach 75%. Original figure courtesy of Dr. Howard Cuckle.

NTD: neural tube defects; CAT: category
UK Collaborative AFP Study, 1977
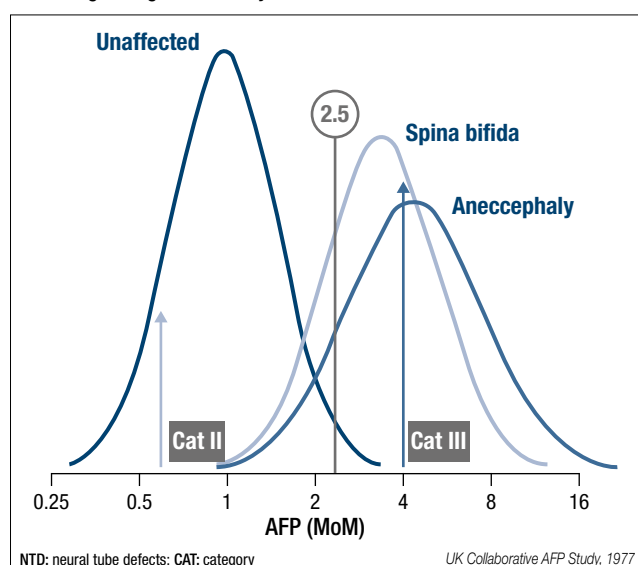
for prioritizing management towards a manageable number of screen positives for whom intervention can be successful. Further to this point, the development of the many proposed, complicated management protocols requiring sophisticated and nuanced interpretations by highly skilled practitioners to manage CAT II EFM is prima facie proof of the validity of our concerns and the failure of the CAT system as screening test and management framework [16]. Indeed, a majority of babies who develop neonatal encephalopathy and CP from the events of labor never have a CAT III tracing appreciated; they are mostly only CAT II. With either cutoff point, the performance is statistically substandard. Clinically, CAT was an improvement upon then existing approaches, but in retrospect, it had significant opportunity for further improvement.

Developing a new approach

We have developed a modified approach to the interpretation of EFM [20-24, 28]. Our risk scoring system formally includes both antepartum and intrapartum risk factors that contribute to adverse neurological outcomes in newborns. Our conceptual notion is that interpretation of FHR should be optimized not for the recognition of asphyxia, but for the prevention of injury and for avoidance, by conservative measures, of the need to "rescue". We defined a new term, the "fetal reserve index" (FRI), which is a weighted calculation of various maternal, obstetrical and fetal risk (MOFR) factors along with quantitative component FHR interpretation and the presence of increased uterine activity (IUA) (Table 4) [20-24, 28]. The FRI categorizes the various risk factors on the basis of their anticipated effect on maternal well-being, placental and cerebral perfusion, and the probability of safe vaginal delivery. All definitions used are standard as per ACOG criteria, except that we define IUA as ≥5 contractions per 10 minutes rather than 6. We have explained these aspects in detail elsewhere [20-24, 28]. The FRI was initially calculated for each 20-minute segment of monitoring. In the calculation, each of 8 categories is assigned a score of "1" if the

Table 4 Components of the fetal reserve index.

COMPONENTS OF THE FETAL RESERVE INDEX
FHR
Baseline variability
Accelerations
Decelerations
Increased uterine activity
Maternal risk factors
Obstetrical risk factors
Fetal risk factors
Each factor scored as 1 if normal, and 0 if not.
Maximum of 8/8 = 100%.
Green zone: >50%
Yellow zone: 50% to 26%
Red zone: ≤25%

category is deemed normal and "0" if it is considered abnormal (Table 4). The MOFR variables are static, that is, once point reductions in each category occur, then they remain until the fetus is delivered. The EFM and IUA variables, however, are dynamic and therefore may change as the characteristics of the FHR tracing change often in response to: 1. the clinical onset of labor complications and progression to the second stage of labor, and 2. the onset of pushing and descent of the fetal head in the lower pelvis. The FRI was calculated for the number of points divided by 8 and multiplied by 100 to give a percentage. All 8 categories being normal would result in an FRI of 100 (8/8). Loss of points would result in FRI values of: 87.5 (7/8), 75.0 (6/8), 62.5 (5/8), 50.0 (4/8), 37.5 (3/8), 25.0 (2/8), 12.5 (1/8), and 0 (0/8). For clinical simplification, the scores were then divided into 3 zones: green >50%, yellow 50% to 26%, and red ≤25%. An abnormal FRI was defined as ≤25 (corresponding to the "red zone"). Entering the red zone is not to be taken as a call for immediate delivery, but rather as a cause for immediate attention by senior staff, who can evaluate the situation. IR efforts should usually be the first course of action, such as: stopping oxytocin, repositioning the patient, increasing IV fluids, and administration of oxygen by mask. As a good analogy, reaching the red zone can be likened to defending a corner kick, as opposed to a penalty kick. Most of the time it will turn

out fine. Entering the red zone should also start a "shot clock" (as in basketball), and our management protocol is to allow up to 40 minutes to get out of the red zone. Failure to do so would start a 30 minute to delivery protocol, as per the ACOG guidelines with, as an overall resulting, delivery CP occurring usually within 1 hr of turning red which is less time than any of our CP cases were in the Red zone [20-24, 28]. To assess the performance of the FRI, our first study was a direct comparison of the postnatal ACOG monograph criteria, CAT III criteria and FRI in a data set of 60 singleton term babies who developed CP — all of whom had entered labor with CAT I tracings [15]. For none of them, even in retrospect, were there other apparent causes of their neurological compromise beyond labor issues. These infants were compared to 200 controls with normal outcomes.

In this study, the Apgar scores of the CP cases were much lower, as were their pH measurements which averaged 7.03. However, only a third 27% of the cases had a pH of <7.00, arguing against the rigidity of the ACOG monograph criteria, which required 7.00 for labor-induced issues to be considered possible [21]. The pattern of the FRI showed substantially lower scores for CP babies than controls. Only 22% of controls reached the red zone, and they were there for an average of 1 hour. CP babies "turned red" earlier in labor and stayed in the red zone for an average of over 5 hours (Figures 2, 3).

Figure 2 Representative control cases. Each column is a different patient. Time proceeds downward in 20 minute intervals.
Case A: 27 y.o. multigravida at 39 weeks with asthma. Following AROM, onset of decreased variability, loss of accelerations, FRI entered the red zone after onset of variable contractions with delayed recovery and IUA. IR performed with discontinuation of oxytocin as FRI reverted back to the yellow zone. Normal spontaneous vaginal delivery (NSVD) of 3650 g, Apgar's 8/9 with pH of 7.23.
Case B: 23 y.o. multigravida at 35 weeks with obesity and oligohydramnios. The yellow zone was entered after an epidural given following AROM. Variable decelerations developed with late recovery in 2nd stage. NSVD of 2690 g, Apgar's 8/9, pH 7.30.
Case C: 19 y.o. multigravida at 40 weeks with SROM. Experienced onset of IUA for 4 hours resulting in decreased variability, prolonged FHR deceleration, and loss of accelerations. Entered the red zone but IR was performed. NSVD of 3720g, Apgar's 8/9, pH 7.22.

Figure 3 Representative cerebral palsy cases.
Case A: 27 y.o. old primigravida at 41 weeks with hypertension and oligohydramnios admitted for induction of labor. Onset of yellow zone after 11 hours with IUA, decreased variability. Entered red zone with meconium, late recovery with decelerations, onset of 2nd stage pushing, then lost FHR accelerations and developed tachycardia before delivery. No IR performed; never reached CAT III. Apgar's 4/7, pH 6.99, BE -17.
Case B: 21 y.o. primigravida at 40 weeks with obesity, PIH, and meconium. Turned yellow with start of MgS04 with decreased variability, absent FHR accelerations. Red zone entered with epidural, onset of prolonged decelerations, and IUA but no IR performed. Five hours later stat c/section performed; birth weight 3220 g, Apgar's 1/5/7, pH 7.18, BE -14.4.
Case C: a 21 y.o. primigravida at 36 weeks with pre-eclampsia oligohydramnios, SROM and meconium. Red zone entered with onset of late decelerations, loss of variability and accelerations without IR being performed. Terminal deceleration noted. NSVD; birth weight 3050 g, Apgar's 0/0/3, pH 7.13, BE 6.00. Developed hypoxic ischemic encephalopathy (HIE) and seizures within 12 hours of delivery.

Representative controls
Time proceeds downward in 20-minute intervals
Score assess at each interval
EFM and IUA are dynamic
Risk factors are unidirectional

EFM: electronic fetal monitoring
IUA: intrauterine activity
AROM: artificial rupture of membranes
VAR: variability
ACL: loss of accelerations

Representative case
Case tend to go "RED" early and stay there for average of 5 hours
All CP cases were RED for at least 2 hours
All would have been delivered much earlier if FRI approach had been used

CP: cerebral palsy

As stated previously, all CP cases were "red" for at least 2 hours unless a sudden sentinel event occurred (i.e. prolapsed cord, sudden bradycardia) in which case, the "shot clock" protocol would have ensured patients were delivered well before the 2-hour threshold for CP damage, as we have seen in our previous studies.

Head-to-head analysis of the same cases showed that the sensitivity obtained using the ACOG criteria (pretending we knew prenatally what could only be determined postnatally) was 28%, CAT III had a sensitivity value of 45%, while for the FRI it was 100%. While ithe FRI will never stay at 100%, it was substantially better than existing methods [21]. We now have six published studies with over 1500 control patients and continue to show that the FRI has far better performance metrics than the CAT system [20-24, 28]. Overall, performing a meta-analysis of our publications and combined database, the FRI strongly outperformed CAT III (Table 5) [20-24, 28].

We have also been able to study other aspects of care. While the prediction and prevention of fetal neurological injury are of utmost importance, the incidence of emergency deliveries (EODs) is much higher and takes its own toll on patients, families, and the entire labor and delivery staff [23]. It is well appreciated that such emergency interventions have higher complication rates and exact a "price" even when everything turns out well. Our data show the FRI can anticipate the need for EODs in that, compared with controls, those needing EOD spent an average of 1 hour in the red zone. Among the cases that did not need EOD, most never reached the red zone or were there for a much shorter period of time.

We also performed an intervention series. For 400 cases, management was conducted as per usual clinical routine. Then, one of us applied the principles of the FRI to management and found that the rate of emergency deliveries was reduced from 17% to 4% (65%), emergency cesarean deliveries decreased from 8.5% to 3.3% (62%) as the utilization of IR more than doubled (20% to 47%) [22].

These findings suggest that one of the principal benefits of the FRI is earlier identification of problems that have a higher likelihood of being neutralized by earlier attention. In another study, we demonstrated that the interaction effect of understanding the risk as the FRI is a capable predictor of the chances of EODs because it accurately identifies the level of malleable risk [24]. When used as a clinical tool, the FRI increases the chances of using IR in stage 1, thereby matching risk and resources, which dramatically reduces the chances of EOD. On the other hand, the earlier one responds to risk signals calling for IR, the better, but this applies only if the risk is high [24].

Implications and expectations

Our studies suggest the FRI provides a more reliable metric for assessing risks of fetal compromise and the need for emergency intervention than those currently provided by existing methods of EFM interpretation. The CAT system is much too complex and subjective for front line management. There are too many variables that have to be informally considered, and there is no clear, straightforward method of management. Anecdotally, some experienced fetal medicine specialists have responded to our system stating that they do not need the FRI because they have always factored in "other factors" in their interpretation. Unfortunately, many physicians are not sufficiently capable of such expert subjective judgements, necessary to overcome the limitations of the CAT system. A good analogy is the diagnosis of myocardial infarction. For decades, the diagnosis was a gestalt incorporating clinical signs and symptoms, interpretation of the ECG, and non-specific blood tests. It was the discovery of the CPK isoenzymes in the 1970s (and later troponin) that turned the diagnosis into a lab test that had considerably improved metrics [6].

In developing the FRI, attention has been paid to each of these issues, the most important of which, we believe, is the notion of the role of EFM in avoiding fetal harm, which includes the need to avoid an emergency delivery during a trial of labor [23]. We have attempted to change the objective of surveillance from trying to decide the severity of asphyxia and "rescue" to "keeping the fetus out of harm's way in the first place". We do this by changing the mindset, switching from focusing attention on diagnosis of the severity of acidemia to instead recognizing the state at which the fetus cannot be guaranteed to be normal but is not yet definitively damaged. As such, EFM parameters can be used as screening criteria before fetal neurological injury actually occurs.

There is a typical pattern of FHR changes and FRI scores as the clinical situation in labor worsens. The parameters (heart rate, variability, accelerations, and decelerations) do not change independently of one another, and the order of EFM deterioration and occurrence of labor events, (e.g. meconium, 2nd stage, need for IR) is not random. Anticipating pathophysiological deterioration, the red zone is reached when at least two of the EFM screening test variables are still normal. This earlier "warning alarm," i.e. the identification of problems earlier in the pathophysiology, is the critical difference between the FRI and the CAT system, as the former generally allows more time for IR to attempt to halt the progression.

We treat the EFM tracing as a language, albeit an imperfect one to be sure. We use this "language" to query the fetus, not asking, "What is your pH? but, rather "How did you like that contraction?" This approach begins at the onset of monitoring. We use the observed pattern to define whether the FRI can distinguish between cases deemed normal vs abnormal on admission. Our approaches in our published studies have, to date, focused on how behaviorally normal neurologically intact

Table 5 Prediction of cerebral palsy.

PREDICTION OF CEREBRAL PALSY		
	FRI	CAT III
Sensitivity	100	44
Specificity	78	78
PPV	53	33
NPV	100	85
PLR	4.55	2.00
NLR	0.00	0.72

fetuses respond to the stresses and events of labor. If the fetus is determined upon admission to be already compromised, then a different set of management approaches apply (not discussed here).

Our studies to date have progressed substantially through the phase of development towards establishing proof of principle. Automation is underway to make our approach practical for frontline use. Then will come large scale studies using data in an electronic medical records format. Finally, there will be live implementation.

A priori, we attempt to safeguard the fetus by paying attention to a lowered FRI due to IUA, especially in the 2nd stage. This is independent of FHR patterns. Similarly, the FRI is lowered when data are missing. We believe it is safer to assume "abnormal" and have data to refute, rather than vice versa. We have previously shown that by focusing on limited interventions earlier in the course of fetal deterioration (especially those involved with pushing in the 2nd stage of labor), we can diminish the need to rescue the fetus for heart rate patterns when significant fetal injury has occurred before labor, and no fetal hypoxia or acidosis is currently present.

Conceptually, our philosophy is that EFM is only a screening test, not a diagnostic one. It must be treated as if it were a lab test with a single score. The more subjectivity there is in reaching a conclusion, the less precise any screening classification will be. The military has its weapon systems designed by geniuses, but they have to be capable of being operated successfully by high school only educated troops. EFM as practiced has failed miserably in this sense. It is time to recognize that just as highly experienced commercial airline pilots routinely use computer directed/assisted landing programs, even experienced obstetricians can benefit from computer assisted management of the complexities of labor and heart rate patterns.

Generalizable concepts from our approach suggest the need to see the "big picture" first and then cone down to specific circumstances. Over a decade ago, we suggested that the protocols of tertiary referrals were generally backwards, i.e. a less trained provider is deciding if a patient should be triaged upwards [29, 30]. We continue to believe and have shown that higher level evaluation and appropriate triage downward produces better, and likely cheaper, care.

This is consistent with the "inverted pyramid" that Nicolaides et al. later suggested for prenatal care [31] The realities of medical care in the current environment require better and cheaper approaches. We must continue to develop technologies to help providers make more accurate assessments of risks to empower earlier interventions.

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Fallopian tube and endometriosis: an ambiguous relationship

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ABSTRACT

Background: The Fallopian tube, or oviduct, plays an essential role in mammalian reproduction. Endometriosis affects 2 to 10% of women of reproductive age. Infertility and nulliparity are regarded as risk factors for endometriosis, therefore an increased prevalence of this affliction in the population of infertile women may be expected. The literature shows that endometriosis affects 30% to 68% of infertile women. However, its prevalence varies considerably depending on the type of infertility (i.e. male or female), the presence of chronic pelvic pain, and whether or not a previous exploratory laparoscopy was performed. The effects of endometriosis on fertility are still debated. While the impact of moderate or severe endometriosis on fertility is well established, especially in the presence of adhesions, the role of minimal or mild lesions, which are the most common in infertile women, is still controversial. Therefore, different possible underlying mechanisms have been proposed, including tubal alterations associated with endometriosis.
Conclusion: Tubal pathologies have an influence on fertility in these patients. This short review analyzes the effect of tubal endometriosis on fertility.

KEYWORDS
Oviduct, tubal endometriosis, infertility, etiopathogenesis.

Introduction

The fallopian tube, or oviduct, plays an essential role in mammalian reproduction. Endometriosis affects 2 to 10% of women of reproductive age [1]. Infertility and nulliparity are regarded as risk factors for endometriosis, therefore an increased prevalence of this affliction in the population of infertile women may be expected. The literature shows that endometriosis affects 30% to 68% of infertile women [1]. However, its prevalence varies considerably depending on the type of infertility (i.e. male or female), the presence of chronic pelvic pain, and whether or not a previous exploratory laparoscopy was performed.

The effects of endometriosis on fertility are still debated. While the impact of moderate or severe endometriosis on fertility is well established, especially in the presence of adhesions, the role of minimal or mild lesions, which are the most common in infertile women, [2,3] is controversial [4]. Therefore, different possible underlying mechanisms have been proposed, including tubal alterations associated with endometriosis.

The aim of this study is to review tubal endometriosis (on which data are limited), in order to evaluate structural and functional alterations associated with endometriosis in the fallopian tube, and determine the contribution of the oviduct to endometriosis etiopathogenesis.

Tubal location of endometriosis is rare

With only a few cases reported in the literature, tubal endometriosis seems to be a rare condition. In a well-known study, the anatomical distribution of endometriotic lesions was evaluated in a population of 182 infertile women [5]. The authors described endometriosis on the left tube in 4.3% of the pelvic locations and on the right tube in only 1.6%; tubal endometriosis thus accounted for about 6% of all the pelvic locations. In a similar study of 1101 patients, conducted by our group, tubal endometriosis was found in 50 patients (4.5%) (superficial implants or cornual occlusive nodules confirmed by pathological analysis) [6]. However, if all kind of lesions possible, superficial implants seem to be the most frequent (78% in our series) (Figures 1 and 2). In all the observed cases, lesions were located either on the ampulla or on the tubal isthmus, but never on the fimbriae. Tubal endometrioma appears to be very rare; only one adolescent case has been reported [7], while we also observed a case of endometrioma at the level of right tubal isthmus in our study (Figure 3). Rare complications associated with tubal endometriosis, such as hemoperitoneum or adnexal twisting, have been reported.

Although the oviduct is the first organ exposed to reflux of endometrial fragments, endoluminal lesions are unusual. This paradox is unexplained. The tubal epithelium is able to produce endometrial-like tissue, as demonstrated by the phenomenon of endometrialization observed in the tubal lumen after tubal sterilization [8]. Some cases of mid-segment occlusion of endometriotic nature origin have also been described. After tubal ligation (and especially after tubal coagulation), histological examination may reveal epithelial inclusions or localized endometriosis [9,10].

Several series, on the basis of the results of histological examinations, have reported a prevalence of endometriosis ranging from 12 to 14.3%. The data come mainly from patients undergoing microsurgical proximal tubal occlusion, and the vast majority of reported lesions were located in the intramural portion of either the fallopian tube or the isthmus. These are better known as proximal or cornual occlusive lesions. Without a histological examination, it is not always possible to distinguish tubal endometrial lesions from isthmic nodular salpingitis (Figure 4).

Impact of pelvic endometriosis on tubal function

The fallopian tube, by means of a still incompletely understood mechanism of communication between spermatozoa and the tubal mucosa [11,12], allows the transport, storage, capacitation and selection of the spermatozoa for fertilization. The tube also allows the uptake and reception of the oocyte. It provides the microenvironment in which fertilization and initial embryonic development take place. Finally, the fallopian tube also allows the embryo to reach the uterine cavity at the appropriate time, when endometrial receptivity is optimal. These physiological processes can be altered by different pathologies, mainly microbial infections and endometriosis.

1. Microenvironment and tubal function alterations

Abnormalities of the tubal environment associated with pelvic endometriosis are difficult to identify, requiring a lavage or an excision of the tube. Cytological analysis is also possible with

Figure 1 Implants located at the tubal level, close to the right infundibulum.

Figure 2 Tiny implants at the right tubal ampulla.

Figure 3 Endometrioma at the level of right tubal isthmus.

Figure 4 Proximal right nodule without methylene blue extravasation.

adequate instrumentation [13]. Several studies have shown modifications comparable to those observed in the peritoneum in a pro-inflammatory environment, namely:

- an increased concentration of inflammatory cells;
- an increased concentration of macrophages in the ampullary portion of the salpinx (compared with the concentration seen in sterilized women) [13,14];
- the presence of immunological cells such as leukocytes;
- the presence of cytokines in the distal portion of the fallopian tube, originating from the peritoneal fluid;
- dysregulation of prostaglandin production, i.e. increased production of prostaglandins E and F in the ampulla and the isthmus, resulting in a change in the PGF/PGE ratio.

Alterations of ciliary activity [15] and of tubal peristalsis have been observed in endometriosis, especially if it is associated with adenomyosis [16]. All these findings suggest that endometriosis may affect tubal functions.

2. Consequences on tubal function

Tubal dysfunction may occur in the presence of tubal or pelvic endometriosis. Observation of radionuclide migration through permeable fallopian tubes in infertile patients has shown that the pathway of these particles is impeded if endometriosis is present [17]. A reduction in GIFT (gamete intrafallopian transfer) in cases of endometriosis has been described. Thus, we can conclude that tubal dysfunctions observed in this condition may contribute to hypofertility, particularly in the presence of minimal or mild lesions.

a. Impact on spermatozoa migration, storage and function

Data on the tubal migration of spermatozoa in patients with endometriosis are contradictory. Spermatozoa seem to be found in smaller amounts in the peritoneal fluid of patients with endometriosis [18] and their presence has some favorable prognostic value. Other authors describe a reduction in their motility [19] or even completely immobile spermatozoa [20]. Prostaglandin F2 may be responsible for this decreased motility [21]. Sperm phagocytosis by macrophages has been reported to be increased in endometriosis [22]. Moreover, endometriosis may also affect the interactions between spermatozoa and the tubal mucosa [23]. The interactions that take place between spermatozoa and the tubal isthmus epithelium are an important step in spermatozoa migration and preparation for fertilization. Attachment of spermatozoa contributes to the storage role of this tubal portion and might reduce the risk of polyspermic fertilization. In the presence of endometriosis, more spermatozoa become attached to the epithelium of the tubal ampulla, suggesting that a consequent reduction in the number of free spermatozoa available to participate in fertilization may contribute to reduced fertility [23].

b. Oocyte uptake and transport

Oocyte uptake is an essential part of reproduction. Although the mechanism of this process is not completely known, contact between the infundibulum and the ovary (thanks to the contraction of muscles in myosalpinx and tubo-ovarian ligaments) is its first step. If this step is not capital (some oocytes are caught after transperitoneal migration), it improves the tubal migration of the oocyte. Direct contact between infundibulum cilia and the cumulus surrounding the oocyte permits oocyte uptake [24]. The journey of the oocyte in the fallopian tube lasts three days in mammals, with oocyte retention in the ampullary portion (the usual place of fertilization) lasting 72 hours. "Dialogue" between the oocyte and the tubal epithelium probably plays a role in this phenomenon, but the underlying molecular mechanisms are still poorly understood. The impact of the tubal microenvironment associated with endometriosis is also unknown.

Ciliary activity might be impaired even in endometriosis cases with apparently healthy fallopian tubes [25]. The presence of a macromolecular inhibitor has also been reported, but never confirmed by further studies [26].

Tubal abnormalities associated with endometriosis

In addition to endometriosis located in the fallopian tube, tubal abnormalities may also occur in association with pelvic endometriosis diagnosed elsewhere. The diagnosis of tubal abnormalities is based on imaging and laparoscopy. In women with endometriosis, imaging has shown contrasting performances, although the studies concerned small numbers of cases. In a study of 35 women, hysterography showed a sensitivity of 40% and a specificity of 83% in identifying tubal abnormalities associated with endometriosis [27]. A study of 42 women with laparoscopic confirmation of associated endometriosis reported better performances of contrast hysterosonography (hysterosalpingo-contrast-sonography), with a sensitivity of 85% and a specificity of 93% [28]. Finally, only laparoscopy and fertiloscopy allow correct assessment of the state of the fallopian tube, and particularly of the infundibulum.

Many fallopian tube abnormalities have been described: abnormalities of the infundibulum (phimosis, agglutination of the fimbriae, peritubal adhesions, for example), hydrosalpinx, diverticulum of the accessory infundibulum and cornual polyp, among others. They are located mainly in the infundibulum, although other parts of the tube may also be altered. Most of the abnormalities observed are considered subtle and their impact on fertility is thus uncertain. A study of 124 women submitted to laparoscopy for infertility associated with pelvic endometrial lesions evaluated their tubal state and arbitrarily classified the impact of lesions on fertility [29]. No impact of the lesions was reported in 75 of them, whereas there was a moderate impact in 32, and in the remaining 16 cases, no procreation seemed possible.

Finally, in a study of 87 women with ovarian endometriosis, surgically removed fallopian tubes were analyzed [30]. Chronic salpingitis scars were found in 33% of the cases. Unfortunately, these data have not been confirmed by other studies, but the presence of salpingitis associated with endometriosis might contribute to hypofertility.

1. Alterations of the tubal infundibulum

In a further study, 315 women with stage I/II endometriosis, no history of infection or pelvic surgery, and negative Chlamydia trachomatis serology were compared with a control group

of 152 infertile women without endometriosis [31]. Abnormalities of the fimbriae were assessed by laparoscopy. This study demonstrated a significantly increased prevalence of infundibulum alterations in the endometriosis group compared with the control group (50.2% vs 17.8%). The authors concluded that these abnormalities might contribute to the hypofertility observed in these women. However, a negative influence of tubal abnormalities on GIFT outcome was also reported [32].

Pregnancy is possible following fimbrioplasty treatment of these abnormalities. A retrospective study compared reproductive outcome after fimbrioplasty in a group of women with unexplained infertility or stage I endometriosis (n = 50) with reproductive outcome in a control group (n = 57) in whom no treatment had been administered [33]. The observed pregnancy rate was 40% in the treated group and 2.7% in the control group.

2. Other lesions found

Hydrosalpinx is usually caused by a microbial infection. There is no study in the literature reporting hydrosalpinx caused by endometriosis, although we have observed one case of hydrosalpinx associated with endometriotic nodules located on the tubal wall (Figure 5). However, an infectious cause could not be excluded in this patient, despite her negative Chlamydia trachomatis serology.

Tubal diverticulum and accessory infundibulum are rare findings, with only a few cases reported in the literature [34]. Accessory infundibulum is more often found in the ampullary portion of the fallopian tube. In a retrospective study involving 1113 women undergoing laparoscopy for infertility, only 21 cases of accessory infundibulum had been reported (1.9%) [35]. Among the 403 women with endometriosis, an accessory infundibulum was discovered in 19 (4.7%), while only 2 cases (0.3%) were identified in a control group of 701 patients without endometriosis (p = 0.001). In the 18 operated cases, 12 pregnancies followed (66.7%).

Cornual polyp (a polyp of the proximal portion of the oviduct) is composed of ectopic endometrial stromal and epithelial cells, which is the definition of endometriosis. According to hysterosalpingography data mainly obtained in infertile women, the prevalence of uterine horn polyps is 2 to 3%; on the other hand, when the diagnosis is based on the pathological analysis of hysterectomy pieces, the prevalence ranges from 1.2% to 33% [36].

In a prospective study conducted in 22 infertile women with uterine horn polyps, 4 cases were associated with anovulation and 6 cases with endometriosis 36. In line with previous observations, the authors concluded that infertile women with uterine horn polyps were more likely to have an associated endometriosis.

Role of the fallopian tube in the genesis of endometriosis

1. Regulation of menstrual reflux by the fallopian tube

Menstrual reflux through the fallopian tubes leading to peritoneal grafting of endometrial fragments is the most validated mechanism and also the one most often cited to explain the genesis of endometriosis. The importance of reflux is correlated with the abundance of menstruation and the presence of an obstacle to menstrual flow (iatrogenic cervical stenosis, obstructive malformation).

The fallopian tube plays an important role in the menstrual debris regurgitation theory (Figure 6), a notion first suggested in 1985 [37]. The reflux results from a functional asynchrony between cervical and utero-tubal junction pressure. Relative hypotonia of the utero-tubal junction has been found in women with endometriosis and the morphology of the intramural portion of the tube seems to be the reason for this hypotonia. Three morphologies have been described: linear, curved and tortuous. Two studies evaluated the risk of endometriosis based on these morphologies. The first one involved 154 dissected oviducts: the tube was tortuous in 74.02% of cases (n = 114), curved in 13.64% (n = 21) and had a linear path in 12.34% (n = 19).

Figure 5 Endometriosis implants located close to a hydrosalpinx adherent to the wall.
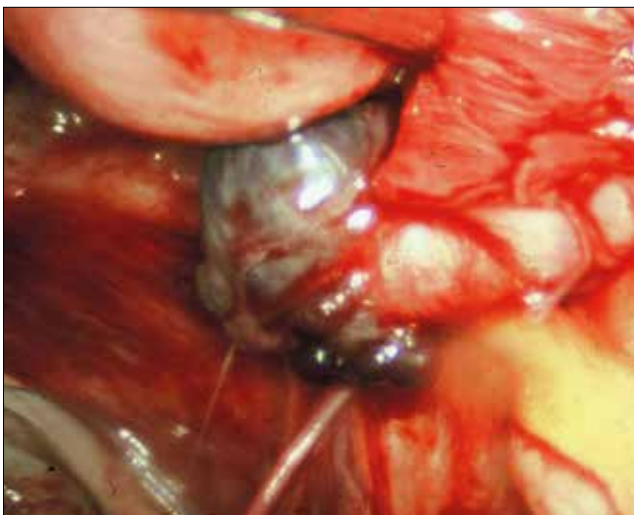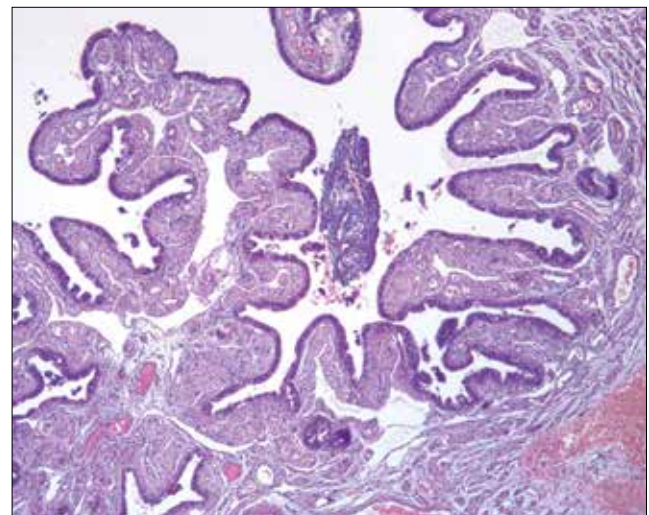

Figure 6 Presence of menstrual debris in the fallopian tube.

Endometriotic lesions were identified in 12 women with either a curved or a linear oviduct on at least one side. [38] Any woman with a bilaterally tortuous course of the interstitial part of the oviducts had no endometriosis. The second study involved 227 patients who underwent a hysterectomy, allowing a retrospective analysis of the tubal morphology and assessment of endometriosis risk [39]. Endometriosis was again less frequent in cases with a tortuous path of the intramural portion of the fallopian tube. These two studies, with concordant results, demonstrated that tortuous fallopian tube morphology diminishes the risk of endometriosis.

2. Role of the oviduct in the histogenesis of endometriosis

The ovary is the site most frequently affected by endometriosis. The hypothesis of a tubal origin of ovarian endometriosis was suggested in a study of epithelial ovarian carcinoma characterized by a tubal phenotype [40]. Other publications have confirmed the role of the fallopian tube in the origin of malignant ovarian lesions [41]. Genes (FMO3 and DMBT1) and their corresponding proteins, strongly expressed in the fallopian tube, were used as biomarkers for analyzing ovarian endometriosis in 32 patients [42]. The authors observed that 60% of ovarian endometriosis may have a fallopian tube origin and 40% an endometrial origin. This endometriotic cells migration is favored by close contact between the infundibulum and the ovary and by desquamation of tubal cells. However, the authors concluded that these data must be confirmed by further studies.

Conclusion

Tubal endometriosis seems to be rare. This location of endometriosis is found in only 4.5 to 6% of women affected by the condition. Tubal endometriosis essentially consists of superficial implants on the ampullary portion of the Fallopian tube or proximal occlusive lesions. In the presence of pelvic endometriosis, several tubal morphological abnormalities have been identified. These findings may help to explain the mechanism of hypofertility associated with endometriosis, especially stage I or II. Some abnormalities of tubal micro-environment and function have also been reported. Tubal abnormalities may disrupt gamete and embryo transport and function. The Fallopian tube also plays a major role in the genesis of endometriosis: the morphology of its proximal portion determines the importance of menstrual reflux and the risk of endometriosis. Finally, tubal cells seem to be at the origin of tubal endometriosis.

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Endometriosis-related spontaneous hemoperitoneum in pregnancy (SHiP): report of two cases and review of the literature

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ABSTRACT

Spontaneous hemoperitoneum in pregnancy (SHiP) is a rare but life-threatening complication, for both the mother and the fetus. Its exact incidence is unknown. Several pathophysiological mechanisms have been suggested. However, the etiology of SHiP remains unknown. Endometriosis, whose incidence is increasing, is currently recognized as a major risk factor in its development.

We report the case of a patient with spontaneous rupture of the right uterine venous plexus revealed by a severe abdominal pain during delivery, and another case with spontaneous rupture of the right uterine artery revealed by hypovolemic shock and fetal distress. In both patients, exploratory laparotomy revealed hemoperitoneum and active bleeding. Hemostasis and hemodynamic stability were obtained after right adnexectomy in the first case, and uterine artery suturing in the second. These two patients with a diagnosis of SHiP had both previously been diagnosed with and treated for endometriotic lesions.

KEYWORDS

Spontaneous hemoperitoneum, pregnancy, endometriosis, severe hypotension.

Introduction

Spontaneous hemoperitoneum in pregnancy (SHiP) is non-traumatic intraperitoneal bleeding which, in most cases, is revealed by the onset of acute abdominal pain. It has been described before, during and early after labor in 61%, 18% and 21% of cases, respectively [1]. Several etiologies have been described in the literature, some of them related to endometriosis [2-11]. The risk of SHiP with endometriosis is currently not predictable. However, this diagnosis must be considered in any pregnant woman with a past medical history of endometriosis and/or suffering from acute abdominal pain, and with signs of hypovolemic shock without external bleeding, and with or without fetal distress. SHiP is a life-threatening condition for both mother and fetus. We report two cases of SHiP recently observed in our department.

Case 1

A 23-year-old woman, G2P1, was admitted to our delivery ward at 39 weeks of pregnancy (WP), in spontaneous labor. Her medical history was significant for a cesarean section (C/S) performed due to an abnormal fetal heart rate, and surgical laparoscopy for right endometrioma.

At full dilatation, she presented severe abdominal pain radiating from the scapula, associated with an episode of hypotension.

Ultrasound did not demonstrate either hemoperitoneum or

placental abruption. Therefore, since the patient was hemodynamically stable, a vaginal delivery was allowed and a healthy, 3435-gram female baby was delivered. The baby had Apgar scores of 8 and 9 at 1 and 5 minutes respectively. Manual delivery of the placenta with uterine examination confirmed an intact C/S scar.

Immediately after delivery, the patient complained of chest pain and fresh onset of acute abdominal pain. In view of this new hemodynamic instability, it was decided to perform emergency exploratory laparotomy using the previous C/S (Pfannenstiel) scar.

At incision, a 1-liter hemoperitoneum and blood clots were observed. Abdominal inspection confirmed an intact uterine scar. However, active bleeding was noted next to the right adnexa. In order to facilitate access to the bleeding site, the right round ligament was sectioned and the ipsilateral broad ligament was dissected until visualization of the right ureter. This dissection allowed us to identify several tears in the right uterine venous plexus, indicating the need for a right adnexectomy. The total blood loss during this operation was estimated to be 2 liters.

Anatomopathological analysis of the right adnexa was performed and the report indicated the presence of cytogenic endometriosis of the right ovarian cortex and the mesoovarium, combined with an underlying arteriovenous malformation of the mesoovarium.

Case 2

A 37-year-old woman, G1P0, was admitted to the labour ward at 41 WP. The pregnancy had been spontaneously conceived following surgical treatment for severe endometriosis followed by 3 months of Gn-RH analog therapy. The endometriosis surgery consisted of laser ablation of ovarian endometriosis and complete resection of deep infiltrating endometrium (DIE), including the right uterosacral ligament up to the right posterolateral parametrium. The latter lesion constricted the right ureter causing right hydronephrosis. During induction of labor, severe hypotension was documented, requiring resuscitation with colloids.

An emergency C/S was performed under general anesthesia due to fetal distress on cardiotocography and hypovolemic shock. During surgery, an abundant hemoperitoneum (2 liters) was noted. A 4-kg baby was delivered. The baby recorded Apgar scores of 2, 6 and 8 at 1, 5 and 10 min respectively. The umbilical arterial pH was 6.8. The placenta showed no signs of abruption and exploration of the uterine cavity was unremarkable.

However, the patient presented a profuse active hemorrhage from the right uterine artery in the parametrial region. The posterior wall of the uterine artery was ripped and stretched by a suspected endometriotic adhesion. Ligation of the uterine artery was performed and successful hemostasis was achieved.

Recovery was uneventful and the patient was discharged five days after delivery.

Discussion

Spontaneous hemoperitoneum in pregnancy (SHiP) is a rare complication that most often occurs during the third trimester [3, 12-17]. It consists of intraperitoneal bleeding that manifests itself, in the absence of trauma, during pregnancy and up to 42 days after delivery [18]. It has been described before, during and early after labor in 61%, 18% and 21% of cases, respectively [1].

In 89.5% of cases described in the literature, SHiP presented as acute or subacute abdominal pain. In almost all patients, the suspicion of hemoperitoneum arose during assessment following a rapid drop in the hemoglobin level and/or because of signs of hypovolemic shock and/or fetal distress on cardiotocography.

The amount of hemoperitoneum is variable, ranging from 150 mL to 4000 mL (median: 2125 mL).

Fifty-seven clinical cases of SHiP have been reported in the literature between 2000 and 2018 and the findings of this literature review can be summarized as follows.

Forty-two patients underwent an imaging examination, which diagnosed hemoperitoneum in 34 of them (80.9%). Ultrasonography examination revealed free fluids in the peritoneal cavity in 29 patients, in seven cases confirmed by MRI or CT scan. Five of the 42 patients were diagnosed with hemoperitoneum by CT scan only.

The nature of the fluid could be identified only during the operative management in all the cases, with the exception of one who was hemodynamically stable and underwent conservative treatment but died during the transfer to a hospital by a MIC (maternal intensive care) service [19]. Eight of the 57 (14%) patients presented a spontaneous hemoperitoneum in the post-partum, up to Eight days after the delivery, occurring at intervals of 35 to 40 weeks.

Fifty of the 57 patients were treated by laparotomy to manage the haemorrhage, and only 31 of them underwent emergency C/S.

In both the cases encountered in our department, the spontaneous hemoperitoneum was observed during labor. In the first case, a salpingo-oophorectomy was needed in order to achieve hemostasis, while in the second, uterine artery suturing enabled us to achieve correct hemostasis. The hemodynamic status of the second patient prompted us to perform an emergency C/S, and this allowed us to make the unsuspected diagnosis of severe hemoperitoneum.

Both of these patients had a history of surgical laparoscopy for endometriosis, and histological analysis of the right adnexa in the first patient demonstrated the presence of cytogenic endometriosis of the ovarian cortex.

The hypothesis that endometriosis may be an etiological factor of SHiP was first raised in 1992 [2]. Under this hypothesis, the pathophysiological mechanisms of endometriosis as a risk factor for SHiP would be multiple:

1) the chronic inflammation caused by endometriosis would make the blood vessels more prone to rupture;
2) the combined presence of pelvic adhesions and an increased uterine volume would put the vessels at increased risk of rupture [9];
3) the increased size of endometriosis lesions during pregnancy, due to the phenomenon of first-trimester decidualization, combined with their resistance to progesterone, could also be responsible for the appearance of SHiP [5].

No correlation between the stage of endometriosis and incidence of SHiP has been demonstrated [5].

The vessels responsible for SHiP are mostly venous, but in rare cases, including our second patient, uterine artery ruptures are described. In 90% of cases the bleeding is located on the posterior surface of the uterus or in the parametrial region [20].

Other causes of spontaneous hemoperitoneum during pregnancy, such as uterine rupture, rupture of the liver and spleen and of their vessels, bowel perforation, placenta percreta and ovarian cyst rupture, have to be excluded.

Conclusion

Physicians should be informed about the risk of obstetric complications associated with a medical history of endometriosis.

In patients with acute abdominal pain, signs of hypovolemia, which may or may not be associated with abnormal

fetal heart rate, should raise the suspicion of hemoperitoneum, especially in women with a history of endometriosis. Appropriate management is required in order to reduce the morbi-mortality of both mother and fetus.

The frequency of spontaneous hemoperitoneum in pregnancy is probably underestimated due to the lack of case reports. To date, the exact incidence of SHiP remains unknown, however several countries have recently decided to establish a common SHiP database for prospective purposes [21]. This initiative would increase our knowledge about the impact and causes of this event. It might also lead to recommendations for primary prevention and optimal management.

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A case of Allen-Masters syndrome in an early pregnant woman: laparoscopic barbed suture repair of an internal small bowel obstruction due to a broad ligament hernia

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ABSTRACT

We here report a case of small bowel obstruction due to a broad ligament hernia treated successfully by laparoscopy in a very early stage of pregnancy. A 33-year-old woman with a previous laparoscopic appendectomy at age 23 presented to the emergency room with acute abdominal pain, non-responsive to analgesic therapy, and without inflammatory signs. After 12 hours, the pain increased and a CT scan revealed an occlusive small bowel syndrome. Laparoscopic exploration revealed a 30 cm loop of small bowel herniated into a left broad ligament defect, and collaterally, a similar, unobstructed, defect on the right side. We performed a successful surgical treatment, without bowel resection, and discharged the patient on day 4, with no postoperative complications. She had her dating scan 5 weeks later and delivered spontaneously following a physiological term labor. CT scan and rapid exploratory laparoscopy enabled both correct diagnosis and prompt treatment in this case of bowel herniation into a broad ligament defect in a pregnant woman.

KEYWORDS

Internal herniation, Allen-Masters syndrome, laparoscopy, pregnancy.

Introduction

Allen-Masters syndrome is a very rare condition due to a defect of the broad and/or utero-sacral ligaments. In general, it is diagnosed only as a result of complications such as acute abdominal pain or intestinal occlusion. In here, we report a case of small bowel obstruction due to a broad ligament hernia treated by laparoscopy in a very early pregnant woman.

Methods

A 33-year-old woman presented to the emergency room with analgesic-resistant acute pelvic pain, associated with nausea and vomiting. The pain had started 5-6 hours earlier. She was on day 28 of her last menstrual period, and had a positive urinary beta-HCG test. The only relevant aspect of her prior medical history was a laparoscopic appendectomy at the age of 23. She had a BMI of 16.8 kg/m² (43 kg, 160 cm) and all vital signs were within the normal limits. She reported regular urination and normal bowel movements. On admission, routine blood tests were normal, including normal white blood count (WBC 7.58 x 10^3/ul). Serum beta-HCG was 61.7 mU/mL. Given the low beta-HCG titer, we excluded a possible ectopic pregnancy as a cause of the acute abdomen and diagnosed a very early stage pregnancy.

Physical examination revealed a soft, flat abdomen with pain located in the right iliac fossa and in the hypogastrium. The Blumberg, Murphy and Giordano signs were negative. A transvaginal ultrasound showed a normal endometrial pattern in the secretive phase with 11 mm thickness, and a corpus luteum located in the right ovary. Vascularization was normal in both ovaries with no evidence of ischemia.

The patient was hospitalized for observation. Over the following 12h period, her condition deteriorated with further episodes of vomiting and the appearance of abdominal rigidity. WBC increased (13 x 10^3/ul) with no fever. Serum beta-HCG rose to 100 mU/mL. We performed abdominal CT scans, both with and without iodinated contrast (Figs. 1-2), which documented a partial obstruction of the distal duodenum and proximal ileum with significant dilation of a large section of the preceding bowel.

Most of the occluded tract was located in the left hemipelvis. Imaging showed multiple air-fluid levels. There was no evidence of pneumoperitoneum. In the perihepatic region, we documented a peritoneal exudate with a depth of 13 mm collecting in the paracolic gutters and associated spaces.

Figure 1 CT scan coronal section (late arterial phase) showing duodenum and proximal ileum obstruction, bowel dilatation, air-fluid levels, minimum peri-hepatic exudate.

Figure 2 CT scan axial section (early arterial phase) showing the majority of the occlusion located in the left hemipelvis.
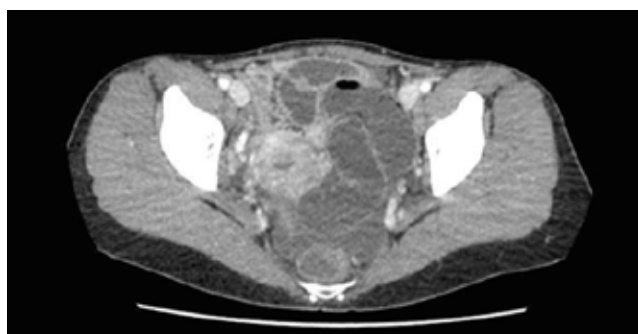
Results

We agreed to perform an urgent laparoscopic exploration. We found a loop of small intestine, which was herniated into a defect in the fascia between the round ligament and the left fallopian tube. The defect measured 3 cm in diameter. A similar defect was found on the opposite side measuring 2 cm in diameter, which thus confirmed the diagnosis of Allen-Masters syndrome. A section of approximately 30 cm of the bowel was extracted from the defective fascial wall, and it did not appear significantly ischemic. No bowel resection was indicated. Then we performed a bilateral surgical repair of the fascial layer with a 3.0 barbed suture, followed by an abdominal lavage. Prior to closure, we reconfirmed that the extracted bowel was in good condition. We did not observe any other anatomical defects.

The patient recovered well following the procedure. On day 4 she was discharged without any postoperative complications, and with a serum beta-HCG of 538 mU/mL.

Five weeks later, she performed her first prenatal ultrasound dating, revealing an intrauterine pregnancy with vital embryo of 7 weeks. The patient delivered spontaneously at full-term pregnancy.

Discussion

Internal hernia is a rare cause of intestinal obstruction, reported in less than 1% of cases. Broad ligament herniation is an even more rare condition, occurring in approximately 0.05% of all hernias. [1] Although the ileum is the most common section of the intestines to herniate, colonic herniation is also reported. [2,3] The etiology of this kind of hernia is still under discussion. Congenital and iatrogenic factors, as well as pelvic inflammatory disease, delivery trauma, underweight status and endometriosis seem implicated. The present case is in line with the report by Garcia-Oria et al., who suggested that very low BMI is a risk factor for spontaneous rupture of the broad ligament due to thinness of the meso-ovarium and mesosalpinx. [4] Guillem et al. [5] described the first laparoscopic repair of a small bowel incarceration in a broad ligament defect. They closed the broad ligament defect with an endoscopic clip, whereas we agreed on a 3.0 running suture, as recommended by Higa et al., in order to avoid possible subsequent internal hernias. [6] We decided to perform a barbed suture because its evenly spaced barbs throughout the strand provide secure closure of the ligament incision by distributing tension across the wound. [3] Considering that the uterus and bilateral broad ligaments would have to endure all the physiological modifications induced by pregnancy, a barbed suture seemed the best approach. We preferred a laparoscopic procedure to ensure a faster and painless postoperative recovery with better functional and esthetic results. [3,6] The laparoscopic approach is safe and feasible in every trimester of pregnancy without differences in perinatal outcomes. [7] To our knowledge, this is the first description of the use of barbed sutures during laparoscopy to repair a bilateral broad ligament defect in a pregnant woman. We consider this method safe, fast and effective. In the present case of bowel herniation into a broad ligament defect very early during pregnancy, CT scan followed by immediate exploratory laparoscopy allowed both correct diagnosis and prompt treatment.

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Modulatory role of D-chiro-inositol and alpha lipoic acid combination on hormonal and metabolic parameters of overweight/obese PCOS patients

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ABSTRACT

Context: Polycystic ovary syndrome (PCOS) is a frequent disease characterized by several endocrine impairments and frequent metabolic abnormality, i.e. compensatory hyperinsulinemia.

Aims: To evaluate the improvements induced by a daily treatment with a combination of d-chiro-inositol (DCI) (500 mg) and alpha-lipoic acid (ALA) (300 mg) for 12 weeks.

Setting: retrospective study

Design: Thirty overweight/obese patients were evaluated. The presence/absence of first-degree diabetic relatives was ascertained. Patients were administered DCI (500mg/day) and ALA (300 mg/day) per os for at least 12 weeks. Only patients completing 12 weeks of treatment (n=30) were included in the study. Patients were evaluated before and after the treatment through measurement of plasma levels of LH (Luteinizing Hormone), FSH (Follicle Stimulating Hormone), estradiol, progesterone, androstenedione, testosterone, insulin, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT). They also underwent an oral glucose tolerance test (OGTT) to evaluate glucose, insulin and c-peptide responses.

Results: The combination treatment improved hormonal and metabolic parameters, as well as insulin and c-peptide responses to OGTT and the HOMA index. On subdividing the patients by presence/absence of familial diabetes, DCI+ALA was found to be more effective, both on metabolic and on hormonal parameters, in PCOS subjects with familial diabetes. PCOS patients with familial diabetes had higher baseline GOT and GPT levels than those with no familial diabetes and the combination treatment significantly reduced these levels.

Conclusions DCI+ALA proved to be an efficient combination that improved insulin sensitivity and hormonal and metabolic profiles in overweight/obese PCOS patients, especially those with familial diabetes, in whom it reduced the GOT and GPT levels. This latter effect might reduce the risk of non-alcoholic fatty liver disease (NAFLD), typical of PCOS patients.

KEYWORDS

PCOS, insulin resistance, NAFLD, anovulation, d-chiro inositol, alpha lipoic acid.

Introduction

Polycystic ovary syndrome (PCOS) is a frequent endocrine disease affecting 4-25% of women of reproductive age [1, 2]. The diagnostic criteria were established at the American Society for Reproductive Medicine and European Society for Human Reproduction and Embryology consensus meeting in Rotterdam [3]. A diagnosis of PCOS requires the presence of at least two of the following criteria: [4] chronic anovulation disorder (oligo or anovulation leading to amenorrhea); [5] clinical (acne, hirsutism) or biochemical signs of hyperandrogenism; and [6] the presence of micro-polycystic ovaries at ultrasound or the presence of 12 or more follicles with a diameter of 2–9 mm in each ovary, and/or increased ovarian volume (> 10 ml) [7].

In the last decade the dysmetabolic state of insulin resistance (IR) and its correlate, compensatory hyperinsulinemia, have been considered important additional aspects [8, 9]. Both are due to a deficiency of a D-chiro-inositol (DCI)-containing phosphoglycan that mediates the action of insulin [10]. Inositol improves insulin sensitivity because it works as a second messenger that may achieve an insulin-like effect on metabolic enzymes [11]. However, the presence of familial predisposition to diabetes in PCOS patients is an important consideration, since it predisposes to lower endogenous conversion of myo-inositol (MYO) to DCI as a result of reduced expression/function of the epimerase enzyme [12]. The use of both these types of inositol as a combination treatment improves insulin sensitivity [11, 12] in hyperinsulinemic PCOS patients and restores more appropriate

metabolic control of glucose and better reproductive functions [12]. However, the use of DCI seems to be more appropriate in PCOS patients who have at least one first-degree relative affected by type I or II diabetes [12, 13]. Interestingly, PCOS women have increased oxidative stress, and this seems to contribute to the IR state [14]. In fact, increased oxidant status is related to central obesity, age, blood pressure, serum glucose, insulin and triglyceride levels, and also to IR [9, 15]. Alpha lipoic acid (ALA) is a potent antioxidant, and controlled-release ALA has been reported to improve glucose control in type II diabetes patients [14], and to improve insulin sensitivity and metabolic disorders in women with PCOS [16]. In addition, a combination of MYO and ALA can be used in insulin-resistant PCOS patients to improve their insulin sensitivity [7] and metabolic and reproductive profiles. The aim of our study was to evaluate the effects of a combination of DCI and ALA on both metabolic and hormonal parameters in a group of obese patients with PCOS.

Materials & Methods

Subjects

Among the many patients seen between January 2015 and December 2017 and recorded in the outpatients' database of our Gynecological Endocrinology Center, a total of 30 overweight/obese patients [22.5 ± 1.7 years, mean ± standard error of the mean (SEM)] was selected. All these patients required treatment for their PCOS condition (n = 30), but they were not willing to have any hormonal therapy. Informed consent was obtained from all individual participants as a standard procedure of the University of Modena and Reggio Emilia, Italy. These patients were selected according to the criteria established by the American Society for Reproductive Medicine and the European Society for Human Reproduction and Embryology for diagnosing the presence of PCOS [3], and at least two of the following criteria had to be present: (a) oligomenorrhea with inter-menstrual intervals longer than 45 days, (b) clinical (acne, hirsutism) or biochemical signs of hyperandrogenism, (c) presence of micro-polycystic ovaries at ultrasound. In addition, patients had to fulfil the following criteria: (d) absence of enzymatic adrenal deficiency and/or other endocrine disease, including diabetes, (e) normal prolactin (PRL) levels (range 5–25 ng/ml), (f) no hormonal treatment during a period of at least 6 months prior to the study, (g) body mass index above 26. None of the subjects enrolled had taken medications and/or steroids, oral contraceptives or metformin within the 3 months prior to the evaluation. All the patients, at the first consultation, were interviewed to establish whether or not they had one or more first-degree relative (parents and/or grandparents) with diabetes. The anamnestic investigation revealed that 18 of the 30 patients (60%) reported first-degree diabetic relatives. All these patients were selected from the database because they had been taking a preparation combining DCI (500 mg) and ALA (300 mg) every morning at around 10 a.m. for at least 3 months (12 weeks). No lifestyle or dietary changes were required of the patients and all were studied, the first time, on day 3–6 of the menstrual cycle, if present. The post-treatment follow-up was performed after at least 12 weeks of treatment, plus a few days if necessary, so that patients were again evaluated on day 3–6 of the menstrual cycle (the first occurring after the treatment). All patients were evaluated for luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2), progesterone (P), androstenedione (A), testosterone (T), insulin, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). HOMA index was computed to estimate sensitivity to insulin [4]. An oral glucose tolerance test (OGTT), for insulin and glucose determinations, was performed sampling before and 30, 60, 90, 120, 180 and 240 min after the oral assumption of 75 g of glucose, before and after the 12 weeks of combination treatment. A hyperinsulinemic response is recognized when insulin plasma levels are above 50 μU/ml within 90 min of glucose load [5]. The mean treatment duration was 97.5 ± 4 days [mean ± standard error of the mean (SEM)], the range being 92–113 days.

Assay

All samples from each subject were assayed in duplicate in the same assay. Plasma LH and FSH concentrations were determined using a previously described immunofluorometric assay [6, 17]. The sensitivity of the assay, expressed as the minimal detectable dose, was 0.1 IU/ml. The cross-reactivities with free and ß-subunits of LH, FSH and thyroid stimulating hormone (TSH) were less than 2% [4]. Intra-assay and inter-assay coefficients of variation were 4.3% and 6.5%, respectively. Plasma E2, A, cortisol and T were determined by radioimmunoassay (Radim, Pomezia, Rome, Italy), as previously described [18]. Based on two quality control samples, the average within- and between-assay coefficients of variation were 3.5% and 8.4%.

Plasma insulin and c-peptide concentrations were determined using an immunoradiometric assay (Biosource Europa S.A., Nivelles, Belgium). Based on two quality control samples, the average within- and between-assay coefficients of variation were 4.0% and 10.2%.

Statistical analysis

After analysis of variance (one-way ANOVA), data were tested for statistically significant differences between the groups (before and after the treatment) by means of Student's t-test for paired and unpaired data, as appropriate. The differences in insulin and c-peptide responses to OGTT were computed as maximal responses (ΔMax). ΔMax was computed as the difference between the maximal hormonal response and the hormonal concentration before the stimulation (time 0). The HOMA index was computed to estimate sensitivity to insulin [4] since it is considered the main index of the metabolic syndrome and a common link between the coexisting abnormalities; it can be calculated by homeostasis model assessment of IR (HOMA-IR) as (fasting insulin mU/l) × (fasting glucose mmol/l)/22.5 [4]. The cutoff value we used is 2.71 as previously stated [4, 5]. Data are expressed as mean ± SEM.

Results

The patients' hormonal and metabolic parameters are reported in Table 1. The administration of DCI plus ALA significantly changed LH, A, insulin and LDL plasma levels. Also, BMI and the HOMA index decreased significantly (Table 1).

As regards the OGTT, the maximal insulin and c-peptide responses (ΔMax) to the glucose load decreased significantly in the whole group of PCOS patients (Fig. 1), thus indicating the positive effects of the combination treatment. With regard to the presence or absence of familial diabetes (Table 2), the group with familial diabetes showed improved plasma LH, A and insulin levels and significantly reduced triglycerides, total cholesterol, LDL, GOT and GPT. Patients with no familial diabetes showed improvements only in plasma LH, insulin and A levels, as well as in the HOMA index (Table 2), while no changes in GOT and GPT or in the lipid profile were observed.

This subdivision of the patients revealed that in baseline conditions PCOS patients with familial diabetes showed higher GOT and GPT levels and a higher HOMA index than the other group, while insulin plasma levels were higher but without the difference reaching statistical significance (Table 2). After the treatment, GOT and GPT plasma levels decreased in PCOS with familial diabetes and became no different from those of patients without familial diabetes (Table 2).

As regards the OGTT results, different responses to glucose load were observed when considering the two subgroups of PCOS patients. Those with familial diabetes showed a significant reduction of insulin (Fig. 2 panel A) and c-peptide ΔMax (Fig. 2 panel B), greater than what was observed in PCOS patients without familial diabetes (Fig. 2 panels C and D). More-

over, the insulin ΔMax of PCOS patients with familial diabetes in baseline conditions was greater than in the other group (Fig. 1 panel A and C), similarly to the c-peptide ΔMax (Fig. 1 panels B and D). Though PCOS patients with no familial diabetes had a reduction of insulin ΔMax (Fig. 1 panel C), no changes in c-peptide ΔMax were observed (Fig. 2 panel C).

Figure 1 Maximal insulin (left) and c-peptide responses (right) (Δmax) to OGTT in all PCOS patients under study. ** $p < 0.005$.
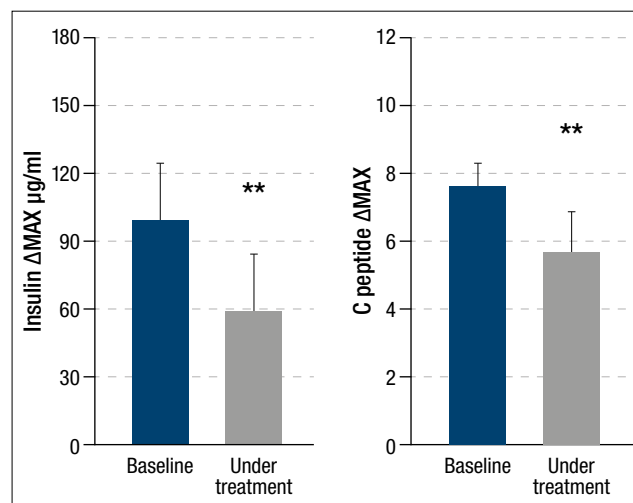

**Table 1** Hormonal characteristics of all PCOS patients under study.

PCOS patients n=30	LH mIU/ml	FSH mIU/ml	Estradiol pg/ml	A ng/ml	Total T ng/ml	Insulin µU/ml	Glucose mg/dl	Tryglice-rides mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	GOT U/l	GPT U/l	BMI	HOMA index
Baseline	13,5±1,5	5,8±0,5	47,1±5,9	26,8±0,15	0,6±0,04	14,1±2,6	88,2±2,7	126,2±22	182,7±9,9	50,3±4,5	112±12,7	24,5±2,6	29,7±4,7	31,5±1,4	3,12±0,6
Under treatment	8,6±0,9	5,6±0,5	62,8±14	2,2±0,15	0,4±0,04	9,5±1,3	84,5±2,2	99±13,3	174,3±7,3	55,5±3,3	104±8,4	19,8±1,8	24,7±2,5	30,4±1,3	2,1±0,3
p level vs baseline	0,01		0,0003			0,003					0,04			0,0006	0,002

Table 2 Hormonal characteristics of PCOS patients according to the presence or absence of diabetic relative(s).

Diabetic relatives n=18	LH mIU/ml	FSH mIU/ml	Estradiol pg/ml	A ng/ml	Total T ng/ml	Insulin µU/ml	Glucose mg/dl	Tryglice-rides mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	GOT U/l	GPT U/l	BMI	HOMA index
Baseline	12.8±1,6	5.4±0.5	56±10.5	280.8±11	0.6±0.03	14±2.8	87,5±3	118,7±18	188,5±10,3	52,8±4,8	116,7±12,2	27.4±2,4	33.5±3.9	32.5±1.5	3.1±0.7
p vs NO diabetic												0.03	0.02		0.05
Under treatment	8.7±1.1	5.2±0.5	62.6±14	240±23	0.4±0.03	11±2.2	87,8±3.2	97,1±13.1	175±10,1	59,1±5	103±12	20.8±1.2	21.5±3	31.1±1.5	2.5±0.6
p level vs baseline	0.02			0.02		0.02		0.009	0.05		0.02	0.006	0.004	0.0001	0.03

Diabetic relatives n=12	LH mIU/ml	FSH mIU/ml	Estra-diol pg/ml	A ng/ml	Total T ng/ml	Insulin µU/ml	Glucose mg/dl	Tryglice-rides mg/dl	Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	GOT U/l	GPT U/l	BMI	HOMA index
Baseline	13.4±2.2	5.9±0.6	48.1±7.1	233.3±18	0.6±0.06	9.5±1.3	83.7±2.5	108.7±24	177.7±12	52,7±2.4	107.2±11.1	19.8±2.4	19.5±3.6	30±2.9	2.2±0.3
Under treatment	8.5±1.6	6.2±0.9	41.7±3.6	193.1±20	0.4±0.08	7.4±1.2	80.1±2.2	101.5±27	173.3±12	51±4	106.1±12.7	17.8±1.2	18.2±2.7	30.7±2.8	1.6±0.3
p level vs baseline	0.05			0.01		0.01									0.01

Figure 2 Maximal insulin and c-peptide response (Δmax) to OGTT in PCOS patients subdivided according to the presence or absence of familial diabetes. Insulin and c-peptide Δmax were found to be higher in PCOS patients with familial diabetes.
Panel A: ** $p<0.01$ vs baseline; § $p<0.03$ vs no diabetes (panel C); Panel B: ** $p<0.03$ vs baseline; § $p<0.05$ and # $p<0.04$ vs no diabetes (panel D).
Panel C: * $p<0.05$ vs baseline.
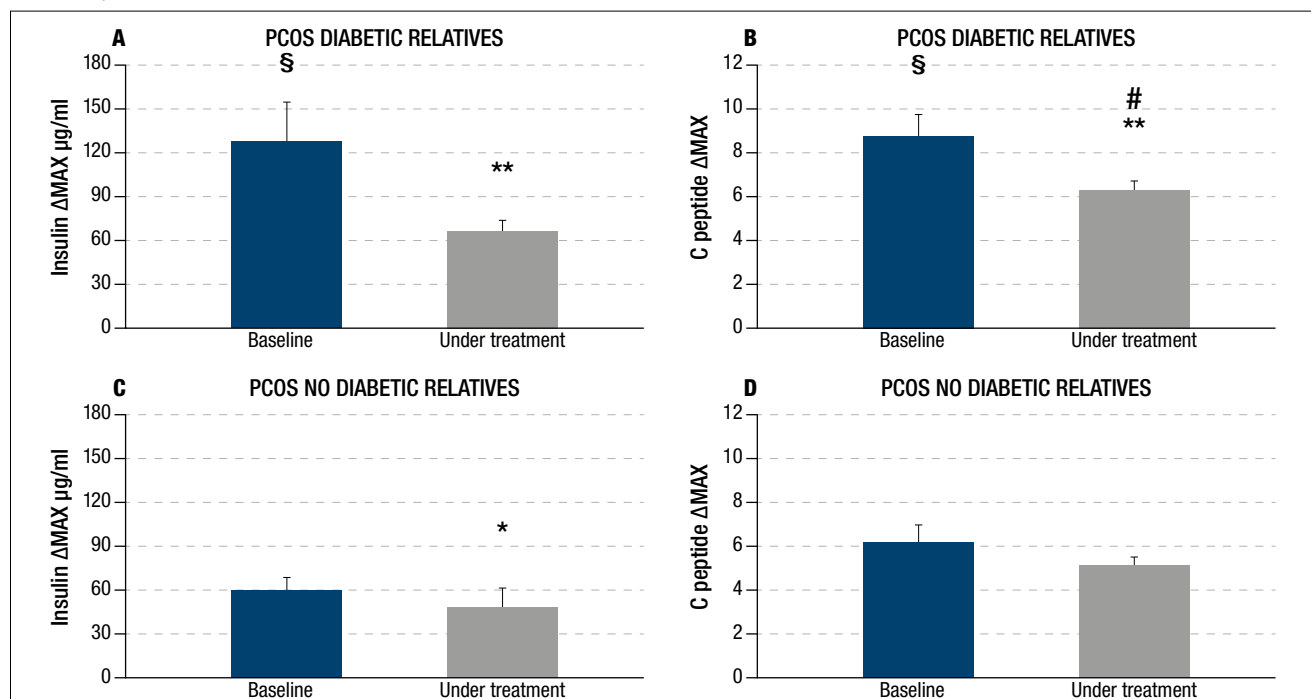
Discussion

The present study reported improvements in hormonal and metabolic parameters in obese PCOS patients administered DCI+ALA. Moreover, our data support the relevance of the presence of familial diabetes, since this predisposes to greater metabolic impairment and liver dysfunction.

Insulin resistance (IR) is a frequent finding in PCOS patients but it is not completely related to being overweight or obese, since it also occurs in normal weight PCOS subjects [12, 19, 20]. In fact, higher occurrence of IR is classically a feature of those patients who have familial diabetes [12, 20]. Metformin has been demonstrated to reduce IR but due to its side effects, especially in subjects needing higher dosages [12], alternative strategies have been developed, such as the use of MYO, DCI and ALA. These compounds have been demonstrated to improve IR by increasing the efficiency of post-receptor signalling of insulin [11, 12, 13, 16, 20]. Previous studies demonstrated that MYO administration was effective in improving insulin sensitivity in hyperinsulinemic PCOS patients [11], similarly to what was observed when using DCI [13] or ALA [16], having no side effects and a marked clinical impact on metabolic dysfunction and hormonal impairment.

The present data are clearly in line with such studies, and add, to their findings, results observed when DCI and ALA are used in combination. According to a recent review MYO or DCI can be clinically helpful whether absence or presence of familial diabetes has been disclosed [14]. Moreover, previous studies reported that DCI administration [13] was able to reduce IR in PCOS subjects, including those with familial diabetes [13], similarly to the use of ALA [16]. Our data are in perfect agreement with previous observations demonstrating that the combination

of DCI and ALA greatly improved insulin sensitivity in all subjects, independently of the presence or absence of familial diabetes [13, 16]. However, subdivision of the patients on the basis of this latter criterion allowed us to disclose greater efficiency of the treatment in PCOS patients with familial diabetes. In the other group, the treatment improved hormonal parameters, but less evidently than in PCOS patients with familial diabetes. In fact, PCOS patients with familial diabetes showed not only improved plasma LH, insulin and A concentrations, but also improved levels of triglycerides, total cholesterol, LDL, GOT and GPT.

Our evaluation of ΔMax for insulin and c-peptide under OGTT revealed that though both insulin and c-peptide decreased after the treatment, PCOS patients with familial diabetes had a higher maximal responses (ΔMax) of insulin and c-peptide to glucose load in baseline conditions, and that the DCI+ALA administration decreased ΔMax at a higher rate in this group of PCOS patients. In fact, PCOS patients with no familial diabetes showed no significant changes in c-peptide ΔMax after the combination treatment, thus suggesting that familial predisposition to diabetes underlies, to a certain extent, the metabolic impairment, worsening the IR and the compensatory hyperinsulinemia, probably by increasing the pancreatic secretion/function of the Langerhans islets, even though impaired insulin metabolic clearance cannot be excluded.

The hormonal and metabolic improvements we observed confirm a previous report by Cianci A et al. [21], even though those authors used higher doses of DCI and ALA. As an additional feature, our report described the efficacy of the treatment on liver function, since we observed a significant reduction of hepatic enzyme levels (i.e. GOT and GPT) in the PCOS patients with familial diabetes. This observation is superimposable on

what was previously described when administering ALA alone [16]. In fact, the presence of type II diabetes downregulates the expression of lipoic acid synthase (LASY), responsible for ALA synthesis in mammalian mitochondria [22, 23], thus reducing endogenous ALA synthesis and leading to the lower glucose uptake in skeletal muscle cells that is at the basis of IR [23]. Endogenous ALA modulates glucose utilization through the increase of adenosine monophosphate-activated protein kinase in skeletal muscles [12], and thus by increasing glucose-transporter-4 levels [24, 25]. These data support the fact that having familial diabetes predisposes to impaired endogenous synthesis of both ALA and DCI, related to defective expression/function of LASY and epimerase [12, 16] respectively.

The combination DCI and ALA modulated, at the same time, both hormonal and metabolic aspects. ALA has recently been reported to act on specific metabolic indexes and to exert a good hepatic protective action with no improvement of reproductive hormonal profiles in PCOS subjects, independently of familial diabetes status [17]. Our data report, for the first time, that the DCI+ALA combination has a full effect in PCOS patients, since it shows a hepatic protective action in addition to metabolic and hormonal effects. Indeed, this combination might be effective in preventing not only the risks related to IR and compensatory hyperinsulinemia, but also the risk of developing non-alcoholic fatty liver disease (NAFLD) [26]. A recent review stated that NAFLD is very frequent in PCOS patients [26] and the combination of PCOS with obesity and IR is a dangerous cocktail that, over time, triggers not only NALFD but also the occurrence of type II diabetes [26, 27].

In conclusion, the combined DCI+ALA regimen, at the low dosages we used, was effective in improving both hormonal (related to DCI) and metabolic (related to both DCI and ALA) parameters. The present study clearly supports the need for an accurate anamnestic investigation, so as to better choose the most effective combination treatment strategy.

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Depression across menopause: severity, symptoms, climacteric and hormonal background

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ABSTRACT

Menopausal depression is a common problem in menopausal women. In this paper we examine the symptoms of menopausal depression in relation to various stages of the menopausal transition, defined according to the STRAW + 10 classification. The present study included 201 women aged 42-65 years admitted to the Department of Gynecological Endocrinology, Poznan University of Medical Sciences, because of climacteric symptoms. The intensity of climacteric symptoms in the studied women was evaluated using the Kupperman index, and depression symptoms were assessed using the Hamilton depression scale and the Beck Depression Inventory. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), 7ß-estradiol (E2), prolactin (PRL), total testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), thyrotropin (TSH), free thyroxin (FT4), progesterone and cortisol 8:00 and 16:00 levels were evaluated in all the studied women. We concluded that depression is most frequent in late menopausal transition, and that depressive symptoms are related to hormones during menopausal transition but not during postmenopause.

KEYWORDS
Depression, menopause.

Introduction

Menopausal women have a three-fold higher risk of depression than women in other periods of life [1]. It is estimated that clinically relevant depression may be diagnosed in about 50% of women seeking medical advice due to climacteric symptoms [2]. Menopausal depression usually has a mild course [2]. The etiology of depressive symptoms during menopausal transition remains unclear, although hormonal, neurotransmitter and neurosteroid changes [3, 4], cerebral blood flow changes [5], and genetic predisposition [6] are reported among etiological factors of climacteric depression. A history of depression and severe premenstrual syndrome as well as disturbed sleep, hot flashes and urinary and sexual dysfunction (thought to be connected with the incidence of depressive symptoms through a "domino effect"), are among the predictive factors of climacteric depression [7].

The Stages of Reproductive Aging Workshop (STRAW) [8] divides reproductive aging into two periods: menopausal transition and postmenopause. Menopausal transition comprises early menopausal transition and late menopausal transition. Early menopausal transition starts several years before the menopause and is characterized by menstrual cycle variability (cycle length > 7 days), increased FSH levels, and low anti-Mullerian hormone (AMH) and inhibin B levels. The late menopausal transition starts about 1-3 years before the menopause and is characterized by a cycle length longer than 60 days, low AMH and inhibin B levels, and a high FSH concentration (usually higher than 25IU/l) on days 2-5 of the cycle. The early postmenopausal period is the period between 1 yaer and 3 years after the menopause, when the main complaint is

the presence of vasomotor symptoms. The late postmenopausal period starts later, when the main complaint is urogenital atrophy-related symptoms.

The aim of this study was to evaluate profiles of depression symptoms and their hormonal background in relation to the various stages of reproductive aging.

Material and methods

The study included 201 women aged 42-65 years admitted to the Department of Gynecological Endocrinology, Poznan University of Medical Sciences, because of climacteric symptoms. The mean age of the studied women was 54.1 ± 4.8 years; 52 of them were still menstruating, or reported a time since last menses no longer than 12 months, whereas in 97 cases at least one year had elapsed since their last menstrual period. The women were divided into four study groups according to the STRAW classification categories:
- early menopausal transition group (EMT): 39 women with a cycle longer than 35 days and shorter than 60 days.
- late menopausal transition group (LMT): 34 women with a cycle longer than 60 days and shorter than 1 year.

- early postmenopause group (EP): 82 women between 1 year and 3 years after menopause.
- late postmenopause group (LP): 46 women at least 3 years after menopause.

The intensity of climacteric symptoms in the studied women was evaluated using the Kupperman index [9] and depression symptoms using the Hamilton depression scale [10] and Beck Depression Inventory [11]. Body mass index (BMI) was calculated using the BMI=body mass/height2 formula. Serum FSH, LH, E2, PRL, total testosterone, SHBG, DHE-AS, TSH, fT4, progesterone and cortisol 8:00 and 16:00 levels were evaluated in all the studied women. In the still menstruating women, blood was drawn between the 8th and 12th day of the menstrual cycle. Serum FSH, LH, 17β-estradiol, total testosterone, SHBG, TSH, fT4, progesterone and cortisol concentrations were tested by immunoenzymatic methods (Roche Diagnostics, Mannheim, Germany). Intra- and interassay coefficient of variation (CV) ranges were 1.2-3.3% and 2.0-5.6%, respectively. DHEAS level was evaluated using the radioimmunological method (Diagnostic Products Corporation, Los Angeles, CA): intra-assay CV and interassay CV values were 5.1% and 11%, respectively.

For the statistical analysis, the Kruskal-Wallis test and a two-tailed test were used to assess the differences between the studied groups and Spearman's test was used to assess correlations between variables. The study was approved by the Poznań University of Medical Sciences ethics committee, and financed by the State Committee for Scientific Research (project no: 50305-01109136-12261-08039). The authors declare no conflict of interest.

Results

Correlations were found between the Hamilton depression scale and Beck Depression Inventory in all the studied groups (EMT: Spearman R=0.83 p<0.05; LMT: Spearman R=0.79 p<0.05; EP: Spearman R=0.67 p<0.05; LP: Spearman R=0.87 p<0.05).

There were also correlations, in all the studied groups, between the Hamilton depression scale and the Kupperman index (EMT: Spearman R=0.66 p<0.05; LMT: Spearman R=0.55 p<0.05; EP: Spearman R=0.71 p<0.05; LP: Spearman R=0.61 p<0.05), and between the Beck Depression Inventory and the

Table 1 Clinical and hormonal characteristics of the studied groups.

Parameter	EMT	LMT	EP	LP	Kruskal-Wallis test
Number of women	39	34	82	46	-
Age (years)	50.0±3.8	52.1±2.9	53.9±6.5	58.6±5.5	(1)
Time since last menstruation/ menopause (m=months, y=years)	1.3m±0.4	5.7m±2.3	3.3y±1.6	10.9y±4.3	-
BMI (kg/m²)	26.2±4.7	26.9±4.0	26.3±6.2	27.2±4.4	NS
Kuperman index	23.9±12.8	30.6±13.6	25.8±13.2	24.4±12.3	NS
FSH (IU/l)	38.7±37.8	68.2±38.8	76.9±33.5	72.1±20.9	(2)
LH (IU/l)	23.8±18.9	42.3±21.2	37.3±14.8	33.5±12.4	(3)
E2 (pg/ml)	101.9±135.9	64.7±114.5	33.0±82.6	17.1±9.7	(4)
PRL (ng/ml)	16.7±15.1	15.1±9.5	12.6±8.6	12.5±7.4	NS
Testosterone (ng/ml)	0.31±0.2	0.29±0.16	0.27±0.17	0.27±0.26	NS
Progesterone (ng/ml)	2.44±4.7	0.44±0.42	0.39±0.23	0.30±0.2	(5)
SHBG (nmol/l)	59.7±34.8	54.5±27.6	57.8±31.3	65.7±53.4	NS
DHEAS (ng/ml)	1.65±1,05	1.36±0.75	1.36±0.69	1.1±0.81	(6)
TSH (mIU/l)	2.9±3.7	2.24±1.6	2.5±3.0	2.06±1,1	NS
Ft4 (pmol/l)	1.09±0.28	1.13±0.18	1.25±0.48	1.23±0.26	NS
Cortisol 8:00 (nmol/l)	110.2±55.1	97.6±53	114.9±51.1	115.2±55.5	NS
Cortisol 16:00 (nmol/l)	67.9±30.5	58.6±31.9	68.5±35.9	67.9±35.7	NS

(1) EMT vs LMT: NS; EMT vs EP: p=0.000002; EMT vs LP: p=0.000001; LMT vs EP: p=0.01; LMT vs LP: p=0.000001; EP vs LP: 0.002.
(2) EMT vs LMT: p=0.01; EMT vs EP: p=0.000001; EMT vs LP: p=0.0002; LMT vs EP: NS; LMT vs LP: NS; EP vs LP: NS.
(3) EMT vs LMT: 0.0001; EMT vs EP: p=0.0003; EMT vs LP: NS; LMT vs EP: NS; LMT vs LP: NS; EP vs LP: NS.
(4) EMT vs LMT: NS; EMT vs EP: p=0.00008; EMT vs LP: 0.0005; LMT vs EP: NS; LMT vs LP: NS; EP vs LP: NS.
(5) EMT vs LMT: NS; EMT vs EP: NS; EMT vs LP: 0.0008; LMT vs EP: NS; LMT vs LP: NS; EP vs LP: NS.
(6) EMT vs LMT: NS; EMT vs EP: NS; EMT vs LP: 0.03; LMT vs EP: NS; LMT vs LP: NS; EP vs LP: NS.

Kupperman index (EMT: Spearman R=0.63 p<0.05; LMT: Spearman R=0.43 p<0.05; EP: Spearman R=0.45 p<0.05; LP: Spearman R=0.53 p<0.05). With regard to the hormonal background to depression, correlations were found, in the EMT group, between the Hamilton depression scale and serum levels of testosterone (Spearman R=0.47 p<0.05), progesterone (Spearman R=0.45 p<0.05) and DHEAS (Spearman R=0.35 p<0.05), while in the LMT group there was a correlation between the Beck Depression Inventory and progesterone (Spearman R=-0.41 p<0.05).

Investigation of the hormonal background to climacteric symptoms revealed, in the EMT group, correlations between the Kupperman index and serum levels of 17β-estradiol (Spearman R=-0.33 p<0.05) and testostosterone (Spearman R=0.38 p<0.05). In the LMT group, there emerged a correlation between the Kupperman index and serum 17β-estradiol level (Spearman R=-0.38 p<0.05). In the LP group there was a correlation between the Kupperman index and serum cortisol 16:00 level (Spearman R=-0.39 p<0.05).

Discussion

The four studied groups (early menopausal transition, late menopausal transition, early postmenopause and late postmenopause) differed in frequency of depression as assessed using the Hamilton depression scale. Depression was found to be most frequent in the late menopausal transition group and least common in the late postmenopause group. Similar data were presented by Maki et al., who reported that the early and late menopausal transition stages as well as the early postmenopause stage are a window of vulnerability for the development of both depressive symptoms and major depressive episodes [12]. The frequency of depression was high in all the groups considered in our study (69.2%, 82.3%, 70.7%, 56.5%), a finding in line with other studies which reveal a high frequency of depression in menopausal women [2, 13, 14]. The most frequent symptoms of depression in all the studied groups were general somatic symptoms, loss of interest in activities, shallow sleep, psychological symptoms of anxiety and fear, and so-

Table 2 Depression and depression symptoms in the studied groups according to the Hamilton scale.

Parameter	EMT	LMT	EP	LP	Kruskal-Wallis test
Hamilton scale score	10.6±6.6	12.1±6.7	11.6±6.7	9.5±6.6	NS
Beck Depression Inventory score	12.4±8.2	13.6±9.3	13.7±9.6	11.7±8.6	NS
Depression on Hamilton scale (≥8 points)	27 (69.2%)	28 (82.3%)	58 (70.7%)	26 (56.5%)	(1)
Depressive mood	13 (33.3%)	15 (43.5%)	35 (42%)	14 (30.8%)	NS
Feelings of guilt	17 (44.2%)	14 (40.6%)	34 (40.8%)	14 (30.8%)	NS
Suicidal thoughts and tendencies	8 (20.8%)	7 (20.3%)	19 (22.8%)	11 (24.2%)	NS
Sleep disorders	22 (57.2%)	21 (60.9%)	45 (54%)	20 (44%)	NS
Shallow sleep	23 (59.8%)	25 (72.5%)	56 (67.2%)	24 (52.8%)	NS
Waking early	20 (52%)	22 (63.8%)	50 (60%)	21 (46.2%)	NS
Loss of interest in activities	24 (62.4%)	20 (58%)	49 (58.8%)	19 (41.8%)	NS
Slowness of movement	10 (26%)	8 (23.2%)	19 (22.8%)	8 (17.6%)	NS
Sensorimotor anxiety	5 (13%)	8 (23.2%)	13 (15.6%)	7 (15.4%)	NS
Psychological symptoms of anxiety and fear	23 (59.8%)	25 (72.5%)	51 (61.2%)	25 (55%)	NS
Somatic symptoms of anxiety and fear	23 (59.8%)	28 (81.2%)	57 (68.4%)	25 (55%)	(2)
Gastrointestinal symptoms	10 (26%)	6 (17.4%)	16 (19.2%)	4 (8.8%)	NS
General somatic symptoms	25 (65%)	27 (78.3%)	53 (63.6%)	26 (57.2%)	NS
Symptoms of the genitourinary system	22 (57.2%)	20 (58%)	46 (55.2%)	23 (50.6%)	NS
Hypochondria	7 (18.2%)	11 (31.9%)	10 (12%)	4 (8.8%)	(3)
Weight loss	1 (2.6%)	1 (2.9%)	4 (4.8%)	1 (2.2%)	NS
Self-criticism	4 (10.4%)	1 (2.9%)	5 (6%)	2 (4.4%)	NS

(1) LMT vs LP p=0.014897.
(2) EMT vs LMT p=0.033092; LMT vs LP p=0.046539.
(3) LMT vs EP p=0.015551; LMT vs LP p=0.016320.

matic symptoms of anxiety and fear. The groups also differed in frequency of somatic symptoms of anxiety and fear and frequency of hypochondria. Both hypochondria and somatic symptoms of anxiety and fear were most frequent in the late menopausal transition group and less frequent in the early menopausal transition, early postmenopause and late postmenopause groups. Our data are similar to the observations of other authors, who report a characteristic pattern of menopausal depression, which includes several physical and psychological symptoms, such as muscle pain, weight gain, low energy levels, decreased self-esteem, feelings of isolation, cognitive impairment and decreased libido [3, 5, 15]. Typical signs of perimenopausal depression are: a milder mood presentation, anger, irritability and paranoia, manifesting as verbal outbursts over minor stressors [16]. Mood changes in perimenopausal depression may last minutes to hours and spontaneously resolve [7]. Perimenopausal depression is associated with increased fatigue and decreased energy levels [15, 17-18]. It is related to psychosocial factors, among which perception of aging and childbearing status, habits, and stressful family/life roles are reported [15]. With regard to the perception of aging, a tendency to value young people more than the elderly can increase the likelihood of depression during menopause [15]. Communities that place more value on young people have higher rates of menopausal depression. Smoking and limited physical activity may also increase the frequency of menopausal depression [19]. The stress of disharmonious family relationships has been linked to higher rates of depressive symptoms during perimenopause [15, 20]. Menopausal depression is a subtype of depression with a unique etiology and specific symptom characteristics. Women with menopausal depression respond differently to antidepressant medications in comparison to other patients with depression [21].

The etiology of perimenopausal depression is related to hormonal changes, which in turn lead to changes in neurotransmitter systems regulating mood modulation [22]. Estrogens and progesterone influence neurogenenesis, neurotransmission and neuronal regeneration and have anti-inflammatory effects at the level of CNS [23]. Correlations between depressive symptoms and hormones in our study were present in early menopausal transition and late menopausal transition groups. Hormones were not found to be related to severity of depressive symptoms in postmenopausal women. As regards single hormones, there emerged a negative correlation between the severity of depressive symptoms and progesterone, and positive correlations between depressive symptoms and androgens (testosterone and DHEAS).

Most studies on the relationship between progesterone and depression report a negative effect of progesterone on mood. Progesterone increases the activity of serotonin-degrading enzymes: monoaminooxydase and catechol metylotransferase. A mood-impairing effect of progesterone has also been observed in studies exploring the etiology of premenstrual syndrome. In depressive patients a correlation between Montgomery-Asberg Depression Rating Scale score and serum progesterone concentration was reported [24]. The negative correlation between depressive symptoms and progesterone observed in our study may be explained by the positive influence of estrogen

on mood. The patients in our sample with higher progesterone levels may ovulated and had higher estrogen levels.

With regard to the effects of androgens on menopausal depression, studies give various results. Testosterone supplementation in women with major depressive disorder significantly improved the clinical state [3], and testosterone treatment reversed mood disturbances and depression in women after surgical removal of the ovaries [25]. A single dose of testosterone reduced anxiety in the fear-potentiated startle response [26]. Transdermal testosterone in women experiencing age-related declines in androgens resulted in improved mood and psychological well-being [27]. On the other hand, Rohr reported that testosterone can negatively impact mood in women, and can even contribute to the onset of major depressive disorder [28]. Androgens may influence the clinical picture of depression, but there is still no clearly reliable method of androgen level evaluation.

Our groups did not differ in relation to the severity of climacteric symptoms measured using the Kupperman index. Climacteric symptoms were found to be dependent on hormone levels only during menopausal transition. Specifically, climacteric symptoms were dependent on E2 (negative correlation) and testosterone (positive correlation). It is worth stressing that the climacteric symptoms were estrogen dependent, whereas the depression symptoms were not. In late postmenopause climacteric symptoms were not dependent on sex steroids but on cortisol (negative correlation).

The groups differed in age and this may have influenced the severity and frequency of the studied symptoms. Older age is related to a higher risk of depression. The prevalence of depression after the age of 60 is estimated to be 13.3% [29]. An older age at menopause was associated with a lower risk of menopausal depression [30].

There were no differences in BMI between the studied groups; in all of them, the mean BMI was in the overweight range. Epidemiological data reveal that 2/3 of postmenopausal women in Poland have overweight or obesity status [31]. Weight gain during menopause is 0.5 kg per year [32]. This is due not only to hormonal changes but also environmental factors [33, 34]. Among these, urbanization, lower education level, higher parity, obesity in the family, lack of physical activity, marriage at a younger age, shift work, lack of sleep and depression are reported [35].

Conclusions

1. Depression is most frequent in late menopausal transition.
2. Depressive symptoms are related to hormones during menopausal transition, but not during the postmenopause phase.

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Factors affecting pain perception in outpatient hysteroscopy

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ABSTRACT

Background and Purpose: The aim of the study was to evaluate pain severity during and 24 hours after office hysteroscopy in an unselected population.

Methods: A total of 200 women undergoing diagnostic hysteroscopy with different indications were enrolled in the study. Immediately after the examination and at 24 hours visual analog scale (VAS) scores for pain severity were collected. Data on patient age, parity, menopausal age, consumption of anti-inflammatory drugs and biopsy of the endometrium were also collected.

Results: Perceived pain was greater at the end of examination than after 24 hours (p<0.01). Associations were found between higher VAS score and age >50 years, menopausal age, and consumption of analgesics after the intervention. Conversely, there was no association with parity or with the indication for the examination.

Conclusions: The present data confirm that ambulatory hysteroscopy is acceptable to most patients and is safe and reliable.

KEYWORDS
Diagnostic hysteroscopy, office hysteroscopy, vaginoscopy, pain, visual analog scale.

Introduction

First performed by Pantaleoni in 1869 to remove a uterine polyp, hysteroscopy became an effective diagnostic tool only in the 1970s [1]. Since then, continuous development of techniques for distension and illumination of the uterine cavity, together with incessant upgrading of instrumentation, has made hysteroscopy the gold standard examination for study of the uterine cavity. It is indeed an easily implementable, economical and, in most cases, safe technique [2].

Although in recent years modern and innovative techniques such as 3D transvaginal ultrasound and magnetic resonance imaging have been included in diagnostic pathways, hysteroscopy remains the standard approach in the study of the uterine cavity [3, 4]. Moreover, an office hysteroscopy can be performed without the use of local or general anesthesia. Indeed, the vaginoscopic approach (intended to eliminate the need for a tenaculum or a speculum), the creation of more flexible instruments, and the use of saline solution have together reduced discomfort during this examination.

Diagnostic hysteroscopy is defined as an exploration of the uterine cavity without biopsy, and it is extremely useful in several situations: for differentiating normal and abnormal endometrium [5], detecting endometrial inflammation [6], and enabling a diagnosis of endometrial carcinoma [7]. Nevertheless, in some cases, as in the presence of an unevenly shaped or thick endometrial mucosa or an anatomically distorted uterine cavity, it is often necessary to perform a biopsy [7]. Pain and low tolerance are the most common causes of failure of diagnostic hysteroscopy. Today, the need for anesthesia or analgesia

during hysteroscopy is still a matter of debate. Several factors, related to the technique used, patient characteristics and the indication for the intervention, explain the lack of agreement concerning the use of anesthesia in hysteroscopy [8-10].

Although local anesthesia is commonly used for gynecological procedures, a multimodal approach may be more effective, and this applies to hysteroscopy [11]. In addition to technical factors, the various instruments and the approach used, operator expertise, duration of the examination, different definitions of diagnostic hysteroscopy, and the possibility of combining the exploration phase with endometrial biopsy or with surgical treatment of the disease diagnosed ("see-and-treat approach") must all be considered. Furthermore, uterine characteristics or abnormalities, such as cervical stenosis, and patient psychological characteristics influence the perception of pain and the acceptability of the technique [12]. The traditional diagnostic hysteroscopy technique involved the use of > 5-mm hysteroscopes, a speculum, a tenaculum, cervical dilators, and carbon dioxide (CO_2) for uterine distension [13-19]. All of these factors contributed to the risk of discomfort and vasovagal reactions, in about 15% of patients [20, 21]. Operative procedures

using instruments 7 mm or larger for hysteroscopy were considered painful, and they required that analgesia be performed in the operating room. In the last decade, however, substantial changes both in the instrumentation and the technique used have made diagnostic hysteroscopy a completely different examination terms of its feasibility and acceptability [22, 23].

Notwithstanding the popularity of hysteroscopy, there currently exist no precise guidelines on the use of anesthesia or analgesia in diagnostic or operative hysteroscopy, and often the same procedures are performed in women under general, local or even no anesthesia. Consequently, the possibility of time-consuming procedures being performed in the office setting is increasing, and this, without adequate evaluation and control of pain, could affect the feasibility and acceptability of the technique. In particular, nulliparous, menopausal and anxious women most often report significant pain symptoms, which can result in interruption of the examination.

Therefore, the aim of our study was to evaluate pain perception, expressed as a visual analog scale (VAS) score, in women immediately after and 24 hours after this examination.

Materials and Methods

The present study was performed at the University of Siena between January 2013 and December 2013. A group of women (n=200) undergoing diagnostic hysteroscopy were enrolled. The indications for performing an office hysteroscopy were multiple: abnormal uterine bleeding, endometrial thickening, suspicion of endometrial polyp or myoma during an ultrasound examination, and infertility. The office hysteroscopy was always performed by a specializing physician. A 5-mm continuous-flow operative office hysteroscope was used. All the patients signed an informed consent document before undergoing the procedure. The hospital ethics committee approved the study.

To evaluate pain perception, VAS scores were collected immediately after the examination and then again 24 hours later (by telephoning the patients the day after surgery). The VAS consisted of questions where the patient chose a number from 0 to 10 to indicate the severity of the pain she perceived, with 0 meaning no pain and 10 the worst possible pain.

Other data that may be associated with hysteroscopy-related pain were also collected, such as patient age, parity, menopausal age, and use of anti-inflammatory drugs before or after the examination (the most frequently used analgesics included paracetamol, nimesulide or butylscopolamine). The execution of biopsy during the examination was also considered, to assess whether or not it was associated with a higher VAS score.

Statistical analysis: normal distribution of quantitative clinical data was assessed using the Kolmogorov-Smirnov test. Data analyzed by descriptive statistics are presented as means ± standard deviations. Comparing the two groups, the paired t test was used to compute statistical significance. Qualitative variables were compared by the Fisher's exact test. Statistical analysis was performed using the GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., San Diego, California, USA). For all analyses, $p < 0.05$ was taken as the level of statistical significance.

Results

Perceived pain was found to be greater during the execution of the examination than 24 hours after it. In particular, during hysteroscopy, 23 patients (12%) reported no pain, with a VAS score of 0; otherwise, 72 patients (36%) reported mild pain, with a VAS score of between 1 and 4; 72 patients (36%) reported moderate pain, with a VAS score of between 5 and 7, and 33 patients (16%) reported severe pain with a VAS score of 8 or more (Figure 1).

Evaluation of pain scores at 24 hours after hysteroscopy revealed that 136 patients (68%) had no pain, as shown by a VAS score of 0, while the remaining 64 patients (32%) all reported mild pain, with VAS scores of between 1 and 4 (Figure 2).

Details of patient age, parity, menopausal age, consumption of anti-inflammatory drugs before and after the intervention, and indication for the examination are shown in Table 1.

Patients aged 50 years or more gave higher VAS scores

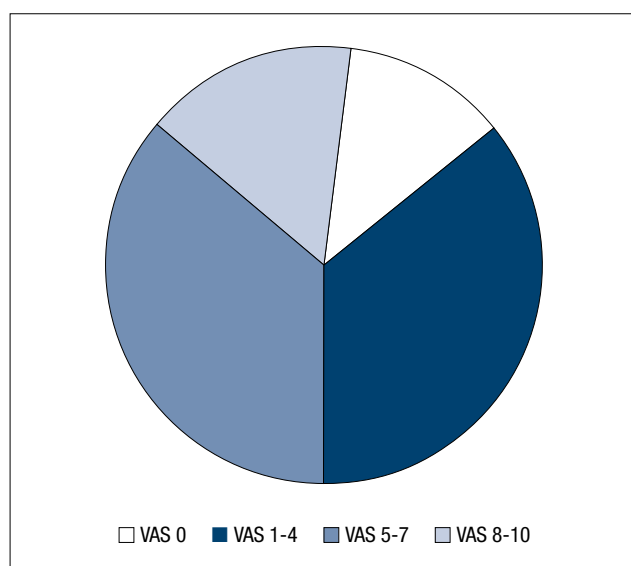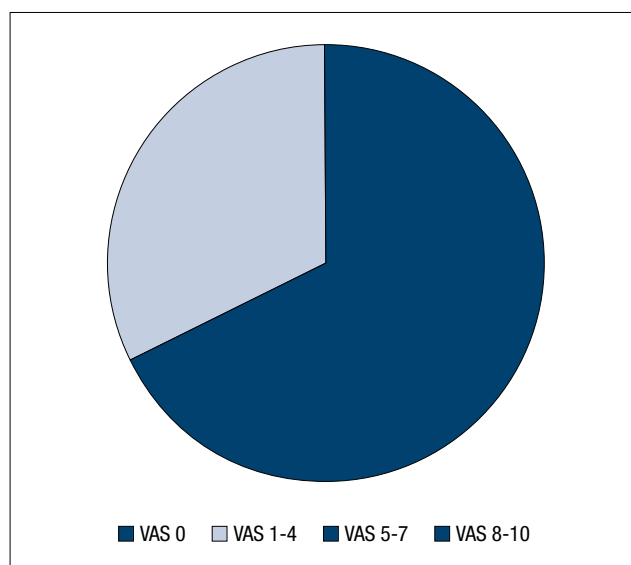**Figure 1** VAS score immediately after hysteroscopy.

☐ VAS 0 ■ VAS 1-4 ■ VAS 5-7 ☐ VAS 8-10

Figure 2 VAS score 24 hours after hysteroscopy.

■ VAS 0 ☐ VAS 1-4 ■ VAS 5-7 ■ VAS 8-10

(4.7) after examination compared with the others (3.7) with p=0.0103. Postmenopausal women gave higher VAS scores (median value of 4.62) than patients of reproductive age (median value of 3.7) with p=0.039 (Figure 3). No difference in VAS score was found between patients with a previous vaginal delivery (median VAS of 4.14) compared with nulliparous patients (median VAS of 4.54).

Furthermore, there was no difference in VAS score between the women who underwent the examination for infertility and those who underwent it for other reasons, such as metrorrhagia, endometrial thickening, or suspicion of endometrial polyp or submucosal myoma. No significant difference was observed between the group of women who took anti-inflammatory drugs and the group of women who did not (p=0.058).

Women who used drugs such as paracetamol, nimesulide or butylscopolamine recorded higher VAS scores after surgery (p< 0.0001) compared with those who did not use these analgesics, while there was no statistical difference in their VAS scores at 24 hours after hysteroscopy.

There were no differences in postoperative VAS and 24 hours VAS between the group undergoing biopsy and the group of women who did not have a biopsy.

Discussion

For the diagnosis and, in some cases, treatment of abnormal uterine bleeding ambulatory hysteroscopy is a safe, reliable alternative that is acceptable to patients when compared with hysteroscopy under general anesthetic [24]. The vaginoscopic approach, in which neither a speculum nor a tenaculum are used, while saline solution at low pressure is used for distending the uterine cavity, has contributed substantially to these improvements. Moreover, thanks to the use of thin endoscopes, diagnostic hysteroscopy is considerably less painful and easier to perform, even for operators with minimal training, and it is becoming a popular technique [25,26]. Due to the innovations in this setting, anesthesia is now finding only limited space, even though several studies over the years have examined the use of various anesthetic techniques, such as transcervical block, paracervical block, intracervical block, topical anesthesia and non-steroidal anti-inflammatory drugs [24, 27-29]. Although miniaturized instruments are making hysteroscopy in the office setting possible in a growing number of women, the primary limitations to its widespread use are pain and low patient tolerance, as severe pain and adverse effects may rarely occur even when using mini-instruments.

Generally, women with a history of cesarean section, chronic pelvic pain or anxiety should be considered at risk of pain perception, whereas in our study we found that older women experienced more pain than younger ones in a statistically significant manner.

Focusing on older age and in particular on menopause in women, the Study of Global Ageing and Adult Health reported an increase in the prevalence of pain with increased age. Old age predisposes to frequent occurrence of chronic pain connected both with involuntary changes of the elder organism and with multiple morbidities characteristic of that period of life [30].

Table 1 Association between higher VAS score and different parameters.

Parameter	Statistical Significance
Menopausal age	p < 0.05
Age > 50	p = 0.01
Parity	Ns
Indication: infertility	Ns
Anti-inflammatory before intervention	Ns
Anti-inflammatory after intervention	p < 0.0001
Biopsy	Ns

Figure 3 Postmenopausal women showed greater VAS score compared to patients of reproductive age.
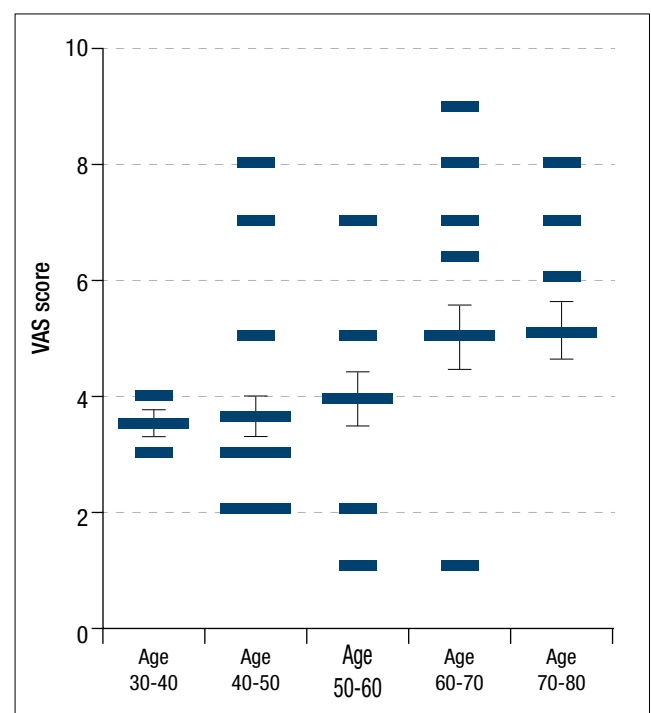
In addition, it is well known that menopause, which is a normal event for women, is associated with several symptoms, such as vasomotor dysfunction and vaginal dryness or mood changes, sleep disturbances, urinary incontinence, cognitive changes, somatic complaints, sexual dysfunction, and, in general, with a reduced quality of life [31]. Pain in menopause is a problem that involves various aspects, e.g. musculoskeletal, sexuality, the cranio-facial region. However, hysteroscopy remains a first-line technique for investigation of abnormal uterine bleeding and other diseases involving the uterine cavity.

Therefore, our study, like most of the literature, suggests that in experienced hands, office hysteroscopy is well tolerated, and analgesia is required only in selected patients. In our experience, these selected patients, who could benefit from an analgesic treatment prior to this fundamental procedure, are the oldest group. With the general aging of the population, old age is becoming an increasingly important focus of aging research

and public health [32].

With more and more people reaching very old age it is necessary to improve treatments and therapies that may protect their health and their quality of life. From this perspective, pain control during hysteroscopy makes sense.

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The LH/AMH ratio as a predictive value for the outcome of assisted reproductive techniques

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ABSTRACT

Purpose: This study was conducted to test the luteinizing hormone/anti-Müllerian hormone (LH/AMH) ratio as a possible new predictive factor for outcomes after controlled ovarian hyperstimulation (COH) in women undergoing in vitro fertilization (IVF) / intracytoplasmic sperm injection (ICSI) treatment.
Methods: This retrospective cohort study included 164 women submitted to their first IVF/ICSI treatment.
Results: Pregnancy and live birth rates were 21.5% and 18.6%, respectively. In a generalized linear model for the prediction of oocyte quantity after COH, age (odds ratio, OR, 0.98, 95% confidence interval, CI, 0.97;0.99), smoking (OR 0.83, 95%CI 0.68;0.99), AMH serum levels (OR 1.06, 95%CI 1.03;1.09) and a higher LH/AMH ratio (OR 0.97, 95%CI 0.96;0.98) were associated with oocyte quantity. For both ongoing pregnancy and live birth rates, only age was found to be predictive (OR 0.92, 95%CI 0.86;0.97, and 0.87, 95%CI 0.78;0.96; respectively).
Conclusion: The LH/AMH ratio is a new predictive parameter for oocyte quantity after COH. However, for the clinically more relevant outcome parameters of ongoing pregnancy and live birth, only patient age was significantly predictive.

KEYWORDS

Ovarian reserve test, anti-Müllerian hormone, luteinizing hormone, follicle-stimulating hormone, IVF, ICSI, outcome, pregnancy rate, live birth.

Introduction

Since there is a wide variation in the ovarian aging process, and even some young women respond poorly to controlled ovarian hyperstimulation (COH) [1], a test or a predictive model able to provide reliable information about the individual woman's chances of achieving pregnancy after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment would certainly be desirable. The identification of potential low and high responders to COH could be of high clinical value.

The term "ovarian reserve" is widely used to refer to the number of oocytes in the ovaries and their quality. Several ovarian reserve tests have been evaluated with the goal of reliably predicting outcome after IVF/ICSI treatment. However, as reviewed in detail, no single ovarian reserve test offering more than modest predictive properties has been identified to date [2]. Several tests have been known for some time and include measurement of early-follicular-phase blood values of follicle-stimulating hormone (FSH), estradiol, inhibin B, and anti-Müllerian hormone (AMH), the antral follicle count (AFC), measurement of ovarian volume and ovarian blood flow, the clomiphene citrate challenge test, the exogenous FSH ovarian reserve test, and the gonadotrophin agonist stimulation test [2]. In addition, early follicular levels of luteinizing hormone (LH) and their relationship to FSH levels have been evaluated in the past few years [3-5]. An elevated day 3 FSH/LH ratio has been demonstrated to be a sign of diminished ovarian reserve and poor response to hyperstimulation [3, 4]. Unfortunately, in an

analysis based on age, day 3 FSH/LH ratio was accurate only in younger women [5]. In women of reproductive age, AMH is secreted by small antral follicles [6, 7]. It is structurally related to inhibin and activin, and, as a protein hormone, it belongs to the transforming growth factor ß- family [8]. The gene responsible for AMH is located on chromosome 19p13.3, and the genetic locus for the AMH receptor II is located on chromosome 12, which has a role in controlling the formation of primary follicles [9]. A large meta-analysis that included 5705 women undergoing IVF demonstrated that AFC and AMH levels, in addition to age, might predict a poor response. However, a combination of both parameters did not improve prediction, and other ovarian reserve tests did not add any information. Moreover, when testing predictive parameters for ongoing pregnancy, only age was of moderate value [10]. Thus, for the main outcome parameters of IVF, namely pregnancy and live birth rates, there are, as yet, no laboratory parameters that can be used for prediction.

Weghofer and Gleicher have suggested that the human reproduction system aims to maintain a "normal equilibrium" in

the "presence of opposing forces". This refers, for example, to the activities of androgens and estrogens, as well as FSH and LH [11]. The rationale behind testing the predictive value of the interaction between LH and AMH was based on previous findings and assumptions. First of all, whereas LH alone does not seem to be predictive of IVF outcome, the FSH/LH ratio does [4,12]; second, we realize that LH and AMH are produced and secreted at totally different sites, and that LH has no effect on AMH expression by lutein granulosa cells in normo-ovulatory women. However, LH does lead to an up-regulation of AMH messenger RNA production in oligo-/anovulatory women with polycystic ovary syndrome. In addition, LH reduces AMH receptor II expression in normo-ovulatory women [13]. These data seem to indicate that there is a pathophysiologically relevant link between LH and AMH, at least in women with an ovulatory imbalance. On the basis of these considerations, we set out to evaluate the clinical value of the LH/AMH ratio for the prediction of oocyte quantity after COH, pregnancy, and live birth rates in our patient population. We also considered the predictive value of several "canonical" ovarian reserve tests.

Materials and Methods

Study population and study design

This retrospective study included 177 women who underwent their first IVF/ICSI treatment cycle at the Department of Gynecologic Endocrinology and Reproductive Medicine of the Medical University of Vienna, between January 2008 and January 2012. Inclusion criteria were the use of a standard antagonist protocol, age <42 years, regular menstrual cycles (every 25–35 days), total antral follicle count of 8–18, and basal day 3 FSH levels <12 mIU/mL in unstimulated cycles.

Data were retrieved by retrospective chart review. The main outcome parameters were oocyte quantity after COH and ongoing pregnancy rate, indicated by a positive heartbeat during week five after embryo transfer (ET), as well as live birth rate. The study was approved by the ethics committee of the Medical University of Vienna, Austria (IRB number 044/2010).

Laboratory determinations

All patients were tested for serum AMH, FSH, LH, estradiol, and thyroid-stimulating hormone (TSH) levels on the second day of the menstrual cycle before COH. All examined serum parameters were determined in the central laboratory of the General Hospital of Vienna, Vienna, Austria, using commercially available assays. Enhanced chemiluminescence immunoassay systems were used to determine serum levels of LH, FSH, and estradiol. An enzyme-linked immunosorbent assay was used to determine AMH levels. Details of these tests applied are provided online at http://www.kimcl.at.

IVF treatment

All women were treated using an antagonist protocol, as published previously [14]. On day 2 of the menstrual cycle, transvaginal sonography was performed and a blood sample was obtained for hormone analyses (AMH, FSH, LH, estradiol, TSH, prolactin), which were performed, using standard protocols, at the Central Laboratory of the General Hospital of Vienna, Department of Laboratory Diagnostics, Medical University of Vienna. The stimulation was started on day 3 with a basal dosage of 200 IU of recombinant FSH (Puregon; AESCA Pharma). Monitoring was performed by transvaginal sonography. When necessary, the FSH dosage was adjusted according to the follicle number and diameter. When adequate stimulation was achieved (≥3 follicles of R18 mm in diameter), a 10,000 IU dose of human chorionic gonadotropine (hCG) (Pregnyl; AESCA Pharma) was administered. Oocyte retrieval was performed 35 hours after hCG injection. Conventional IVF following standard techniques was used for fertilization. A maximum of two embryos were transferred through a Wallace catheter between days 3 and 5 after oocyte retrieval. All patients received 10 mg of dydrogesterone (Duphaston; Solvay Pharma) orally twice daily and 200 mg of progesterone (Utrogestan; Meda Pharma) vaginally three times daily for luteal support. Biochemical pregnancy was defined as a positive urinary hCG test on day 14 after transfer. A clinical pregnancy was defined as an intrauterine pregnancy with an embryo with positive heartbeat, verified by transvaginal sonography five weeks after ET.

Parameters analyzed

We included the following parameters in the multivariate models to predict the number of retrieved oocytes, as well as the rates of pregnancy and live birth: age; body mass index (BMI); female smoking; FSH; LH; estradiol; AMH; and the LH/AMH ratio. For the latter, AMH values <0.01 were analyzed as 0.01 to allow calculation of the LH/AMH ratio.

Statistical analysis

Nominal variables are reported as numbers and frequencies, and continuous variables as medians and interquartile ranges (IQR). Statistical analysis was performed using a logistic regression model to test the statistical significance of all coefficients for the prediction of pregnancy and live birth. A generalized linear model with a Poisson link function was used for the prediction of oocyte quantity. Odds ratios (OR) are given, including the 95 per cent confidence interval (95% CI). P-values <0.05 were considered statistically significant. Statistical analyses were performed with the SPSS software package, version 19 (SPSS, Chicago).

Results

Table 1 provides details of patient characteristics. The median number of retrieved oocytes was 5 (IQR 3–8). Pregnancy and live birth rates were 21.5% and 18.6%, respectively. In a generalized linear model for the prediction of oocyte quantity after COH, increasing age, smoking, a lower AMH value, and a higher LH/AMH ratio were associated with a lower oocyte quantity (Table 2). We then calculated two logistic regression models for the prediction of (i) ongoing pregnancy (Table 3), and (ii) live birth after IVF (Table 4). In both analyses, only age was significantly associated with IVF outcome (OR 0.92 and 0.87, respectively), with increasing age leading to lower pregnancy and live birth rates.

Table 1 Basic patient characteristics.

Number of patients	177
Age (years) [a]	34 (30-38)
Body mass index (kg/m²) [a]	23.0 (20.4-26.3)
Tubal factor [b]	32 (18.1)
Male factor infertility [b]	123 (69.5)
Endometriosis [b]	30 (16.9)
Polycystic ovary syndrome [b]	19 (10.7)
Idiopathic infertility [b]	4 (2.3)
Number of retrieved oocytes [a]	5 (3-8)
Length of stimulation (days) [a]	11 (10-12)
ICSI [b]	71 (47.0)
Pregnancy rate [b]	38 (21.5)
Live birth rate [b]	33 (18.6)

Data are provided as *a* median (interquartile range) or *b* n (%)
Multiple citations possible

Table 2 Generalized linear model using a Poisson link function for prediction of oocyte quantity after controlled ovarian hyperstimulation.

	Odds ratio	95% confidence interval	Wald's chi square	LR test*
Smoking	0.83	(0.68;0.99)	3.812	0.050
Age	0.98	(0.97;0.99)	5.612	0.018
Body mass index	1.00	(0.98;1.01)	0.138	0.711
FSH	0.99	(0.95;1.02)	0.659	0.071
LH	1.02	(0.99;1.05)	2.258	0.133
Estradiol	1.00	(1.00;1.01)	1.295	0.255
AMH	1.06	(1.03;1.09)	19.100	<0.001
LH/AMH ratio	0.97	(0.96;0.98)	4.464	0.035

* LR test = likelihood ratio test

Table 3 Multivariate logistic regression model for the prediction of ongoing pregnancy after IVF/ICSI.

	Pregnancy (n=38)	No pregnancy (n=139)	Odds ratio	95% confidence interval	Wald's chi square	LR test [a]
Age (years) [b]	30 (26;34)	33 (29;38)	0.92	(0.86;0.97)	3.528	0.030
BMI (mg/m²) [b]	23.2 (20.2;28.9)	23.5 (20.9;26.3)	1.01	(0.92;1.11)	0.023	0.879
Smoking [c]	9 (23.7)	31 (22.3)	0.88	(0.30;2.58)	0.050	0.823
Endometriosis [c]	8 (21.1)	22 (15.8)	1.18	(0.28;5.07)	0.051	0.821
FSH (U/l) [b]	6.5 (5.5;8.6)	6.7 (5.4;8.3)	1.01	(0.83;1.23)	0.015	0.903
LH (mIE/ml) [b]	5.2 (3.5;8.8)	5.2 (4.0;7.1)	1.10	(0.93;1.30)	1.206	0.272
E2 (pg/ml) [b]	37.0 (26.0;43.0)	37.0 (27.8;52.0)	0.99	(0.97;1.02)	0.617	0.432
AMH (mg/dl) [b]	1.9 (1.2;3.0)	1.9 (0.8;3.5)	0.93	(0.73;1.18)	0.359	0.549
LH/AMH ratio [b]	2.1 (1.1;4.9)	2.7 (1.6;5.3)	0.95	(0.83;1.10)	0.425	0.515

a LR test = likelihood ratio test; data are provided as *b* median (interquartile range) or *c* n (%)

Table 4 Multivariate logistic regression model for the prediction of live birth after IVF/ICSI.

	Live birth (n=33)	No live birth (n=144)	Odds ratio	95% confidence interval	Wald's chi square	LR test [a]
Age (years) [b]	29 (25;33)	33 (29;38)	0.87	(0.78;0.96)	7.124	0.008
BMI (mg/m²) [b]	23.4 (21.5;29.2)	23.4 (20.6; 26.3)	1.04	(0.93;1.15)	0.442	0.506
Smoking [c]	7 (23.3)	33 (22.9)	0.61	(0.18;2.12)	0.603	0.437
Endometriosis [c]	7 (23.3)	23 (16.0)	2.07	(0.45;9.63)	0.863	0.353
FSH (U/l) [b]	6.1 (5.4;8.6)	6.7 (5.4;8.4)	0.97	(0.79;1.20)	0.080	0.777
LH (mIE/ml) [b]	6.1 (3.0;10.9)	5.2 (4.0;7.0)	1.15	(1.97;1.36)	2.729	0.099
E2 (pg/ml) [b]	37.0 (26.0;43.0)	37.0 (28.0;51.8)	0.98	(0.95;1.01)	1.314	0.252
AMH (mg/dl) [b]	1.9 (1.2;3.8)	1.9 (0.9;3.5)	0.95	(0.77;1.18)	0.218	0.641
LH/AMH ratio [b]	2.2 (1.0;5.9)	2.7 (1.6;5.1)	0.93	(0.93;1.05)	0.059	0.808

a LR test = likelihood ratio test; data are provided as *b* median (interquartile range) or *c* n (%)

Discussion

This retrospective study demonstrated that a lower LH/AMH ratio was associated with higher oocyte quantity during IVF/ICSI treatment, making this a new predictive parameter for oocyte quantity, in addition to classical predictive parameters, such as age, smoking, and AMH. To the best of our knowledge, this is the first study to evaluate the LH/AMH ratio from this perspective.

The measurement of AMH in reproductive medicine is the subject of some debate [15]. It is now evident that AMH concentrations correlate with the number of retrieved oocytes. However, many studies were unable to find an association between AMH serum levels and the outcome of IVF and ICSI, in terms of pregnancy rate and live birth rate [14-17]. Only a few trials suggested the opposite: Merhi and colleagues, for example, found that an AMH cut-off level of 0.2 ng/ml seemed to be a meaningful threshold for the prediction of the clinical pregnancy rate in women with severely diminished ovarian reserve [18]. Moreover, the combination of abnormally high FSH and AMH levels was found to reflect highly beneficial outcomes of IVF [19]. In women undergoing ICSI, AMH was also highly predictive for pregnancy and live birth [20].

However, the observation of many previous studies, namely that AMH was not reliable in the prediction of pregnancy and life birth rates, is in accordance with our results. Our study also confirmed the correlation between AMH serum levels and the number of retrieved oocytes (OR 1.06, p< 0.001). In our study, we were unable to find a correlation between AMH serum levels on day two of the cycle and pregnancy rate or live birth rate. Many factors can play a role here. One is the fact that AMH levels in serum do not predict the chromosomal abnormalities of oocytes or embryos that may occur with ovarian aging and diminishing ovarian reserve, as Tremellen also reported [21, 22]. These findings suggest that the most determinant factor for oocyte quality is not the size of the ovarian pool, which is measured by AMH, but the age of the oocyte. Moreover, the fact that AMH levels decline with age is independent of oocyte aging and is likely due to the reduction of the follicle pool [23, 24].

We combined LH and AMH serum levels by calculating the LH/AMH ratio. The connection between LH and oocyte maturation is not fully understood, but it is known that the LH peak in the middle of the cycle causes a resumption of meiosis, the rupture of the follicular wall, cumulus-oocyte expansion, and transformation of granulosa cells into luteal cells through a signaling pathway dependent on epidermal growth factor and amphiregulin. Moreover, LH promotes the growth and differentiation of ovarian granulosa cells, and thereby, the formation of the corpus luteum [23, 25-27]. Although, hypothetically speaking, higher LH levels at the start of the cycle may result in better maturation of ovarian granulosa cells, and thus, in better function of the corpus luteum after IVF treatment (which is already the case for AMH) [28, 29], this presumably better function of the corpus luteum did not lead to a higher rate of implantation after IVF (Table 3). Notably, LH was not an independent predictive parameter, which is in accordance with a previous study in women undergoing long-protocol COH that failed to demonstrate LH as a predictive parameter for ovarian response,

conception, or pregnancy outcome [30].

However, the new parameter, LH/AMH ratio, adds additional information to the multivariate model for the prediction of oocyte quantity (OR 0.97, p=0.035). As demonstrated in Table 2, the predictive value of AMH is obviously higher than that of the LH/AMH ratio. However, the latter is still an independent predictive factor in the multivariate model. Notably, the lower the LH/AMH ratio was (in other words, the higher the AMH and the lower the LH in the ratio), the better. One might argue that although a certain baseline LH level is needed for high-quality maturation of ovarian granulosa cells, higher AMH levels, indicating a sufficient ovarian pool, are beneficial to the patient. Hypothetically, the LH/AMH ratio could be representative of egg quality, since oocyte number and egg quality have been suggested to be directly related to each other [29]. Of note, the LH/AMH ratio did not predict pregnancy and live birth rates, and, thus, we do not consider it to be associated with the chance of implantation.

Despite the fact that we found various factors influencing the number of retrieved oocytes, our data provide a further demonstration that there is no serum parameter able to predict clinical pregnancy and live birth rates after IVF treatment. Unfortunately, the LH/AMH ratio did not meet our expectations as only age was associated with these outcome parameters, which stands in accordance with another report [10].

This study must be interpreted within the context of its retrospective design. In conclusion, we provided the first demonstration of LH/AMH ratio as a new predictive parameter for oocyte quantity after COH, in addition to age, smoking, and AMH. However, for the clinically more relevant outcome parameter of ongoing pregnancy rate, only patient age was significantly predictive. Future studies are warranted to confirm our results and, should they be confirmed, elucidate the reason for the association between the LH/AMH ratio and oocyte number, which could hypothetically be linked to egg quality.

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What is the relationship between visceral adiposity index (VAI) and total and free sex hormones in aging polish women?

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ABSTRACT

The visceral adiposity index (VAI) is a novel and very sensitive cardiometabolic predictor. The number of cardiovascular diseases in women increases with climacteric transformation. In 505 women aged over 65 years, randomly selected from the whole of Poland, we investigated whether VAI score was associated with sex hormones. We split the women into two groups on the basis of a VAI cut-off score of 2.0. In the group with normal VAI scores, we found significantly higher serum testosterone levels and a lower free estradiol index vs the group with abnormal VAI scores. VAI correlated positively with fasting glucose and insulin levels and with homeostatic model assessment of insulin resistance (HOMA-IR). Our data suggest a cardiometabolic protective role of testosterone in aging women.

KEYWORDS
Visceral adiposity index, cardiometabolic risk.

Introduction

Cardiovascular diseases are the most frequent cause of mortality in aging women. The term "cardiometabolic risk" was coined by the American Diabetes Association and American Heart Association to describe the overall risk of developing type 2 diabetes and cardiovascular diseases [1].

Today, waist circumference (WC) is the measure most commonly used to identify visceral obesity [2]. Yet we are still looking for a more precise cardiometabolic risk predictor [3]. Amato et al. proposed the visceral adiposity index (VAI) — a mathematical model based on simple parameters, both anthropometric (body mass index – BMI, WC) and functional (serum triglycerides – TG and high-density lipoprotein cholesterol – HDL-C) — as a marker of insulin resistance [4]. It has been demonstrated that an elevated VAI is associated with cardiometabolic risk predictors.

It must be also considered that total and free sex hormones as well as sex hormone-binding globulin (SHBG) play an important role in fat mass and fat distribution in women [5, 6]. This is best exemplified by fat redistribution during the climacteric transition, when visceral fat deposition increases and gynoid fat deposition decreases [7, 8]. As a consequence of the association of visceral obesity with insulin resistance, the majority of postmenopausal women presented increased cardiometabolic risk predictors and cardiovascular disease [9]. In contrast to the reproductive years and menopause, periods in a woman's life in which sex hormones are known to play a cardioprotective role, the associations between sex hormones and cardiometabolic risk predictors in aging women are not clear [6, 10].

The goal of our study was to examine how VAI score was associated with serum levels of SHBG, estradiol (E2) and testosterone (T) and with the free estrogen index (FEI) and free androgen index (FAI) in aging women.

Material and methods

The research sample comprised 505 women aged 65-103 years recruited from the whole of Poland. The recruitment procedure has been described in a previously published paper [11]. We measured BMI and WC in all the women according to World Health Organization recommendations [12]. Blood samples were drawn from the cubic vein in the morning after an overnight fast and immediately centrifuged and stored at -20°C.

The biochemical and hormonal parameters investigated included: serum fasting glucose (FG), total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C) and TG, measured by an enzymatic method using Architect Plus 4100 by Abbott Diagnostica, Illinois, USA.

Fasting insulin (FI), T, E2, and SHBG levels in serum were estimated by a chemiluminescent method using Immulite 2000 by Siemens Healthcare, Erlangen, Germany. The FAI was cal-

culated by the formula: 100 x testosterone [nmol/l] / SHBG [nmol/l]. To convert testosterone to nanomolar values, the nanogram per mililiter value is multiplied by 3.47. Likewise, the FEI was calculated using the following formula: 100 x estradiol [nmol/ml] / SHBG [nmol/l]. To convert E2 to nanomolar values, the picogram per milliliter value is multiplied by 0.00367 [13, 14]. The VAI score was calculated as described elsewhere [15] using following formula: Female VAI = [WC/ 36.58 + (1.89 x BMI)] x (TG/0.81) x (1.52/HDL-C). The homeostatic model assessment – insulin resistance (HOMA-IR) value was calculated from the formula: (FG [nmol/l] x FI [mUI/l])/22.5 [16]. We divided the whole group into two subgroups on the basis of a VAI cut-off score of 2.0, which was suggested by Amato et al. for women over 65 years of age [4]. VAI scores above 2.0 were present in 151 (30%) women, while 354 (70%) had scores below 2.0. In both groups, we compared the mean values of serum E2, FEI, T, FAI, SHBG, FG, FI and HOMA-IR. Also, correlations between VAI score and sex hormones as well as carbohydrate metabolism parameters were estimated.

Ethical approval

Ethics Committee approval was obtained. The PolSenior project was approved by the Bioethics Commission of the Medical University of Silesia in Katowice, Poland. This study was performed after the patients, who had been informed in detail about the study, had signed an informed consent form, prepared according to the principles of the Helsinki Declaration [17].

Statistical analysis

The Statistical Packages for Social Sciences SPSS version 17 and MedCalc version 11.3 were used for data analysis. Baseline characteristics were presented as mean ± standard deviation (SD) for continuous variables. Normality of distribution for quantitative data was assessed by the Kolmogorov-Smirnov test. Differences between groups in univariate analysis were detected by the unpaired Student's t-test. Spearman's rank correlation test was used to estimate the associations between sex hormones and cardiovascular risk predictors.

Results

The mean values ± SD of BMI, WC, biochemical parameters (TC, HDL-C, LDL-C, TG, FG, FI, HOMA-IR) and hormonal parameters (E2, FEI, T, FAI) in a group of aging women are presented in table 1. The mean values ± SD of serum T, E2, SHBG and FG, FI as well as the FAI, FEI and HOMA-IR values calculated in a group of aging women with VAI scores > or < 2.0 are presented in table 2. Women with VAI scores above 2.0 presented statistically significantly lower serum T levels and FAI scores, and higher FEI scores. Also, serum SHBG levels were significantly lower in the group with VAI > 2.0. With regard to carbohydrate metabolism, women with VAI > 2.0 showed significantly higher serum FG, FI and HOMA-IR values.

Table 1

	Mean±SD
Age (years)	75.57±11.47
BMI (kg/m²)	27.55±4.42
WC (cm)	92.27±1.44
TC (mg/dl)	210.52±43.63
HDL-C (mg/dl)	59.39±13.25
LDL-C (mg/dl)	126.86±37.69
TG (mg/dl)	116.29±48.45
FG (mg/dl)	91.96±11.38
FI (IU/ml)	6.30±4.1
HOMA-IR	1.47±1.04
E₂ (pg/ml)	6.51±2.56
FEI	0.04±0.02
T (ng/ml)	0.3±0.18
FAI	1.24±1.28
SHBG (nmol/l)	69.00±32.52

- To convert E2 to nanomolar, the picogram per milliliter value is multiplied by 0.00367.
- To convert T to nanomolar, the nanogram per mililiter value is multiplied by 3.47.
- To convert FG to mmol/l, the milligram per deciliter value is multiplied by 0.056.
- To convert TC, HDL-C, LDL-C to mmol/l, the milligram per deciliter value is multiplied by 0.011.
- To convert TG to mmo/l, the milligram per deciliter value is multiplied by 0.026.

Table 2

	VAI > 2.0 (n=131)	VAI < 2.0 (n=354)	Statistical significance
T (ng/ml)	0.15±0.09	0.22±0.20	p=0.0001
E₂ (pg/ml)	6.46±2.45	6.53±2.63	NS
SHBG	59.72±27.55	72.90±33.93	p=0.00001
FG	95.52±10.35	90.44±11.56	p=0.00001
FI	7.16±4.46	5.96± 3.90	p=0.002
FAI	1.11±0.09	1.30±1.24	p=0.04
FEI	0.05±0.03	0.04±0.02	p=0.00001
HOMA-IR	1.73±1.12	1.37±0.99	p=0.0003

We also estimated correlations between VAI score and sex hormones, as well as between VAI score and serum FG, FI and HOMA IR. We did not find any statistically significant correlation between VAI score and serum T, E2, FAI and FEI values in aging women. In contrast, VAI score was significantly correlated with FG (r=0.17; p=0.04), FI (r=0.24; p=0.04) and HOMA-IR (r=0.24; p=0.003) in aging women.

Discussion

It has been shown that VAI can be a useful tool for early detection of a condition of cardiometabolic risk, before it develops into an overt metabolic syndrome. It has also been observed that VAI correlates independently with insulin resistance in various endocrine diseases, such as acromegaly, poly-

cystic ovary syndrome (PCOS), type 2 diabetes, non-alcoholic fatty liver disease, viral hepatitis C and prolactinoma [15, 18, 19, 20].

The VAI is a useful indicator for evaluation of cardiometabolic risk with higher sensitivity and specificity than classical parameters such as WC, BMI and lipids, which are routinely used in order to evaluate visceral adipose function [21, 22, 23].

In the whole group of aging women investigated in this study, 30% presented elevated VAI scores, which were associated with lower serum T and SHBG levels compared with those recorded in the group with VAI scores < 2.0. It can be suggested that serum T may play a cardiometabolic protective role in aging woman.

However, normoglycemic women with PCOS and hyperandrogenism have been shown to present comparable VAI scores vs women with pre-diabetes [22]. Also, in another paper, it was shown that VAI scores in women with PCOS and in healthy controls were comparable. However VAI scores in obese vs non-obese women were significantly higher in the obese group [20, 24].

We also found higher serum FG, FI and HOMA-IR values in aging women with VAI scores > 2.0. Our results seem to be supported by data which show significantly higher HOMA-IR values in women with PCOS and elevated VAI scores [21, 22]. In contrast to Amato et al. data, we did not find correlations between biological age and VAI score [4]. However, this was a different ethnic population.

Investigating VAI score associations with serum T and E2, as well as FAI, FEI and SHBG, we did not find any correlations. Only in one study was FAI score found to correlate positively with VAI in women with PCOS, but it did not correlate with sex hormones, as it does in our study [22]. So, the association of VAI score with sex hormones in aging women needs further study. Similarly to other investigators, we found statistically significant correlations between VAI score and HOMA-IR [21, 22].

In conclusion, our original data show that the use of a VAI cut-off score of 2.0 for aging women can be a simple and useful method for identifying women with cardiometabolic risk. Also, it must be considered that serum T levels play a cardiometabolic protective role in aging women, in contrast to FEI, and this is an aspect that needs further study.

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Management of thyroid disorders in pregnancy: an antenatal clinic audit

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ABSTRACT

Background and purpose: Optimization of maternal thyroid hormone levels is of paramount importance specifically during the first trimester of pregnancy, due to the relatively delayed maturation of fetal thyroid gland. This audit evaluated the referral process of pregnant women to the antenatal endocrinology clinic, as per local guidelines.

Methods: A total of 100 pregnant women referred to the antenatal endocrinology clinic were evaluated between April 2017 and April 2018. Demographic parameters, obstetric/endocrine history, timing of referral and thyroid status were documented by reviewing the handwritten and electronic records of each patient.

Results: Among the evaluated women, 90% were referred directly by general practitioners. One third of women were referred during the first trimester. The reasons for referral were: overt/subclinical hypothyroidism (74%), current/previous hyperthyroidism (17%), history of thyroidectomy (5% due to thyroid cancer; 4% for other reasons), thyroid nodule (1%). Only 34% of the women had TSH levels below 2.5mU/L before the first appointment, while subclinical hypothyroidism was evident in 41%. In 25% of cases, the TSH test was performed only at the time of the first appointment, while readjustment of levothyroxine dose was evident in approximately half of the hypothyroid cases.

Conclusion: These pregnant women were appropriately referred to the antenatal endocrinology clinic. However, failure to up-titrate the levothyroxine dose was relatively frequently observed, while many cases were seeking specialist care late in their pregnancy. The management of women with thyroid disorders could be improved through educational programs targeting those who wish to conceive or have recently conceived.

KEYWORDS

Thyroid disorders, treatment algorithm, pregnancy, gestational age.

Introduction

Fetal growth and development are dependent on bioavailable levels of thyroxine and triiodothyronine [1]. Given the delayed maturation of the fetal thyroid gland, which is estimated to take place between the 14th and the 18th week of gestation, the bioavailability of thyroid hormones in the fetal circulation is directly linked to the optimal function of the maternal hypothalamus-pituitary-thyroid axis and the resulting levels of thyroid hormones [2,3]. In fact, the greatest demand in thyroid hormones occurs very soon after gestation starts, increases until mid-gestation and thereafter stabilizes up until delivery [4].

Thyroid hormones exert anabolic effects on fetal metabolism. Their primary role consists of stimulating fetal oxygen consumption and controlling the effectiveness of other hormones and growth factors that impact on fetal development, such as insulin-like growth factors1. From a physiological point of view, thyroid hormones induce fetal brain and somatic tissue development, and promote general accretion of fetal mass. Closer to term, thyroid hormones trigger terminal differentiation of fetal tissues and mediate the maturational effects of glucocorticoids that ensure neonatal viability [1].

Disorders of thyroid function complicating pregnancy are not uncommon. The prevalence of overt hypothyroidism during pregnancy ranges from 0.3 to 0.5%, whereas rates of subclinical hypothyroidism (SCH) with either positive or negative thyroid peroxidase antibodies are estimated to range from 4 to 6%. On the other hand, overt hyperthyroidism is evident in 0.1-0.4% of pregnancies, with Grave's disease accounting for up to 85% of cases. Finally, subclinical hyperthyroidism is observed in 2-5% of pregnant women [5].

A significant body of evidence has indicated that thyroid dysfunction may have an adverse impact on the maternal-fetal unit. Hypothyroidism is associated with a higher risk of gestational hypertension, pregnancy loss, premature birth, low birth weight and lower neonatal IQ. Moreover Instead, inadequate control of maternal hyperthyroidism might result in pregnancy loss, intrauterine growth restriction, stillbirth, maternal congestive heart failure, thyroid storm, as well as prematurity and low birth weight [6,7]. As described in a meta-analysis of 2,532,704 women, pregnant women with overt hypothyroidism or hyperthyroidism

had up to 1.19 or 1.24 higher odds of preterm delivery compared with the reference group [8]. However, evidence regarding the implications of SCH in pregnancy outcomes remains conflicting, with meta-analyses reporting either a direct association [9] or no link with preterm birth [8]. In vivo studies exhibited impaired memory performance and spatial learning in the offspring of animals with maternal SCH during pregnancy. [10]

The joint endocrinology/obstetrics clinic at the Royal Free Hospital is very active, monitoring the health of future mothers throughout their pregnancy until the time of delivery, and forming individual healthcare plans according to the needs of every patient. In 2017, the Royal Free London NHS Foundation Trust published guidelines to help general practitioners and obstetricians with the management of pregnant women with thyroid disorders, which provide a clear stepwise approach [7].

The aim of the present audit was to evaluate the referral process of pregnant women to the hospital's antenatal endocrinology clinic, considering the standards set by the Trust guidelines [7]. Moreover, we screened the management of hypothyroid women by primary care physicians from the time of their first pregnancy test until their appointment at the clinic.

Methods

In this audit, 100 women with thyroid disorders referred to the antenatal endocrinology clinic at the Royal Free Hospital, North London, between April 2017 and April 2018, were consecutively screened. During this screening process, reference was made to the Trust guidelines for the management of patients with thyroid disorders in pregnancy [7]. Table 1 lists the local criteria for referral to the antenatal endocrinology clinic. The audit was registered in the database of the Royal Free Hospital.

For each patient, we recorded: age, gestational age, and reason for referral. Details of previous endocrine disorders were retrieved by reviewing handwritten patients' notes as well as electronic medical records. Moreover, we recorded the current therapeutic dose of levothyroxine and any dose readjustments as per the guidelines. Hormonal status was recorded around the time of the first appointment, consulting the women's electronic records to retrieve serum levels of thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3).

Table 1 Criteria for referral to antenatal endocrinology clinic according to local guidelines.

REFERRAL CRITERIA
Grave's disease with (or without) thyrotoxicosis.
Thyroid cancer.
Lump in thyroid first discovered in pregnancy.
Large multinodular goiter (may present problems if anesthetic is required).
Newly diagnosed primary hypothyroidism or subclinical hypothyroidism.
Newly diagnosed thyrotoxicosis.
Patients with known autoimmune thyroid disease (positive thyroid peroxidase antibodies) who are not currently on thyroxine treatment.
Patients on thyroxine replacement for secondary (central) hypothyroidism due to pituitary / hypothalamic disease.

Thyroid status was classified as hypothyroid, euthyroid and hyperthyroid, on the basis of the TSH and FT4/FT3 levels indicated in the latest American Thyroid Association guidelines6. Considering that the pregnancy-specific reference ranges for thyroid hormone levels in pregnancy are only indicative, we considered TSH<2.5mU/L as the most rational cut-off level for optimal thyroid function [7]. Subclinical hypothyroidism was defined as TSH levels higher than the pregnancy-specific reference range, i.e. higher than 2.5mU/L, in the context of normal FT4 levels. Subclinical hyperthyroidism was defined as TSH levels lower than the laboratory reference range in the context of normal FT4 levels [6,11].

Statistical analysis

Statistical analysis was performed using SPSS version 22.0. The descriptive analysis was performed presenting quantitative data as mean values ± SD, while qualitative data were presented as frequency (percentage, %) values. On the basis of the thyroid hormone levels recorded at the time of the first appointment in the antenatal endocrinology clinic, the cases were classified as euthyroid, hyperthyroid or hypothyroid. Moreover, the euthyroid women were further classified into those with TSH levels within or outside the accepted pregnancy-specific range (i.e. 2.5mU/L).

Results

The mean age of the women referred to the endocrine antenatal clinic was 34.9 years. The average gestational age at the time of the first appointment was 14.2 weeks and 2.8 days. The pregnant women were mainly referred directly by primary care practitioners, while some were referred by other physicians, such as obstetricians (7%), general endocrine clinic staff (2%) or the respiratory team (1%). The first TSH test was done prior to referral in 75% of the cases, and after the first visit to the antenatal clinic in the other 25% of the cases. Moreover, we found a documented increase in levothyroxine dose only in half of women treated for thyroid hypofunction (prevalence, 52%).

Table 2 presents the reasons for the referrals to the antenatal endocrinology clinic. Rates of referral per trimester were as follows: 33%, first trimester; 59%, second trimester; and 8%, third trimester. The reasons for referral were compatible with the Trust guidelines in 99% of cases. Only one woman was referred due to detection of a thyroid nodule, for which the

Table 2 Frequency of the different reasons for referral to the antenatal endocrinology clinic.

REASONS FOR REFERRAL	FREQUENCY (%)
Hypothyroidism, current or previous	74%
Hyperthyroidism, current or previous	17%
Thyroidectomy due to cancer	5%
Thyroidectomy for other reasons	4%
Thyroid nodule	1%

primary care practitioner was seeking further advice on management.

As regards the thyroid status of these pregnant women at their first appointment at the antenatal endocrinology clinic, normal thyroid hormone levels were observed in 34% of cases, SCH was documented in 41% of cases, clinical hypothyroidism was evident in 2%, subclinical hyperthyroidism was identified in 13% and clinical hyperthyroidism was documented in 10%. Finally, among the hyperthyroid women, more than half of the cases showed only biochemical evidence of subclinical hyperthyroidism (57%) and the remaining cases (43% of hyperthyroid subgroup) exhibited clinical thyroid disease.

Discussion

According to the results of this audit, all pregnant women referred to the antenatal endocrinology clinic were at high risk of thyroid disorders, requiring specialist input as per local guidelines. However, the referral was mainly delayed until the second or even third trimester of pregnancy. Moreover, a considerable proportion of the women had never had their thyroid function tested prior to the first appointment. Additionally, TSH levels were found to be outside the pregnancy-specific range in many cases. Finally, only half of the women previously under treatment with levothyroxine had had their dose increased by the time of their first positive pregnancy test.

Almost all indications for referral were compatible with the criteria set by Trust guidelines, and the main problem was current or previous hypothyroidism. Only one case was referred for specialist advice on management of a newly identified thyroid nodule. Moreover, 90% of cases were referred directly to the antenatal endocrinology clinic, while up to 2% of women (2/100) were initially referred to the general endocrinology clinic. These results are generally reassuring as regards the level of awareness within the primary sector of the need for specialist management of women with more complicated thyroid disorders [7], and they showed up to 99% compliance with criteria set by local guidelines.

However, it should be noted that only 33% of the cases screened were referred within the first trimester. This implies either delayed presentation of pregnant women to primary care practitioners or difficulties, for primary healthcare providers, in optimizing the management of thyroid hypofunction in early pregnancy, which might result into hypothyroidism later on in pregnancy. Currently available guidelines [6,7] do not specify the optimal timing of referral to an antenatal endocrinology clinic, however, considering that such clinics deal with complicated thyroid disorders, a prompt referral might be considered to be warranted. NICE guidelines support access to the general antenatal care ideally by 10 weeks of gestation [12]. Previous audits and studies described results similar to our findings, namely appointments within the first trimester were confirmed in only half of evaluated cases, [13] or the majority of women were referred during the second trimester [14].

This audit showed that in 75% of the women thyroid function was tested following confirmation of pregnancy. Given that 90% of the cases were referred directly by their general practitioners, on the basis of a known history of thyroid disorder, the proportion of women who had already had thyroid tests performed by the time of conception was surprisingly low. In fact, our results are comparable with those of other studies and audits that indicate suboptimal screening of thyroid function early in pregnancy in women at high risk of thyroid disorder, given that thyroid hormones were evaluated in 17.8-27% of cases [13-15]. Unfortunately, this issue is longstanding and underlines the need for further education in the context of pre-partum management.

Conflicting advice is provided by different authorities regarding the ideal timing of thyroid function testing in a pregnant woman [16]. Many of the available guidelines recommend increased surveillance of thyroid function in women considered to be at high risk of developing thyroid disease during pregnancy [6,11,17,18]. An elevated risk of thyroid dysfunction should be expected in women with a known history of thyroid disorders, women residing in areas with moderate to severe iodine insufficiency, women with type 1 diabetes, a history of head or neck radiation, severe obesity, fertility issues or clinical symptoms or signs suggestive of either thyrotoxicosis or hypothyroidism, women treated with lithium or amiodarone, as well as women recently exposed to iodinated radiological contrast agents [6,11,17,18]. Since evidence supporting universal screening remains limited [16,18], a case-finding approach is currently implemented. As reported by international authorities, the optimal timing for testing thyroid function should be by the time of confirming the pregnancy, at least in high-risk cases [11,19].

Almost one quarter of the women included in this study had TSH levels higher than the pregnancy-specific reference range at the time of the first appointment. The Trust guidelines recommend a 25% increase in the thyroxine dose immediately after confirmation of pregnancy [7]. However, the proportion of women who actually increased their levothyroxine dose prior to their first appointment in the specialist clinic was surprisingly low, estimated to be 53% of previously treated patients with hypothyroidism. Comparable results were described in the TEARS study (Thyroid, Epidemiology, Audit and Research Study) from Scotland, where an increase in levothyroxine dose was observed in approximately 60% of cases, 34% of whom were within the first trimester [20]. Significantly lower rates were reported in a similar Australian study, where 21% of women had their levothyroxine dose increased prior to their first appointment in the specialist clinic [14]. The THERAPY trial confirmed that increasing the levothyroxine dose by two tablets weekly in women with previously treated hypothyroidism is sufficient to cover the physiologically increased levothyroxine requirements during pregnancy [21]. Interestingly, the additional levothyroxine requirements of a hypothyroid pregnant woman seem to differ according to the actual cause of the hypothyroidism [22].

With regard to its clinical implications, this audit highlights some important issues that could be readdressed, aiming to enhance the management of thyroid disorders during pregnancy. Improved counseling of women with hypothyroidism wishing to conceive would ensure a more prompt adjustment of medication and likely reduce the risk of a maternal thyroid hormone imbalance developing within the first crucial weeks

of gestation. On a secondary level, educational sessions could involve repeated presentation of the Trust guidelines and place emphasis on algorithms for managing those pregnant hypothyroid women who may not require specialist intervention. Primary care physicians should become aware of the importance of targeted testing, at least in cases that might warrant referral to a specialist antenatal clinic.

In conclusion, the results of this audit indicate that primary care practitioners are correctly referring pregnant women to the antenatal endocrinology clinic; however, referrals are often delayed until the second trimester. Further education is required to ensure optimal management of women with hypothyroidism prior to gestation, with simple measures that could be implemented by the individual as well as by the GP. Finally, further studies are required to establish firm guidelines regarding the management of women with SCH especially in early pregnancy.

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Bone density and body weight is associated with MTHFR677 polymorphism in girls with anorexia nervosa

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ABSTRACT

Introduction: Anorexia nervosa (AN) is associated with dysfunction of the hypothalamus-pituitary-gonadal axis, and thus with adverse effects on skeletal integrity. We aimed to identify the combination of polymorphisms linked to deterioration of bone health in a sample of adolescent girls with AN.

Patients and Methods: The sample of this cross-sectional study consisted of 40 young girls (12-21 years old), diagnosed with AN according to the American Psychiatry Association criteria. A detailed medical history was recorded for each participant and blood samples were taken for hormonal evaluation and genotyping. Lumbar spine bone density (bone mineral density, BMD) was evaluated using dual energy X-ray absorptiometry.

Results: Lower BMD values were observed in girls with: i) presence of the CTR-AluI polymorphism vs wild type (BMD, CC&CT vs TT genotypes: $0.84\pm0.21g/cm^2$ vs $0.96\pm0.12g/cm^2$, p-value=0.028); ii) presence of the MTHFR677T polymorphism vs wild type (BMD, TT&CT vs CC genotypes: $0.86\pm0.23g/cm^2$ vs $0.94\pm0.11g/cm^2$, p-value=0.047, adjusted for age, BMI, amenorrhoea). BMD measures exhibited a graded stepwise decrease with the addition of the CTR-AluI and/or the MTHFR677 polymorphic variant (BMD, wild type vs one vs two polymorphic variants: $0.97\pm0.11g/cm^2$ vs $0.90\pm0.11g/cm^2$ vs $0.75\pm0.33g/cm^2$, p-value for linear trend 0.011). Girls carrying the MTHFR677 polymorphism had 5.67-times higher odds of having a higher BMI (BMI >$16.4kg/m^2$ vs ≤$16.4kg/m^2$, MTHFR677 polymorphism, CT&TT vs CC genotypes: OR=5.667, 95% CI: 1.254—25.606, p-value=0.024).

Conclusion: Combined presence of the MTHFR677 and CTR-AluI polymorphisms is associated with lower bone density in young girls with AN, implying a dose-response effect. The association between MTHFR677 and bone metabolism is likely mediated by body weight.

KEYWORDS

Bone density, body mass index, CTR-AluI genetic polymorphism, MTHFR C677T genetic polymorphism, anorexia nervosa.

Introduction

Even though rarely encountered among the general population, eating disorders are relatively common among teenage girls and young women. Anorexia nervosa (AN) is an eating disorder of unknown aetiology [1]. The incidence rate of AN in adolescent girls has been reported as 270 cases per 100,000 person years, while the lifetime prevalence has been reported as 2.2% [2]. This state of low energy availability results in dysfunction of the hypothalamus-pituitary-gonadal axis and hypothalamus-pituitary-adrenal axis [3]. The hormonal consequences of this are hypoestrogenism, hypercortisolemia and insulin growth factor-1 deficiency [3]. These hormonal alterations have been linked to significant adverse effects on health and well-being, including impaired skeletal integrity [3,4]. The link between AN and bone health has been investigated extensively [4,5]. This chronic state of malnutrition has been linked to osteopenia in up to 51.7% of affected cases and with osteoporosis in up to 34.6% 6. Moreover,

females with AN have an approximately 1.6-times higher risk of fracture at all ages [7]. However, the deterioration of bone density is not uniform among affected individuals [6,7], implying that the association between bone health and AN might be influenced by other factors, not as yet explored. Secondary osteoporosis or bone density below the expected range for age is commonly linked to genetic background.

Single-nucleotide polymorphisms (SNPs) have been linked to genes encoding important pathways of bone metabolism. These genes include the calcitonin receptor (CTR) [8], estrogen

receptor alpha (ESR1) [9,10], collagen type 1 (COL1A1), and methylenetetrahydrofolate reductase (MTHFR) genes [11-14]. As previously shown, presence of the ESR1 polymorphic variants is an important predicting factor of lower bone density in girls with AN [15]. We hypothesized that a combination of SNPs linked to one or more genes that represent important regulators of bone metabolism might predispose girls with AN, already in a hypoestrogenic state, to further loss of bone mass.

Therefore, this study aimed to evaluate the link between the CTR-AluI polymorphism, the COL1A1-Sp1 polymorphism, the ESR1-PvuII polymorphism, the ESR1-XbaI polymorphism, and the MTHFR C677T polymorphism and bone density in a sample of girls with diagnosed AN

Methods

Study sample

This pilot study recruited a total of 40 young girls (14-17 years old) from the Child and Adolescent Gynaecology Outpatient Clinic of the 2nd Department of Obstetrics and Gynecology, Aretaieio Hospital, Athens, Greece. All participants had been diagnosed with AN according to the American Psychiatry Association criteria (proposed DSM-V, 2012) [16]. The inclusion criteria included body mass index (BMI) values of 12.5-18.5kg/m^2, corresponding to values below the 5th percentile for age, as well as physical exercise amounting to no more than 2 hours per week. The exclusion criteria included the presence of current or previously diagnosed metabolic bone disease, use of hormone supplements or contraceptives, malnutrition or current smoking. All participants as well as their legal guardians signed an informed consent document and the study was approved by the Ethics Committee of Aretaieio Hospital.

Protocol study procedures

A detailed medical history was obtained from all the participants at the time of their first visit to the clinic. Moreover, all the girls completed questionnaires collecting demographic and socioeconomic characteristics, which included questions regarding self-esteem in relation to their body. Weight and height were measured using an electronic scale and a wall height meter, in the early morning with the participants dressed in light clothing. BMI was calculated using the algorithm BMI = body weight (kg) / height^2 (m).

The age-related BMI z-score was calculated using the online calculator of the Baylor College of Medicine, Children's Nutrition Research Center [17]. Fasting blood samples were collected in the morning, between 8:30 a.m. and 9:30 a.m. Blood specimens used to assess polymorphisms were collected in K3EDTA (3mL) and stored at -20°C, while those used to assess oestradiol (E2) levels were centrifuged, and the serum was stored at -80°C until assessment. In participants with normal menstruation, blood samples were collected during the 3rd–5th day of the cycle, while in participants with amenorrhoea they were collected randomly. Serum levels of E2 were determined using standard laboratory techniques.

Bone densitometry

Lumbar spine bone mass density (LBMD) was measured on the same day by means of dual energy X-ray absorptiometry (anteroposterior projection at L2-L4) performed using a Norland Excell Plus densitometer (Cooper Surgical Inc., Fort Atkinson, WI, USA). Age- and gender-matched LBMD z-scores (corrected for age and gender) were calculated using reference data from the study by Zanchetta et al. [18]. Bone density lower than expected for age was defined as BMD z-score values less than -2 [19].

DNA preparation and pyrosequencing

DNA was extracted using the Nucleospin Blood QuickPure kit (Macherey-Nagel GmbH & Co, Düren, Germany), and SNPs were detected by pyrosequencing. The primers used are shown in the supplementary table. The polymerase chain reaction (PCR) technique was applied, in 50mL reaction volumes with 2mL DNA, 1mL of each primer and 25mL Hot Start Master Mix (GE Healthcare Biosciences, Pittsburgh, PA, USA). After the initial denaturation at 95°C for 5min, amplification consisted of 30 cycles of denaturation at 95°C for 30s, annealing at 58°C for 30s and elongation at 72°C for 30s, followed by a final elongation step at 72°C for 4min. Reactions were carried out in microplates using the PyroMark Q24 system (Qiagen GmbH Hilden, Germany), and results were analysed using the PyroMark Q24 software. The following polymorphisms were analysed: the AluI polymorphism in the CTR gene (CTR-AluI, rs1801197), the Sp1 binding site polymorphism in the COL1A1 gene (COL1A1-Sp1, rs1800012,), the PvuII and XbaI polymorphisms in the ESR1 gene (ESR1-PvuII, rs2234693; ESR1-XbaI, rs934079), and the C677T polymorphism in the MTHFR gene (MTHFR C677T, rs1801133).

Statistical analysis

Statistical analysis was performed using SPSS v.25 (SPSS Inc., Chicago, IL, USA). The Chi-square test was utilized to test for the Hardy-Weinberg equilibrium, in each of the evaluated polymorphisms. All polymorphisms were evaluated comparing individuals carrying at least one mutated variant (homozygous or heterozygous) vs girls homozygous for the wild-type genotype. Qualitative variables were expressed as absolute frequencies (%) and quantitative variables were expressed as mean values and standard deviation (mean±SD) or median values and interquartile range. Differences between qualitative variables were assessed using the Chi-square test, whereas quantitative variables were compared using Student's t-test for independent variables.

Linear regression analysis was performed to determine the ability of genetic polymorphisms to predict change in BMD, which was set as the primary outcome measure of this study. The multivariate models were adjusted for age and BMI. Partial correlation coefficients were evaluated to identify, as secondary outcome measures, potential correlations between variables assessed in the multivariate analysis (i.e. genetic polymorphisms, age, BMI). In the event of significant partial correlation, we continued the investigation for a potential mediation effect of the primary hypothesis, by using simple correlation analysis

between parameters of interest (Spearman's correlation coefficient) and by evaluating differences between quantitative variables. BMI values were treated both as continuous variables and as dichotomous variables, with the median of 16.4 kg/m² taken as the cut-off value. Statistical significance was set at the level of p-value<0.05.

Results

This sample consisted of 40 adolescent girls, previously diagnosed with AN. Table 1a presents the descriptive analysis of the anthropometric and demographic parameters as well as BMD mean values. The genotypes of the CTR-AluI genetic polymorphism showed the following frequencies: CC vs CT vs TT: 5% (1/40) vs 30% (12/40) vs 67.5% (27/40). The genotype frequencies of the assessed genetic polymorphisms were as follows: in ESR1-PvuII, CC vs CT vs TT (wild type) was estimated as 10% (4/40) vs 55% (22/40) vs 35% (14/40); in COL1A1, TT vs GT vs GG (wild type) was estimated as 5% (2/40) vs 17.5% (7/40) vs 77.5% (31/40); in ESR1-XbaI, GG vs AG vs AA (wild type) corresponded to 7.5% (3/30) vs 47.5% (19/40) vs 45% (18/40); in MTHFR C677T, TT vs CT vs CC (wild type) was estimated as 7.5% (3/40) vs 25% (10/40) vs 67.5% (27/40).

The participants had a mean age of 15.3±1.64 years and a mean BMI of 16.3±1.41kg/m². In total, 30% (12/40) of the girls had bone density values lower than expected for age (BMD z-score of less than -2). Using the Chi-square test, we observed that the assessed genotypes for all polymorphisms were in Hardy-Weinberg equilibrium (p-value >0.05).

Table 1b shows mean BMD values according to the presence or absence of the evaluated polymorphic variants. Lower BMD values were observed in girls carrying the polymorphic C allele of the CTR-AluI gene when compared with those carrying the wild type (CTR-AluI polymorphic variants, CC&CT genotype vs TT genotype: 0.84±0.21g/cm² vs 0.96±0.12g/cm², p-value=0.028). Similarly, lower mean values of BMD were observed in girls carrying the mutated T allele of the MTHFR C677T polymorphism vs the wild type (MTHFR677 polymorphism, TT&CT genotype vs CC genotype: 0.86±0.23g/cm² vs 0.94±0.11g/cm², p-value=0.047, adjusted for age, BMI, presence of amenorrhoea). Aiming to further evaluate the impact of the genetic polymorphisms on bone density, we compared BMD values between girls carrying one polymorphic variant (either the CTR-AluI or the MTHFR677 polymorphism) and those carrying two polymorphic variants (both the CTR-AluI and the MTHFR677 polymorphism). As presented in Figure 1, BMD values showed a graded stepwise decrease with the addition of each polymorphic variant (BMD values, wild type vs carriers of the CTR-AluI or the MTHFR677 polymorphism vs carriers of both the

Table 1a Mean demographic/anthropometric parameters and mean values of bone density measures of the 40 adolescent girls comprising our sample.

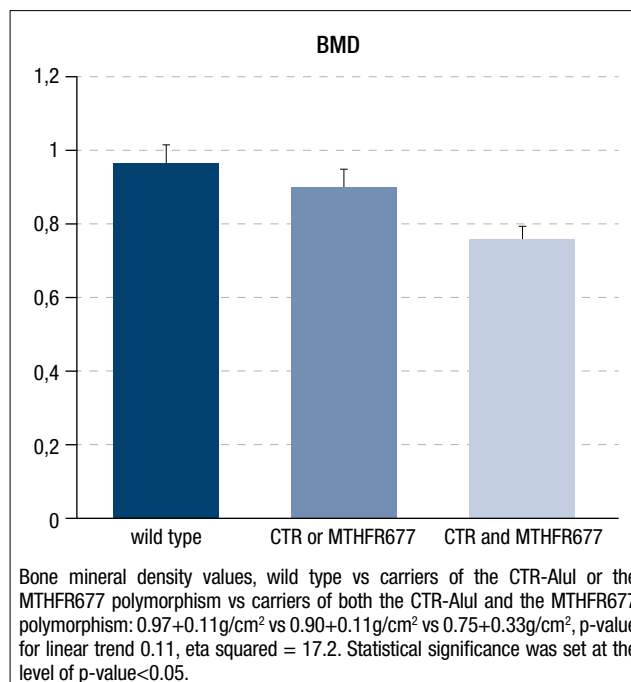| DEMOGRAPHIC/ANTHROPOMETRIC	MEAN±SD OR FREQUENCY (%)	MEDIAN	IQR
Age (years)	15.3±1.64	15	14 – 17
BMI (kg/m²)	16.3±1.41	16.4	15.4 – 17.4
BMI z-score	7.71±9.29	4.85	0.38 – 12.68
Prevalence of amenorrhoea	80%		
Bone density measures			
BMD (g/cm²)	-0.92±0.16	0.93	0.84 to 0.99
z-score	-1.58±0.92	-1.54	-2.4 to -1.06
Bone density less than expected for age	30%		
BMD=bone mass density; SD=standard deviation; IQR=interquartile range.			

Table 1b Mean values of bone density according to the presence of genetic polymorphisms in the 40 girls with anorexia nervosa.

	BONE MASS DENSITY (g/cm²)	MEAN	SD	P-VALUE ANOVA OR ANCOVA
CTR AluI	Presence of T allele (wt)	**0.96**	**0.12**	**0.028**
	Presence of C allele	**0.84**	**0.21**	
COL1A1-Sp1	Presence of G allele (wt)	0.91	0.17	0.657
	Presence of T allele	0.94	0.12	
ESR1-PvuII	Presence of T allele (wt)	0.92	0.12	0.885
	Presence of C allele	0.91	0.18	
ESR1-XbaI	Presence of A allele (wt)	0.90	0.07	0.674
	Presence of G allele	0.93	0.21	
MTHFR677	Presence of C allele (wt)	**0.94**	**0.11**	**0.047***
	Presence of T allele	**0.86**	**0.23**	
*Adjusted for age, BMI and presence of amenorrhoea; Bold indicates statistical significance; Statistical significance was set at the level of p-value<0.05.				

CTR-AluI and the MTHFR677 polymorphism: 0.97 ± 0.11g/cm^2 vs 0.90 ± 0.11g/cm^2 vs 0.75 ± 0.33g/cm^2, p-value for linear trend 0.011, eta squared = 17.2). Multivariable linear regression analysis models were fitted to evaluate the effect of each of the significant genetic polymorphisms on bone density, adjusted for age and BMI (Table 2). We observed that presence of the CTR-AluI polymorphism associated significantly with BMD measures (CTR-AluI, CC&CT vs TT genotype, b-coefficient= -0.399, p-value=0.012), an effect also mediated by age and BMI. Similarly, the MTHFR677 polymorphism showed a borderline association with BMD measures (MTHFR677, TT&CT vs CC genotype, b-coefficient= -0.321, p-value=0.050), an association also mediated by BMI. Finally, the girls with both the CTR variant and the MTHFR677 polymorphic variant showed significantly lower BMD values than those carrying wild-type polymorphisms (CTR-AluI and MTHFR677 polymorphisms vs wild type: b-coefficient = -0.514, p-value=0.001), an effect also associated with BMI but independent of age. Partial correlation coefficients were indicative of a direct association between BMI values and the evaluated genetic polymorphisms. Aiming to further explore the direction of these associations between BMI and genetic polymorphisms in a univariate approach, we performed a simple correlation analysis using Spearman's correlation coefficient. The results showed an almost significant correlation between presence of the MTHFR677 polymorphism and BMI values (r-coefficient = 0.286, p-value = 0.074). The presence of the CTR-AluI polymorphic variant did not correlate with BMI values (r-coefficient = 0.171, p-value = 0.293). Comparing girls with the TT genotype vs the CC genotype of the MTHFR C677T polymorphism, we observed significantly higher BMI values in carriers of the T allele (TT vs CC genotype, BMI: 17.2 ± 0.6kg/m^2 vs 16.0 ± 1.4kg/m^2, p-value=0.045, t-test for independent samples), as well as higher values of the BMI z-score adjusted for age (TT vs CC genotype, BMI z-score adjusted for age: -1.2 ± 0.26 vs -2.16 ± 1.35, p-value=0.005, t-test for independent samples). We then compared the prevalence of genetic polymorphisms according to BMI, taking the median BMI value of 16.7kg/m^2 as the cut-off value. No association was observed between the presence of the CTR genetic polymorphism and BMI values. However, the prevalence of the MTHFR677 genetic polymorphism was found to be associated with higher BMI values (BMI >16.4kg/

Figure 1 Mean values of bone mineral density between girls carrying either two polymorphic variants or one polymorphic variant, or only wild type polymorphisms. Genetic polymorphisms evaluated include CTR-AluI and MTHFR C677T.

Bone mineral density values, wild type vs carriers of the CTR-AluI or the MTHFR677 polymorphism vs carriers of both the CTR-AluI and the MTHFR677 polymorphism: $0.97+0.11$g/cm^2 vs $0.90+0.11$g/cm^2 vs $0.75+0.33$g/cm^2, p-value for linear trend 0.11, eta squared = 17.2. Statistical significance was set at the level of p-value<0.05.

m^2 vs ≤16.4kg/m^2, prevalence of MTHFR677 genetic polymorphism: 50% vs 15%, p-value=0.018 for Chi-square test, Figure 2). Multivariable regression analysis showed that presence of the T allele of the MTHFR677 genetic polymorphism as opposed to the wild type was associated with 5.7 times higher odds of "healthier" BMI (BMI >16.4kg/m^2 vs ≤16.4kg/m^2, MTHFR677 genetic polymorphism, TT&CT vs CC genotype: OR 5.667, 95% CI: 1.254 to 25.606, p-value=0.024, data not shown).
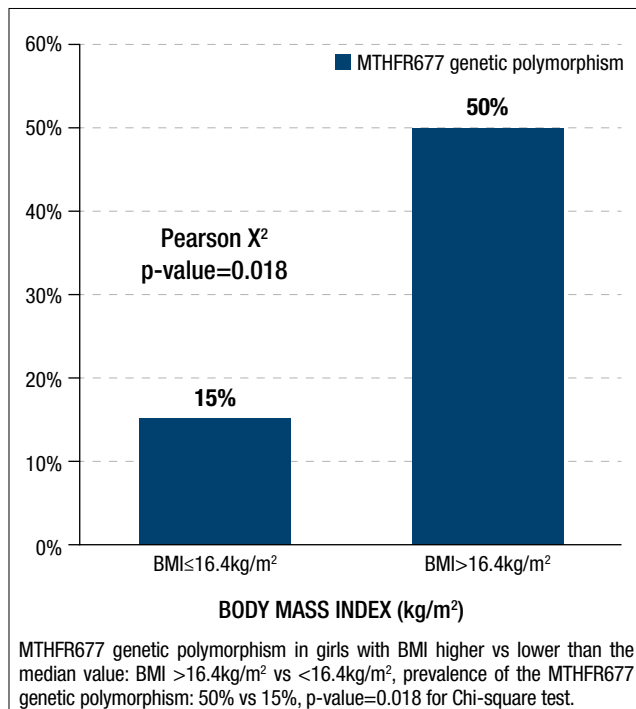
Discussion

The results of this study indicate that presence of two polymorphic variants, namely CTR-AluI and MTHFR677, is associated with lower bone density in girls with AN. Carriers of at least

Table 2 Linear multivariable regression analysis including genetic polymorphisms as independent variables and bone mineral density levels as dependent variables in 40 girls with anorexia nervosa. The models were adjusted for age and body mass index.

BONE MASS DENSITY (g/cm²)	R²	B-COEFFICIENT	95% CI	P-VALUE	PARTIAL CORRELATION
CTR-AluI		**-0.399**	**-0.242 to -0.032**	**0.012**	**-0.404**
Age (years)	15.9%	0.027	-0.027 to 0.033	0.856	0.030
BMI (kg/m²)		**0.325**	**0.003 to 0.472**	**0.036**	**0.342**
MTHFR677		**-0.321**	**-0.220 to 0.000**	**0.050**	**-0.321**
Age (years)	9.8%	0.006	-0.030 to 0.032	0.971	0.006
BMI (kg/m²)		**0.339**	**0.002 to 0.576**	**0.037**	**0.339**
CTR-AluI and MTHFR677		**-0.514**	**-0.602 to -0.050**	**0.001**	**-0.504**
Age (years)	25.1%	0.068	-0.023 to 0.095	0.635	0.080
BMI (kg/m²)		**0.401**	**0.009 to 0.676**	**0.009**	**0.420**
Bold indicates statistical significance, which was set at the level of p-value<0.05.					

Figure 2 Frequency of MTHFR677 genetic polymorphism (T polymorphic variant) according to body mass index values, using the median value of 16.4kg/m² as cut-off.

MTHFR677 genetic polymorphism in girls with BMI higher vs lower than the median value: BMI >16.4kg/m² vs <16.4kg/m², prevalence of the MTHFR677 genetic polymorphism: 50% vs 15%, p-value=0.018 for Chi-square test.

one polymorphic variant have higher BMD values compared with carriers of both genetic polymorphisms, implying a dose-response effect. In addition, the underlying genetic background and, mainly, the presence of the MTHFR677 polymorphism are related to higher BMI among girls with AN. Significantly lower measures of bone density were observed in girls with AN carrying the CTR-AluI polymorphic variant when compared with girls carrying the wild type. The link between the CTR-AluI polymorphism and BMD has been described in different populations; interestingly, a difference in the prevalence of CTR genotypes according to the ethnic background has been noted [20], implying an effect of demographics in the association between CTR polymorphisms and BMD. Evaluating pathophysiological implications of genetic polymorphisms with aging, an Italian study of 663 postmenopausal and 52 perimenopausal women described an impact of the CTR genotypes on the process of acquiring peak bone mass, which as expected was more pronounced in younger women, rather than on the process of age-related bone loss [8]. Studies in postmenopausal populations described conflicting results, with evidence supporting lower BMD values in postmenopausal carriers of the unfavourable T allele [21,22], while other authors were unable to identify any association in young perimenopausal women [23]. Finally, no association between the CTR genotype frequencies and indices of bone metabolism, such as BMD and osteocalcin levels, was observed in a sample of healthy Japanese premenopausal women [20], a result that might be related to the different ethnic background and thus different frequency of the evaluated polymorphism compared with our sample. The results in our sample of girls with AN indicate a borderline association between the presence of the MTHFR C677T polymorphic variant and BMD values. In this sample, the effect of this polymorphism on bone metabolism seems to be mediat-

ed by other confounders, such as BMI. A possible contribution of the MTHFR C677T polymorphism on bone metabolism has been described by Li et al. [24], in a meta-analysis of 5,833 post-menopausal women. Their study highlighted that women with the mutated MTHFR gene had lower BMD at the femoral neck, but not at the lumbar spine, compared with women carrying the wild-type MTHFR gene [24]. A meta-analysis of 3,525 cases and 17,909 controls 12 identified a modestly elevated fracture-risk for individuals with the TT genotype of the C677T polymorphism (TT vs CT and CC genotypes, age<60 years: OR 1.51, 95% CI: 1.10 to 2.07). Similarly, the rare TT genotype has been found to be more common in women with vertebral fractures vs controls [12,13]. A study of 346 postmenopausal Thai women found that heterozygous carriers of the unfavourable T allele of the MTHFR C677T polymorphism had a 5.66-times higher risk of osteopenia at all sites combined compared with carriers of the wild type 11. Furthermore, McLean et al. [14] highlighted that the link found between the MTHFR C677T polymorphism and lower hip BMD levels is prevalent only in individuals with folate deficiency, following evaluation of 1,632 male and female participants of the Framingham Offspring Study.

Furthermore, our results highlight a possible interplay between genetic background and BMI values, mainly prevalent for carriers of the MTHFR C677T polymorphic variant. To our knowledge, ours is the first study to show an association between genetic background and both BMI and bone density values in young girls with AN. The impact of the MTHFR C677T polymorphism on weight gain remains conflicting, with studies of overweight and obese individuals both supporting [25] and rejecting this association [26]. Interestingly, individuals with a higher prevalence of metabolic abnormalities but normal body weight showed, in comparison with obese individuals, a higher prevalence of the favorable CC genotype of the MTHFR C677T polymorphism [27]. In contrast with our findings, a regulatory effect of the CTR-AluI polymorphic variant on weight gain has been described in a study of a sample of healthy Japanese pre-menopausal women, where presence of the unfavourable T allele was linked with a higher tendency to gain weight compared to women who were carrying the wild type genotype [20].

According to our findings, bone disease in girls with AN is particularly evident in those carrying both the CTR-AluI and the MTHFR C677T polymorphism, implying a possible interaction and dose-response association between these polymorphisms. The association between the MTHFR C677T polymorphism and worsening bone density is not surprising considering that the ensuing hyperhomocysteinemia has been shown to be linked to reduced collagen synthesis as well as disruption of collagen cross-linking within bone tissue [28]. The latter is likely due to a direct mechanistic effect of homocysteine on the enzyme lysyl oxidase, which mediates the process of collagen cross-linking [29]. Interestingly, in the present sample of girls with AN, the association between the MTHFR C677T genetic polymorphism and BMD values became non-significant when adjusting for BMI, suggesting an interaction between genetic background and weight gain. The link between presence of the MTHFR C677T polymorphism and body weight is difficult to explain. However, as suggested by others, the MTHFR C677T polymorphism is associated with higher levels of homocysteine, which has been pro-

posed to exert an epigenetic effect on the expression of genes regulating body fat storage [26]. Interestingly, according to data from in vitro, in vivo and genetic studies, homocysteine metabolism is closely related to the methylation process, hence affecting the methylation of DNA and amino acid residues on histones [25-27]. Limitations of our study should be acknowledged. First, the sample size is relatively small. Second, the cross-sectional design does not permit detection of casuality. Third, we did not evaluate z-score values. However, as suggested by the international guidelines on clinical densitometry, z-score values are not indicative of fracture risk, which is instead determined by clinical evidence of fracture, in this young subset of women [19]. Fourth, we did not evaluate folate levels, a possible regulator of the function of the MTHFR gene. Finally, the association between the evaluated MTHFR677 genetic polymorphism and BMI was only a secondary outcome measure. Therefore, our results should not be generalized to all women with AN but should rather be regarded as indicative of a possible mediation effect of BMI in the link observed between MTHFR677 and bone metabolism.

In conclusion, we observed that bone disease in young girls with AN is associated with presence of the CTR-AluI and MTHFR C677T genetic polymorphisms. Moreover, the combined presence of these polymorphisms is linked to further deterioration of bone density, implying a possible underlying genetic interaction and dose-response relationship between the identified polymorphisms. Finally, presence of the association between MTHFR C677T and bone density seems to be mediated by BMI values. Larger cohort studies are required to estimate the significance of our findings.

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Pregnancy outcome in women with polycystic ovary syndrome: a retrospective study on the influence of clomiphene stimulation versus laparoscopic ovarian drilling after clomiphene resistance

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ABSTRACT

Purpose: We aimed to retrospectively evaluate adverse outcomes of singleton pregnancies in women with polycystic ovary syndrome (PCOS) after clomiphene (CC) stimulation versus laparoscopic ovarian drilling (LOD) after CC resistance.
Methods: This retrospective study included 67 PCOS women who had conceived naturally within 12 months after LOD and 67 matched pregnant women who had conceived after CC stimulation. In addition, 134 matched non-PCOS controls who had conceived naturally were included.
Results: The controls had higher rates of pregnancy complications than the PCOS cases. Neither CC responsiveness nor CC resistance was associated with any higher risk of pregnancy complications such as gestational diabetes mellitus (46.3% vs. 38.3%, p=0.485), pregnancy-induced hypertension (23.9% vs. 28.4%, p=0.694), preeclampsia/HELLP syndrome (10.4% vs. 16.4%, p=0.448), or preterm delivery (20.9% vs. 14.9%, p=0.500).
Conclusion: Singleton pregnancies in PCOS women do not carry higher risks after LOD for CC resistance compared with pregnancies after successful CC stimulation.

KEYWORDS

Pregnancy complications, polycystic ovary syndrome, ovarian drilling, clomiphene citrate.

Introduction

As demonstrated in a recent meta-analysis performed by Yu et al. [1], women with polycystic ovary syndrome (PCOS) are more likely to develop pregnancy complications, including gestational diabetes mellitus (GDM), pregnancy-induced hypertension (PIH), preeclampsia, and preterm delivery. However, evidence on the impact of different artificial reproductive techniques that lead to pregnancies in women with PCOS is scarce, according to Bahri Khomami et al. [2]. Ott et al. [3] demonstrated that in metformin-treated PCOS women, clomiphene citrate (CC) stimulation might increase the overall risk of pregnancy complications compared with laparoscopic ovarian drilling (LOD). However, this analysis was limited by the small sample size and poor matching. From a clinical perspective, it would be interesting to know the risks associated with specific PCOS treatment modalities. Moreover, according to Ellakwa et al. [4], CC-responding and CC-resistant women reveal different characteristics that could also explain differences in their pregnancy-associated risk profiles. In view of these considerations, we aimed to retrospectively evaluate pregnancy complications in singleton pregnancies of PCOS women, also comparing those who conceived after CC stimulation with those who conceived after LOD for CC resistance.

Materials and methods

This study included 67 PCOS women who had conceived naturally within 12 months after undergoing LOD for CC resistance and delivered at ≥23+0 weeks at the Medical University of Vienna (January 2010 - December 2016). These women were matched 1:1 for age (±0.5 years), body mass index (BMI; ±1.0 kg/m²), metformin use before pregnancy, and parity with 67 PCOS women who had conceived after CC stimulation. Thus, 134 PCOS women were included. These were matched for age (±0.5 years), BMI (±1.0 kg/m²), and parity with 134 non-PCOS controls who had conceived naturally. The revised Rotterdam criteria were applied for PCOS definition; all the women in the study sample showed polycystic ovaries on ultrasound [5].

CC was the first-line tool for ovulation induction, while LOD was recommended in cases of CC resistance, i.e., after three to six CC cycles that had failed to induce ovulation. Bilateral standard electrocoagulation LOD was performed with five to eight incisions in accordance with previous reports [3].

Data on risk were acquired using the PIA Fetal Database (GE-Viewpoint, Wessling, Germany) and SAP-based AKIM software. Using common definitions as Ott et al. [3] did, we focused on: the development of GDM, diagnosed using the 75g, 2h oral glucose tolerance test (OGTT), routinely performed in the third trimester. Patients were diagnosed with GDM if one of the following parameters reached or exceeded the 97.5th percentile: fasting venous blood glucose concentration (4.5mmol/l), at 1h (9.1mmol/l) and 2h (7.9mmol/l).

The indications for performing the OGTT in the second trimester were as follows: glucose in any of the monthly urine samples; GDM in a previous pregnancy; diabetes in the immediate family; and fetal macrosomy (>2 standard deviations); insulin-dependent GDM (IGDM); PIH, defined as gestational hypertension (blood pressure >140/90 mmHg without proteinuria at a gestational age >20 weeks on two or more occasions at least 6 h apart); preeclampsia, defined as blood pressure >140/90 mmHg in combination with proteinuria >0.3 g/24 h after 20 weeks' gestation; the HELLP syndrome, defined as hemolysis, elevated liver enzymes (serum lactate dehydrogenase, LDH ≥ 600 IU/L or total bilirubin ≥ 1.2 mg/dL), and low platelet count (≤ 100.000 cells/μL); premature delivery, defined as delivery between the 22nd and 37th week of gestation.

In addition, we evaluated general patient-, PCOS-, and pregnancy-related characteristics, including basal testosterone, anti-Mullerian hormone (AMH), luteinizing- (LH), and follicle-stimulating hormone (FSH) levels on days 2-5 of the first CC cycle. Insulin resistance was defined as a homeostasis model assessment, HOMA index (= fasting insulin x fasting glucose / 22.5x18) ≥2.5.

Variables are reported as numbers (frequencies) or mean values ± standard deviation. Differences between groups were tested using the Welch test and Fisher's exact test. A binary logistic regression model was performed to test the impact of patient characteristics on the development of IGDM. For this analysis, odds ratios (OR) with 95% confidence intervals (95%CI) and p-values of the likelihood ratio tests are provided. Statistical analysis was performed using SPSS 24.0 (SPSS Inc., 1989–2017). P-values< 0.05 were considered significant. The study was approved by the Institutional Review Board (number: 2203/2016).

Results

The controls and PCOS patients did not differ in terms of age (31.6 ± 4.8 versus 31.6 ± 4.8 years, respectively; p= 0.922), BMI (26.8 ± 4.9 versus 26.8 ± 4.9 kg/m^2, respectively; p=0.947), or parity (0.1 ± 0.3 versus 0.1 ± 0.3, respectively; p=1.000). With regard to pregnancy complications, the PCOS women revealed higher rates of GDM (57/134, 42.5% versus 12/134, 8.9%, respectively; p< 0.001), IGDM (38/134, 28.4% versus 8/134, 6.0%, respectively; p< 0.001), PIH (35/134,

26.1% versus 15/134, 11.2%, respectively; p=0.003), preeclampsia/HELLP syndrome (18/134, 13.4% versus 5/134, 3.7%, respectively; p=0.008), and preterm delivery (24/134, 17.9% versus 5/134, 3.7%, respectively; p> 0.001). No differences between PCOS patients and controls were found for preterm premature rupture of the membranes (5/134, 3.7% versus 2/134, 1.5%, respectively; p=0.447), cervical insufficiency/preterm labor (13/134, 9.7% versus 9/134, 6.7%, respectively; p=0.505), or intrauterine growth retardation (6/134, 4.5% versus 9/134, 6.7%, respectively; p=0.597). While birth weight was the same in both groups (PCOS patients: 3139.0 ± 906.4 g versus controls: 3242.0 ± 613.2 g, p=0.278), gestational age at delivery was higher in the controls (PCOS patients: 37.5 ± 4.3 weeks versus controls: 38.5 ± 2.6 weeks, p=0.029).

Table I shows the basic characteristics of the PCOS patients divided by final PCOS treatment. CC-resistant women who underwent LOD revealed higher baseline LH and AMH levels, LH:FSH ratios, and amenorrhea rates.

In a subsequent step, a multivariate binary logistic regression model for the prediction of IGDM was calculated. A higher age (OR: 1.226, 95%CI: 1.206,1.464; p=0.025) and a higher presence of insulin resistance as indicated by the HOMA index during diagnostic evaluation of PCOS (OR: 29.542, 95%CI: 5.727,152.393; p<0.001) were associated with development of IGDM during pregnancy, while primary versus secondary sterility (OR: 1.601, 95%CI: 0.323,7.937, p=0.564), LOD for CC resistance (OR: 3.182, 95%CI: 0.579,17.490, p=0.183), BMI (OR: 1.159, 95%CI: 0.974,1.379, p=0.096), parity (OR: 1.158; 95%CI: 0.079,16.881, p=0.915), LH:FSH ratio (OR: 1.068, 95%CI: 0.614,1.857; p=0.815), total testosterone (OR: 1.296, 95%CI: 0.074,22.569; p=0.859), and AMH (OR: 0.915, 95%CI: 0.797,1.1050; p=0.206) were not.

Discussion

In our retrospective dataset, PCOS women revealed higher rates of GDM, IGDM, PIH, preeclampsia/HELLP syndrome, and preterm delivery. These data are in line with previous reports summarized in a recent meta-analysis [1]. However, the main focus of our study was differences in rates of complications in singleton pregnancies after successful CC stimulation versus LOD for CC resistance.

On comparing these two PCOS groups, it became evident that the CC-resistant women had higher baseline LH and AMH levels, LH:FSH ratio and amenorrhea rates than the CC responders. This is in line with previous publications, like that of Ellakwa et al. [4]. It can be assumed that these findings reflect the more severe clinical state of CC-resistant PCOS patients. However, as regards pregnancy outcome, in our dataset of age-, BMI-, and parity-matched PCOS women, neither CC responsiveness nor CC resistance was associated with higher risk of any pregnancy complication (Table I).

Thus, it seems reasonable to assume that pregnancy risks in the PCOS population are affected by basic patient characteristics, such as obesity, age, and insulin resistance, as stated by Bahri Khomami et al. [2], rather than by CC stimulation or LOD. We were able to prove this in relation to the risk of developing

Table 1 Patient characteristics and pregnancy complications in women who achieved singleton pregnancy after CC stimulation or after LOD.

	SUCCESSFUL CC STIMULATION (N=67)	LOD (N=67)	P
Patient characteristics			
Age (years)*	31.6±4.8	31.6±4.8	0.974
Pre-pregnancy BMI (kg/m²)*	27.0±5.0	26.6±4.8	0.679
Parity*	0.1±0.3	0.1±0.3	1.000
Secondary sterility#	29 (43.3)	21 (31.3)	0.211
Amenorrhea#	15 (22.4)	27 (40.3)	0.040
Number of CC cycles*	2.3±0.9	3.4±0.7	<0.001
LH (IU/l)*	8.8±4.7	14.3±9.4	<0.001
Maximum CC dose 50mg	55 (82.1)	0	
100mg	10 (14.9)	42 (62.7)	<0.001
150mg	2 (3.0)	25 (37.3)	
LH/FSH ratio*	1.6±1.1	2.6±1.5	<0.001
Testosterone (ng/ml)*	0.44±0.18	0.47±027	0.565
AMH (ng/ml)*	7.1±5.3	9.7±6.0	0.010
Insulin resistance#	16 (23.9)	18 (26.9)	0.843
Metformin treatment#	18 (26.9)	17 (25.4)	1.000
Pregnancy complications			
GDM#	31 (46.3)	26 (38.3)	0.485
Insulin-dependent GDM#	20 (29.9)	18 (26.9)	0.848
PIH#	16 (23.9)	19 (28.4)	0.694
Preeclampsia/HELLP#	7 (10.4)	11 (16.4)	0.448
Preterm premature rupture of membranes#	2 (3.0)	3 (4.5)	1.000
Cervical insufficiency/preterm labor#	8 (11.9)	5 (7.5)	0.561
Intrauterine growth retardation#	3 (4.5)	3 (4.5)	1.000
Preterm delivery#	14 (20.9)	10 (14.9)	0.500
Birth weight (g)*	3136.8±1038.7	3141.3±759.4	0.977
Gestational age at delivery (completed weeks)*	37.2±4.7	37.9±3.8	0.355

Data are provided as *mean values ± standard deviation or # numbers (frequencies). Differences between groups were calculated using the Welch-test and the Fisher's exact test.

IGDM. In our multivariate analysis, a higher patient age and an abnormal pre-pregnancy HOMA index significantly increased this risk, whereas the type of fertility treatment that led to pregnancy did not. Notably, about one fourth of the patients in our study were insulin resistant; the patients' mean BMI was about 27 kg/m², and thus within the range indicating overweight. The first of these findings seemed to have particular relevance for the observed rates of GDM and IGDM (42.5% and 28.4%, respectively), although more women developed GDM than had been considered insulin resistant based on the HOMA index before pregnancy.

However, in a previous study these surrogate indices were found to be of limited accuracy in identifying insulin resistance. In other words, they could only "rule in" but could not "rule out" insulin resistance as evaluated by the clamp test, which was considered the gold standard [6].

Interestingly, it has also previously been pointed out that not PCOS itself, but associated factors including higher age and BMI put the patient at risk of developing of GDM [7]. A study in more than 1800 pregnant women revealed that by using a modified two-step screening strategy which included clinical risk factors, the number of oral glucose tests needed would be decreased substantially [8].

We acknowledge that the retrospective design and the fact that all the women undergoing LOD had been pre-treated with several CC stimulations constitute limitations of the present study, although the latter aspect seems of minor relevance, given that a mean of 57.7 ± 27.2 days elapsed between the last CC application and LOD.

In conclusion, in PCOS women, neither CC stimulation nor LOD for CC resistance carries higher risks for pregnancy-associated complications, at least in singleton pregnancies. Thus, treatment-depending pregnancy risks should not influence physicians when choosing the type of infertility treatment. Moreover, obstetricians need not fear increased risks for their patients in the event of LOD/CC resistance

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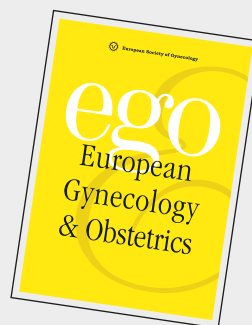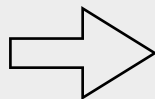
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