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Editorial

ENDOMETRIOSIS - DIAGNOSTIC AND THERAPEUTIC CHALLENGE

Endometriosis represents an important health problem, affecting women of reproductive age. It requires often multidisciplinary approach, involving gynecologists, urologists and general surgeons as well.

Endometriosis, the presence of endometrial tissue outside the uterus, affects up to 6%-10% of fertile women, being usually located inside the pelvis[1]. Furthermore, up to 30% of patients have primary or secondary infertility issues related to endometriosis[2]. There are some rare cases of unusual location like pulmonary endometriosis, implying thoracic surgery. Small or large bowel could be a primary location of the disease, as we previously published[3].

Abdominal wall endometriosis is a rare entity, usually developed in the fertile period of women. It means endometriosis outside the peritoneum, located to abdominal wall, related or not to a scar. Parietal endometriosis include, beside abdominal wall endometriosis, perineal endometriosis, related to an episiotomy scar or de novo[4].

Symptoms are typically cyclic, synchronous with menstra. Any symptomatic association with menstrual period should be suspected of this disease.

The treatment of endometriosis could be medical, surgical, or both (combined treatment). Till now, there is no consensus or an optimal established treatment for endometriosis. Resection in healthy tissue, with free margins, seems to remain the best option. Resection could imply multiple organ approach, often in difficult conditions, with organ sacrifice and requiring of reconstruction methods. Intraoperative bleeding of endometrial tissue and dissection difficulties due to lesion infiltration is the rule. All these aspects justify the interdisciplinary approach and collaboration between surgeons of different specialties.

Endometriosis has an old history of referring in the literature. The first historical reference of endometriosis dates back since 1500 BC from a discovery of an ancient

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Egyptian papyrus which described a treatment for a painful disorder of menstruation. In 1690, Daniel Shroen described in his book titled "Disputatio Inauguralis Medica de Ulceribus Ulceri", a more detailed presentation of the peritoneal endometriosis referring to the adhesions and endometriomas as complications of the disease[5].

According to the depth of invasion and anatomic location, endometriosis is usually classified as: ovarian endometriosis, superficial peritoneal endometriosis and the most severe form of endometriosis, deep infiltrating endometriosis. The severest form of the disease is that of deep infiltrating endometriosis involving pelvic organs, such as rectum, urinary bladder, small or large bowel and uterosacral ligaments.

General surgeons, urologists and especially gynecologists should consider the diagnosis of endometriosis when encountering the typical symptoms. It is also important to get trained in a dedicated center for excellence in endometriosis surgery to improve diagnosing and managing the disease or referring the patients to specialized centers where they can get a holistic approach from a multidisciplinary team.

Laparoscopic surgery is considered the first choice for diagnosis and treatment of infertility related to endometriosis[6]*. Laparoscopic adhesiolysis is also very important and constitutes as a part of the treatment*[7].

Bringing together specialists from various fields under the auspices of the endometriotic pathology at the Second National Congress dedicated to this pathology, in Sinaia, between 20th and 22nd of June, this year, proved to be welcomed and the exchange of ideas of high academic level was very useful. The best and most elaborate works are included in extenso in this supplement of the journal.

It would be of great interest to have more consensus conferences and to elaborate practical guidelines for diagnosing and treating this disease.

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Medicine

ABDOMINAL WALL ENDOMETRIOSIS - A REVIEW AND PERSONAL EXPERIENCE

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ABSTRACT:
ENDOMETRIOSIS IS CHARACTERIZED BY THE PRESENCE OF UTERINE MUCOSAL TISSUE OUTSIDE THE UTERUS. ABDOMINAL WALL ENDOMETRIOSIS (AWE) IS A RARE ENTITY, USUALLY DEVELOPED IN THE FERTILE PERIOD OF WOMEN. IT MEANS ENDOMETRIOSIS OUTSIDE THE PERITONEUM. PARIETAL ENDOMETRIOSIS INCLUDE, BESIDE AWE, PERINEAL ENDOMETRIOSIS. IT CAN BE PRIMARY ENDOMETRIOSIS (WHICH IS QUITE RARE, SUCH AS UMBILICAL), OR, MORE OFTEN, SECONDARY, AFTER SURGICAL OR GYNECOLOGICAL PROCEDURES. SCAR ENDOMETRIOSIS IS DUE TO INTRAOPERATIVE DISEMINATION AND IS MORE FREQUENTLY LOCATED TO C-SECTION SCAR. THE SYMPTOMS INCLUDE CATAMENIAL PAIN, PALPABLE MASS WITH OR WITHOUT CYCLIC VARIATIONS, AND EVEN EXTERNAL HAEMORRAGE SYNCRONOUS WITH MENSTRA. CYCLICITY OF PAIN OR OF MASS DIMENSIONS' VARIATION IS NOT PRESENT IN ALL CASES. DIFFERENTIAL DIAGNOSIS SHOULD BE MADE WITH STITCH GRANULOMA, ABSCESS, LIPOMA, HEMATOMA, SEBACEOUS CYST, SEROMA, HERNIA, OR EVEN MALIGNANT TUMORS. IMAGISTIC METHODS, SUCH AS ULTRASOUND, MRI AND CT SCAN ARE USEFULL FOR DIAGNOSIS. SURGICAL EXCISION OF ENDOMETRIAL MASS IS UNANIMOUSLY CONSIDERED TO BE THE ONLY CURATIVE TREATMENT.

KEY WORDS: ABDOMINAL WALL ENDOMETRIOSIS, PARIETAL ENDOMETRIOSIS

INTRODUCTION

Endometriosis could be an important issue concerning women of reproductive age due to its debilitating painful symptoms. The severest form of the disease is that of deep infiltrating endometriosis involving pelvic organs, such as rectum, urinary bladder, small or large bowel, uterosacral ligaments[16]. This form poses complex treatment problems.

The presence of the uterine mucosa outside the organ is rather rare. However, there are cases of endometriosis both in the abdominal wall, perineum, and even located in the internal organs,

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such as the large bowel[17]. The diagnosis in such cases could be difficult. Endometriosis affects 6%-10% of fertile women, being usually located in the pelvis[18].

The treatment of endometriosis could be medical, surgical or both (combined treatment). There is no optimal treatment for endometriosis. The medical treatment is based on synthetic progestin pills. It represents a solution for the women who do not intend to become pregnant. The long term medical treatment could be applied preoperatively or postoperatively[19]. The surgical excision of the lesions, in healthy tissue (with histopathologicaly confirmed free margins), remains the only curative treatment.

MAIN TEXT

The etiopathogenesis of endometriosis was not clearly elucidated and remains controversial. There are several theories supposed to be involved, including metaplasia, direct dissemination or transplantation, retrograde menstruation, vascular or lymphatic metastasis, and aerosolization[20].

Two theories have been suggested for explaining abdominal wall endometriosis (AWE)[21]. The first suggests that endometrial cells may be translated to ectopic sites (particularly during surgical procedures that require opening the uterus). This is the most accepted theory that can explain why many patients with scar location of the disease do not present signs or personal history of peritoneal endometriosis. The second theory supposes that, primitive pluripotential mesenchymal cells may undergo specialized differentiation to form endometriomas. This mechanism explains the rare cases of AWE non-scar reported in the literature - patients without a surgical history.

Different imaging methods (ultrasound, MRI, CT) are useful in positive diagnosis and determining the extent of disease. On imagistic bases, also, one can plan the extension of the surgical resection, especially for recurrent and large lesions. In lesions involving multiple organs, we have to plan the resection and the reconstruction, as well. The surgical treatment also implies adhesiolysis[22]. The diagnosis is non-specific, there are no pathognomonic imagistic findings for endometriosis. The only certain positive diagnostic is established by the hystopathological exam.

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The pelvin tumoral endometrial masses should be imagistically differentiated from other genital tumors, benignant or malignant[23].

Although rare, it is important that clinicians, even gynecologists or surgeons, and sonographers are familiarized with this pathology. Considering the increasing rate of caesarean deliveries all over the world[24], it is expected that this pathology will be encountered more and more frequently in daily practice.

The histopathological diagnosis is based on recognition of at least two from three following criteria: endometrial stroma, endometrial glands and hemosiderin pigment[25].

In a two years interval (between 2017 and 2018), in the surgery clinic of "Sf. Pantelimon" Emergency Hospital, Bucharest, we surgically treated 11 women with parietal endometriosis. In 9 cases, the women had personal history of surgical interventions in the gynecological area. This was the reason why we supposed that the main ethiopathological mechanism was direct dissemination of the endometrial tissue during previous surgical interventions that involved uterus, since all those patients had scar lesions. All the 9 women did not have any peritoneal history of endometriosis, thus they confirmed the most accepted theory, of intraoperative dissemination. All these patients had lesions adjacent to cesarean-delivery scars, in 7 cases Phannenstiel incision and in the other 2 median sub-umbilical incisions. These data are concordant to those in the literature[26]. Some authors hypothesized that suboptimal closure of either the uterine or abdominal wall could be at the origin of the implantation theory[27]. The closure becomes more difficult in obese patients, this being a risk factor, together with the higher levels of estrogens synthesized in the adipose tissue.

Only isolated cases of AWE without any previous surgery are reported in the literature[28]. We had one case of umbilical spontaneous endometriosis (Villar node) and one case located in right iliac fossa. This seems to confirm that, under right circumstances, primitive pluripotential mesenchymal cells could differentiate to endometriomas. This is the second theory that explains the rare cases of endometriosis in women with negative surgical history.

All our patients had preoperative ultrasound soft tissue examination. Ultrasound examination represents the first step in evaluating a soft tissue mass. It showed unhomogenous abdominal wall nodules with hypoechoic content and blurred outer margins. The mainly preoperative established

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diagnosis was stitch granuloma in 6 from 11 cases. It is well known that a correct diagnosis of scar endometriosis is preoperatively made only in a minority of 20%-50% of patients[29].

Only 2 of our patients had preoperative CT scan and only one underwent a MRI examination. Neither these imagistic examinations could established the diagnosis. The right diagnosis was supposed only in 3 of our cases, based on the personal surgical history and the cyclical variations of symptoms.

The typical clinic triad consists of a history of cesarean delivery, cyclic pain associated with menses, and palpable nodules near a surgical scar[30].

Spontaneous umbilical endometriosis, also known as Villar's nodule, was previously described in literature and represents an unusual location of the endometrial tissue[31]. Its frequency represents less than 1% of all endometriosis locations.

The large excision in some of our cases involving the fascia and muscular aponeurosis (4 cases) required an alloplastic repair of the abdominal wall, using a polipropylene mesh. The technique was cited before in the literature as a solution for parietal reconstruction after large excisions[32]. We did not encountered complications related to mesh usage in cases of endometriosis. The use of mesh was neither followed by common postoperative complications related to synthetic meshes, such as seroma and infection of the prothesis, nor by a higher rate of recurrence of the disease. For all cases in which we used synthetic material, we placed a subcutaneous aspirative drain, which was maintained several days, depending on the quantity of drained fluid per day. The mesh usage was considered to protect against incisional hernia, which was not encountered for our patients in the follow-up period of one year. All the patients underwent postoperative anticoagulant therapy (prophylactic)[33] and no thrombotic complications were noticed. Intensive care units admission was made only under objective criteria[34].

CONCLUSIONS

Even if rarely encountered by the general surgeon, abdominal wall endometriosis must be take into account in all fertile women with or without previous surgical or obstetrical interventions, that complain of pain synchronous with mensa at the level of abdominal wall. The diagnosis should be sustained by imagistic methods (the simplest being soft tissue ultrasound) and confirmed by histopathology exam. The only curative treatment remains large excision of PE with disease-free margins. It is recommended a free margin of 1 centimeter from the surrounding tissues. Radical

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extended excisions may be followed by abdominal alloplastic wall reconstructions, that do not negatively affect the morbidity and mortality of patients.

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UPDATE ON CLINICAL DIAGNOSIS AND MANAGEMENT OF ADENOMYOSIS

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ABSTRACT:
INTRODUCTION
IN THIS ARTICLE WE SUGGEST A MODERN APPROACH TO MANAGING A PATIENT WITH A CHRONIC ILLNESS SUCH AS ENDOMETRIOSIS AND PARTICULARLY ADENOMYOSIS OF THE UTERUS.
MAIN TEXT
ADENOMYOSIS OFTEN COEXISTS WITH OTHER UTERINE PATHOLOGIES LIKE LEIOMYOMA AND, ESPECIALLY, DEEP AND SUPERFICIAL PERITONEAL AND OVARIAN ENDOMETRIOSIS. FOR PROPER MANAGEMENT OF THESE INTRICATE DISEASES, HEALTH CARE SPECIALISTS AND GYNECOLOGISTS NEED TO BE TRAINED IN DETECTING ENDOMETRIOSIS.
CLEAR AND COMPLETE DIAGNOSIS REPORTING IS HELPFUL FOR NETWORKING AND ESSENTIAL TO ANY PROFESSIONAL MANAGING THIS DISEASE, FOR A BETTER INTERSPECIALTY COOPERATION.
MANAGEMENT OF ADENOMYOSIS SHOULD BE TAILORED FOR THE SPECIFIC SYMPTOMS AND NEEDS OF THE PATIENT.
LONG TERM FOLLOW-UP SHOULD TAKE PLACE PREFERABLY IN SPECIALIZED CENTERS OF EXCELLENCE IN ENDOMETRIOSIS SURGERY. COMPLETE CARE SHOULD BE PROVIDED BY THE MULTIDISCIPLINARY TEAM.
CONCLUSION
INCREASING AWARENESS FOR THIS DISEASE BOTH AMONGST MEDICAL CAREGIVERS AND PATIENTS CAN IMPROVE EARLY DIAGNOSIS, BETTER CARE AND PRESERVATION OF FERTILITY.

KEY WORDS: ADENOMYOSIS, ENDOMETRIOSIS, DEEP INFILTRATING ENDOMETRIOSIS, ADENOMYOMA

INTRODUCTION

In this article we suggest a modern approach to managing a patient with a chronic illness such as endometriosis and particularly adenomyosis of the uterus.

Adenomyosis is a form of endometriosis affecting the myometrium, morphologically described as endometrial glands and stroma within the uterine musculature. It was first mentioned by an anatomopathologist - Rokitansky in the 18th century as 'cystosarcoma adenoids uterinum'. It is generally estimated that adenomyosis is present in about 20-25% of women[13]. Most specialists agree that adenomyosis is present in women in the later reproductive years, and rarely in youg adolescent women – based on histerectomy specimens.

Little to no progress has been made for decades both in understanding pathophysiology and treatment of the associated pain and infertility. To support this idea, there is no consensus on management or guideline for this particular disease in Romania. Because of the particularities of diagnosis and treatment of endometriosis, a lot of patients are diagnosed with a delay of as much as 10 years since the beginning of the symptoms[14]. In this delay there are multiple attributing factors such as the belief that the disease cannot affect young adolescents, physicians not

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considering the disease, lack of medical education in schools for young girls, who should be thought about normal menses and sharing their concerns and symptoms with the general practitioner[15].

MAIN TEXT

Adenomyosis often coexists with other uterine pathologies like leiomyoma and, especially, deep and superficial peritoneal and ovarian endometriosis. Because of the frequent coexistence with endometriosis, we must always look for peritoneal endometriosis lesions in patients with adenomyosis and vice-versa. For proper management of these intricate diseases, health care specialists and gynecologists need to be trained in detecting endometriosis[16]. Guided by the principle that you only see what you recognize, this disease is still very much underdiagnosed and consequently sub optimally treated.

The best **training** for interested specialists would be in a center of excellence in endometriosis, specifically a center of excellence in endometriosis surgery, with the emphasis on "surgery" due to the fact that without the operating part, a center would lack the quality control and complete management of the illness[17]. Typically the endometriosis patient gets through an

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integrated approach involving multiple different specialties like gynecologyst, infertility specialists, pain management, nurses, physiotherapists, nutritionists, patient support organisations and also non traditional practitioners if needed[18]. These endometriosis centers or networks of excellence are accredited periodically by professional bodies to ensure continued performance.

Women with suspected adenomyosis should have thourough evaluation consisitng of detailed history, pelvic evaluation and imaging. Laboratory tests are used only for differential diagnosis and for diagnosing anemia.

The medical history should contain detailed obstetric, gynecological and other significant medical history. The questions should focus on dismenoreea, meno/metrorrhagia, dispareunia, dischezia, disuria, chronic pelvic pain, other cyclic or non-cyclic pain and infertility. The scoring of pain reporting should use the visual analog scale (VAS) detemining the intensity of pain from one to ten. Pain scores higher than 7 are associated with poor quality of life, altered mental and social status and usually necessitate the admnisitration of painkillers.

Clinical examination of a patient with suspicion of adenomysis is comprised of a bimanual evaluation of the pelvis typically showing a mobile, difusely enlarged (usually globular) soft and possibly tender uterus. Particular cases can present with fixed uterus (association with deep infiltrating endometriosis)[19].

Transvaginal ultrasound (TVUS) is the first line imaging choice for evaluation of adenomyosis. The two week learning programme proposed by Guerriero S, Pascal MA, Ajossa S, et al.[20] for TVUS detection of lesions is fesible to improve diagnosis of deep infiltrating endometriosis. TVUS evaluation of the pelvis should include: uterus size, shape, description of the myometrium and endometrium, mobility - sliding sign, both adnexa (including mobily, position, cysts), rectovaginal septum, rectosigmoid/bowel lesions, uterosacral ligaments, anterior compartment – bladder. If suspected abdominal ultrasound can diagnose a ureteral obstruction.

Other imagining options are Magneric Resonance Imaging (MRI) but it should be recommended for patients with inconclusive TVUS, when accurate diagnosis is essential for further management. Also to aid surgical planning of adenomyosis excision MRI is often performed.

Both imaging tehniques evaluate the myometrial changes the same way: ‘asymmetric thickening of the myometrium (with the posterior myometrial typically thicker), (2) myometrial cysts[21], linear striations radiating out from the endometrium[22], loss of a clear endomyometrial border, and[23] increased myometrial heterogenity. With MRI, some quantitation of the thickening

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of the junctional zone is possible, with >12 mm generally considered diagnostic of the disease and <8 mm excluding adenomyosis'[24].

To note that endometrial byopsy is not informative in the diagnosis since this is a myometrial disease[25]. Definitive diagnosis is made based on anatomopathology evaluation of the myometrium using a histerectomy specimen.

The second part of our recommendations for the clinician consulting adenomyosis is directed to proper **diagnosis reporting**. The Romanian national guideline for endometriosis approves the revised American Society of Reproductive Medicine guideline (rASRM) for endometriosis staging but there is no mention of adenomyosis in this classification. Completing this intra operatory evaluation score (rASRM) is the Enzian score - which takes into account the deep infiltrating endometriosis lesions and endometriosis lesions outside the small pelvis or infiltrating adjacent organs. This classification mentions that whenever adenomyosis is diagnosed it should be marked as FA (far, adenomyosis). Both the Enzian and rASRM classifications fail to describe adenomyosis completely (localization, depth of penetration, interaction with the endometrium, vascularity, number of lesions, etc.) and when reported they do not help other physicians make a treatment plan.

In 2015 the consensus opinion of the MUSA (Morphological Uterus Sonographic Assessment - [26]) group issued a statement that gave us an important tool to correctly describe and report ultrasonographic aspects of various uterine pathologies, including adenomyosis. Based on this generally accepted guidelines for sonographic assessment the group differentiates uterine adenomyosis from fibroids. Regarding adenomyosis MUSA group gives an exhaustive description of the lesions: adenomyosis can be present in one or more sites (i.e. focal adenomyosis) within the uterine wall or involve most of the myometrium in a dispersed pattern (i.e. diffuse adenomyosis). A rare form is represented by a cystic structure (described as an adenomyotic cyst or adenomyoma).

The MUSA group also discusses two different phenotypic adenomyosis forms. The first type, described as 'classic adenomyosis' is the result of the invasion of the endometrium into the muscularis uteri and the other one is the result of the invasion of the peritoneal lesions into the uterine serosa[27].

Clear and complete diagnosis reporting is helpful for networking and essential to any professional managing this disease, for a better interspecialty cooperation. Unfortunately, in clinical practice, the implementation of these recommendations have not progressed as expected and there is a big interobserver variability in describing myometrial pathology.

Management of adenomyosis should be tailored for the specific symptoms and needs of the patient.

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Historically the definitive treatment for adenomyosis was total or supracervical hysterectomy, being a disease limited to the myometrium, the ovaries and cervix are usually conserved.

For women with mild to severe symptoms and who have future plans for pregnancy, one can either choose no treatment with observation of progression every 6 months, or hormonal treatment - starting with oral combined contraceptives, progestin only pills or a levonorgestrel intrauterine device. For severe disease and prior to artificial reproductive techniques (ART), gonadotropin modulating hormones can be used to induce a menopause-like state and regression of myometrial lesions.

For women with severe symptoms who completed childbearing, hysterectomy is the treatment of choice with the alternative of uterine artery embolization. During surgery, all sites of endometriosis should be thoroughly examined and, if possible, excised completely.

Uterus sparing resection is a surgical procedure chosen for fertility preservation purposes. It is a very demanding surgical procedure intended to excise the adenomatous tissue from the myometrium and perform a uterine reconstruction using a triple flap method as described by Osada. Reported uterine rupture rates with the tehnique are approximately 4%[28]. Uterine reconstruction tehniques are laborious, time consumptive, high risk and are performed by abdominal open route only, as to obtain better suturing and approximation of tissue in multiple layers.

Seldom used techniques are radiofrequency ablation and high intensity frequency ultrasound (HIFU)[29].

Being a long-term, chronic disease, periodic follow up is the key for a quality management. Postoperatively patients should be reevaluated at 1, 3, 6 and 12 months by a standard gynecological evaluation, assessment of painful symptoms, recurrence of disease and very importantly quality of life assessment. Patients with adenomyosis should have a periodic follow up every 6 months or in case of emergency. Also in selected cases couples who desire future pregnancy are referred to artificial reproductive techniques (ART) for counseling. These couples should be informed that clinical pregnancy rates are lower in patients with adenomyosis – a retrospective analysis was carried out by an Indian group of scientists[30] comparing clinical pregnancy rates in patients with adenomyosis, endometriosis and control groups – the clinical pregnancy rate 22.72% in women with endometriosis and adenomyosis, 23.44% in women with adenomyosis only and 34.55% in healthy patients. Also miscarriage rates are higher in patients with adenomyosis: 35% in women with endometriosis and adenomyosis, 40% in women with adenomyosis only and 13.04% in healthy patients.

As we mentioned long term follow-up should take place preferably in specialized center of excellence in endometriosis surgery. Complete care should be provided by the multidisciplinary team of gynecologyst, infertility specialist, pain management doctor, physiotherapist, nutritionist and possibly non traditional practitioner. The multidisciplinary team offers a core of experts who adress this complex illness with the goal of reducing reccurence of disease, improving pain scores

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of dysmenoreea, reducing menorrhagya, improving social life, improving fertility outcome. No less important the patient is encouraged talk to other with the same disease via patient support organisations because it gives them hope and a sense of belonging.

CONCLUSIONS

Uterine adenomyosis is a widespread disease affecting approximately 20-35% of women across the reproductive years. Symptoms may vary in intensity and can be heavy menstrual bleeding, dysmenorrhea, chronic pelvic pain, dyspareunia, bloating - all of which can determine a low quality of life, depression, anxiety and infertility.

General practitioners and especially gynecologists should consider the diagnosis of adenomyosis when encountering the symptoms mentioned above. It is also important to get trained in a dedicated center for excellence in endometriosis surgery to improve diagnosing and managing this disease or referring the patients to these specialized centers where they can get a holistic approach from a multidisciplinary team.

Diagnosing and reporting should be done by the international standards to aid in follow-up and also in disease management. Regular check-ups every six months with a pelvic exam, imaging and also pain symptoms assessment is recommended for a good control of recurrence and pain.

For a better compliance and medical care, patients with endometriosis should be well informed about their disease and about treatment options, including fertility preserving surgery. Artificial human reproduction is an important part in the management of infertile couples suffering from adenomyosis.

Increasing awareness for this disease especially in young adolescents can improve early diagnosis, better care and preservation of fertility.

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Medicine

RUPTURED OVARIAN ENDOMETRIOTIC CYST IN A 28-YEAR-OLD WOMAN - CASE REPORT

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ABSTRACT:
ENDOMETRIOSIS IS A COMMON BENIGN DISEASE, WITH DIFFICULT QUANTIFICATION OF INCIDENCE GIVEN BY THE DELICATE SONOGRAPHIC DIAGNOSIS, WITH LOW SENSITIVITY FOR SMALLER IMPLANTS. THE MAIN METHOD OF DIAGNOSIS IS LAPAROSCOPIC SURGERY WITH BIOPSY AND HISTOPATHOLOGY POSITIVE RESULT. USING THIS STANDARD 1.6/1000 WOMEN BETWEEN 15 AND 49 SUFFER FROM ENDOMETRIOSIS, BUT THE UNDIAGNOSED CASES ARE PROBABLY MUCH MORE. PRIOR, IT WAS BELIEVED THAT WHITE WOMEN REPRESENTED THE MOST AFFECTED ETHNICITY, BUT RECENTLY VARIABLE RESULTS WERE OBSERVED. WE REPORT A CASE OF A 28-YEAR-OLD WOMAN WITH A HISTORY OF ENDOMETRIOTIC CYST WHO PRESENTED AT THE EMERGENCY ROOM WITH ACUTE ABDOMEN AFTER SEXUAL INTERCOURSE. MAKING A REVIEW OF THE LITERATURE, WE FOUND FEW CASES OF RUPTURED LARGE ENDOMETRIOTIC CYSTS. THE POSTOPERATIVE EVOLUTION OF THE PATIENT WAS FAVORABLE, WITH GOOD RESPONSE OF TREATMENT AT LATER FOLLOW-UP.

KEY WORDS: ENDOMETRIOTIC OVARIAN CYST, ENDOMETRIOSIS, OVARIAN CYST

INTRODUCTION

Endometriosis represents the presence of endometrial glands and stroma in other locations than the uterine cavity[13]. Such locations with abnormal implants are pelvic peritoneum, ovaries, uterosacral ligaments as the most common ones[14].

Even though endometriosis is frequently a nonmalignant process, such abnormal implantations of endometrial tissue can cause chronic pain, dysmenorrhea, dyspareunia and infertility[15], many of them also present in other conditions. This symptoms can even reach paralyzing pain, affecting the day to day life of the patient[16]. Itis an estrogen-dependent, inflammatory illness, which affects women during their premenarcheal, reproductive, and postmenopausal hormonal stages[17]. Not much is known about prevalence and risk factors when it comes to endometriosis, accurate assessment of epidemiological findings of these disease is held back by the incapacity to detect it in general population[18]. There are some theories when it comes to pathogenesis such as retrograde menstruation, coelomic metaplasia and Müllerian remnants,

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neither explaining all types of endometriosis[19]. Risck factos may include nuliparity, low BMI[20], late menopause, shorter menstrual cycle (< 27days), heavy menstrual bleeding, obstruction of menstrual outflow[21], early menarche[22], sexual abuse as child or teenager[23], high intake of trans saturated fat[24].

Clinical examinations in woman with endometriosis is variable, sometimes the clinician can observe vaginal tenderness, fornix nodules, pelvic mass which may place the uterus laterally[25].

There are no specific laboratory findings, but as a routine all woman suspected of endometrioma are required CA125 marker, which can be elevated[26].

For preoperative diagnoses sonography magnetic resonance imaging can be used to detect ovarian cysts, rectovaginal nodules and bladder nodules[27], some abdomen wall lesion can be seen as hipoecogenic vascular lesions with irregular outline[28].

Considering symptoms, response to medication, clinical and paraclinical findings, sometimes surgery is required, laparoscopic surgery is usually the preferred method but emergency situations may indicate laparotomy[29]. Staging: I - minimal disease is characterized by isolated implants and no significant adhesions, II - superficial implants that are less than 5 cm in aggregate and are scattered on the peritoneum and ovaries, no significant adhesions are present, III - both superficial and deeply invasive, peritubal and periovarian adhesions may be evident, IV - multiple superficial and deep implants, including large ovarian endometriomas, filmy and dense adhesions are usually present[30].

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Endometriotic foci most often appear as punctate red, brown or white areas[31]. The age and functional state determines their colour as following: the earliest lesions present as yellow-red surface stains, while the red ones are also early forms of the disease, only actively growing; these are followed by black lesions, most commonly seen by pathologist in operation specimens, in which the bleeding has resolved; some lesions can be brown to slightly yellow, reflecting hemosiderin deposits, while the oldest of them are white, with fibrosis and scarring[32]. At some sites, mostly in the ovary, lesions can become cystic and reach impressive sizes, with diameters up to 15cm[33]. Endometriotic cysts (endometriomas) have fibrotic walls with a smooth or ragged brown to yellow lining and semifluid or thickened brown, chocolate-coloured contents[34].

Microscopically, endometriosis consists of endometrial glands and stroma, with the diagnosis being often possible with only component identified[35]; however, they should both be definitively identified for avoidance of diagnosis errors. The glandular epithelium is one cell thick consisting of columnar cells, with cigar-shaped, vertically oriented nuclei and eosinophilic cytoplasm; cilia can sometimes be identified. Serous (tubal), clear cell, mucinous and even suqamous metaplasia can occur[36]. Usually, endometrial-type glands are associated with fibrosis, hemosiderin deposits and histiocytes[37]. The stroma is composed of small, elongated cells with scant cytoplasm and a delicate reticulin network[38]. Decidualization of the stroma and gestational changes can occur.

Endometriomas usually feature an extremely attenuated epithelial lining who can be totally replaced by granulation tissue, fibrous tissue and pseudoxanthoma cells[39]. These findings along with hemosiderin deposits and islands of residual cuboidal glandular epithelium or endometrial stroma can lead to a diagnosis of endomotriotic cyst.

The most important entities to be considered in the differential diagnosis of endometriosis are solitary follicle or corpus luteum cysts, tubo-ovarian abscesses, surface epithelial cysts/cysadenomas, unilocular cystic granulosa cell tumours and secondary neoplasm (especially

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clear cell or endometrioid carcinoma). Immunhistochemically, the epithelium of the endometriotic glands is positive for ER and PR, while the stroma shows tipical positivity for ER, PR and CD10.

Malignancy can arise in up to 1% of the cases, the most common being clear cell and endometrioid carcinomas[40].

Generally the prognosis of patients with endometriosis is good. It is important to understand the high possibility of recurrence and the long term medical treatment, as it is considered a chronic disease.

Treatment is a complex problem in this pathology, depending on the age, symptoms and fertility preservation. It may include medical treatment of the pain with nonsteroidal antiinflamatory drugs, combination hormonal contraceptives, progestins, GnRH agonists, GnRH antagonists, aromatase inhibitors, selective progesterone – receptor modulators, androgens or surgery[41].

Patients are advised to have a long-term follow-up.

The aim of this case report is to show the diagnostic stages and treatment of a rare event, which is encountered in less than 3% of women of childbearing age who are known to have endometriomas[42].

Our case was appealing for 2 reasons: the size of the endometrioma with thick wall and the fact that it ruptured.

CASE PRESENTATION

A 28-year-old woman, C.A.M., was admitted in the Department of Obstetrics and Gynaecology "St. Pantelimon" Emergency Clinical Hospital, Bucharest, Romania, on June 2017, for acute abdominal pain and muscular defense after intercoursein order to determine therapeutic specialist conduct. From the patient's personal history, we take note of 0 pregnancies and bilateral ovarian endometriomas.

At admission in our department, the patient was cooperative, blood pressure was 130/75 mmHg, pulse 110 beats/minute. The gynaecological examination with the speculum showed tenderness, no macroscopic lesions on the cervix and no bleeding coming from the uterine cavity. On the bimanual examination we could identify the uterus, in intermediary position, highly painful with mild palpation, with difficulties in assessing the size, right adnexa increased in volume, highly painful at palpation, with impossible to determine shape and consistency because of muscle defense, left adnexa painful at mild palpation, increased in volume, vaginal fornix bulges. Laboratory exams were completed and were in normal range. Transvaginal ultrasound was performed and revealed uterus in intermediary position, surrounded by hypoechoic fluid, on the right of the uterus and continuing to the Douglas an ovoidal formation can be seen, with irregular shape, thick wall and non-homogeneous hypoechoic content; on the left ovary a cystic formation

[40] Nucci, Marissa R; Oliva, Esther; series editor Goldblum, John R; *Gynecologic Pathology - a volume in the series Foundations in Diagnostic Pathylogy*, 2009, Elsevier.
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with a 3 cm diameter and non-homogeneous hypoechoic content could be observed (Figure 1., Figure 2., Figure 3., Figure 4.).

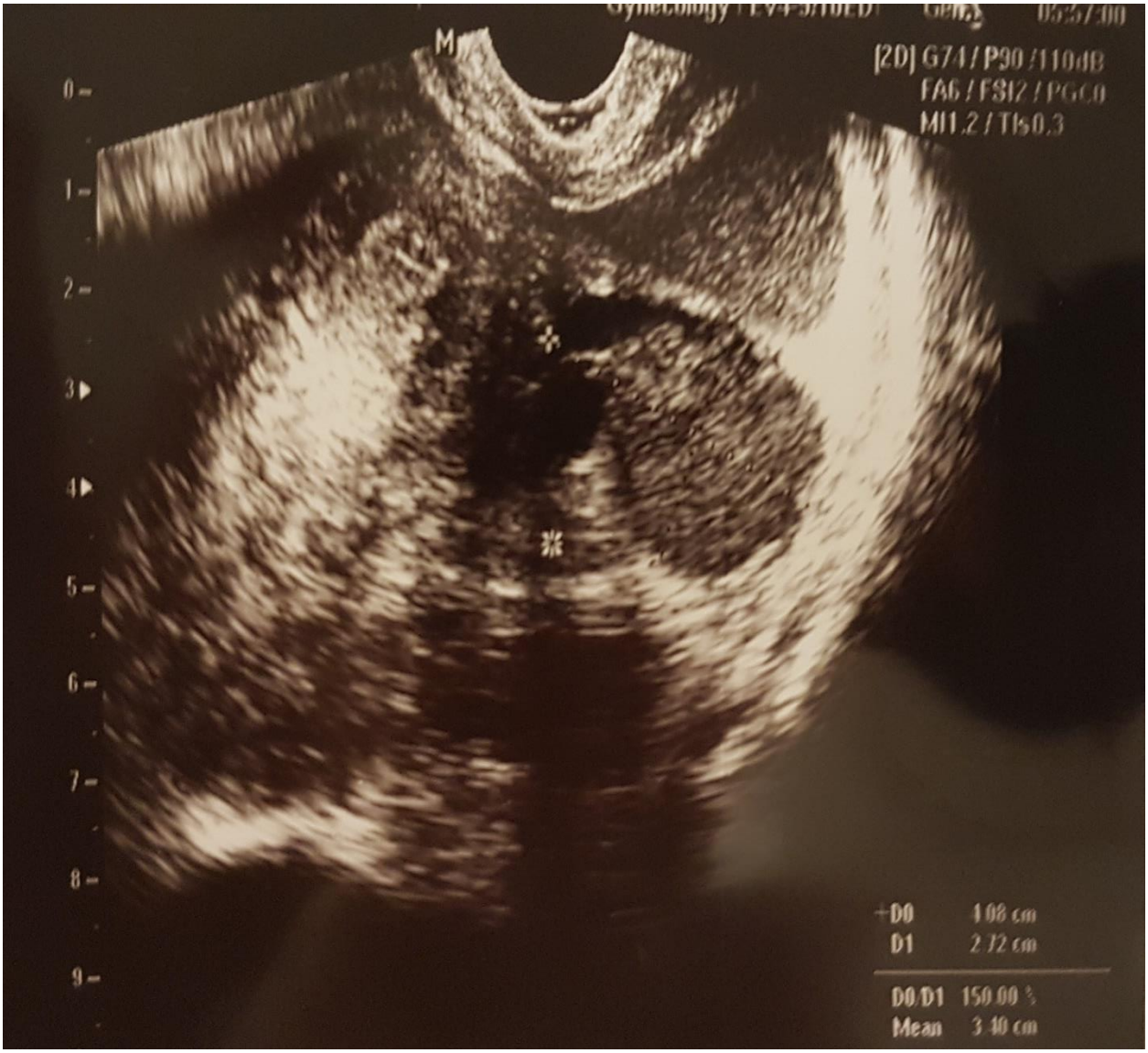

Figure 1) Transvaginal sonography.

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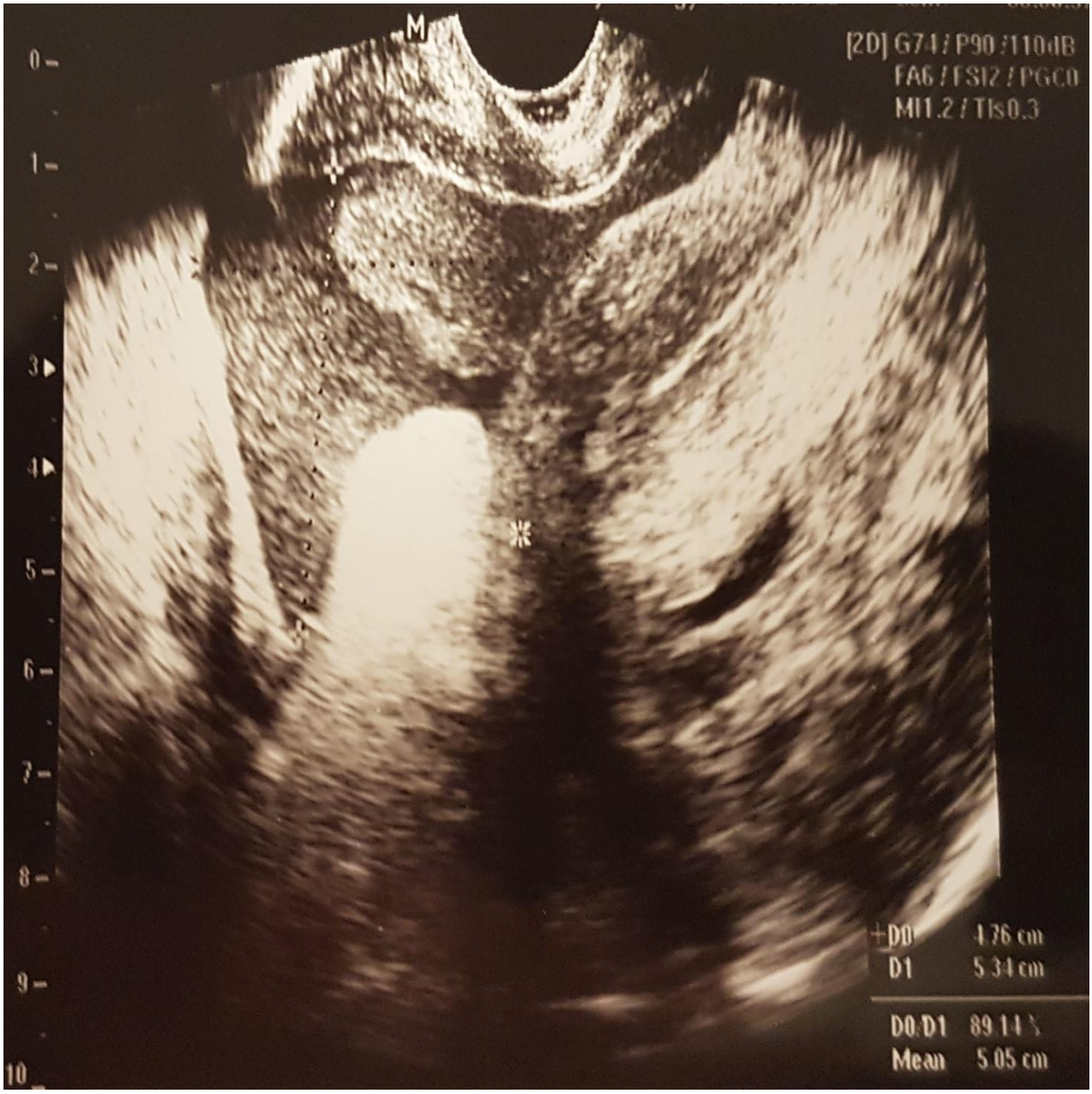

Figure 2) Transvaginal sonography.

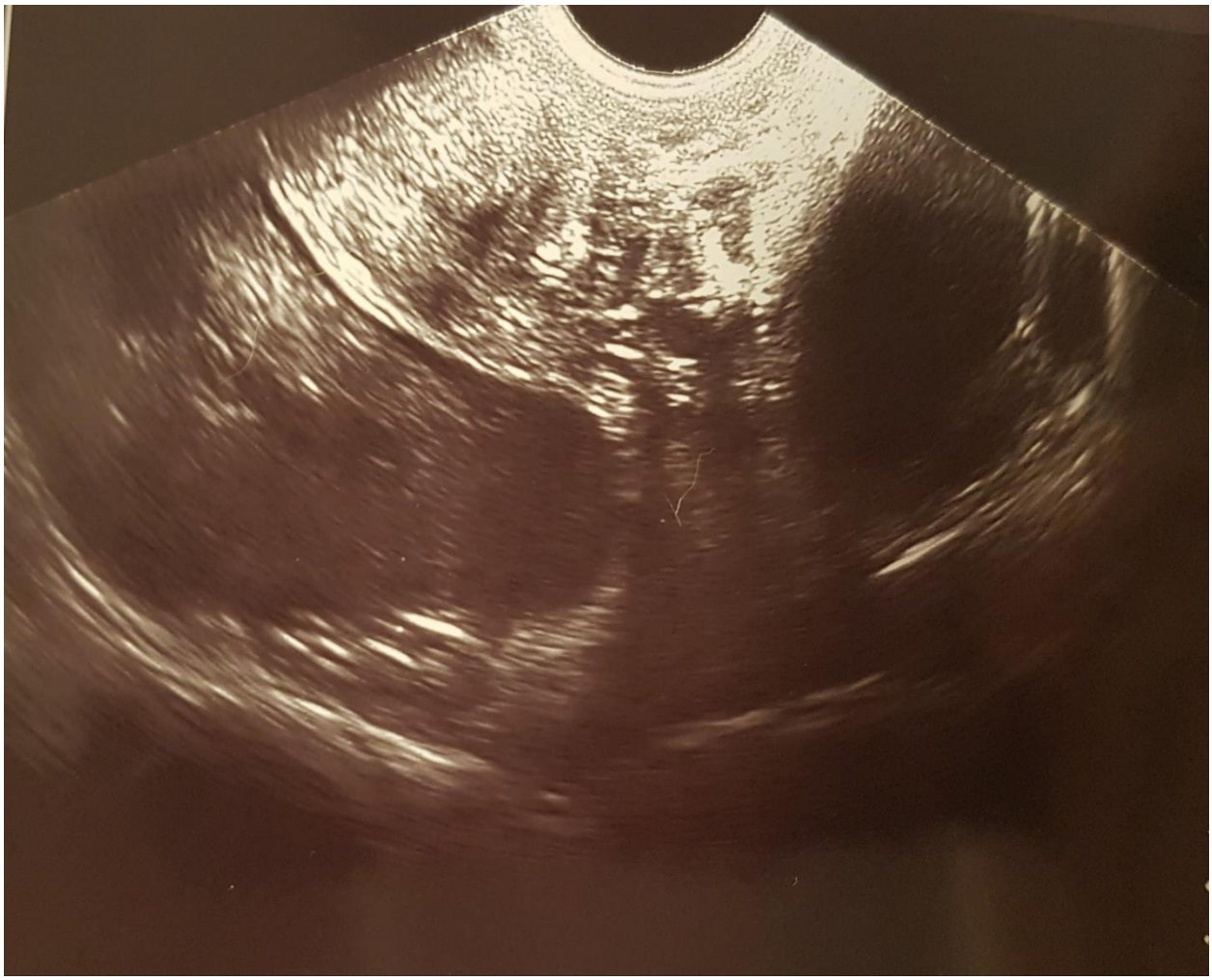

Figure 3) Transvaginal sonography.

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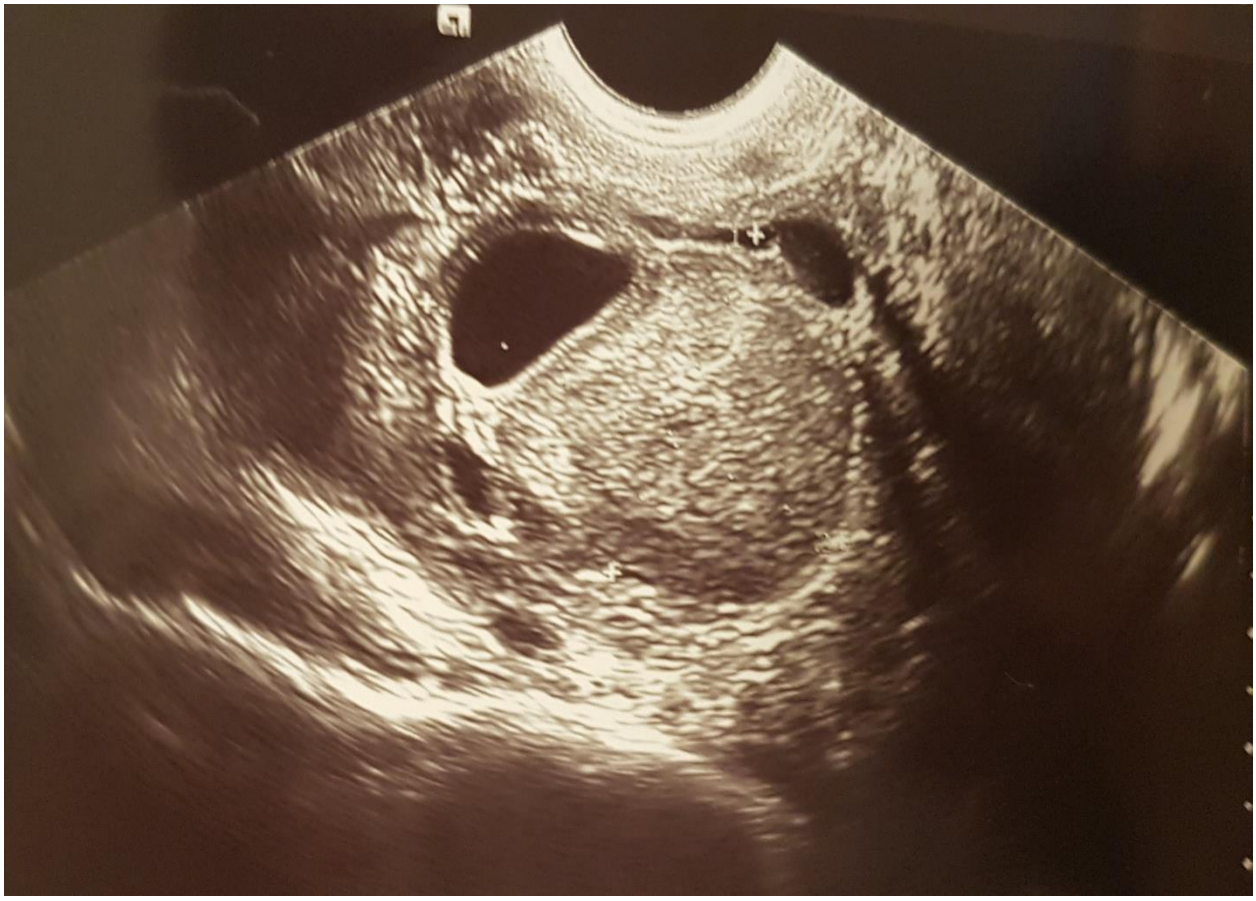

Figure 4) Transvaginal sonography.

The informed consent was obtained and exploratory surgery through laparotomy was performed under general anaesthesia, followed by aspiration of 400 ml of blood and chocolate-like fluid. Upon inspection of the peritoneal cavity on the right side of the uterus one could observe a large ruptured ovarian endometrioma, approx. 15 cm in diameter, adherent to the peritoneum, uterus and rectum; on the left side there was an intact 3 cm ovarian endometrioma. The surgical team decide to perform right cystectomy and left cystectomy and send the specimens for histopathological evaluation.

The postoperative evolution was favourable, without any complications. On day 5, the patient was released from the hospital with good general condition and afebrile. Progestins were given for the next 6 months after surgery.

The histopathological result showed a flattened columnar epithelial lining and typical endometriotic stroma with hemosiderin deposits and foci of granulation tissue.

At the 6 months follow-up of the patient - no complications occurred and the serum investigations were in normal limit. Progestins were replaced with combination birth control until the patient wishes for a pregnancy.

DISCUSSION

Clinical diagnosis is difficult if no sonographic signs are discovered[43]. Most of the times the patient presents symptoms which are described in other conditions as well and differential diagnoses is difficult to make. In our case the patient was had a known history of ovarian endometriomas and presented with acute abdomen and a positive sonographic diagnose which led to emergency laparotomy.

It is preferred to exclude the endometrioma without rupturing it, to prevent spreading the content in the peritoneal cavity as it is believed this increases the likelihood of a relapse. In our case it was not possible and lavage with sodium chloride was done multiple times.

Periodical reevaluation in such cases is mandatory as this is a chronic disease with a positive outcome if managed correctly.

Conduction of patients with infertility and wish for a pregnancy is difficult due to the higher possibility of having a more severe extension of the disease. Managing such cases and obtaining a pregnancy has proven to be a difficult task. Severe cases (stage IV) are able to obtain pregnancies by in vitro fertilisation, with poorer implantation rates than stage III[44].

CONCLUSIONS

Ruptured ovarian endometriomas are rare cases which must be treated carefully by experienced gynaecologists. Surgery is required and the type of the surgery is selected by the doctor taking into consideration the experience and the size of the ruptured endometrioma. CA125 may be elevated and the extracted specimen must be sent to the histopathologic department to exclude other possibilities and correctly conduct treatment afterwards.

Fertility can be preserved, but caution must be implied. The majority of the patients have a good outcome.

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BLADDER ENDOMETRIOSIS – A SHORT REVIEW

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ABSTRACT
BLADDER ENDOMETRIOSIS (BE) IS DEFINED AS THE PRESENCE OF ENDOMETRIAL GLANDS AND STROMA IN THE DETRUSOR MUSCLE, USUALLY LOCATED IN THE TRIGONE AND THE DOME OF THE BLADDER. IT IS A DISEASE WITH AN UNKNOWN PREVALENCE, BEING FREQUENT UNDERDIAGNOSED AND HAVING UNSPECIFIC MANIFESTATION, THAT TENDS TO APPEAR IN A CYCLICAL MANNER. MULTIPLE HYPOTHESES REGARDING BLADDER ENDOMETRIOSIS HAVE BEEN PROPOSED, WITH MIGRATION AND TRANSPLANTATION THEORIES BEING THE MOST ACCEPTED. A THOROUGH EXAMINATION OF THE PATIENT IS RECOMMENDED, AND ONCE THE DIAGNOSIS OF BE HAS BEEN MADE, CLINICAL MANAGEMENT CAN BE CONSERVATIVE, USING HORMONALTHERAPIES, OR SURGICAL.

KEY WORDS: BLADDER ENDOMETRIOSIS, CONSERVATIVE MANAGEMENT

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INTRODUCTION

Endometriosis represents the presence of endometrial glands and stroma outside the uterine cavity[9]. The lesions are usually located in the pelvis, but can occur in other locations such as bowel, ovaries, urinary tract and rarely diaphragm, pleural cavity, lungs and central nervous system. It has been observed that most women suffering from endomestriosis have multiple areas of involvement[10]. The presence of extra-uterine endometrial tissue can cause local inflammation with secondary dysmenorrhea, dyspareunia, chronic pain, and infertility, with symptoms varying from mild to severely debilitating[11].

Due to the fact that endometriosis is a histologic diagnosis and that women have varied presentations, ranging from asymptomatic to non-specific symptoms the real, to determine the real incidence and prevalence of this disease is challenging. In one paper it has been stated that endometriosis affects 5-15% of premenopausal females[12]. Ballard et al in their case control study of more than 5500 women found that the prevalence of endometriosis varied from 1.2-1.5%[13]. Furthermore, a retrospective study on 9500 women undergoing hysterectomy for benign tumors, Mowers et al found that up to 15% of patients were diagnosed with endometriosis[14]. Up to 50 % of women with infertility and 70% of patients with chronic pelvic pain (CPP) have been diagnosed with endometriosis[15].

A series of risk factors have been associated with an increased risk of developing endometriosis: nulliparity, early menarche, late menopause with prolonged exposure to endogenous estrogens, shorter menstrual cycle, obstruction of menstrual outflow, heavy menstrual bleeding, lower body mass index.

According to the depth of invasion and anatomic location, endometriosis is usually classified as: ovarian endometriosis, superficial peritoneal endometriosis and the most severe form of endometriosis, deep infiltrating endometriosis - more than 5 mm deep (DIE).

These ectopic endometrial implants can sometimes affect the urinary tract, with the bladder and ureter being the most commonly affected. The prevalence of disease regarding specific sites,

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among women with urinary tract endometriosis (UTE), is as follows: bladder 85-90%, ureter 10%, kidney 4% and urethra 2%[16]. The incidence of UTE seems to vary from study to study, ranging from around 1% to approximately 12% of women affected by endometriosis[17]. Most of the time, UTE is diagnosed because of the complaint of urinary symptoms during gynecologic follow-up for endometriosis. UTE can be classified as primary, spontaneously appearing in the urinary tract or secondary, after pelvic surgeries, such as hysterectomy or cesarean delivery. Approximately half the patients with UTE underwent a pelvic surgery in the past[18]. The reason why the incidence of UTE varies widely between different studies might be the underdiagnosis of this disease in the preoperative evaluation and during laparoscopic surgery[19].

Bladder endometriosis (BE) is defined as the presence of endometrial glands and stroma in the detrusor muscle. It can affect the full thickness of the bladder muscle or it can be partial. BE usually affects premenopausal women, with an average age of 35 years old, while postmenopausal BE is extremely rare, endometriosis being an estrogen-dependent disease[20]. Typically, BE lesions are located in the trigone and the dome of the bladder and the disease usually spreads from the serosal surface of the bladder towards the mucosa.

PATHOGENESIS

Despite the fact that the pathogenesis of bladder endometriosis is still debated, there are four hypotheses that are generally accepted: embryonic, migration, transplantation, and iatrogenic theories.

The embryonal theory suggests that BE could originate from remnants of the Mullerian ducts, mainly located in the vesicouterine and vesicovaginal septum[21]. Proposed for the first time by Donnez et al[22], this theory could explain the existence of endometriosis in girls prior to

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menstruation, and thus not yet exposed to retrograde menstruation, the mechanism proposed in the migration theory.

The migration theory states that endometrial cells flow retrograde through the fallopian tubes and into the peritoneal cavity during menses, and from here they implant themselves into the bladder wall[23]. This process is facilitated by the presence of the vesico-uterine pouch, which creates a sheltered "pocket" in which endometrial cells are protected from the usual peritoneal clearance, thus allowing endometrial implants to develop[24]. This shelter effect can explain why BE is not usually seen in women having a retroverted uterus and why ureteral endometriosis is seen more frequent on the left, where the sigmoid colon creates a shelter on the left side of the pelvis[25]. Furthermore, it has been observed that women with genital tract obstructions that prevent drainage of menstrual blood through the vagina, thus having an increase tubal reflux, have an increased risk of developing endometriosis, further supporting the migration theory[26]. However, due to the fact that up to 90% of women have retrograde menstruation and most of them do not develop endometriosis, additional factors could be involved[27].

According to the transplantation theory, endometrium cells are displaced through the lymphatic and circulatory system and then implanted into the urinary system[28].

Intraoperative dissemination of endometrial cells following pelvic surgery represents the basis of the iatrogenic theory[29].

Once endometriosis is established, the process appears to cause symptoms through inflammatory changes. The mechanism of endometriosis-related infertility seems to involve anatomic distortion from pelvic adhesions and the production of cytokines, prostanoids and other substances that are "hostile" to normal ovarian function, fertilization and implantation.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Women suffering from bladder endometriosis usually presents with bladder pain, suprapubic discomfort, dysuria, urinary urgency, increased urinary frequency and less frequent, hematuria, with symptoms depending on the location and size of the lesions. Urinary incontinence is a very rare symptom associated with BE[30]. However, approximately one third of women remain

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asymptomatic, or presents minor complaints, making the diagnosis an incidental finding[31]. In most of these asymptomatic cases, the lesions measure approximately 1-2 cm in diameter[32]. In the literature, dysuria has been reported in 21-69% of patients with BE. Hematuria has been described in only 20-30% of women suffering from BE, due to the fact that endometriosis rarely infiltrates the mucosal layer. Furthermore, approximately half the patients with BE have been describing a cyclic manner in which these symptoms tend to occur, with predominanceduring the premenstrual period[33].

Bimanual examination of the patient can reveal a thickened area, a palpable nodule or cystic expansion with topographic-anatomical correlation to uterosacral ligaments, vagina, rectovaginal

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space, pouch of Douglas, the rectosigmoid and the posterior wall of the bladder, findings highly suggestive of endometriotic infiltration of the pelvis[34].

All patients should have their renal function evaluated by blood creatinine levels, as a silent loss of renal function is not so rare[35]. An urine examination should be performed for evidence of hematuria and to rule out an infectious etiology for the irritative voiding symptoms[36]. Urine cytology should be performed because of the necessity of differential diagnosis with bladder cancer[37].

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In terms of imaging studies, ultrasonography (abdominal, transvaginal, or transrectal) is the first line tool in diagnosing BE, mainly due to low cost, ready availability, and lack of radiation exposure[38]. On ultrasonography, with the bladder full of anechoic urine, endometriosis lesions appear as a filling defect on the posterior wall, with variable grades of protrusion, with an iso/hypoechoic aspect. These nodules, usually, are spherical or comma-shaped with regular contours, and they display few blood vessels on Doppler examination[39]. Both abdominal and transvaginal ultrasound can be used to detect BE, with the latter being preffered in the gynecological clinical practice. According to literature, transvaginal ultrasonography has a sensitivity of about 60-70%, with a specificity of 100%[40]. Thonnon et al compared the performance of 3D transvaginal ultrasonography with color Doppler to MRI and cystoscopy in the diagnosis of bladder endometriosis and found that ultrasonography was superior to cystoscopy and at least as effective as MRI in the diagnosing and planning surgery for BE[41].

On the MRI, BE appears to be high signal intensity on T1-weighted images and low signal intensity on T2-weighted images. A conventional MRI protocol for diagnosing BE needs to include sagittal and axial T1 and T2-weighted images, before and after fat supression. MRI seems to have a sensitivity reaching 80-90% with a specificity of up to 98%. Due to the fact that it is more expensive than transvaginal unltrasonography, and that in experienced hands, the latter, has approximately similar accuracy in detecting BE, MRI should not be routinely performed. In case of cancer suspicion and in complex cases of endometriosis with extensive adhesions it is more helpful due to its higher contrast resolution,better delineation of bladder wall layers, better tissue characterization, and better multiplanar capability[42].

Due to the fact that the endometriosis lesions progress from the serosal layer to towards the mucosa, thus having an intraperitoneal origin, cystoscopic findings may be normal. However, it can identify in some cases a nodular mass on the posterior bladder wall or on the dome. Scheduling cystoscopy immediately before or during menstruation is important for a best characterization of the nodule. During this period the nodule is larger and more congested, thus the cystoscopic

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visualization is clearer. Endometriotic lesions appear to be an adenomatous and irregular nodular masses,with different colors, with blue-red, blue-black, or blue-brown being the most frequent types. The bladder mucosa is usually unaffected.

Cytoscopy is a very useful tool, allowing the estimation of the distance between the ureteral orifices and the nodule borders, thus contributing to the decision of the most appropriate surgical approach. Furthermore, cystoscopy is very effective in excluding bladder cancer, but it should be noted that, with the exception of transurethral resection (TUR), a simple biopsy is frequently not diagnostic for endometriosis[43]. Some authors recommend that cystoscopy should not be routinely performed, except in those cases when there is a suspicion of bladder cancer or when the distance from the nodule to the ureteral orifices cannot be clearely evaluable using other imaging techniques[44].

Differential diagnosis should be made with urinary tract infections, interstitial cystitis/bladder pain syndrome, urinary tract stones, bladder cancer[45].

TREATMENT

Treatment of BE can be pharmacological, surgical or a combination of both procedures. Due to the relative rarity of this disease, there are no substantial guidelines regarding the treatment of BE.

The objective of the medical treatment is to cause a regression of the endometrial tissue. The most common medical therapies used in the treatment of BE are: gonadotrophin-releasing hormone (GnRH) agonists and antagonists, progestogenes and combined oral contraceptives.

Some authors recommend the use of medical therapy, such as combined oral contraceptives (COC) or progestogenes, regardless of the method of administration, as a first line treatment for DIE (rectovaginal, colorectal, and bladder endometriosis), being efficacious, safe and well tolerated[46]. Noel et al, in their study on estrogen and progesterone receptors in smooth muscle

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component of deep infiltrating endometriosis, found that these receptors were well represented in the detrusor lesions, with progesterone ones outnumbering the estrogen type, thus these lesions could be responsive to hormonal treatment[47].

Westney et al reported partial or complete resolution of symptoms in 12 out of their 13 women with BE treated with low-dose COC for a period of 8-24 months. In some cases progesterone was added to the current regimen of treatment[48].

In a study comparing gonadotropin-releasing hormone agonist and a continuous oral contraceptive pill in the treatment of BE, Fedele et al, treated five women with a COC used continuously and five patients with a GnRH agonist and stated that, at the end of the treatment, cytoscopy showed nearly complete resolution of the lesions in patients treated with GnRH agonists. The patients treated with a COC had a significant regression of the lesions, but not complete[49].

A frequently studied medical therapy for BE is dienogest, a 19-nortestosterone derivative with anti-androgenic properties. Multiple authors treated women with BE with this agent, all having the same result. Takagi et al managed a remarkable relieve in symptoms and a significant reduction in the size of the lesion after a 6 months treatment with oral dienogest in a 39-yr-old woman with a positive histologic diagnosis of BE. The patient was symptom-free at 1 year after drug discontinuation[50]. The same result was found by Harada et al, after 11 months of treatment[51]. Angioni et al treated 6 women who opted for a medical therapy with dienogest for 1 year and their symptoms improved very quickly and the nodule decreased remarkably in size[52].

Hormonal treatment is very effective in supressing, but not treating BE, thus, if the decision is taken to opt for a medical treatment, long-term therapy should be planned. Patients should be counceled on the risk of progression under medical therapy and that regular follow-up is necessary.

Surgical treatment should be performed after a thorough diagnostic workup to rule out bladder cancer, to define the exact location of the endometriotic nodule and its relationship with the ureteral orifices. Two surgical techniques have been proposed: trans-urethral resection (TUR) or partial cystectomy.

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Even though evidence supporting the efficacy and safety of TUR in the management of BE is scarce, this procedure has been proposed as a method of treatment for this disease. Furthermore, this procedure does not apply as a method of treatment for BE from a pathogenic point of view, since the nodules evolves from the serosa of the bladder towards the mucosa and complete resection of the lesion is not achievable without the risk of perforating the bladder[53].

Partial cystectomy is a bladder-preserving surgical technique for the treatment of BE, that can be performed via laparotomy or laparoscopy (with or without robotic assistance). Concomitant cystoscopy can be performed to for better defining the margins of the endometriotic lesion and, depending on the surgeon experience, cystoscopic catheterizations of the ureters may be advisable. Ureteral stenting is recommended when the distance between the caudal border of the endometriotic lesion and the interureteric ridge is < 2 cm[54]. For women with bladder lesions that are less than 2 cm away from the interureteric ridge some authors recommend ureteroneocystostomy to prevent ureteral obstruction or stricture. Partial cystectomy is generally a safe and simple procedure, vesical sutures heal easily because of rich vascularization, and prolonged urine drainage usually prevents fistula formation[55].

Different surgeons have been proposing a combined TUR and laparoscopic partial cystectomy for BE. The aim of this double approach is to overcome the limitations of both surgical techniques. Stopiglia et al presented their experience with a cystoscopy-assisted laparoscopy for bladder endometriosis, in which a partial videolaparoscopic cystectomy was performed with cystoscopy-assisted vesical reconstruction throughout the entire surgical time. They stated that this technique provides adequate identification of the lesion limits, intra or extravesically and it also allows a safe reconstruction of the organ aiming for its maximum preservation. The median operative time was 138 minutes and patients were evaluated for a period of 12-78 months, with clinical evaluation and a control cystoscopy performed every six months. No relapse was observed during the follow-up period[56].

The main goal of partial cystectomy is to completely remove the endometriotic nodule, thus minimizing the risk of recurrence.

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There are some urogenital malformations that could be associated[57]. For eldery patients we should take care of postoperative dementia[58] and, on certain objective criteria, admission in intensive care units could be salutary[59].

CONCLUSIONS

Bladder endometriosis is frequent in the context of DIE, and usually coexists with other forms of endometriosis. BE has unspecific symptoms, thus a thorough differential diagnosis should be made. Women of reproductive age complaining of urinary symptoms, usually during the menstrual cycle, should always be investigated for the presence of BE. Transvaginal ultrasonography should be considered a first line technique to assess endometriotic nodules, followed by pelvic MRI, if the lesion is not well described on US. Once a diagnosis of BE has been established, clinical management can be conservative (hormone therapy) or surgical. When surgical treatment is chosen, TUR alone should be avoided.

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ENDOMETRIOSIS: WHAT ARE THE MECHANISMS RESPONSIBLE FOR INFERTILITY?

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ABSTRACT:
INTRODUCTION: ENDOMETRIOSIS IS ONE OF THE MOST IMPORTANT CAUSES OF INFERTILITY IN REPRODUCTIVE AGE PATIENTS, BUT WHAT ARE THE MECHANISMS INVOLVED? THIS ARTICLE DESCRIBES STEP BY STEP THE PSYSIOPATHOLOGY BACKGROUND THAT LEADS TO PREGNANCY ACHIEVING IMPAIRMENT.
METHODS: WE PERFORMED A REVIEW OF THE INTERNATIONAL SPECIALTY LITERATURE.
RESULTS: ENDOMETRIOSIS DETERMINES MODIFICATION OF NEOANGIOGENESIS AND CELLULAR MEDIATED RESPONSE BY HIGH CONCENTRATIONS OF INFLAMMATORY CYTOKINES (IL1, IL6, TNF ALPHA, PROSTAGLANDIN, PROTEASES) AND ANGIOGENIC FACTORS (IL8 AND VEGF). THESE CAUSE ALTERATION OF THE PELVIC ANATOMY AND, IN ADDITION TO LOWERING AVB3 INTEGRIN ADHESION MOLECULE AND THE L-SELECTIN LIGAND, CONTRIBUTES TO ASSOCIATED IMPLANTATION FAILURE. NEVERTHELESS, OVULATION IMPAIRMENT BY MECHANICAL DISTRUCTION OR ALTERATION OF GRANULLAR CELLS FUNCTION WITH ESTROGEN AND PROGESTERONE SECRETION DEFICIENCY AND LUTEAL PHASE MODIFICATIONS HAVE A FERTILITY NEGATIVE IMPACT. THE EMBRYOTOXICITY EFFECT COMES IN ADDITION TO THIS WITH AN INCREASED ABORTION RATE. ALTERATION OF SPERMATOZOA MOBILITY AND ENDOMETRIAL RECEPTIVITY, INCREASED UTERINE CONTRACTILITY AND FALLOPIAN TUBES OBSTRUCTION COMPLETE THE PICTURE OF INFERTILITY IN THE ENDOMETRIOSIS PATIENT.
CONCLUSIONS: ENDOMETRIOSIS AFFECTS FERTILITY THROUGH MULTIPLE MECHANISMS, AND IN ORDER TO TREAT AND GET PREGNANCY IN THESE PATIENTS, IT IS NECESSARY TO UNDERSTAND THEM.

KEY WORDS: ENDOMETRIOSIS, INFERTILITY, IMPLANTATION, EMBRIOTOXICITY, *INFLAMMATORY CYTOKINES.*

INTRODUCTION

Infertility poses more and more negative impact in the modern society, both by the psychical effect upon the couples, but by the social and financial society implications as well, as the rate of natural increase is negative in many developed countries, and in Romania it has been negative for many years now. This determines population aging, with fewer young working people that can support the elderly, with negative financial impact on the society. In addition to this, endometriosis patients suffer from frequent severe pain episodes that prevent them from working, affects their personal and professional relationships, nevertheless causing them fertility problems as well[9].

Recent studies demonstrate that almost 50% of infertile women have endometriosis and that 30-50% of endometriosis patients are infertile[10]. Endometriosis is a chronic disease present in 6-10% of fertile aged women[11], that alters fertility due to alteration of all reproductive system

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components[12]. By simply comparing the pregnancy rates (PR) we can observe the differences: PR per month in normal couples is 15-20 %[13], and in endometriosis patients, the monthly PR is 2-10 %[14].

OBJECTIVE

The exact physiopathological mechanism is not yet completely established[15], but there are many well known modifications related to endometriosis[16]. In order to be able to treat these patients we must take into account its physiopathology and in this paper we studied the international specialty literature and we will present the findings.

MATERIALS AND METHODS

This paper is a review of the international specialty literature regarding the physiopathological mechanisms of endometriosis that determine infertility, including cohort studies and reproductive societies guidelines (ESHRE, ASRM).

RESULTS

Inflammation and infertility

Endometriosis patients have been shown to have a larger amount of peritoneal fluid containing significant concentrations of inflammatory cytokines such as IL1, IL6, TNF alpha and angiogenic factors such as IL8 and VEGF[17], causing alteration of neoangiogenesis and cellular mediated response[18]. These may affect the fallopian tubes, the oocyte function, the embryo and even the sperm. First of all, we must understand why this aspect is clinically important. Peritoneal inflammation leads to the modification of the pelvic anatomy[19], it influences the tubal peristalsis[20]

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and creates adhesions that affect the permeability of the salpinges[21], can impair oocyte release and inhibit ovum pickup or disturb ovum transport[22].

Non-invasively, the peritoneal fluid can be visualized by ultrasound, CT scan or MRI examination, or its presence can be indirectly presumed in case of diagnosing other endometrial lesions. The first investigation to perform, after clinical examination[23], is ultrasound examination[24], but this detects basically only endometriomas or adenomyosis. Transvaginal ultrasound can detect hydrosalpinx as well, when tubal obstruction is present and fluid builds up in the salpinges. To determine modifications of normal anatomy in endometriosis patients that can lead to infertility[25], there are additional investigations that must be performed, in order to have a correct surgery indication if necessary[26]. Sonovaginography with ultrasound gel is a useful investigation in detecting endometriotic lesions in the posterior pelvic compartment[27] that usually are accompanied with tubal obstruction and ovulation impairement. Sonovaginography with ultrasound gel, in case of endometriotic lesions of the uterosacral ligaments has a 78.5 % sensitivity and a 96 % specificity. In addition to this, endometriosis in the vaginal and rectovaginal wall[28] can be diagnosed with a very good sensitivity (79%, respectively 94%) and a specificity of 99%, respectively 97%. In regards of the Douglas pouch, were very frequently endometriosic adherences are present, the sensitivity of the method is 81%, and it can diagnose rectosigmoid endometriosis with a 94% sensitivity. Lower sensitivity (64%) of sonovaginography with ultrasound gel has been observed in detecting urinary bladder lesions[29] and not to forget, special attention must be considered in case of possible urinary tract anomalies[30]. Another important investigation in endometriotic patients is Magnetic Resonance Imaging that can be improved by Computed

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Tomography Based Virtual Colonoscopy for better accuracy of assessment in colorectal endometriosis[31].

Endocrine dysfunction, follicle development and steroidogenesis

Secondly, ovulation is affected either by ovarian endometriomas that destroy the follicular reserve or by altering the function of granullar cells. The ovarian reserve markers are affected is the endometriosis patients, it has been shown that these patients have lower AMH and higher FSH levels, even in the absence of any prior ovarian surgical treatment[32]. Nevertheless, endometriosis patients have statistically significantly higher proportion of AMH<1, compared with infertile patients with no endometriosis. Besides this affecting of the ovarian reserve, the function of the ovary is affected as well, with consequent follicular fluid alteration (low levels of progesterone, estrogen and androgens and increased activin concentrations[33] that prevent ovocyte maturation and stimulate growth of the endometriotic implants). It has been shown that the follicular fluid of the patients with endometriosis inhibit the proliferation of granular cells and the secretion of progesterone[34]. The dysregulation of steroidogenesis is an effect of decreasing of the expression of P450 aromatase in patients with ovarian or pelvic endometriosis[35]. Normally, the granulosa cell-derived paracrine factors promote P450 aromatase activity, that is the key enzyme of estrogen and E2 (17β-estradiol) production. Without sufficient E2, the follicular development is affected and so is the production of competent oocytes, that are able to reach mature metaphase II (MII) in order to be fertilized.

Endometriosis affect the normal steroidogenesis by modifying cell cycle, increasing apoptosis and dysregulating the molecular pathways involved. The low levels of E2 are present in preovulatory stage and at the LH (luteinizing hormone) surge, at ovulation time. Nevertheless, progesterone secretion is lower in postovulatory stage, additional affecting oocyte maturation[36] and the luteinized unruptured follicle syndrome has been discussed to be present in endometriosis as well. In addition to altered development of sex-hormones, the expression of estrogen and

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progesterone receptors is modified as well, by the pro-inflammatory citokines IL-1, IL-6 and TNF-alfa.

Endometriosis affects the follicular oxidative stress status, with reactive oxygen species that determine meiotic abnormalities and chromosomic instability, suplimentary reducind oocyte quality[37]. This is an effect of spindle dysruption, arresting the oocyte in prophase I, the cytoplasmatic and nuclear maturation being impaired[38]. The oxidative stress is the reason studies are being made about supplementation with Vitamin C and E in endometriosis infertility, with controversial finding though[39].

Oocyte maturation and spindle are affected by the high concentration of pro-inflammatory cytokines (IL-8, IL-12), found both in the follicular fluid and in the peritoneal fluid of endometriosis patients[40]. The oocyte function has been shown to be altered as well. In endometriosis women it has been observed incresed cortical granular loss and zona pellucida hardening, that affects fertilization and the ability of the embryo to undergo hatching and implantation[41]. The peritoneal fluid in endometriosis patients has high levels of products that also affect MII oocyte maturation, besides pro-inflammatory cytokines there are very-long-chains ceramides (sphingolipids) mediating through mitochondrial superoxide[42].

Endometriosis patients may have other endocrine and ovulatory disorders. Besides affected folliculogenesis and unrupted follicle syndrome, there may exist luteal phase defects or impairement of LH surge (premature or multiple surges). Luteal phase modifications or the fact that more than one LH peak may appear during a menstrual cycle[43] can be caused by the reduction of follicular LH receptors, with late LH peak and luteal phase, which prevents follicular growth, estrogen secretion and progesterone secretion[44].

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Embryo implantation

Thirdly, endometriosis affects embryo implantation. This may happen on the one hand due to the high concentrations of inflammatory factors[45] (increased number of activated macrophages, increased production of TNF-α, prostaglandin, IL-1, IL-6, and proteases), which have a toxic effect on implantation[46] and a negative impact upon the expression of estrogen and progesterone receptors. Reduced expression of progesterone B receptors, essential in decidualization, have an important role in implantation failure[47]. Inflammation also leads to increased uterine contractility, that may be a significant infertility factor[48]. On the other hand, implantation failure may be caused by lowering the αvβ3 integrin adhesion molecule[49]. Nevertheless, endometriosis impairs implantation by lowering the quality of L-selectin ligand (LSL) and by affecting LSL expression[50]. L-selectin ligand is a marker of endometrial receptivity, an endometrium expressed glycoprotein that binds to the expressed trophoblast L-selectin. Its defects thereby affect embryo attachment to the endometrium and implantation failure. Last but not least, endometrial receptivity and embryo implantation may be affected by elevated levels of IgA and IgG antibodies, autoantibodies to endometrial antigens, and lymphocytes, that have been observed in the endometrium of endometriosis women[51]. The immune system in these patients is disrupted, with high concentrations of immune cell types, such as neutrophils, macrophages, dendritic cells, natural killer cells, T helper cells, B cells[52].

Mobility of spermatozoa

In addition to these, endometriosis affects the mobility of spermatozoa. Pro-inflammatory cytokines and the oxidative stress mentioned above affect the sperm in the uterus and when travelling through the fallopian tubes[53].

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Embryotoxicity

Another important aspect is that endometriosis alters embryonic development, it can have embryotoxicity and leads to an increased abortion rate. Studies showed that the high inflammation from the peritoneal fluid, that flows in the uterus through the fallopian tubes, slows down the growth rate of the embryo, increases the apoptosis rate and the DNA fragmentation[54]. An interesting aspect is that administration of dexamethasone has shown to reduce the embryotoxic effect[55].

Immunohistochemical profile, aggressiveness and recurrence risk

Last but not least, an important aspect in endometriosis patients is how to appreciate the aggressiveness and recurrence risk of endometriosis, in order to advise the young patient how long can she wait until having a pregnancy, if she does not desire one at the moment. The clinical implications are not directly proportional with the severity of the disease, as there can be patients with acceptable pain and no urinary[56] or gastrointestinal symptoms that actually have a severe and aggressive endometriosis that in a couple of years will reduce almost to zero her chances of conceiving. Studies have been made to assess the immunohistochemical profile of endometriosis implants. The expression of anti-apoptotic markers (Bcl-2) and cell proliferation marker (Ki-67) has been linked to the progression and dissemination capacity of endometriosis[57]. Ki-67 marker has been showed to be not correlated with the stage of endometriosis, but with the aggressiveness and dissemination potential. When cell dissemination activity is high, cells become independent and affect the neighbouring tissues. This leads to important adhering process between the surrounding tissues and the anatomy of the area is altered. Bcl-2 proteins have been observed to be involved in disrupting the process of apoptosis, leading to abnormal cell dissemination. The expression of estrogen and progesterone receptors[58] is related to response of endometriosis patients

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to treatment[59], the low expression explaining the persistence of symptoms[60], progression and recurrence rate under treatment[61].

CONCLUSION

Endometriosis affects fertility through multiple mechanisms and in order to effectively treat these patients it is essential to have them in mind. Inflammation may be considered the background, affecting the peritoneal fluid, the ovaries, the follicular fluid, the fallopian tubes and the endometrial cavity, with consequent damages. We may start with the well-known adherence syndrome, with tubal obstruction, peristalsis impairment and defects in oocyte release, pickup or transport. In addition to this, the disorder in follicular function is important, with negative impact upon oocyte quantity and quality, observed by the reduced number of MII oocytes retrieved and lower fertilization rates. The endocrine dysfunction plays another important role, with low follicular growth, affected estrogen and progesterone concentrations, impaired steroidogenesis, disrupted LH surge patterns and low LH concentration in follicular fluid[62]. Reduced fertilization rates by low oocyte quality and quantity, associated with low implantation rates due to altered endometrial receptivity and high uterine contractility complete the picture of infertility related to endometriosis. In addition to this, dysfunctional immune response associated with high pro-inflammatory cells, sperm motility impairment and embryotoxic effect complete the question: why endometriosis patients have fertility problems? This is why fertility preservation should be taken into account in the patients with severe endometriosis[63], best pregnancy rates giving embryo cryopreservation (but with the ethical and legal involvement between the parteners afterwards[64]), next oocytes (that also gives independency to the woman), and ovarian tissue prelevation (that may restore ovarian endocrine function in case of drastical surgery needed).

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HPV VACCINATION PROGRAMMES: HOW EFFICIENT AND SAFE?

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ABSTRACT:
THE PURPOSE OF THIS PAPER IS TO ASSESS THE DATA CURRENTLY AVAILABLE IN LITERATURE REGARDING THE EFFECTIVENESS AND HARMS OF HPV VACCINATION PROGRAMMES. THERE ARE NOW MORE THAN FIFTEEN COUNTRIES WHICH HAVE DATA REGARDING VACCINE EFFECTIVENESS AND SHOW FALLS IN TARGETED TYPES FOLLOWING VACCINATION. THERE ARE CURRENTLY THREE FDA-APPROVED MULTIVALENT PROHYLACTIC HUMAN PAPILLOMAVIRUS VACCINES AND ALTHOUGH THERE IS RIGUROUS PROOF OF THE NEED FOR IMPLEMENTING VACCINATION NATIONAL SCHEDULES, CONCERNS REGARDING THEIR SAFETY CONTINUE TO EMERGE.

KEY WORDS: HPV VACCINATION, EFFECTIVENESS, SAFETY

INTRODUCTION

It has been clearly proven that persistent infection with human papillomavirus oncogenic types is a necessary cause of cervical cancer and other types of neoplasia[6]. Case-control studies,

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case series and prevalence surveys have unequivocally shown that HPV DNA can be detected in adequate specimens of cervical cancer in 90-100% cases in comparison with a prevalence of 5-20% in cervical specimens from women identified as suitable epidemiological controls[7]. In consequence, in public health terms this finding is equally important as the discovery of the association between cigarette smoking and lung cancer or between chronic infections with hepatitis B virus or hepatitis C virus and the risk of liver cancer.

As a consequence of these findings, three FDA- approved multivalent prophylactic vaccines composed of virus-like particles (VLPs) have been developed.

HPV VACCINE DEVELOPMENT

The public health impact of cervical cancer was the aspect that practically obliged for a vaccine to be created. Most licensed vaccines against infectious agents are preventive, as it has proven easier to use the immune system to prevent a new infection or the disease rather than to treat the established infection or disease[8]. Drugs that are used in HPV infection act as immunomodulators, thus trying to help the host fight against the viral strain and prevent the occurrence of cervical dysplasia[9].

The latest figures from the International Agency for Research on Cancer (IARC) show that an estimated 570.000 new cases of cervical cancer were diagnosed worldwide in 2018, making it the fourth most common cancer in women globally. Moreover, every year more than 310.000 women die from this preventable disease. IARC′S projections show that unless preventive measures are implemented promptly, the burden of cervical cancer is expected to increase to almost

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460.000 deaths per year in 2040, an increase of nearly 50% over the estimated number of deaths in 2018[10].

Cervarix® and Gardasil® were the first vaccines for the prevention of cervical cancer. Cervarix®targets types 16 and 18 HPV, which are responsible for 70% of all cervical cancer[11], while Gardasil® adds also activity against typed 6 and 11 HPV, which cause 90% of anogenital warts[12]. The nonavalent HPV vaccine contains additionally types 31, 33, 45, 52 and 58 antigens. Interesting enough, as of May 2017 Gardasil 9 is the only HPV vaccine available for use in the United States (while Cervarix and Gardasil are still used in other countries).

The current CDC recommendations for Gardasil 9 vaccination in the USA are:

- All children aged 11 or 12 years should get two HPV vaccine shots 6 to 12 months apart. If the two shots are given less than 5 months apart, a third shot will be needed.
- HPV vaccine is recommended for young women through age 26 and young men through age 21.
- Adolescents who get their first dose at age 15 or older need three doses given over 6 months
- Persons who have completed a valid series with any HPV vaccine do not need any additional doses[13].

According to the health care system′s organisation the coverage rate and the programmes of vaccination differ: some of them are offered through schools (Australia, UK) while others are provided in private clinics or public primary care (United States)[14]. In Romania, despite the implementation of two HPV vaccination programmes, the uptake remained extremely low and the programmes were discontinued. A content analysis of mass media reports regarding HPV vaccination showed that while 23.6% of the materials were positive towards the vaccine, 28% were negative or extremely negative and 31.4% were neutral; side effects and insufficient testing were the main vaccine-related concerns[15].

HOW EFFICIENT?

The initial controlled clinical triales observed that VLPs were highly immunogenic, even without adjuvant, and well tolerated[16]. The bivalent vaccine was evaluated in a phase III trial of

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girls and women aged 15-25 years and its efficacy against CIN2+ was 94.9%[17]. The efficacy against 6-month and 12-month persistent HPV 16 or 18 cervical infections in the per-protocol cohort was 94.3% (96.1% CI=91.5-96.3) and 91.4% (96.1% CI=86.1-95.0), respectively[18]. The bivalent vaccine was also highly efficient (90%) for prevention of CIN 2/3 among participants who were DNA-positive to one vaccine HPV type[19].

The quadrivalent vaccine also proved high efficacy (> 98%) for prevention of HPV 6-, 11-, 16-, and 18-related CIN 2/3 or AIS, grade 2 or 3 vulvar intraepithelial neoplasia (VIN 2/3) and grade 2 or 3 vaginal intraepithelial neoplasia (VaIN 2/3)[20]. The qHPV vaccine also provided effective and durable protection against low-grade CIN, VIN and VaIN[21].

In a phase III efficacy trial among men, qHPV also showed great results in preventing genital warts among 4.055 males aged 16 through 26 years; there was no clear evidence of protection from disease caused by HPV typed for which boys and men were DNA-positive regardless of serostatus at baseline[22]. In a sub-study of the phase III efficacy trial the authors randomly assigned 602 healthy men who have sex with men (MSM), 16 to 26 years of age, to receive either qHPV or placebo. The primary efficacy objective was prevention of anal intraepithelial neoplasia (AIN) or anal cancer related to infection with HPV-6, 11, 16 or 18. The rate of grade 2 or 3 AIN was reduced by 54.2% in the intention-to-treat population and by 74.9% in the per-protocol efficacy population. No vaccine- related serious adverse events were reported[23].

The nonavalent HPV vaccine was studied in 6 clinical trials including more than 13.000 individuals aged 9-26 years and it was proved to be safe, with a 93% efficacy against anogenital lesions/ cancers caused by the included HPV types[24]. A randomised, double-blind, efficacy, immunogenicity, and safety study of the 9vHPV vaccine was undertaken at 105 study sites in 18 countries. The results showed that the 9vHPV vaccine prevents infection, cytological abnormalities, high-grade lesions and cervical procedures related to HPV 31, 33, 45, 52 and 58. In comparison to qHPV vaccine, both of them had a similar immunogenicity profile with respect to HPV 6, 11, 16 and 18. Vaccine efficacy was sustained for up to 6 years. Moreover, the 9vHPV

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vaccine could potentially provide broader coverage and prevent 90% of cervical cancer cases worldwide[25].

Regarding the long-term effectiveness of the vaccines, a recent study assessed the combined incidence of CIN 2,3, AIS and cervical cancer related to HPV 16 and 18. Statistical power was sufficient to conclude that qHPV vaccine effectiveness remains above 90% for at least 10 years, with a trend for continued protection through 12 years of follow-up[26].

Three studies reported immunogenicity data comparing 9vHPV to qHPV in females aged 16-26 years[27], in females aged 9-15 years[28] and in males aged 16-26 years. The 9vHPV vaccine prevented infection and disease related HPV-31, 33, 45, 52 and 58 in a susceptible population and generated an antibody response to HPV-6, 11, 16 and 18 that was noninferior to that generated by the qHPV vaccine.[33]Same results were obtained for the group of girls aged 9-15 years, with a similar safety profile between the two vaccines[29].

A recent Cochrane Database review evaluated the harms and protection of prophylactic HPV vaccines against cervical precancer and HPV 16/18 infection in adolescent girls and women[30]. The authors included 26 randomised controlled trials (73.428 participants) and the primary outcomes were: histologically- confirmed high-grade cervical intraepithelial neoplasia (CIN 2, CIN 3 and AIS) or worse associated with the HPV types included in the vaccine or any lesions irrespective of HPV type, invasive cervical cancer and safety/ occurrence of adverse effects. The latter include local adverse effects (redness, swelling, pain, itching at the injection site), mild and serious systemic effects, mortality and pregnancy outcomes observed during the trials, in particular occurrence of congenital anomalies.

The results showed that there is clear evidence that HPV vaccines protect against cervical precancer in adolescents and young women aged 15 to 26 and the effect is higher for lesions associated with HPV 16/18 than for lesions irrespective of HPV type.

In addition, a pivotal efficacy study was conducted to analyse the level of HPV types 6/11 antibodies in peripartum maternal blood and in cord blood of infants born to women who received 9vHPV or qHPV vaccine. The results indicate that antibodies induced by the 9vHPV vaccine cross the placenta which could potentially be beneficial against HPV 6/11 infection and related disease such as recurrent respiratory papillomatosis[31]. In addition, vaccination has the potential to

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massively reduce the burden that cervical dysplasia has during pregnancy regarding both diagnostic means and treatment[32].

Most post-licensure studies report highest effectiveness with a three-dose regimen; some of them found no statistically significant difference between two and three doses and almost half found some effectiveness with one dose[33]. This is especially important for many sub-Saharan African countries where the HIV burden is high but where the high cost of HPV vaccine programmes has to date proved a deterrent to introduction. A study revealed that at 90% coverage of females age 9 years with 80% lifelong vaccine efficacy, single dose HPV vaccination was projected to reduce cervical cancer incidence by 74% and mortality by 71% in the general female population at 70 years after the start of the vaccination program[34].

HOW SAFE?

In a statement issued in February the current year, IARC fully endorsed the position of the World Health Organization (WHO) on HPV vaccination and confirmed that the vaccine is safe, efficacious and critical in the fight against cervical cancer. Unfounded rumours about HPV vaccines continue to unnecessarily delay or impede the scaling up of vaccination, which is so urgently needed to prevent cervical cancer[35].

A safety review by the FDA and Centers for Disease Control and Prevention (CDC) considered adverse side effects related to Gardasil immunization. The rates of adverse side effects were consistent with what was seen in safety studies carried out before the vaccine was approved and were similar to those seen with other vaccines.[20] Still, a higher proportion of syncope and venous embolic events were observed with Gardasil than are usually seen with other vaccines. On the other hand, a large cohort study performed in Denmark and Sweden found no evidence to support the association between exposure to qHPV vaccine and autoimmune, neurological, and venous thrombembolic adverse events[36].

Regarding the 9-valent HPV vaccine, efficacy, immunogenicity and safety outcomes were assessed in Latin American participants enroled in 2 international studies. The results showed that the most common adverse effects of vaccination were injection- site related, mostly of mild and moderate intensity[37]. In studies assessing concomitant vaccines administration, injection-site adverse effects of swelling after 9vHPV and Tdap-IPV were more frequent in concomitant arms as compared with non-concomitant ones (after 9vHPV 14.4% vs. 9.4%, after Tdap-IPV was 21.7%

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vs. 31.3%), the risk difference between the groups being statistically significant. This strategy would minimize the number of visits required to deliver each vaccine individually[38].

CONCLUSIONS

We now know that the combination of HPV vaccination and cervical screening provide the best protection against cervical cancer. Various markers have been developed in order to detect cervical lesion which benefit of prompt treatment[39]. Also, vaccination is the approved public health intervention for reducing the risk of developing HPV-associated cancers at sites other than the cervix[40]. Widespread vaccination has the potential to reduce cervical cancer incidence around the world by as much as 90%[41].

The 9-valent HPV vaccine appears to be non-inferior to other HPV vaccines in terms of safety, short-term immunogenicity and efficacy against common HPV types. However, the impact on reducing cervical cancer burden depends greatly on vaccine uptake and coverage, availability and affordability. It is therefore extremely important that international and national health authorities engage in planning and implementing immunization programmes to increase the level of knowledge and awareness of HPV prevention among providers, parents and people receiving the vaccine[42].

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THE IMPORTANCE OF ANAMNESIS IN PEDIATRICS

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ABSTRACT:
ANAMNESIS, OR PHYSICIAN-PATIENT DISCUSSION, HAS REMAINED AN ESSENTIAL AND PRELIMINARY STEP FOR ESTABLISHING A CORRECT DIAGNOSIS SINCE HIPPOCRATES. THE RISK OF POSSIBLE ERRORS SHOULD BE MINIMIZED BY PROVIDING SOME PARAMETERS: ACCURACY, GOING THROUGH "CHRONOLOGICAL" INTERVIEWING PHASES, EMPATHY TOWARDS THE PATIENT AND CARETAKERS, PROVIDING A CALM ENVIRONMENT WITHOUT DISTURBANCE, WITHOUT DISTURBANCE, ADAPTING MEDICAL LANGUAGE AND USING DISCERNING MEDICAL TERMS ACCORDING TO THE PARTICULARITIES INTELLECTUALS OF THE INTERLOCUTOR.
NOT COMMITTING TO ANY STAGE, ACCURATE AND USEFUL INFORMATION COLLECTION HELPS TO QUICKLY APPLY PROPER TREATMENT, SHORTER HOSPITALIZATION, AND EASIER RECOVERY OF THE PATIENT, THE MORE IT IS ABOUT A CHILD.

KEYWORDS; ANAMNESIS, CHILD.

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Anamnesis is all the information about the patient or his / her entourage related to the state of health or disease obtained by the physician, following the dialogue. Pediatrics, as a distinct specialty, having a child, an immature body with no discernment until the age of 18, attaches great importance to the collection of in-depth information about the patient, information obtained almost exclusively from other family members, often marked of great subjectivity.

The doctor-patient binomial relationship, common to all other medical activities and specialties, is no longer respected in Pediatrics.

Interactions are multiple, subjective reports, deliberate neglect or not communicating important information, and implicitly, precious information may be lost to elucidate the diagnosis. The work and medical thinking are aggravated, the work of the doctor takes over detective notes, and the time spent in the anamnesis may increase significantly, possibly with returns in the coming days or during hospitalization[9].

The high degree of subjectivism, the stress caused by the child's illness, alters the attitude of the parents (the relatives) in relation to the treating physician.

Reticence, communication of precious information (other family affections, poisoning, child abuse, etc.) can direct medical thinking in a dangerous, non-conforming direction, delay or stop the correct diagnosis and the uninterrupted establishment of appropriate treatment[10].

The pediatrician, in addition to doctors in other specialties, must show empathy, translate into the excitement of the parent, pay attention to it, repeat fragments of phrases in order to gain confidence and make sure he understands description made. The language will be appropriate to the degree of training, intelligence and emotion of the parent-interlocutor. The doctor must be a fine psychologist capable of "molding" the intellectual and emotional abilities of the dialogue partner[11].

The doctor should be calm, quiet, undisturbed, in a pleasant and relaxed setting. The tone of the conversation must be kept “stable", the dialogue must flow fluidly, without syncope, in order not to create suspicion. The doctor should be able to avoid time and energy-consuming "traps", focusing on the "steps" needed to complete the medical interview.

The escalation of subjectivism and mistrust can be done by apparently giving up the questions considered "inconvenient" by the family and returning to the subject, in another form and in another register. For example, many families show maximum reluctance in chronic clumsiness, and pulmonary tuberculosis is, almost without exception, hidden and not communicated for the purpose of pediatric anamnesis, not necessarily of ill-will, notably unconscious of implications or sensation culpability and guilt or a sense of negation of assuming a chronic illness and a lasting treatment. The question, in this case, will not be "Do you have

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tuberculosis in the family?" But "Are there adult members of the family, friends, frequent visitors with persistent or chronic coughing in contact with the child?"[12].

The anamnestic interview, in essence, is standardized and structured according to some "steps", valid in all specialties. Reasons for admission refer to the symptoms (objectives) that mobilized the family to bring the child to the doctor. The age of the child (newborn, infant, preschool, school child, etc.) is a major factor of subjectivism in perceiving symptoms by the family. Parental anxiety is inversely proportional to the age of the child and, often false, the pathology agglomerates the guard rooms in the pediatric wards. The normal manifestations of age (eg regurgitation of the newborn or small baby collics) are hyperbolically perceived by the family / mothers without experience, education, previous documentation or uninformed in the maternity[13].

The doctor is obliged and responsible for thoroughly consulting each child for family reassurance and counseling of parenting tips and guidance. This seems to be appropriate for primary care or in the form of counseling hours, organized antepartum or maternity. Newborn physiology, the role of natural nutrition, incidents and physiological variations must be explained but also easy to understand for mothers. This can limit the agglomeration in waiting rooms of pediatric wards, the contact of healthy children with the sick, and last but not least the overwork and the appearance of the "burnout" syndrome that places pediatricians in the top of professions affected by major stress.

The admissions reasons will be briefly and succinctly stated in the field of the observation sheet dedicated to them.

The heredocolateral antecedents refer to questions about existing affections within the family, to third degree relatives. Questions will address hereditary genetic disorders (thalassemia, haemophilia, muscular dystrophy, dysmetabolic diseases, familial disorders, etc.).

Personal physiological history refers to the "constellation" of factors that are related to the physiological condition of the child.

To ease the interview and avoid syncope or return to dialogue, it will be structured on the principle of chronology. Preconception, conception, pregnancy, birth, newborn, diet, diversification, childhood, puberty, menarche (girls), vaccination schedule, etc. will be covered.

It concerns the condition of preconception for both partners of the couple, the gynecological problems of the mother (urogenital infections) that can affect the local balance and cause changes in the structure of the local flora and even the risk of premature birth (the association of M. hominis, Gardnerella vaginalis, Atopobium vaginae).

The pregnancy modality (natural or fertilization procedures), the pathology of the pregnant woman, knowing the increased risk of fetal malformations during the organogenesis period (the first 2 weeks), the occurrence of gestational diabetes, eclampsia, preeclampsia, the risk of abortion, interest in environmental factors, stress, pregnancy activity. Birth time will be quantified in a number of ways: pregnancy duration, birth rate (the higher the risk associated with low birth weight or early anemia), birth weight, Apgar score (a 5-point cumulative which shows the condition of the skin, muscular reactivity, breathing, cardiac activity, is assessed at birth and at 5 minutes A score below 8 suggests a neonatal adaptation deficit and requires neuropsychiatric consultation

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from the age of 3 months later. If necessary, an Apgar score under 5 means multiple impairment with neonatal adaptive deficiency. It is important the type of diet (natural or artificial), the moment of diversification, the presence of physiologic jaundice, the prophylaxis of rickets (duration, doses), the vaccination schedule.

Personal pathological antecedents should have the same chronological path: prenatal, perinatal, and postnatal.

Prenatal is potentially interested in the "uterus" solved pathology (reducing the total number of fetuses in multiple pregnancies or suppression of an aberrant embryonic vessel, in twin transfusion ratio / transfused twin, or correction of severe cardiac malformations, etc.). Perinatally interested in reanimation maneuvers, applied treatments, prolonged hospitalization, oxygen therapy, incubator placement, prolonged phototherapy, the risk of retinopathy of prematurity. Postnatal concerns the totality of the pathology the child suffered until the time of admission. Emphasis is placed on repeated hospitalized diseases, infectious-contagious diseases, congenital malformations, chronic conditions (eg bronchial asthma), surgical interventions (adenoidectomy, appendectomy, corrected congenital heart defects, etc.)

The living and working conditions refer to the number of persons / room, type of dwelling, heating system (wood stoves, gas, electricity), noxes (smoke, dampness, moisture, dust), hygiene, bath placement inside the dwelling or type of latrine.

Family behaviors will be specified (smokers, alcohol users, drugs), food taboos (vegetarian or raw vegans) with multiple deficiencies in children (anemia, dystrophy, protein-calorie malnutrition). Treatments administered until the time of admission concern the type of substance administered, doses, duration, administration of therapeutic regimens, chronic disease therapy.

The history of the disease refers to the chronological description of events, signs and symptoms from the time of the disease until the time of admission. Also here is the way in which the admission is made (urgent, through the clinic, from GP with a referral ticket).

The time spent on the anamnesis is 15-20 minutes, the climate must be neutral, the calm, relaxing tone, the empathic and understanding attitude and the discussion adopted at the intellectual level of the interlocutors. The chronological interview should be conducted in order not to miss important steps and to guide medical thinking to establish a correct and rigorous diagnosis and to establish uninterrupted treatment. Any anamnesis "trap" may adversely affect the physician and the patient at the same time in order to establish an erroneous diagnosis[14].

The following steps: physical examination, paraclinical investigations, appropriate treatment, depend on the accuracy of the first stage - anamnesis.

CONCLUSIONS

The importance given to the anamnesis is undervalued in all specialties, especially in pediatrics. It should be reconsidered and repositioned correctly, the pediatric patient being "sensitive" in terms of age, compliance, discernment, anamnestic and immunological particularities.

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The homogeneity and coherence of information shapes the clinician's thinking, reduces the risk of diagnosis and treatment errors, implicitly shortens the duration of hospitalization and the risk of nosocomial infections, complications of hospitalization in general.

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NEW PERSPECTIVES IN THE PATHOGENESIS OF ENDOMETRIOSIS – POTENTIAL TREATMENT STRATEGIES TARGETING THE SMART ADULT STEM CELLS

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ABSTRACT:
POPULATIONS OF CLONOGENIC EPITHELIAL AND STROMAL CELLS WERE FIRST REPORTED IN 2004 AND SINCE THEN, DIFFERENT POPULATIONS OF STEM/PROGENITOR CELLS HAVE BEEN IDENTIFIED. THIS REVIEW IS AIMED AT BRINGING NEW PERSPECITVES FOR ENDOMETRIOSIS DEVELOPMENT, TO PROVIDE A BETTER UNDERSTANDING OF PATHOGENIC THEORIES, ALONG WITH POTENTIAL TREATMENT TARGETS. STUDIES SHOW THAT ENDOMETRIUM IS RICH IN STEM CELL POPULATIONS, SUCH AS ENDOMETRIAL MESENCHYMAL STEM CELLS (EMSC), ENDOMETRIAL EPITHELIAL PROGENITOR CELLS (EEP) AND SIDE POPULATIONS (SP). THEIR ROLE IS IMPORTANT IN PHYSIOLOGY, REGENERATION AND REPAIR, BUT ALSO IN THE GENERATION OF ENDOMETRIOSIS. ENDOMETRIOSIS MAY ARISE FROM DISLOCATED OR ABERRANT STEM CELLS, FROM THE ENDOMETRIUM OR EXOGENOUS SOURCES, SUCH AS BONE-MARROW. MORE FINDINGS SUPPORT THE BIDIRECTIONAL MOVEMENT OF CELLS BETWEEN EUTOPIC AND ECTOPIC ENDOMETRIAL TISSUE THROUGH SIGNALING PATHWAYS. EMSC RESIDE IN A

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PERIVASCULAR NICHE AND ARE LIKELY TO MEDIATE ANGIOGENESIS AND STROMAL REGENERATION. TREATMENT OPTIONS FOCUSE ON THE INHIBITION OF THE ECTOPIC EMSC MIGRATION, PROLIFERATION AND ANGIOGENESIS. THE MAIN PURPOSE FOR THE FUTURE CLINICAL PRACTICE IS TO ESTABLISH ACCURATELY THE DIAGNOSIS OF ENDOMETRIOSIS AND POTENTIAL THERAPEUTIC TARGETS. THE ADVANCEMENTS IN OUR KNOWLEDGE ABOUT DIFFERENT TYPES OF ENDOMETRIAL STEM/PROGENITOR CELLS PROVIDE THE BASIS FOR A BETTER UNDERSTANDING OF ENDOMETRIOSIS PATHOGENESIS.

KEYWORDS: STEM CELLS, ENDOMETRIOSIS, PATHOGENESIS, MESENCHYMAL STEM CELLS

INTRODUCTION

Endometriosisis a pathological condition defined by the presence of endometrial glands and stroma in extrauterine locations[9]. The most common location for the ectopic endometrial tissue is the pelvic peritoneum, but there is not impossible to also find extrapelvic involvement, because endometriosis has been described in almost every area of the female body[10]. The prevalence varies between 6%-12% in asymptomatic women and 35%-50% in women with pelvic pain or infertility[11]. The variety of molecular differences between endometriosic lesions and the eutopic endometrium creates difficulties in developing new targets and therapeutic regimens[12]. In this regard, multiple researches have been made regarding the presence of progenitor stem cells in the endometrium and the correlation of this phenomenon with endometrial regeneration and the menstrual cycle[13]. The endometrium has a huge proliferation potential, quantified by the tissue growth of more than 14 days, being able to cycle through cellular proliferation, differentiation, shedding and regeneration of the functional layer by approximately 300-400 times during the

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[13] Abreu, Jaqueline Pedroso; Rebelatto, Carmen Lucia Kuniyoshi, Savari, CA; Capriglione, LGA; Miyague, Lye; Noronha, L; Amaral, VF; *The effect of mesenchymal stem cells on fertility in experimental retrocervical endometriosis.* Rev Bras Ginecol Obstet, 2017, 39(5): 217-223. doi: 10.1055/s-0037-1601484.

reproductive life of a woman[14]. The first evidence of endometrial stem cell survival was provided by Padykula et al., who demonstrated that primates possess a germ cell compartment located in the deep basal layer where intense mitotic activity of the epithelial cells persists after ovulation and menstruation[15]. Chan et al. (2004) was the first to demonstrate the clonogenicity in the human endometrium, defined as the ability of a single endometrial cell to produce a colony, harvesting small populations of endometrial cells (0,22%) and stromal cells (1,25%) which possessed clonogenic activity[16]. Since then, more and more stem cells/progenitor cell populations have been identified and consistent with this aspect, mesenchymal cells derived from the hematogenous bone marrow that could participate in the endometrial regenerative process. and progression of endometriosis, have been studied[17]. The initial theories of endometriosis pathogenesis are the retrograde menstrual theory, neonatal uterine bleeding, coelomic metaplasia, Mullerian ducts and immune and could support new hypothesis of endometrial stem cells. Moreover, the origin of endometrial stem cells is continuously debated, being believed that they originate either from fetal stem cells or by periodically sowing with the source of the hematogenous bone marrow in response to injuries[18]. This review focuses on the characteristics of endometrial stem/progenitor cells and examines their role in the pathogenesis of endometriosis.

STEM CELLS

Adult stem cells are non-differentiated cells that have the ability to generate multiple differentiated cell types identical to those from the tissue they reside while maintaining their ability to renew[19]. Unlike embryonic stem cells, which have the ability to generate cells from all the three layers (endoderm, mezoderm, exoderm), adult stem cells are either unipotent (capable of generating a single cell type) or multipotent (capable of generating several cell types in a particular tissue)[20]. Adult stem cells resides in niches, anatomical structures that constitute a suitable microclimate that favors signaling between the side cells and the stem cell population and the maintenance of the local homeostasis[21]. The involvement of the stem cells in the pathogenesis of endometriosis has been studied especially *in vitro* by harvesting tissue samples collected from women with histopathologically confirmed endometriosis by surgical approach or by experimental models with endometriosis. Thus, the clonogenic, renewal, multipotential properties ande valuation of the gene profiles expressed by them, together with the markers and the transcription factors, were tested. Originally, the genes that determined the stem cell character were identified in embryonic stem cells, while adult stem cells not expressing the same „stem" genes. By

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[18] Dhesi, AS; Morelli, SS; *Endometriosis: a role for stem cells.* Women`s Health, 2015, 11(1): 35-49.
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comparison between endometriosic lesions and endometrial tissues, it was found that the UTF1, TCL1 and ZFP42 genes express more intense activity in endometriotic implants ($p<0,005$ for UTF1) and GDF3 expresses more intense activity in the endometrium[22]. By immunohistochemical analysis, SALL4 showed increased expression in the stromal cells from the periglandular areas of the endometriosic lesions, although the expression of the corresponding mRNA from both types of samples suggested the post-transcriptional involvement of this factor in the pathogenesis of endometriosis[23]. Regarding Oct-4, it was highlighted both in the endometrial stromal cells and in ectopic lesions, even stimlating cell migration[24]. Pacchiarotti et al. demonstrated that Oct-4 has significantly greater expression in endometriosic tissues (32.3%), unlike the endometriotic tissue of women without endometriosic disease, supporting the ability of endometriosic cells to renew, maintain, and survive[25]. Another transcription factor associated with the Notch1 transmembrane receptor is the RNA-binding protein, Musashi-1, a factor associated with the maintenance and asymmetric division of neural and epithelial progenitor cells. Its expression is significantly increased both during the proliferative phase of the endometrium and in the endometriotic lesions[26]. Other transcription factors have been shown to have increased expression in endometrioti clesions, such as: SOX2, c-kit, NANNOG and SALL4[27].

HUMAN STEM/PROGENITOR ENDOMETRIAL CELLS

a. ENDOMETRIAL MESENCHYMAL STEM CELLS (EMSC)

They are non-haematopoietic stem cells that can be found in the haematogenous bone marrow and many other tissues and can differentiate into cells derived from mesodermal lines (eg. cartilage, bone, muscle, adipose tissue). They can be found in both basal and functional endometrium layers. Their isolation can be accomplished using dual specific markers, either CD146 + and PDGFRβ (CD140b) cells, or Sushi Domain containing 2-SUSD2 positive cells (formerly W5C5), identifying the mesenchymal cells as pericytes or perivascular cells from the basal and functional endometrium *layers*[28]. eMSC have also been found to some extent in the

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menstrual blood, justifying a wider membership in the endometrial layers than limitation to the endometrial basal layer. In contrast, the analyzed gene profiles were similar between the premenopausal and postmenopausal basal cell layers, suggesting that the stem/progenitor cells would rather originate from the endometrial basal layer, playing an important role in the cyclic regeneration of the epithelium and the endometrial stroma that occurs after each menstruation[29]. eMSC, which are located in the microclimate of ectopic lesions determine a selection process that leads to the survival of the clones with pronounced migratory, proliferative and angiogenic activities. It is considered that extrauterine microclimate found in ectopic lesions could modulate epigenetic eMSC by changing their characteristics[30]. Moreover, the ectopic eMSC from endometriomas express higher levels of IL-1β and COX-2 in comparison with eMSCs from eutopic endometrium[31]. The properties of eMSC from endometriotic lesions appear to be enhanced against eutopic eMSC, with higher proliferative capacity with significantly longer doubling time and significantly higher proliferative cumulative proliferation, with much enhanced invasiveness and migratory ability, as well as stimulation of angiogenesis. It is considered that extrauterine microclimate found in ectopic lesions could modulate epigenetic eMSCs by changing their characteristics[32]. The properties of CSMs from endometriotic lesions appear to be potentiated against eutopic CSMs, with higher proliferative capacity with significantly longer doubling time and significantly higher proliferative cumulative proliferation, with much enhanced invasive and migratory ability as well as stimulation of angiogenesis[33]. The CSM-e may be identified by the following markers: CD146, PDGFRβ, CD29, CD44, CD73, CD90, CD31, CD34, CD45[34]. Bone marrow mesenchymal stem cells (CSM-mo) are considered to have the ability in vivo to form a single cell, a bone heterotopic tissue or organs derived from the bone marrow (auditory bones: malleus, incus, stapes). The analogy definition for CSM-e would be the generation of the vascularized stroma with the ability to differentiate into a deciduous stroma when transplanted. This has not yet been demonstrated, but when CSF-2 SUSD-2 + clonal derived cells and were incorporated into subcapsular renal parenchymal vessels in immunocompromised mice, they produced endometrial stroma[35]. Schwaband Gargett argued that the eMSC CD149 + PDGFRβ +

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subpopulation represents 1.5% of stromal endometrial cells[36]. A single specific marker for endometrial tissue was identified for isolation of CSM-e clonogenic cells, namely SUSD2 + 2, with perivascular location, which are mainly characterized as Side Population cells (SP cells), which will be disscused in detail.

MESENCHYMAL-EPITHELIAL TRANSITION AND STEM CELL MIGRATION BETWEEN ENDOMETRIOTIC AND ENDOMETRIAL LESIONS

There is clear evidence from many studies that have shown that stem cells are capable of massive trafficking from endometrial lesions to the endometrium. These cells produce factors that are able to alter the uterine receptivity. Studies were conducted on models of mice transplanted with green fluorescent protein (PVF) tagged tissue. The protein chain reaction (PCR) has denied the presence of PVF in the control group and confirmed the presence of PVF in the group of endometriotic mice. Immunofluorescence was used to locate cells in the endometrium[37]. Cells that expressed PVF in the tissues of the experimental group were mainly located in the basal endometrium layer and were never identified in the luminal epithelial layer or in the glandular cell consistency. Uterine cells that originated in ectopic lesions expressed a distinct genetic profile compared to the eutopic endometrium, such as Snail1, Snail3, Goosecoid and downregulation of the Zeb2 gene. This is the exact gene profile that is associated with epithelial-to-mesenchymal transition[38]. The proccess implies the loss of the epithelial cells polarity and the conversion to the mesenchymal phenotype. The migration is realised as mesenchymal stem cells. The cells that engraft into the uterine stroma display the activation of Wnt signaling pathway, which is an absolute indicator for epithelial identity, even though these cells were not located in the epithelium[39]. Wnt signalling is essential for the development of many tissues including the endometrium and is required for the maintenance of stem populations in adult organs. Wnt7a is envolved in postmenstrual endometrial regeneration, while Wnt4 is observed in normal development of the endometrial glands and also in the stromal decidualization when embryo implanting[40]. These endometriosis-derived cells were found in the stroma despite secreting signaling molecules of epithelial cells. All this abnonmalities lead to a disruption with decreased endometrial receptivity with further consequences, in endometriosis patients. The ectopic Wnt signaling distorts the optimal endometrial developing[41].

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ENDOMETRIOSIS-DERIVED CELLS CIRCULATION WITH EMSC MARKERS AND THEIR IMPLANTATION

There are studies that demonstrated cells with endometriosic lesions provenience express MSC markers and enter the circulation with subsequent ability to differentiate into type II alveolar cells in vivo. Li et al. identified cells from circulation that expressed endometrial stem cell markers CD140b and CD146 in subjects with donor tissue that comprised the endometriosis. This suggest that populations of MSC scan spread over large distances [26]. Similarly, Becker et al. showed that endothelial progenitor cells (eEPCs) were found elevated in the circulating blood after disease induction[42]. The presence of both types of stem/progenitor cells are highly suggestive for active disease and may serve as biomarkers for detecting the pathology in cause[43].

b. ENDOMETRIAL EPITHELIAL PROGENITOR CELLS (EEPCS)

These clonogenic epithelial cells reside in the basalis endometrium layer. The specific marker for the endometrial basalis epithelium is the stage-specific embryonic antigen-1 (SSEA-1 or CD15). When examining in vitro, the cultured cells expressed increased telomerase activity and longer telomers than SSEA-1- cells, which represents a characteristic of the stem/progenitor cells. There were also found larger spheroids and fewer ER-α and progesterone receptors, which might suggest that *in vivo*, SSEA-1+ cells may be found close to the junction between the functionalis and basalis[44].

Recently, N-cadherin was identified as a specific marker for humane EPCs using gene profiling to differentiate between premenopausal and postmenopausal endometrial epithelial cells. There were found 11 upregulated genes in postmenopausal endometrial epithelium, including CDH2 and CDH3, which encode for the N-cadherin and P-cadherin respectively. The expression of the N-cadherin gene was found to be associated with greater self-renewal and more population doublings than N-cadherin- endometrial epithelial cells[45]. Changing the cell phenotypes within endometriotic lesions may be responsible for the invasiveness of endometriotic cells. A well-differentiated CK+E-Cadherin-N-Cadherin+ population is found in the epithelial population, similar to carcinoma micrometastasis[46]. In line with the early carcinoma, endometriosic lesions regress during estrogen depletion therapy but reoccur when cessationing the therapy, suggesting that stem/progenitor cells in the lesion remain dormant and then reactivate under estogen exposure[47].

The relationship between vasculogenesis in endometriosis and endothelial progenitor cells is not well established. There are some studies though that showed the recruitment of eEPCs are

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highly important in the process of creation of blood vessels. The origins of eEPCs are from hematopoietic stem cells, myeloid cells and multipotent bone marrow progenitors. The first step represents the mobilization of EPCs from the bone marrow through VEGF (vascular endothelial growth factor), FGF-2 (fibroblast growth factor) and estradiol[48]. VEGF expression by endometrial cells varies with the menstrual cycle, with higher levels in the proliferative phase than in the secretory phase, in the peritoneum[49]. It is well-known that endometriosis is associated with high-levels of VEGF and FGF-2 and that it is an estrogen-dependent condition. In return, mobilized EPCs secrete angiogenic factors, such as VEGF and IL-8 which promote recruitment of further EPCs and involve them into forming *de novo* microvessels[50].

c. SIDE POPULATION CELLS (SPC)

SPC is represented by a mixed population of epithelial, stromal and endothelial cells, with a predominance of endothelial cells. It represents a specific phenotype that results from high expression of plasma membrane transporters (e.g. ABCG2), which transport organic molecules out of cells, including DNA-binding dye Hoechst 33342, which allows them to be identified through flowcytometry[51]. The endometrial SPC were described using specific markers as it follows: 27% EpCAM+ epithelial, 51% endothelial, 15% CD10+ stromal and 25% CD146+ endothelial/eMSC[52]. The presence of thee MSCs and endothelial phenotypes (CD146+PDGFRβ+) suggest that the SPC play an important role in vasculogenesis during endometrial regeneration[53]. The studies expressed some important differences regarding the type of isolating the cells, showing different stem cell functions *in vivo* and *in vitro*. *In vitro*, epithelial and stromal SPC express typical MSC characteristics, evolving to decidualization, with an enhanced capacity to undergo osteogenic and adipogenic differentiation, similarly to MSCs and to clonogenic endometrial stromal cells[54]. While *in vivo*, when transplanted under the kidney capsule or skin of immunocompromised mice, the SP

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cells from human produce endothelial tissue (46%), epithelial tissue (00.02-8%) and stromal structures (13%)[55].

HOMING AND MOBILIZATION OF STEM CELLS

The traffic of the stem cells is mediated and regulated by low molecular weight cytokines that attract different cells through chemotactic mechanisms. The most investigated pathway in this regard is CXCR4-CXCL12, which was first investigated from the pathogenesis of cancer, when promoting invasion and cellular migration, as well as angiogenesis in the tissue samples that express CXCL12 and communicate in a paracrine manner. CXCL12 is mostlye xpressed in sites where there is injury and inflammation and can be found in the stroma and the epithelium of the endometrium[56]. CXCR4 is the receptor of CXCL12. The difference between serum CXCL12 in women with and without endometriosis is less than observed in the murine models; this thing may be due to the more importance of the microenvironment of the endometriosis lesions, that abund from CXCL12[57]. The CXCR4/CXCL12 axis in involved in stem cell metastasis as well and represent a multistep process as CXCR4 positive cells first leave their stem cell niche, then are transported to the tissues which express high concentrations of CXCL12 via peripheral blood or the lymphatic system[58]. The levels of CXCL12 mRNA are in concordance with the endometriosis derived stem cells in animals with and without endometriosis. Serum CXCL12 is elevated in active disease, independent from estrogen regulation and endometriosis can develop from CXCL12 which mediate the ectopic endometriosis-derived stem cell mobilization. There is strong evidence that CXCR4-CXCL12 may contribute to the endometriosis and angiogenesis in ectopic lesions and that circulating endometriosis stem cells would propagate the disease through lymphatic or vascular dissemination, which may also serve as markers for active lesion establishment[59]. Also, as a therapy tool, the CXCR4-CXCL12 axis, as a primary pathway involved in the recruitment of the bone marrow-derived cells, may represent a novel approach to control the spread of this common disease, inhibiting the abnormal pathways of cell migration between the uterus and the ectopic foci[60].

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POTENTIAL ANGIOGENIC MANAGEMENT ON ENDOMETRIOSIS AND STEM CELL TARGETED THERAPY

The antiangiogenic drugs have been tested in many diseases that include the angiogenesis mechanism and this could also be incriminated in endometriosis. Sorafenibis a protein tyrosine kinase inhibitor that target Raf kinases and growth factors that include PDGFRβ, c-Kit and eMSC markers. In endometriosis, Sorafenib induces regression in lesion volume in heterologous mouse model of endometriosis and autologus rat model[61]. The results showed that however the target therapy, the lesions still persisted in the endometriosis foci and the pain was not alleviated[62]. On the other hand, studies demonstrated that *in vitro*, the angiogenetic properties, as well as migration and proliferation of ectopic eMSCs were reduced through HIF-1αand VEGF inhibition[63]. Another approach was described by inhibiting the delivery of the miRNA when targeting VEGF. This method was used *in vitro* showing the decrease in motility and proliferation when expressing miR-199a-5p downregulated VEGFa of eMSCs, with promising results in both heterologous and homologous mouse models of endometriosis, with lesion suppresion[64]. The mechanism of action of the Sorafenib is by inhibiting the phosphorylation of ezrin in ectopic MSCs, with consequent limitation of the ectopic MSCs migration, proliferation and VEGF release[65].

Another promising tool for endometriosis are SERMs (selective estrogen receptor modulator), which have an agonistic/antagonistic activity over ER (estrogen receptors), depending on the type of SERMs. Bazedoxifene (BZA) is a new type of SERM therapy which does not stimulate the endometrium in postmenopausal women and is best able to counteract the effect of estrogens on the proliferation. The mechanism of action is related to the suppresion of the ER expression of estrogen-mediated cell proliferation. There are studies that show BZA treatment

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leads to decreased stem cell engraftment and recruitement in endometriosis, as well as decreasing the lesion size[66].

CONCLUSION

Several different populations of the endometrial stem/progenitor cells, including CD140+, CD146+, or SUSD2 eMSCs, N-cadherin+ eEPs and SP cells may be used to establish the diagnosis of endometriosis or as therapeutiuc targets.There are hypothesis that support the idea that early onset endometriosis may be due to endometrial stem/ progenitors cells impairment. Some studies show that aberrant or dislocated stem cells, either from ectopic or eutopic endometrial tissue, may play an important role in the endometriosis onset and genesis. After both *in vivo* and *in vitro* studies, the endometrial stem cells represent the new targets in the pharmacologic arsenal and promise potential therapeutic methods. Further research is required in order to evolve and develop new strategies over endometriosis pathology.

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Medicine

FREEZE-ALL STRATEGY: PREGNANCY RATE, OBSTETRICAL PROGNOSIS AND ETHICAL CONSIDERATIONS – REVIEW EVALUATING 278.000 NEWBORNS AFTER ART

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ABSTRACT:
INTRODUCTION: CONTROLLED OVARIAN HYPERSTIMULATION (COH) MAY HAVE A NEGATIVE IMPACT UPON THE ENDOMETRIAL ENVIRONMENT, THUS, A "FREEZE-ALL" (FET) STRATEGY WAS CONSIDERED.
METHODS: REVIEW INCLUDING 44 STUDIES EVALUATING 278.000 NEWBORNS AFTER ART, COMPARING THE RESULTS OF THE CLASSIC VERSUS FREEZE-ALL STRATEGY.
RESULTS: THE CUMULATIVE LIVE BIRTH RATE (LBR) AND PREGNANCY RATE (PR) WERE SIGNIFICANTLY HIGHER IN THE "FREEZE-ALL" (FET) VERSUS ET (LBR: 60.55% VS. 45%, PR: RR 1.30 [CI 95%]). FET IS ASSOCIATED WITH A LOWER OBSTETRIC RISK [CI 95%]: THE FETUS HAS A LOWER RISK OF BEING SMALL FOR GESTATIONAL AGE RR: 0.59, LOW GESTATIONAL WEIGHT RR: 0.74, PREMATURE BIRTH RR: 0.74 BUT MORE INCREASED RISK OF CESAREAN RR: 1.10 AND LARGE FOR GESTATIONAL AGE RR: 1.49. WITH REGARD TO THE RISKS OF ANTEPARTUM HAEMORRHAGE, PLACENTA PRAEVIA, PERINATAL MORTALITY, CONGENITAL ANOMALIES AND SPONTANEOUS ABORTION RATE, THERE ARE NO CONSISTENT FINDINGS: SOME STUDIES CONCLUDED THAT THE RISK IS LOWER IN FET (0.67, 0.68, 0.8 AND, RESPECTIVELY 0,83), BUT MOST CONSIDER THAT THERE IS NO SIGNIFICANT STATISTICAL DIFFERENCE. GESTATIONAL HYPERTENSION IS CONTROVERSIAL, SOME STUDIES HAVE FOUND THAT IT IS MORE COMMONLY LINKED TO FET (RR: 1.29), BUT THE RESULTS ARE INSIGNIFICANTLY STATISTICALLY DIFFERENT. HOWEVER, FET IS AN INDEPENDENT RISK FACTOR FOR PLACENTA ACCRETA, 3 TIMES HIGHER THAN ET. MONOZYGOTIC MONOCHORIONIC PREGNANCY AFTER SINGLE-EMBRYO IS LOWER IN FET IN GENERAL (0.8%), BUT MATERNAL AGE BELOW 35 YEARS IS A RISK FACTOR IN FET CYCLES.
CONCLUSIONS: "FREEZE-ALL" IS AN ELIGIBLE PROTOCOL.

KEY WORDS: CRYOPRESERVATION, EMBRYO TRANSFER, FREEZE-ALL, IN VITRO FERTILIZATION, OBSTETRIC COMPLICATIONS

INTRODUCTION

Assisted Reproductive Techniques (ART) generally involve the transfer of a fresh embryo (ET) during the controlled ovarian hyperstimulation cycle (COH) and the subsequent transfer of one or more cryopreserved (frozen and then thawed) embryos (FET) into the following cycles. The basic principle of infertility treatment, in short, is the use of products that modify hormonal levels in the body and determine and control the process of ovarian follicular growth and maturation of the oocytes resulting from these follicles. These oocytes are than extracted by transvaginal puncture of the ovaries under anesthesia, placed in the embryology lab in contact with the sperm[11] and so there will result a number of embryos (3 days old) or blastocysts (5 days old). Fertlization can be made classically, called in vitro fertilization (IVF) or by intracytoplasmic sperm injection,

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especially when there are male pathology involved [12] [13] [14] [15] [16] (ICSI). At this point, the patient, under the guidance of the attending physician and the embryologist, has the following options: the transfer of a fresh embryo/ blastocyst or the cryopreservation of all of them and transfer them in subsequent cycles.

Ovarian hyperstimulation treatment has positive effects on the ovaries but has been shown to also have a negative impact on the endometrial uterine environment, which may affect the implantation or the development of pregnancy[17]. Thus, a "freeze-all" strategy has been considered by many clinics around the world[18]. What is this strategy basically? Freezing all the resulting embryos and transferring them sequentially, in the next cycles, leaving the body to "recover" after ovarian hyperstimulation. So the "freeze-all" approach consists of two steps and can be schematized as follows: in the first menstrual cycle the focus is on the ovaries: ovarian stimulation, follicle and oocyte development, puncture, oocyte harvesting. In step 2, in the next cycle, emphasis is on the uterus: developing and analyzing the endometrium, improving and monitoring the place where the pregnancy will be implanted[19].

OBJECTIVE

In infertility, an event of maximum emotional intensity is represented by the moment of embryo transfer, the transfer of the resulting embryo to the mother's uterus. Most couples hope that this is the moment when their struggle has come to an end and they will find out that they are parents. Unfortunately, on average, only 1 out of 3 embryo transfers will result in the birth of a living fetus (36%)[20]. It is an interesting subject, because both couples and us as physicians, feel involuntary disappointment at the moment when a pregnancy is not obtained after the transfer of the embryo.

[12] Bratu, O; Spinu, D; Oprea, I; Popescu, R; Marcu, D; Farcas, C; Dinu, M; Mischianu, D. Complications of radical retropubic prostatectomy-our experience. Romanian Journal of Military Medicine 2015;118(3):23-25.
[13] Bratu, O; Oprea, I; Marcu, D; Spinu, D; Niculae, A; Geavlete, B; Mischianu, D. Erectile dysfunction post-radical prostatectomy – a challenge for both patient and physician. Journal of Medicine and Life 2017;10(1):13-18.
[14] Marcu, D; Bratu, O; Spinu, D; Oprea, I; Niculae, A; Mischianu, D. Therapeutic approaches in premature ejaculation. Modern Medicine 2016;23(4): 270-278.
[15] Minciună, V; Crețu, O; Spînu, D; Marcu, D; Bratu, O. The impact of testicular cancer on fertility - literature review. Modern Medicine 2017;24(4):195-198.
[16] Marcu, D; Bratu, O; Spinu, D; Oprea, I; Niculae, A; Mischianu, D. Therapeutic approaches in premature ejaculation. Modern Medicine 2016;23(4): 270-278.
[17] Mehedintu, C; Bratila, E; Brinduse, LA; Cirstoiu, MM; Berceanu, C; Bordea, A; Comandasu, DE; Carp Veliscu, A; Bratu, O; Sava, C; Bumbu, AG. Controlled ovarian stimulation with urinary gonadotrophins and recombinant gonadotrophins in current practice. Rev Chim (Bucharest) 2018;69(12): 3611-3615.
[18] Chang, JC; Chen, MJ; Guu, HF; Chen, YF; Yi, YC; Kung, HF; Chen, LY; Chou, MM. Does the "freeze-all" policy allow for a better outcome in assisted reproductive techniques than the use of fresh embryo transfers? - A retrospective study on cumulative live birth rates. Taiwan J Obstet Gynecol. 2017;56(6):775-780. doi:10.1016/j.tjog.2017.10.013
[19] Shapiro, BS; Daneshmand, ST; Garner, FC; Aguirre, M; Hudson, C and Thomas, S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen thawed embryo transfer in normal responders. Fertil Steril 2011;96:344–348.
[20] Silea, C; Cucu, IA; Zarnescu, O; Stoian, AP; Motofei, IG; Bratu, OG; Pircalabioru, GG; Chifiriuc, MC. Influence of age on sperm parameters in men with suspected infertility. Rom Biotechnol Lett 2019;24(1):82-90.

The main purpose of the study is to improve the chances of achieving a pregnancy in in vitro fertilization programs by improving the embryo transfer method[21]. At the same time, the study aims to reduce maternal and fetal risks associated with pregnancy obtained from assisted human reproduction (ART) programs by analyzing comparatively the pregnancies obtained after the transfer of fresh embryos versus cryopreserved embryos[22].

MATERIALS AND METHODS

We performed a review including 44 studies evaluating 278.000 newborns after ART, comparing the results of the classic versus freeze-all strategy. In this paper, we analyze and compare, based on international studies, the live birth rates and pregnancy rates of fresh embryo transfer and defrosted embryo transfer, the obstetric complication rates in both categories, the rate of abortion, small for gestational age or macrosomia, hypertension, chromosomal anomalies, placenta accreta, monochorionic twin pregnancy after single embryotransfer, in order to quantify the advantages and disadvantages of the pregnancies obtained after fresh and cryopreserved embryotransfer. In addition to this, special infertile groups are taken into account, as low ovarian reserve patients and endometriosis patients, where the issue of fertility preservation and social freezing is raised as well, alongside with the ethical implications of cryopreservation that one must always have in mind while performing freezing of the embryos.

RESULTS

1. Pregnancy rates

There are currently studies that concluded that the life birth rate is higher if all embryos are frozen after fertilization, and they will be transferred to subsequent cycles (LBR: 60.55% vs. 45%)[23]. The cumulative pregnancy rate was also significantly higher in the “freeze-all” group (FET) versus fresh embryo transfer group (ET)[24] (PR: RR 1.30 [95% CI]).

This is because during the ovarian hyperstimulation cycle that results in oocyte production, the medication has a negative influence on the quality of the endometrium[25], thus affecting the implantation, and so influencing the number of pregnancies[26]. Uterine contractility can also be

[21] Weinerman, R and Mainigi, M. Why we should transfer frozen instead of fresh embryos: the translational rationale. Fertil Steril 2014;102:10–18.

[22] Spijkers, S; Lens, JW; Schats, R; Lambalk, CB. Fresh and Frozen-Thawed Embryo Transfer Compared to Natural Conception: Differences in Perinatal Outcome. Gynecol Obstet Invest 2017;82:538–546.

[23] Roque, M, Valle, M, Kostolias, A, Sampaio, M, Geber, S. Freeze-all cycle in reproductive medicine: current perspectives. JBRA Assist Reprod 2017;21(1):49-53. doi: 10.5935/1518-0557.20170012. PMID: 28333033.

[24] Roque, M, Valle, M, Guimarães, F, Sampaio, M, and Geber, S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. Fertil Steril 2015; 103: 1190–1193.

[25] Wong, K, van Wely, M, Mol, F, Repping, S, Mastenbroek, S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database of Systematic Reviews 2017, Issue 3. Art. No.: CD011184. DOI: 10.1002/14651858.CD011184.pub2

[26] Roy, TK; Bradley, CK; Bowman, MC; and McArthur, SJ. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil Steril 2014; 101: 1294–1301.

increased in the same cycle, and the implantation window is sometimes missed at the time of transfer[27].

2. Placentation and associated obstetric complications

In addition to the number of pregnancies that are lower in the fresh embryo group, it appears that ovarian hyperstimulation drugs also affect the development of pregnancy, placenta is altered in the case of ET in the same stimulation cycle[28] and so many obstetric complications associated with this process occur[29]. Thus, in the case of frozen embryos, a better pregnancy prognosis [95% CI] was observed: the fetus had a lower risk of being small for gestational age RR: 0.59, reduced gestational weight RR: 0.74, premature delivery RR: 0.74, but higher risk of RR: 1.10 cesarean and larger for gestational age RR: 1.49[30].

Gestational hypertension is controversial, some studies have found that it is more commonly linked to FET (RR: 1.29), but the results are insignificantly statistically different[31].

With regard to the risks of antepartum haemorrhage[32], placenta praevia[33], perinatal mortality[34], congenital anomalies[35] and spontaneous abortion rate[36], there are no consistent findings: some studies concluded that the risk is lower in FET (0.67, 0.68, 0.8 and, respectively 0.83), but most of the studies consider that there is no significant statistical difference[37] .

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[27] Maheshwari, A; Pandey, S; Shetty, A; Hamilton, M; and Bhattacharya, S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: A systematic review and meta-analysis. Fertil Steril. 2012; 98: 368–377.

[28] Aflatoonian, A; Karimzadeh Maybodi, MA; Aflatoonian, N; et al. Perinatal outcome in fresh versus frozen embryo transfer in ART cycles. International Journal of Reproductive Biomedicine. 2016;14(3):167-172.

[29] Imudia, AN; Awonuga, AO; Kaimal, AJ; Wright, DL; Styer, AK; and Toth, TL. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: A preliminary study. Fertil Steril. 2013; 99: 168–173.

[30] Roque, M; Lattes, K; Serra, S; Sola, I; Geber, S; Carreras, R; et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril. 2013;99:156–162.

[31] Imudia, A.N., Awonuga, A.O., Doyle, J.O., Kaimal, A.J., Wright, D.L., Toth, T.L. et al. Peak serum estradiol level during controlled ovarian hyperstimulation is associated with increased risk of small for gestational age and preeclampsia in singleton pregnancies after in vitro fertilization. Fertil Steril. 2012; 97: 1374–1379.

[32] Wennerholm, UB; Henningsen, AK; Romundstad, LB; Bergh, C; Pinborg, A; Skjaerven, R; Forman, J; Gissler, M; Nygren, KG; Tiitinen, A. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. Hum Reprod. 2013 Sep;28(9):2545-53. doi: 10.1093/humrep/det272. Epub 2013 Jul 5.

[33] Ozgur, K; Berkkanoglu, M; Bulut, H; Humaidan, P; Coetzee, K. Perinatal outcomes after fresh versus vitrified - warmed blastocyst transfer: retrospective analysis. DOI: http://dx.doi.org/10.1016/j.fertnstert.2015.06.031

[34] Vidal, M; Vellvé, K; González-Comadran, M; Robles, A; Prat, M; Torné, M; Carreras, R; Checa, MA. Perinatal outcomes in children born after fresh or frozen embryo transfer: a Catalan cohort study based on 14,262 newborns. Fertil Steril. 2017 Apr;107(4):940-947. doi: 10.1016/j.fertnstert.2017.01.021. Epub 2017 Mar 11.

[35] Ginström Ernstad, E; Wennerholm, UB; Khatibi, A; Petzold, M; Bergh, C. Neonatal and maternal outcome after frozen embryo transfer: increased risks in programmed cycles. Am J Obstet Gynecol. 2019 Mar 22. pii: S0002-9378(19)30487-9. doi: 10.1016/j.ajog.2019.03.010. [Epub ahead of print].
https://www.ncbi.nlm.nih.gov/pubmed/30910545

[36] Maheshwari, A; Pandey, S; Amalraj, Raja, E; Shetty, A; Hamilton, M; Bhattacharya, S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Hum Reprod Update. 2018 Jan 1;24(1):35-58. doi: 10.1093/humupd/dmx031. https://www.ncbi.nlm.nih.gov/pubmed/29155965

[37] Roque, M; Valle, M; Kostolias, A; Sampaio, M; Geber, S. Freeze-all cycle in reproductive medicine: current perspectives. JBRA Assist Reprod. 2017 Feb 1;21(1):49-53. doi: 10.5935/1518-0557.20170012. PMID: 28333033

3. The risk of fetal macrosomia and the rate of cesarean operations

Fetus resulting from cryopreserved embryo transfer are at risk of being macrosomes (large for gestational age) RR: 1.49 and at higher risk of posterm delivery, and mothers have an increased incidence of birth by cesarean section RR: 1.10[38].

4. Embryo transfer or blastocyst?

Generally, studies suggest that blastocyst transfer has a higher rate of achieving a pregnancy[39]. However, there are many contradictory but we should mention low quality studies due to the lack of a clear design. Some studies suggest that the transfer of cryopreserved blastocysts would be the optimal option to reach the largest number of live newborns, but other studies point out that these findings would not be statistically significant[40]. To assess whether the cumulative pregnancy rate and live birth rate is influenced by intrauterine embryo transfer at the cleavage stage (day 2, 3) or blastocyst (day 5, 6), we used a Cochrane meta-analysis that included 27 randomized clinical trials involving 4031 women[41]. Comparing the fresh group, the live newborn rate after fresh blastocyst was higher than after fresh embryo transfer: (OR) 1.48, 95% confidence interval (CI) 1.20 to 1.82, 13 RCTs, 1630 women, I2 = 45%, low quality records)[42]. There were no differences in the cumulative pregnancy rate after a single ovarian puncture between the fresh and the cryopreserved group (OR 0.89, 95% CI 0.64 to 1.22, 5 RCTs, 632 women, I2 = 71%, very low quality evidence). Comparing the blastocyst group, the clinical pregnancy rate was higher for fresh blastocysts (OR 1.30, 95% CI 1.14 to 1.47, 27 RCTs, 4031 women, I2 = 56%, moderate quality evidence). There were no significant differences in abortion rate or multiple group loads (low quality evidence). The main problems encountered were the lack of standard randomization and analysis methods and the high risk of bias. Studies are needed to analyze the same results first (eg cumulative pregnancy rate or live birth rate or pregnancy rate per transfer) and including and comparing patients with the same characteristics, knowing that ovarian reserve and age of the patient are essential features that can dramatically alter the results of assisted human reproductive techniques, independent of the transfer method.

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[38] Ishihara, O; Araki, R; Kuwahara, A; Itakura, A; Saito, H; and Adamson, GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril. 2014; 101: 128–133.

[39] Ishihara, O; Araki, R; Kuwahara, A; Itakura, A; Saito, H; Adamson, GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fert Steril 2014 Jan; 101(1):128-33, DOI: https://doi.org/10.1016/j.fertnstert.2013.09.025

[40] Glujovsky, D., Farquhar, C., Quinteiro Retamar, A.M., Alvarez Sedo, C.R., and Blake, D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev. 2016; : CD002118DOI: http://dx.doi.org/10.1002/14651858.CD002118.pub5

[41] Alviggi, C; Conforti, A; Carbone, IF; Borrelli, R; de Placido, G; Guerriero, S. Influence of cryopreservation on perinatal outcome after blastocyst - vs cleavage-stage embryo transfer: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2018 Jan; 51(1):54-63. doi: 10.1002/uog.18942.
https://www.ncbi.nlm.nih.gov/pubmed/29077229

[42] Wang, X; Du, M; Guan, Y; Wang, B; Zhang, J; Liu, Z. Comparative neonatal outcomes in singleton births from blastocyst transfers or cleavage-stage embryo transfers: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2017 May 4;15(1):36. doi: 10.1186/s12958-017-0255-4.
https://www.ncbi.nlm.nih.gov/pubmed/28472983

5. Congenital fetal abnormalities

Regarding the risk of fetal abnormalities[43], it is known that fetuses obtained by assisted human reproductive techniques may statistically have a higher risk of congenital malformation than those in the population of spontaneously obtained pregnancies[44]. This is mainly due to the fact that, in the general population, pregnancies with various malformations often stop spontaneously in their incipient stages, while IVF / ICSI pregnancies are medically supported and closely monitored. However, the risk of pregnancy with fetal abnormalities is no longer increased but is similar to that of the general population[45] , in the group of patients with cryopreserved embryo transfer[46]. We can attribute this to the fact that not all embryos survive to the freezing / thawing process, so that a "natural selection" of the highest quality embryos will be achieved, which will then be implanted and thus will result in pregnancies with healthy fetuses. Several studies have compared the risk of birth defects associated with defective blastogenesis in IVF pregnancies with fresh ET and frozen versus spontaneous pregnancies. It has been shown that the risk may be 3 times higher in the case of the transfer of fresh embryos to spontaneous pregnancies, but in the case of cryopreserved and defrosted embryo transfer this risk is not increased, but it is the same as in the spontaneously obtained pregnancies[47]. In addition to this, cryopreservation provides the possibility of preimplantation genetic screening. It has been observed that the biopsy should be performed in the blastocyst stage, not to interfere with the normal development of the embryo. So, in the case of couples at risk of genetic syndromes, conduct should be genetic testing on day 5 to 6 followed by cryopreservation of the blastocyst and then making a decision on implantation in the next cycle.

6. Monochorionic monozygotic twin pregnancy risk after single fresh or cryopreserved embryo transfer

Even after single embryo transfer, assisted human reproduction presents a 2-fold higher risk of monochorionic twin pregnancy compared to spontaneous pregnancies. To evaluate this, we made a review of retrospective cohort studies[48]. Although it was postulated that the culture medium

[43] Halliday, J.L., Ukoumunne, O.C., Baker, H.W., Breheny, S., Jaques, A.M., Garrett, C. et al. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. Hum Reprod. 2010; 25: 59–65.

[44] Schoolcraft, W.B. and Katz-Jaffe, M.G. Comprehensive chromosome screening of trophectoderm with vitrification facilitates elective single-embryo transfer for infertile women with advanced maternal age. Fertil Steril. 2013; 100: 615–619.

[45] Chang, JC; Chen, MJ; Guu, HF; Chen, YF;Yi, YC; Kung, HF; Chen, LY; Chou, MM. Does the "freeze-all" policy allow for a better outcome in assisted reproductive techniques than the use of fresh embryo transfers? - A retrospective study on cumulative live birth rates. Taiwan J Obstet Gynecol. 2017 Dec;56(6):775-780. doi: 10.1016/j.tjog.2017.10.013.

[46] Maheshwari, A., Pandey, S., Shetty, A., Hamilton, M., and Bhattacharya, S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: A systematic review and meta-analysis. Fertil Steril. 2012; 98: 368–377.

[47] Roy, T.K., Bradley, C.K., Bowman, M.C., and McArthur, S.J. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil Steril. 2014; 101: 1294–1301.

[48] Knopman, J; Krey, LC; Lee, J; Fino, ME; Novetsky, AP; Noyes, N. Monozygotic twinning: an eight-year experience at a large IVF center. Fertil Steril. 2010 Jul;94(2):502-10. doi: 10.1016/j.fertnstert.2009.03.064. Epub 2009 May 5.

would influence the monozygotic twin pregnancy rate after single embryo transfer and that implicitly the blastocysts, having 5 days in the culture medium, are at increased risk because they have been exposed for a longer time, the latest studies show that the culture medium do not influence the rate of monozygotic pregnancy, but instead, the quality of the embryo plays an important role[49]. Thus, the interesting discovery is that intrinsic factors rather than extrinsic factors influence the embryo's division process and the occurrence of the twin pregnancy after single transfer[50]. The highest rates of monochorionic monozygotic pregnancies were reported in single embryo transfer obtained with donated oocytes, so the superior quality oocytes (3.3%). Logistic regression analysis showed that patients under 35 years old with high quality recovered oocytes and increased number of day 3 or 5 embryos of superior quality had the highest monozygotic pregnancy rates (3.1%), regardless of use ICSI or assisted hatching [p <0.01].

Thus, the hypothesis regarding the damage to the pellucid area and hernia of the blastomere with the subsequent embryo division and the monozygotic pregnancy outcome is negated. Other studies have concluded that blastocyst transfer is a risk factor in fresh cycles (2.6% versus 1.2% fresh 3-day embryos), while maternal age under 35 is a risk factor in cryopreserved cycles. Generally, however, cycles using cryopreserved and thawed FET embryos had a lower rate of monozygotic pregnancies (0.8%). As a conclusion, monozygotic pregnancy rates are higher in ART, even after single transfer, especially in women under 35 years old. The risk is directly proportional to the number of available quality embryos, unaffected by the pellucid penetration procedures, and cryopreservation is a protective factor[51].

7. The risk of placenta accreta after cryopreserved embryo transfer

Assisted human reproduction poses a higher risk of placental pathology than spontaneous pregnancies, but an additional increased risk has been reported recently after the transfer of cryopreserved embryos (FET)[52]. For this reason, we performed a retrospective review to evaluate the correlation between cryopreserved and thawed embryo transfer and increased placenta accreta risk. We noticed that the prevalence of placenta accreta is 3 times higher after the transfer of cryopreserved embryos than after the transfer of fresh embryos (aOR, 3.2, P = 0.03). The physiopathological mechanisms are still in the debate[53]. The freezing method (slow freezing or

[49] Knopman, JM; Krey, LC; Oh, C; Lee, J; McCaffrey, C; Noyes, N. What makes them split? Identifying risk factors that lead to monozygotic twins after in vitro fertilization. Fertil Steril. 2014 Jul;102(1):82-9. doi: 10.1016/j.fertnstert.2014.03.039. Epub 2014 Apr 29.

[50] Wu, D; Huang, SY; Wu, HM; Chen, CK; Soong, YK; Huang, HY. Monozygotic twinning after in vitro fertilization/intracytoplasmic sperm injection treatment is not related to advanced maternal age, intracytoplasmic sperm injection, assisted hatching, or blastocyst transfer. Taiwan J Obstet Gynecol. 2014 Sep;53(3):324-9. doi: 10.1016/j.tjog.2014.07.001.

[51] Busnelli, A; Dallagiovanna C; Reschini, M; Paffoni, A; Fedele, L; Somigliana, E. Risk factors for monozygotic twinning after in vitro fertilization: a systematic review and meta-analysis. Fertil Steril. 2019 Feb;111(2):302-317. doi: 10.1016/j.fertnstert.2018.10.025.

[52] Takeshima, K; Jwa, SC; Saito, H; Nakaza, A; Kuwahara, A; Ishihara, O; Irahara, M; Hirahara, F; Yoshimura, Y; Sakumoto, T. Impact of single embryo transfer policy on perinatal outcomes in fresh and frozen cycles-analysis of the Japanese Assisted Reproduction Technology registry between 2007 and 2012.
Fertil Steril. 2016 Feb;105(2):337-46.e3. doi: 10.1016/j.fertnstert.2015.10.002. Epub 2015 Oct 27. PMID: 26518122

[53] Ishihara, O; Araki, R; Kuwahara, A; Itakura, A; Saito, H; Adamson, GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril. 2014 Jan; 101(1):128-33. DOI: https://doi.org/10.1016/j.fertnstert.2013.09.025

vitrification) seems to have no different effect, neither does the cleavage stage (embryo or blastocyst) or the fertilization technique used (FIV or ICSI). The risk appears to be related to the intrauterine environment, which is not hyperstimulation in cryopreserved transfer cycles. In women with placenta accreta, a lower level of estradiol and a 2 mm thinner endometrium have been reported. Another hypothesis is the implication of microRNA expression in the placenta, which may increase epigenome exposure to external influences. There have been 39 miRNAs that may be involved in this placental pathology. In conclusion, cryopreserved embryo transfer (FET) is an independent risk factor for placenta accreta, 3 times higher than for pregnancies using fresh embryos (ET), although the exact mechanism is not yet known[54]. The estradiol level, endometrial thickness and microARN appear to play an important role.

8. Ethical implications of human embryo cryopreservation

The development of assisted reproduction technologies (ART) has provided millions of infertile couples around the world with the hope of realizing their dream: having children. However, in most cases, more than one embryo result. Through cryopreservation techniques, the excessive number of embryos is kept until the family has achieved the number of children they want. Deciding what will happen with surplus embryos that have remained cryopreserved is of major ethical importance. The couple can either choose to dispose the embryos by defrosting them, or engage in compassionate transfer, donate them to other couples or give them for "adoption", or donate them for research[55]. Some couples simply "abandon" the embryos.

This subject is very sensitive, as embryos are not only simple transplanted tissues but have the potential to generate the life of a new human being[56]. In all the above-mentioned decisions the principles of bioethics must be taken into account. The principles of bioethics are: respect for autonomy (respect for the ability of the person to choose), non-maleficence (avoidance of harm), beneficence (the well-being of a person) and justice (equitable distribution of benefits and costs)[57]. In the decision-making process, there are specific laws that must be respected. We should mention: Human Reproduction and Embryology Societies laws[58], the Human Rights Declaration[59],[60] and the various available Civil and Penal codes that differ from country and state worldwide[61]. Christian religion does not support assisted reproductive techniques and especially not

[54] Hiura, H; Hattori, H; Kobayashi, N; Okae, H; Chiba, H; Miyauchi, N; Kitamura, A; Kikuchi, H; Yoshida, H; Arima, T. Genome-wide microRNA expression profiling in placentae from frozen-thawed blastocyst transfer. Clin Epigenetics. 2017 Aug 3;9:79. doi: 10.1186/s13148-017-0379-6. eCollection 2017.
[55] Guideline of the European Society of Human Reproduction and Embryology (ESHRE): art.11.6, Revised guidelines for good practice in IVF laboratories (2015).
[56] Lomax, GP; Trounson, AO. Nature Biotechnology volume. 31. 2013. p. 288-290.
[57] Beauchamp, TL; Childress, JF. Principles of Biomedical Ethics. Oxford University Press 2001;454. ISBN 0-19-514332-9
[58] Ethics Committee of the American Society for Reproductive Medicine. Disposition of abandoned embryos: a committee opinion 2013;99(7):1848–1849. DOI: http://dx.doi.org/10.1016/j.fertnstert.2013.02.024.
[59] The Universal Declaration of Human Rights, 1948. art 16. http://www.un.org/en/universal-declaration-human-rights/
[60] The Universal Declaration of Human Rights, 1948. art 3. http://www.un.org/en/universal-declaration-human-rights/
[61] Douglas, T; Savulescu, J. Destroying unwanted embryos in research. Talking Point on morality and human embryo research. EMBO Rep. 2009 Apr; 10(4): 307–312. Science and Society Talking Points. doi: 10.1038/embor.2009.54. PMCID: PMC2672894.

cryopreservation. Medical researchers by the other hand emphasize that embryonic stem cells have great potential to treat diseases such as cancer, genetic disorders and infertility.

One important aspect is concerning the fundamental right of people – the right to life – but from what point do we consider the product of conception as a human being with consciousness[62], sensitivity[63], the ability to feel the pain[64]? Neurologic, embryologic and biologic studies show that at the cell level the embryo has not the characteristics in order to consider it as a full righted human being. Is it ethical or not in this case to harvest stem cells, study embryos donated for research or destruction?[65] European legislation in this respect is not homogeneous[66], and Romanian legislation[67] is even more restrictive as the field of assisted human reproduction is under the legislation on organ transplantation[68].

An important point to remember is to strictly decide before starting any ART procedure what will happen with the surplus embryos and the "parents" to sign an informed consent and a contract concerning this issue, to avoid legal problems afterwards. A second point that I would like to highlight is that as doctors, we should explain the importance of the wider picture to the couple, taken into account donating the embryos for adoption or for research development[69]. It is not a simple decision to make but by emphasizing the concept of "beneficence," we could help improve treatments, relieve the suffering caused by different illness, or help other infertile couples to realize their dream family[70].

9. Pregnancy rate after fresh and cryopreserved ET in patients with low ovarian reserve

A particular category of patients with infertility problems is women with low ovarian reserve. The reason we assign a separate chapter is that their response to ovarian stimulation is poor, with few oocyte retrieved and of low quality.[71]. In this regard, we conducted a research with

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the objective of assessing whether the treatment protocol or the embryo transfer method influences IVF prognosis in patients with low ovarian reserve. We performed a review of retrospective cohort studies with a total of 8,556 stimulation cycles for approximately 3,000 patients with AMH <1.1 ng / mL[72],[73],[74],[75],[76].

Stimulating protocols were long or short, using GnRH antagonists, FSH, hMG, GnRH agonists, and included fresh (ET) or thaw (FET) embryotransfer. The implantation rates and cumulative pregnancy rates were similar (approximately 17%). Depending on the age of the patient, it was noted that under 35 years old, even after one transferred embryo the pregnancy rate was 33%, regardless of AMH. This rate decreased with age and required an increasing number of embryo transfers. According to this, it was observed that after 3 embryo transfer cycles, a cumulative pregnancy rate of 25.7% was observed in patients <40 years, including those with very low AMH, while in patients ≥40 years the cumulative pregnancy rate was 17.6% and in patients over 42 years old 7.4%. By dividing the patients into 2 similar mean age groups, only by AMH level (AMH 0.2-1ng / mL and AMH <0.2 ng / mL) the cumulative pregnancy rate was 20% after 5 stimulation cycles in both groups. Regarding the number of live newborns, it was noted that up to 35 years, there was no statistical difference between AMH levels between the group with and without live births. Over 35 years the AMH was significantly higher in the group with pregnancies with live newborns.

As a conclusion, AMH can not be used as an independent marker. In patients <35 years AMH is not a predictor, even with AMH <0.2ng / mL they have a good cumulative pregnancy rate. Age of the patient significantly influences the pregnancy rate, in patients ≥40 years, a higher AMH value shows a better prognosis. The number of cycles increases the chances of pregnancy in these patients, recommended to be at least 3 embryo transfers. Regarding the use of a particular treatment protocol, IVF or ICSI technique, and the pathology causing decreased ovarian reserve, there were no differences. Also, the use of fresh or cryopreserved embryos or blastocysts did not show statistically significant differences.

10. Patients with endometriosis – the need for social freezing

Endometriosis patients are at high risk of infertility[77]. Since endometriosis usually occurs at young ages, when the patient does not desire yet a pregnancy, the subject of fertility preservation

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is essential[78]. Progesterone treatment[79] proved its benefits on improvement of the symptoms, dimensions of the cysts and intraoperative conditions[80] [81] [82]. Endometriosis patients have an even higher risk of infertility and premature ovarian failure when the pathology begins at earlier ages[83], due to the reduction in ovarian reserve, both by the direct effect of ovarian endometriomas or pelvic[84] and follicular high inflammation factors but also following repeated surgery, especially if the lesions are bilateral[85]. To this it is added the peritoneal inflammatory effect of endometriosis, resulting in pelvic adherence syndrome, with tubal obstruction and ovulation impairment[86] [87].

The rate of pregnancy decreases directly proportional with the duration and severity of endometriosis, the time until the pregnancy is desired to happen and the need for repeated surgery[88]. In case of deep endometriosis, the symptomatology was correlated with the disease stage[89]. Early consultation with ART specialists to determine the need to use fertility preservation will increase the chances of this patients to eventually conceive at the wished moment. They must take into consideration age, ovarian reserve, the stage of endometriosis, IHC implants profile, the number of previous operations, the desire of procreation and the symptomatology[90]. In order to

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establish a correct diagnosis, a sonovaginography with ultrasound gel is essential [91]. Depending on age, ovarian reserve and the severity of endometriosis, one can opt for cryopreservation of ovarian tissue, oocytes or embryos[92]. The rate of pregnancy is highest after embryo preservation, but cryopreservation of oocytes gives the patient independence, as maybe at the moment they do not have a stable partner. The cryopreservation of ovarian tissue can be done during the laparoscopic procedure of diagnosis or treatment of endometriosis and it is the only method that gives the patient the possibility of getting a spontaneous pregnancy and restoring ovarian function, if extensive surgery is required due to an advanced endometriosis. It is important to note that the quality of oocytes and embryos can be affected in endometriosis, independent of the ovarian reserve, so the time of action is extremely important for a successful outcome.

CONCLUSION

The cumulative live birth rate (LBR) and pregnancy rate (PR) were significantly higher in the "freeze-all" versus fresh embryo transfer (LBR: 60.55% vs. 45%, PR: RR 1.30 [CI 95%]). The freeze-all strategy is associated with a lower obstetric risk [CI 95%]: the fetus has a lower risk of being small for gestational age RR: 0.59, low gestational weight RR: 0.74, premature birth RR: 0.74 but more increased risk of cesarean RR: 1.10 and large for gestational age RR: 1.49. With regard to the risks of antepartum haemorrhage, placenta praevia, perinatal mortality, congenital anomalies and spontaneous abortion rate, there are no consistent findings: some studies concluded that the risk is lower in freeze-all group (0.67, 0.68, 0.8 and, respectively 0,83), but most consider that there is no significant statistical difference. Gestational hypertension is controversial, some studies have found that it is more commonly linked to cryopreservation (RR: 1.29), but the results are insignificantly statistically different. However, cryopreservation is an independent risk factor for placenta accreta, 3 times higher than fresh embryo transfer. Monozygotic monochorionic twin pregnancy after single-embryo transfer is lower in freezed cycles in general (0.8%), but consider maternal age below 35 years a risk factor in the cryopreserved cycles.

All this information seems to make the "freeze-all" strategy an eligible protocol in the future.

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STAGING ENDOMETRIOSIS – A CONTINUOUS CHALLENGE

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ABSTRACT:
ENDOMETRIOSIS IS SUCH A COMPLEX DISEASE, WITH VARIOUS CLINICAL MANIFESTATIONS AND MORPHOPATHOLOGICAL PRESENTATIONS, THAT MOST OF THE TIME, THE INTRAOPERATORY FINDINGS DON'T MATCH THE PATIENT'S SYMPTOMATOLOGY. IN THAT REGARD, FOR MANY YEARS, GYNECOLOGISTS AROUND THE WORLD STRUGGELED TO CONCEIVE MULTIPLE CLASSIFICATION AND STAGING SYSTEMS IN ORDER TO CONSIDERATE AND TO INCLUDE ALL THE ASPECTS OF ENDOMETRIOSIS FROM QUALITY OF LIFE, PAIN, INFERTILITY, TO BETTER RESEARCH APPLICATIONS AND BETTER MANAGEMENT OF THE DISEASE.

KEYWORDS: CLASSIFICATION SYSTEMS, STAGING SYSTEMS, ENDOMETRIOSIS

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INTRODUCTION

Endometriosis is an enigmatic disease with many facets. The endometrial-like tissue has the ability to proliferate outside the uterine cavity and create great disturbances not only at the *site of implantation, but also at the eutopic endometrium, partially on behalf of its functional capacity to respond to hormonal stimuli (exogenous, endogenous or local)*[8].

The pelvic or extrapelvic endometriosic implants produce the well known "endometriosis effects". Excessive vaginal bleeding, pain (with different grades of intensity), infertility, intestinal occlusion, urinary symptoms, malignancies or, in rare cases, thoracic or neurologic symptoms are only some of the complications associated with endometriosis.

This pathology is an important economic burden in society and a continuous source for frustration among clinicians due to the difficulty in alleviating pain, reducing and preventing the infertility rates or improving the quality of life unaffected women[9].

Endometriosis has a prevalence of 8 – 10% among women of reproductive age, up to 30% of patients having primary or secondary infertility issues related to endometriosis [1]. Its prevalence is higher among women with subfertility (up to 50%) than fertile women (5–10%)[10].

The first historical reference of endometriosis dates back since 1500 BC from a discovery of an ancient Egyptian papyrus which described a treatment for a "painful disorder of menstruation". More than 3000 years later, Daniel Shroen described in his 1690 book titled *"Disputatio Inauguralis Medica de Ulceribus Ulceri"*, a more detailed presentation of the peritoneal endometriosis describing the adhesions and endometriomas as complications of the disease.

Since then, until the early 20th century, scientists from Germany, Holland, England or Scotland provided extensive studies on endometriosis, introducing in the medical dictionary terms like "chocolate cyst" or endometriomas[11]. At that point, the main concern shifted towards the pathogenesis of endometriosis, thus emerging theories[12] like the lymphatic dissemination of the endometrial tissue, the persistence of Wolffian rests on retrograde menstruation and implantation theory[13].

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Giving that, gynecologists from around the world deal with such a chameleonic disease (with no pathognomonic signs or symptoms[14]), the diagnosis of endometriosis can be delayed many years[15], ranging from 7 to 12[16].

One of the biggest challenges consists in establishing the best way to treat the disease and to find the perfect validated staging systems for helping the doctors manage the disease accurately[17].

Staging systems are important in order to create a common use, to evaluate the prognosis, the therapy response or the risk of recurrence, to evaluate the quality of life in women with endometriosis and to facilitate research applications[18].

Despite the struggle in developing staging systems, the classification of endometriosis has remained controversial, due to the many forms of the disease, the focusing on the anatomy and histology for 'surgical staging' and recently, on the prognostic value[19].

In 1921, Sampson elaborated the first classification of endometriosis, followed by similar attempts from Albrecht et al. in 1955, Acosta et al. in 1973 (fig. 1)[20], Kistner in 1977 (fig. 2) and Buttram in 1978 (fig. 3) [10,13].Their systems were criticized for many reasons, *including their inability to predict clinical outcomes*[21]*, especially the pregnancy rates in infertile patients*[22].

The American Society for Reproductive Medicine was founded in 1944 in Chicago with the name of American Society for the Study of Sterility and later renamed as American Fertility Society and includes today members from over 100 countries worldwide[23]. In 1979, the society published the AFS score which, following its multiple revisions from 1985 (rAFS score) and finally 1996 (rASRM) (fig. 4), became the most used classification worldwide[24].

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[23] https://en.wikipedia.org/wiki/American_Society_for_Reproductive_Medicine

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In order to understand why additional systems for endometriosis staging were created, a brief presentation of the rASRM Classification is required. Although the rASRM score meets the requirements only partially, for an endometriosis classification, it has the advantages of being relatively easy to be used by the clinicians and to be understood by the patients[25]. Its disadvantages include the lack of information about the retroperitoneal structures with deep infiltrating endometriosis (DIE) and a poor correlation with pain[26] and sterility, this criteria not being included in the scoring system[27].

Mild

1. Scattered, fresh lesions lie, implants not associated with scarring or retraction of the peritoneum) in the anterior or posterior cul-de-sac or pelvic peritoneum.
2. Rare surface implant on ovary, with no endometrioma, without surface scarring and retraction, or small endometrioma.
3. No peritubular adhesions.

Moderate

1. Endometriosis involving one or both ovaries, with several surface lesions, with scarring and retraction, or small endometrioma.
2. Minimal periovarian adhesions associated with ovarian lesions described.
3. Minimal peritubular adhesions associated with ovarian lesions described.
4. Superficial implants in the anterior and/or posterior cul-de-sac with scarring and retraction. Some adhesions, but not sigmoid invasion.

Severe

1. Endometriosis involving one or both ovaries with endometrioma > 2 x 2 cm (usually both).
2. One or both ovaries bound down by adhesions associated with endometriosis, with or without tubal adhesions to ovaries.
3. One or both tubes bound down or obstructed by endometriosis; associated adhesions or lesions.
4. Obliteration of the cul-de-sac from adhesions or lesions associated with endometriosis.

Endometriosis Society consensus on the classification of endometriosis. Hum Reprod, 2017, 32(2): 315-324; Haas, D; Shebl, O; Shamiyeh, A; Oppelt, P; *The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses.* Acta Obstet Gynecol Scand. 2013 Jan; 92(1): 3-7. doi: 10.1111/aogs.12026; Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

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5. Thickening of the uterosacral ligaments and cul-de-sac lesions from invasive endometriosis with obliteration of the cul-de-sac.
6. Significant bowel or urinary tract involvement[28].

The rASRM score represents the sum of the values assigned when evaluating the size of the endometriosic lesions which involves the peritoneum and the ovaries, the adhesions that affect the ovaries and the Fallopian tubes and the degree (partial or complete) of posterior cul-de-sac obliteration. The resulting number classifies endometriosis into four grades of severity, as it follows: minimal (stage I - 1 to 5 points), mild (stage II - 6 to 15 points), moderate (stage III– 16 to 40 points) and severe (stage IV > 40 points)[29].

The deficiencies in the rASRM score regarding fertility are covered by Adamson and Pasta's 2010 Endometriosis Fertility Index (EFI score), while the lack of description of retroperitoneal structures affected by DIE is surpassed by the Enzian score[30] and pain is described by the American Association of Gynecological Laparoscopists (AAGL) classification[31].

Stage I

Areas of endometriosis are present on the posterior pelvic peritoneum (cul-de-sac, uterosacral ligaments} or on the surface of the broad ligaments but do not exceed 5 mm in diameter. Avascular adhesions may involve the tubes, but the fimbriae are free. The ovaries may show a few avascular adhesions, but there is no ovarian fixation. The surfaces of the bowel and the appendix are normal.

Stage II

A. Areas of endometriosis are present on the posterior pelvic peritoneum (cul-de-sac, uterosacral ligaments; and the broad ligaments but do not exceed 5 mm in diameter. Avascular adhesions may involve the tubes, but the fimbriae are free. Ovarian involvement by endometriosis has been subclassified as follow:

IIA-1: Endometrial cyst or surface is 5 cm or less
IIA-2: Endometrial cyst or surface is over 5 cm.
IIA-3: Ruptured endometrioma; the bowel and the appendix are normal.

[28] Roberts, CP; Rock, JA; *The current staging system for endometriosis: does it help?* Obstet Gynecol Clin North Am. 2003 Mar; 30(1):115-32.
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Stage IIB

The posterior leaf of the broad ligament is covered by adherent ovarian tissue. The tubes present adhesions not removable by endoscopic procedures. The fimbriae are free. The ovaries are fixed to the broad ligament and show areas of endometriosis over 5 mm in diameter. The cul-de-sac presents multiple implants, but there is no adherent bowel nor is the uterus in fixed position. The bowel and the appendix are normal.

Stage III

The posterior leaf of the broad ligament may be covered by adherent tube or ovary. The tubal fimbriae are covered by adhesions. The ovaries are adherent to the broad ligament, and tube may or may not show surface endometriosis or endometriomas. The cul-de-sac shows multiple areas of endometriosis, but there is no evidence of adherent bowel or uterine fixation. The bowel and the appendix are normal.

Stage IV

Endometriosis involves the bladder serosa, and the uterus is in fixed, third-decree retroversion. The cul-de-sac is covered by adherent bowel or is obliterated by the fixed uterus. The bowel is adherent to the cul-de-sac, uterosacral ligaments, or uterine corpus. The appendix may be involved by the endometriotic process[32].

THE REVISED ENZIAN CLASSIFICATION

The Enzian staging system was developed in 2005[33]. It was designed with the purpose to complete the rASRM score and to fulfil the classification of DIE lesions with the involvement of retroperitoneal structures and other organs[34]. It also correlates the clinical symptoms (pain and dysmenorrhea) with the severity grades of the disease[35]. Because of some overlaps with the rASRM score, the Enzian classification was revised (2010 and 2011) in order to optimize the system and to create a separate classification for DIE[36]. Despite of being simplified, this scoring system has

[32] Roberts, CP; Rock, JA; *The current staging system for endometriosis: does it help?* Obstet Gynecol Clin North Am. 2003 Mar; 30(1):115-32.

[33] Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1; Adamson, GD; *Endometriosis Fertility Index: is it better than the present staging systems?* Curr Opin Obstet Gynecol 2013, 25: 186–192. doi:10.1097/GCO.0b013e32836091da.

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still a low acceptance at the international level and is currently used almost exclusively in German-speaking countries. It is more complicated than rASRM Classification and it is more difficult to be understood by the patients. Also, there is currently no data available about the correlation of the Enzian classification with sterility and infertility[37].

Stage I (Peritoneum)

A. No peritoneal involvement.

B. Scattered superficial surface endometrial implants on the pelvic peritoneum (anterior or posterior cul-de-sac, uterosacral ligaments, or the broad ligaments), which do not *exceed* 5 mm in diameter. Neither tubal nor ovarian involvement.

C. Same as for B, but invasive endometriosis or plaques or endometrial implants > 5 mm in diameter. Fine, filmy adhesion may be present that may be lysed without great danger of resultant adhesions.

Stage II (Ovarian): 1. Right; 2. Left; 3. Bilateral

A. No ovarian involvement.

B. Superficial surface endometrial implants of ovary of < 5 mm in diameter, which can be removed by scraping or f taiga ration without great danger of resultant adhesions. Fine, filmy adhesions may be present and lysed without great danger of resultant adhesions.

C. Invasive endometriosis (plaques or endometrioma) > 5 mm but < 2 cm that require surgical removal. Fine, filmy adhesion may be present, which may be lysed without great danger of resultant adhesions.

D. Invasive endometriosis > 2 cm that requires surgical removal or a ruptured endometrioma of any size. Fine, filmy adhesion may be present, which may be lysed without great danger of resultant adhesions.

E. B, C, or D with sufficient dense adhesions to fix ovary to adjacent tissue (usually posterior leaf of broad ligament).

Stage III (Tuba): 1. Right; 2. Left; 3. Bilateral

A. No tubal involvement.

B. Superficial endometrial implants on tube that do not *exceed* 5 mm in diameter and can be removed by scraping or fulgaration without great danger of resultant adhesions. Fine, filmy adhesion may be present, which may be lysed without great danger of resultant adhesions.

C. Invasive endometriosis (plaques or endometrioma > 5 mm but < 2 cm that require surgical removal. Fine, filmy adhesion may be present, which may be lysed without great danger of resultant adhesions.

D. Tube involved with adhesions that distort tuba I anatomy and\or limit tubal movement. Fimbriae are free and tube is patent. C may be present.

[37] Haas, D; Shebl, O; Shamiyeh, A; Oppelt, P; *The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses.* Acta Obstet Gynecol Scand. 2013 Jan; 92(1): 3-7. doi: 10.1111/aogs.12026.

E. Fimbriae are covered by adhesions or distal end of tube is occluded. B, C, or D may be present.

Stage IV (Cul-de-sac)

A. Neither B nor C is present.

B. Invasive endometriosis of bladder or colon.

C. Posterior cul-de-sac obliterated and for uterus fixed and retroverted. Bowel or adnexa may be adherent to cul-de-sac area. B is usually present[38].

After excluding from the original classification, the minor peritoneal lesions, the revised Enzian classification includes three degrees of severity (grade 1: invasion <1 cm, grade 2: invasion 1–3 cm, grade 3: invasion > 3 cm)[39].

The severity is rated after evaluating the lesions from each compartment[40], giving that retroperitoneal structures are divided into three compartments (Compartment A: rectovaginal septum, vagina; Compartment B: Sacro uterine ligament to the pelvic wall; Compartment C: rectum, sigmoid colon).

Deep invasion beyond the lesser pelvis and invasion of the organs are registered separately as it follows: FA = adenomyosis, FB = involvement of the bladder, FU = intrinsec involvement of the ureter, FI = bowel disease cranially to the rectosigmoid junction, and FO ("others") = other locations - for example abdominal wall endometriosis[41].

[38] Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

[39] Haas, D; Shebl, O; Shamiyeh, A; Oppelt, P; *The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses.* Acta Obstet Gynecol Scand. 2013 Jan; 92(1): 3-7. doi: 10.1111/aogs.12026; Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

[40] Haas, D; Shebl, O; Shamiyeh, A; Oppelt, P; *The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses.* Acta Obstet Gynecol Scand. 2013 Jan; 92(1): 3-7. doi: 10.1111/aogs.12026; Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

[41] Haas, D; Shebl, O; Shamiyeh, A; Oppelt, P; *The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses.* Acta Obstet Gynecol Scand. 2013 Jan; 92(1): 3-7. doi: 10.1111/aogs.12026; Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

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The Enzian classification is not a scoring system (like the rASRM score), but more a morphological description of the endometriosic lesions, with size included (fig. 5)[42]. It has the advantage that it can also be used as a clinical classification, similarly to the TNM classification[43].

The number that follows represents the size of the lesion and the following lowercase letter indicates the location or the involved compartment, while two letters indicate bilateral disease[44]. For example, when the clinical evaluation suspects rectovaginal septum involvement, the lesion is referred to as Enzian: A1 B0 C0 ("c" for "clinical"). If the suspicion is histologically confirmed, the lesion is graded Enzian: A1 B0 C0. Thus, this nomenclature allows a more accurate surgical planning and a suspicion over the involvement of retroperitoneal structures before the postoperative histological confirmation[45]. The prefix „E" indicates the presence of an endometriosic tumor[46].

THE EFI SCORE

Laparoscopic surgery is considered the first choice for diagnosis and treatment of infertility related to endometriosis[47].

Adamson and Pasta developed in 2010 the EFI score, in order to predict the probability of pregnancy following the surgical staging and treatment of endometriosis in patients who attempt spontaneous non-IVF conception[48].

The EFI score emerged due to the need of filling the gap of rASF score when predicting postoperative pregnancy rates in women with endometriosis[49]. One factor identified to be correlated with pregnancy rate, but not included in the EFI is uterine abnormality, this simply because uterine abnormality is very uncommon in infertile patients with endometriosis[50]. However,

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[42] Adamson, GD; *Endometriosis classification: an update*. Curr Opin Obstet Gynecol. 2011 Aug; 23(4): 213-20. doi: 10.1097/GCO.0b013e328348a3ba; Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

[43] Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

[44] Adamson, GD; *Endometriosis classification: an update*. Curr Opin Obstet Gynecol. 2011 Aug; 23(4): 213-20. doi: 10.1097/GCO.0b013e328348a3ba.

[45] Haas, D; Wurm, P; Shamiyeh, A; Shebl, O; Chvatal, R; Oppelt, P; *Efficacy of the revised Enzian classification: a retrospective analysis. Does the revised Enzian classification solve the problem of duplicate classification in rASRM and Enzian?* Arch Gynecol Obstet. 2013 May; 287(5): 941-5. doi: 10.1007/s00404-012-2647-1.

[46] Adamson, GD; *Endometriosis classification: an update*. Curr Opin Obstet Gynecol. 2011 Aug; 23(4): 213-20. doi: 10.1097/GCO.0b013e328348a3ba.

[47] Zeng, C; Xu, JN; Zhou, Y; Zhou, YF; Zhu, SN; Xue, Q; *Reproductive performance after surgery for endometriosis: predictive value of the revised american fertility society classification and the Endometriosis Fertility Index.* Gynecol Obstet Invest. 2014; 77(3): 180-5. doi: 10.1159/000358390.

[48] Adamson, GD; Pasta, DJ; *Endometriosis fertility index: the new, validated endometriosis staging system.* Fertil Steril. 2010 Oct; 94(5): 1609-15. doi: 10.1016/j.fertnstert.2009.09.035.

[49] Zeng, C; Xu, JN; Zhou, Y; Zhou, YF; Zhu, SN; Xue, Q; *Reproductive performance after surgery for endometriosis: predictive value of the revised american fertility society classification and the Endometriosis Fertility Index.* Gynecol Obstet Invest. 2014; 77(3): 180-5. doi: 10.1159/000358390.

[50] Nada, Elena-Silvia; Brinduse, Lacramioara; Bratu, Ovidiu; Marcu, Dragos; Bratila, Elvira; *Endometriosis-associated infertility*, Modern Medicine, 2018, 25 (3): 132.

when this condition is discovered, it needs to be taken into consideration in predicting pregnancy rates. Defective reproductive function of the uterus or gametes will clearly affect prognosis and need to be taken into consideration as fertility factors, just as they would with any patient with any other pathology[51].

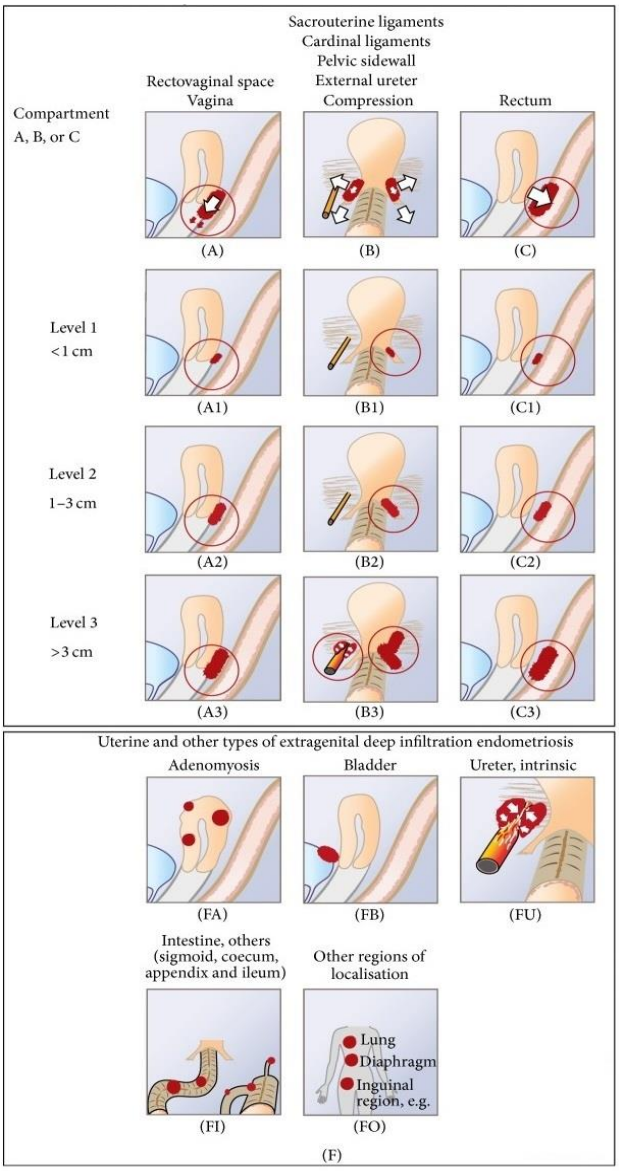

Fig. 5 – The Enzian Classification 2012 of deep infiltrating endometriosis (according to the Endometriosis Research Foundation, SEF)[52]

[51] Haas, D; Shebl, O; Shamiyeh, A; Oppelt, P; *The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses.* Acta Obstet Gynecol Scand. 2013 Jan; 92(1): 3-7. doi: 10.1111/aogs.12026; https://en.wikipedia.org/wiki/American_Society_for_Reproductive_Medicine

[52] Klugsberger, B; Shamiyeh, A; Oppelt, P; Jabkowski, C; Schimetta, W; Haas, D; *Clinical outcome after colonic resection in women with endometriosis.* Biomed Res Int. 2015; 2015:514383. doi: 10.1155/2015/514383.

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The ovarian reserve, quantified either by the plasmatic levels of antimullerian hormone (AMH), or by the ultrasonographic count of ovarian antral follicles[53] also, was not included in the EFI score[54]. *Alongside the ovarian reserve, others factors responsible for infertility in patients with endometriosis are omitted, including adenomiosis and digestive or urinary lesions not treated during laparoscopy for adnexal endometriosis involvement*[55].

EFI score can be useful in deciding the type, the duration and the costs of treatment following endometriosis surgery, anterior to recurring to assisted reproductive technologies in patients with good prognosis. It also allows clinicians to avoid wasting time and treatment in patients with poor prognosis[56].

When calculating EFI score the following factors are to be considered: historical factors (age, duration of infertility, previous pregnancy) and surgical factors (least function score, AFS score for endometriosis, total AFS score)[57].

Surgical findings that predict pregnancy rates were identified and used to develop the "Least Function Score", that, combined with elements of the AFS endometriosis score and historical factors statistically identified to predict pregnancy rates, was used to create the Endometriosis Fertility Index[58]. The least function score evaluates anatomical and functional

[53] Bruja, Alexandra; Brinduse, Lacramioara; Bratu, Ovidiu; Diaconu, Camelia; Bratila, Elvira; *Methods of transvaginal ultrasound examination in endometriosis*. Modern Medicine. 2018, 25 (3): 111-116.

[54] Boujenah, J; Hugues, JN; Sifer, C; Bricou, A; Cédrin-Durnerin, I; Sonigo, C; Monforte, M; Poncelet, C; *Endometriosis Fertility Index ou classification de l'American Society of Reproductive Medicine pour les patientes infertiles endométriosiques operées. Lequel est le plus pertinent?* Gynecol Obstet Fertil. 2015 Dec; 43(12): 806-9. doi: 10.1016/j.gyobfe.2015.10.006 ; Boujenah, J; Bonneau, C; Hugues, JN; Sifer, C; Poncelet, C; *External validation of the endometriosis fertility index in a french population.* Fertil Steril. 2015 Jul; 104(1): 119-23.e1. doi: 10.1016/j.fertnstert.2015.03.028.

[55] Boujenah, J; Hugues, JN; Sifer, C; Bricou, A; Cédrin-Durnerin, I; Sonigo, C; Monforte, M; Poncelet, C; *Endometriosis Fertility Index ou classification de l'American Society of Reproductive Medicine pour les patientes infertiles endométriosiques operées. Lequel est le plus pertinent?* Gynecol Obstet Fertil. 2015 Dec; 43(12): 806-9. doi: 10.1016/j.gyobfe.2015.10.006 ; Boujenah, J; Bonneau, C; Hugues, JN; Sifer, C; Poncelet, C; *External validation of the endometriosis fertility index in a french population.* Fertil Steril. 2015 Jul; 104(1): 119-23.e1. doi: 10.1016/j.fertnstert.2015.03.028; Boujenah, J; Poncelet, C; Madelenat, P; *The Endometriosis Fertility Index (EFI) is simple to use.* Gynecol Obstet Fertil. 2016 May; 44(5): 259-62. doi: 10.1016/j.gyobfe.2016.03.013; Stanimir, M; Chiutu, LC; Wese, S; Milulescu, A; Nemes, RN; Bratu, O. *Mullerianosis of the urinary bladder: a rare case report and review of the literature.* Rom J Morphol Embryol. 2016; 57(2 Suppl): 849-852; Socea, Bogdan; Constantin, Vlad; Carâp, Alexandru; Moculescu, Cezar; Pădeanu, Nicolae; Popa, Florin; *Rare urogenital malformation coupled with complex vascular malformation – a case report.* Chirurgia, 2012, 107(5): 659-663.

[56] Adamson, GD; *Endometriosis classification: an update*. Curr Opin Obstet Gynecol. 2011 Aug; 23(4): 213-20. doi: 10.1097/GCO.0b013e328348a3ba; Adamson, GD; Pasta, DJ; *Endometriosis fertility index: the new, validated endometriosis staging system.* Fertil Steril. 2010 Oct; 94(5): 1609-15. doi: 10.1016/j.fertnstert.2009.09.035.

[57] Adamson, GD; Pasta, DJ; *Endometriosis fertility index: the new, validated endometriosis staging system.* Fertil Steril. 2010 Oct; 94(5): 1609-15. doi: 10.1016/j.fertnstert.2009.09.035; Boujenah, J; Poncelet, C; Madelenat, P; *The Endometriosis Fertility Index (EFI) is simple to use.* Gynecol Obstet Fertil. 2016 May; 44(5): 259-62. doi: 10.1016/j.gyobfe.2016.03.013.

[58] Adamson, GD; Pasta, DJ; *Endometriosis fertility index: the new, validated endometriosis staging system.* Fertil Steril. 2010 Oct; 94(5): 1609-15. doi: 10.1016/j.fertnstert.2009.09.035.

features of the tubes and ovaries[59], thus becoming a significant predictor of fertility[60]. The functional score is determined by the surgeon bilaterally for the tube, fimbria and ovary, where 0 = absent or non-functional; 1, 2 and 3 = severe, moderate, and mild dysfunction, respectively; and 4 = normal with respect to the reproductive functionality of the organ/structure (fig. 6).

Structure	Dysfunction	Description
Tube	Mild	Slight injury to serosa of the fallopian tube
	Moderate	Moderate injury to serosa or muscularis of the fallopian tube; moderate limitation in mobility
	Severe	Fallopian tube fibrosis or mild/moderate salpingitis isthmica nodosa; severe limitation in mobility
	Nonfunctional	Complete tubal obstruction, extensive fibrosis or salpingitis isthmica nodosa
Fimbria	Mild	Slight injury to fimbria with minimal scarring
	Moderate	Moderate injury to fimbria, with moderate scarring, moderate loss of fimbrial architecture and minimal intrafimbrial fibrosis
	Severe	Severe injury to fimbria, with severe scarring, severe loss of fimbrial architecture and moderate intrafimbrial fibrosis
	Nonfunctional	Severe injury to fimbria, with extensive scarring, complete loss of fimbrial architecture, complete tubal occlusion or hydrosalpinx
Ovary	Mild	Normal or almost normal ovarian size; minimal or mild injury to ovarian serosa
	Moderate	Ovarian size reduced by one-third or more; moderate injury to ovarian surface
	Severe	Ovarian size reduced by two-thirds or more; severe injury to ovarian surface
	Nonfunctional	Ovary absent or completely encased in adhesions

Fig. 6 - Description of least function terms[61].

[59] Boujenah, J; Hugues, JN; Sifer, C; Bricou, A; Cédrin-Durnerin, I; Sonigo, C; Monforte, M; Poncelet, C; *Endometriosis Fertility Index ou classification de l'American Society of Reproductive Medicine pour les patientes infertiles endométriosiques operées. Lequel est le plus pertinent?* Gynecol Obstet Fertil. 2015 Dec; 43(12): 806-9. doi: 10.1016/j.gyobfe.2015.10.006.

[60] Adamson, GD; Pasta, DJ; *Endometriosis fertility index: the new, validated endometriosis staging system.* Fertil Steril. 2010 Oct; 94(5): 1609-15. doi: 10.1016/j.fertnstert.2009.09.035.

[61] Adamson, GD; Pasta, DJ; *Endometriosis fertility index: the new, validated endometriosis staging system*. Fertil Steril. 2010 Oct; 94(5): 1609-15. doi: 10.1016/j.fertnstert.2009.09.035.

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ENDOMETRIOSIS FERTILITY INDEX (EFI) SURGERY FORM

LEAST FUNCTION (LF) SCORE AT CONCLUSION OF SURGERY

Score		Description
4	=	Normal
3	=	Mild Dysfunction
2	=	Moderate Dysfunction
1	=	Severe Dysfunction
0	=	Absent or Nonfunctional

To calculate the LF score, add together the lowest score for the left side and the lowest score for the right side. If an ovary is absent on one side, the LF score is obtained by doubling the lowest score on the side with the ovary.

	Left	Right	
Fallopian Tube			
Fimbria			
Ovary			
Lowest Score		+	=
	Left	Right	LF Score

ENDOMETRIOSIS FERTILITY INDEX (EFI)

Historical Factors			Surgical Factors		
Factor	Description	Points	Factor	Description	Points
Age			LF Score		
	If age is ≤ 35 years	2		If LF Score = 7 to 8 (high score)	3
	If age is 36 to 39 years	1		If LF Score = 4 to 6 (moderate score)	2
	If age is ≥ 40 years	0		If LF Score = 1 to 3 (low score)	0
Years Infertile			AFS Endometriosis Score		
	If years infertile is ≤ 3	2		If AFS Endometriosis Lesion Score is < 16	1
	If years infertile is > 3	0		If AFS Endometriosis Lesion Score is ≥ 16	0
Prior Pregnancy			AFS Total Score		
	If there is a history of a prior pregnancy	1		If AFS total score is < 71	1
	If there is no history of prior pregnancy	0		If AFS total score is ≥ 71	0
Total Historical Factors			Total Surgical Factors		

EFI = TOTAL HISTORICAL FACTORS + TOTAL SURGICAL FACTORS: Historical + Surgical = EFI Score

ESTIMATED PERCENT PREGNANT BY EFI SCORE

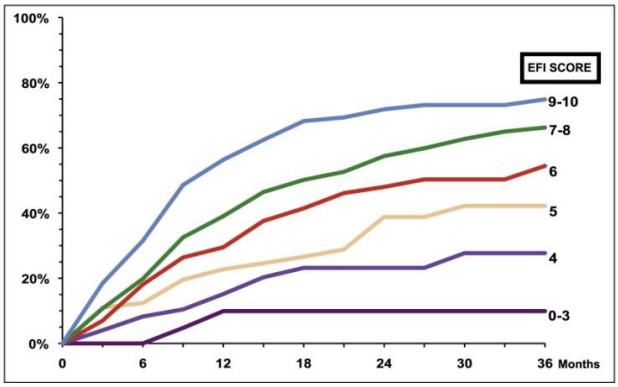

Fig. 7 – Endometriosis fertility index[62]

[62] Brătilă, Elvira; Ionescu, Oana-Maria; Badiu, Dumitru-Cristinel; Berceanu, Costin; Vlădăreanu, Simona; Pop, Doina Mihaela; Mehedinţu, Claudia; *Umbilical hernia masking primary umbilical endometriosis.* Rom J Morphol Embryol, 2016, 57(2): 825-829.

The total least function score is obtained by adding the lowest score from each side to give a combined total of potential for reproductive function of the adnexa. A completely normal pelvis would have a score of 4 + 4 = 8 and corresponding to an excellent reproductive potential. *A completely non-functional pelvis with no reproductive potential would have a score of 0 + 0 = 0.* Because pregnancy requires the functioning of all three segments (tube, fimbria and ovary) the lowest score of those three structures determines the ability of that side to function.

The EFI score ranges from 0 to 10, with 0 representing the poorest prognosis and 10 the best prognosis (Fig. 7). The estimated cumulative percentage of pregnant patients according to the value of the EFI score is presented in the graphic[63].

EFI EXTERNAL VALIDATION

Yacoub et al. in France studied whether EFI is a good pregnancy predictive tool in patients with surgically documented endometriosis followed by intrauterine insemination (IUI) or in vitro fertilization (IVF). The authors concluded that their study showed that the AFS score is not a good tool *of predicting the potential of obtaining pregnancies*, in contrast with the simple and reliable EFI score in patients with surgically documented endometriosis followed by IUI or IVF management[64]. These findings were also supported by Wang et al.[65] in 2013 and Garavaglia et al. in 2015[66] who acknowledged in their studies the superiority of EFI over rASF score in IVF outcomes in patients with endometriosis.

Three other studies that validated the EFI score are available online: Wei et al. in 2011[67], Tomasetti et al. in 2013[68] and Boujenah et al. in 2015[69].

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[63] Adamson, GD; Pasta, DJ; *Endometriosis fertility index: the new, validated endometriosis staging system.* Fertil Steril. 2010 Oct; 94(5): 1609-15. doi: 10.1016/j.fertnstert.2009.09.035.

[64] Adamson, GD; *Endometriosis Fertility Index: is it better than the present staging systems?* Curr Opin Obstet Gynecol 2013, 25: 186–192. doi:10.1097/GCO.0b013e32836091da.

[65] Boujenah, J; Poncelet, C; Madelenat, P; *The Endometriosis Fertility Index (EFI) is simple to use.* Gynecol Obstet Fertil. 2016 May; 44(5): 259-62. doi: 10.1016/j.gyobfe.2016.03.013; Stanimir, M; Chiutu, LC; Wese, S; Milulescu, A; Nemes, RN; Bratu, O. *Mullerianosis of the urinary bladder: a rare case report and review of the literature.* Rom J Morphol Embryol. 2016; 57(2 Suppl): 849-852; Socea, Bogdan; Constantin, Vlad; Carâp, Alexandru; Moculescu, Cezar; Pădeanu, Nicolae; Popa, Florin; *Rare urogenital malformation coupled with complex vascular malformation – a case report.* Chirurgia, 2012, 107(5): 659-663.

[66] Garavaglia, E; Pagliardini, L; Tandoi, I; Sigismondi, C; Viganò, P; Ferrari, S; Candiani, M; *External validation of the endometriosis fertility index (EFI) for predicting spontaneous pregnancy after surgery: further considerations on its validity.* Gynecol Obstet Invest. 2015; 79(2): 113-8. doi: 10.1159/000366443.

[67] Boujenah, J; Hugues, JN; Sifer, C; Bricou, A; Cédrin-Durnerin, I; Sonigo, C; Monforte, M; Poncelet, C; *Endometriosis Fertility Index ou classification de l'American Society of Reproductive Medicine pour les patientes infertiles endométriosiques operées. Lequel est le plus pertinent?* Gynecol Obstet Fertil. 2015 Dec; 43(12): 806-9. doi: 10.1016/j.gyobfe.2015.10.006.

[68] Tomassetti, C; Geysenbergh, B; Meuleman, C; Timmerman, D; Fieuws, S; D'Hooghe, T; *External validation of the endometriosis fertility index (EFI) staging system for predicting non-ART pregnancy after endometriosis surgery.* Hum Reprod. 2013 May; 28(5): 1280-8. doi: 10.1093/humrep/det017. Epub 2013 Mar 5.

[69] Boujenah, J; Hugues, JN; Sifer, C; Bricou, A; Cédrin-Durnerin, I; Sonigo, C; Monforte, M; Poncelet, C; *Endometriosis Fertility Index ou classification de l'American Society of Reproductive Medicine pour les patientes infertiles endométriosiques operées. Lequel est le plus pertinent?* Gynecol Obstet Fertil. 2015 Dec; 43(12): 806-9. doi: 10.1016/j.gyobfe.2015.10.006.

THE AMERICAN ASSOCIATION OF GYNECOLOGICAL LAPAROSCOPISTS (AAGL) CLASSIFICATION[70]

In 2007, the AAGL initiated a project to develop an endometriosis scoring system to document the morphology of endometriosis seen during surgery. The aim was to obtain a clinically useful classification system developed from analysis of the descriptions. After developing the tabulation format, AAGL continued with a research in which 30 endometriosis experts gave a weighted score to different anatomical features considered to be important with respect to pain and infertility.

The AAGL special interest group (SIG) is now about to propose a new classification system in which surgical difficulties are categorized in four levels:

Level 1: Excision or desiccation of superficial implants, and simple thin avascular adhesions.

Level 2: Stripping of ovarian endometriomas; appendectomy; DIE not involving vagina, bladder (not requiring suture), bowel or ureter; dense adhesions not involving the bowel and/or the ureter.

Level 3: Dense adhesions involving the bowel and/or the ureter; bladder surgery requiring suture; ureterolysis; bowel surgery without resection (shaving).

Level 4: Bowel resection with end-to-end anastomosis; ureteral reimplantation or anastomosis.

The AAGL-SIG reported that preliminary results correlate well with pain, infertility and surgical difficulty. AAGL results show that this new classification appears to be better than the existing staging systems in correlating stage of disease to pain intensity and level of surgical difficulties. To date no studies shown that this classification is able to predict pregnancy rates in endometriosis patients with infertility[71].

CONCLUSIONS

Even though many efforts were made during the last decades, the perfect classification for endometriosis still eludes medical professionals while the disease itself remains a challenge prone to generate frustration among clinicians. Efforts should continue to be made in order to develop a classification worldwide accepted that also offers information regarding fertility and quality of life of affected patients.

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All authors report no potential conflict of interest.

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[71] Bodean, Oana; Bratu, Ovidiu; Bohiltea, Roxana; Munteanu, O; Marcu, Dragos; Spinu Dan A; Vacaroiu, IA; Socea, Bogdan; Diaconu, Camelia C; Fometescu Gradinaru, D; Cirstoiu, Monica; *The efficacy of synthetic oral progestin pills in patients with severe endometriosis.* Rev Chim (Bucharest), 2018, 69(6): 1411-1415.

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Medicine

NEW-BORN HYGIENE BETWEEN "TOO LITTLE" AND "TOO MUCH"

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ABSTRACT:
HYGIENE, A WELL-STRUCTURED BRANCH OF PREVENTIVE MEDICINE, HAS DEMONSTRATED ITS CRUCIAL ROLE IN MAINTAINING AN OPTIMAL HEALTH STATUS OF THE POPULATION SINCE ANTIQUITY. CURRENTLY IN THE POLLUTED AND EXCESSIVELY INDUSTRIALIZED ENVIRONMENT IN WHICH WE LIVE, BOTH DEFICIT AND EXCESS HYGIENE ARE CONSIDERED HARMFUL.
A STUDY STARTED ON A BATCH OF NEWBORN INFANTS REVEALED THE RELATIONSHIP OF DIRECT PROPORTIONALITY IN THE CORRECT ACQUISITION OF HYGIENE RULES WITH THE SOCIO-ECONOMIC AND EDUCATIONAL LEVEL OF THE FAMILY AND INVERSELY PROPORTIONAL TO THE RISK OF GERM INFECTION ACQUIRED THROUGH DIRECT SKIN CONTACT OR FECAL ORAL TRANSMISSION.

KEYWORDS: NEWBORN, HYGIENE, INFECTION.

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Hygea was called the goddess of health and cleanliness in Greek mythology. She was watching for people's health, preventing illness, relieving suffering or pain. She demonstrated the role of healthy eating in disease prevention, set up public baths showing people how to use them. She was the daughter of Asclepius, the god of medicine and Panacea' sister, the goddess of healing, of curative medicine. The representation of the goddess in the sculpture was that of a woman with a snake wrapped around her and a cup in her hand, the symbol of the serpent and the cup crossed the time and its significance is now attributed to pharmacies and pharmaceuticals products.

The legends help us look retrospectively and appreciate that the basics of ancient medicine have been put together with the primacy of hygiene rules.

Hippocrates, the father of medicine, who liberated it from the domination of the gods, the superstition or the intransigence of the gods. He is the first to highlight the importance of hygiene in maintaining an optimal state of health, and relies on the role of natural factors (water, sun, air) in cultivating and maintaining the well-being of the body.

In our time, hygiene is an elemental prophylaxis for disease prevention; hygiene education has to start from a very young age (from the baby) to life.

Presently, hygiene is a branch of preventive medicine that studies the action of different environmental factors on people's health, has the purpose of disease prevention, the efficient use of resources by the population in order to reduce its exposure to risk factors capable of causing illness[9].

The newborn and the infant have somatic and physiological features requiring special care.

As soon as the newborn appears in the world, its hygiene needs to be keep in some rigorous parameters[10].

Vernix caseosa is a cheese-like white substance that covers the newborn is best revealed in the flexion folds and is composed of sebaceous secretions, petechial cells that have been accumulated. It has a protective role of the newborn skin from the macerating action of amniotic fluid, moisturizer, lubricant, softening the act of birth and has a protective role in the case of a newborn hemolytic syndrome; giving thermal protection, insulating the baby's skin and preventing heat loss. WHO recommendations are to postpone the newborn's first bath at 24 hours at birth to allow Vernix caseosa to fully replenish.

The bath requires correct and complete hygiene of entirely newborn, realized daily, with water at 37-38 ° C, hypoallergenic gels, dermatocosmetics that preserve the lipid skin layer and does not aggresse skin[11].

Insist on sensitive areas - flexion folds, retro-auricular area. The genital region is given a special attention to both sexes, with easy reduction of foreskin in boys and inspecting the folds from the vulvar region to the girls, the perianal region of both genders will be carefully cleansed. The ear pavilion is cleaned with a towel without the use of ear plugs that can push the posterior cerumen and form wax or epithelial plugs adhering to the eardrum[12].

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At the end of the bath, oils or protective creams are used. Besides the daily bath to any defecation or change of diaper, the perianal and genital toilet with hypoallergenic soap and water is made[13].

Abuse of degreasing, antiseptic, emollient substances can cause allergic reactions, atopic dermatitis, eczema or eczematid[14].

After the age of one month, will be used the same periodicity of the bath.

Practically, the rhythmic routine in hygiene must go into the elementary education of each child.

MATERIAL AND METHOD

A study carried out on 88 newborn babies highlighted the following: 39 of the cases (44.32%) were not washed daily, 75% of the mothers performed the daily hygiene incorrectly, 20 mothers (only) used adequate hygiene products (22.74 %).(Figure 1)

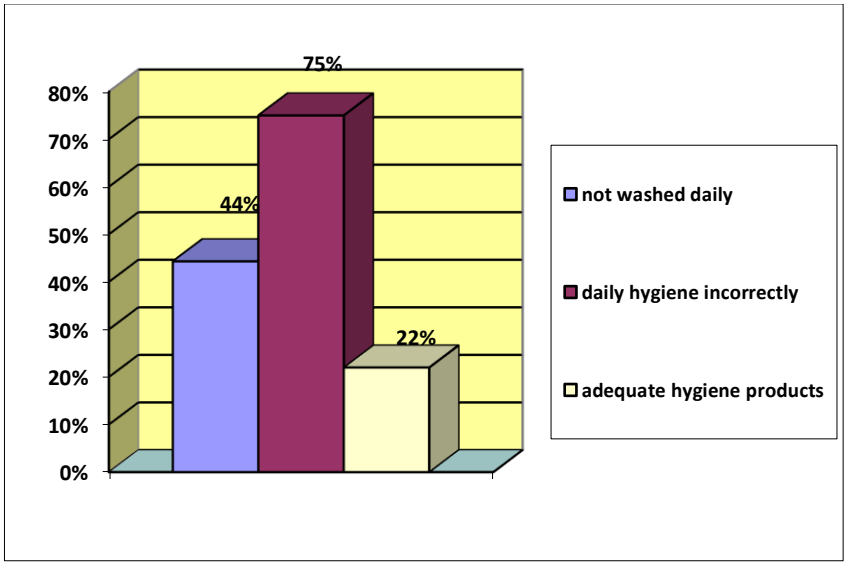

Figure 1

In the respondent range, 17 patients were hospitalized in the first 8 days of postpartum, at which the umbilical plague was not healed.

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7 cases out of 17 (41.18%) presented omphalitis (due to local hygiene), the microbial etiology being varied: 3 were with Pseudomonas aeruginosa, 2 with hemolytic staphylococcus aureus, 1 with Proteus, 1 with E. Coli, all children requiring antibiotic antibiotic therapy. (Figure 2)

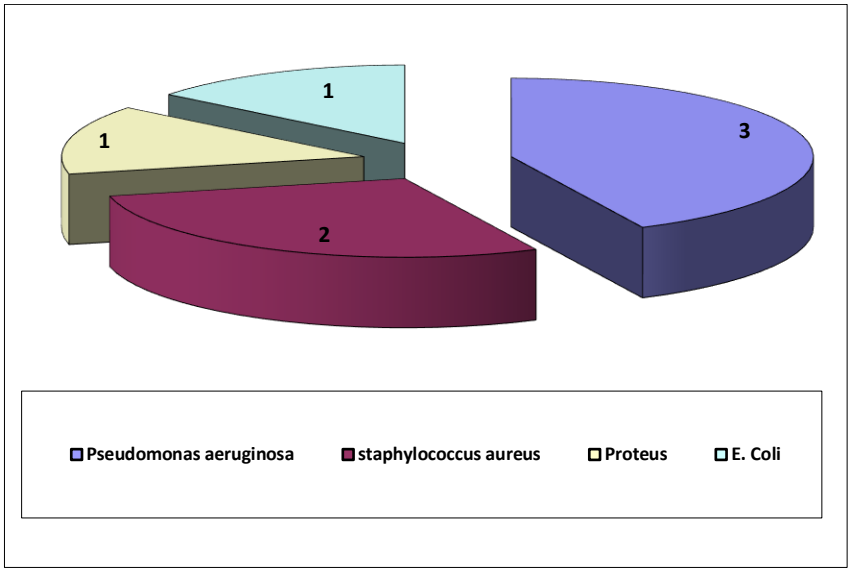

Figure 2

The socio-economic and cultural degree of mothers was another suggestive parameter. Of the 88 mothers, 24 had higher education, 27 secondary studies; the remaining 37 had a maximum of 8 grades (30) or were illiterate. (Figure 3)

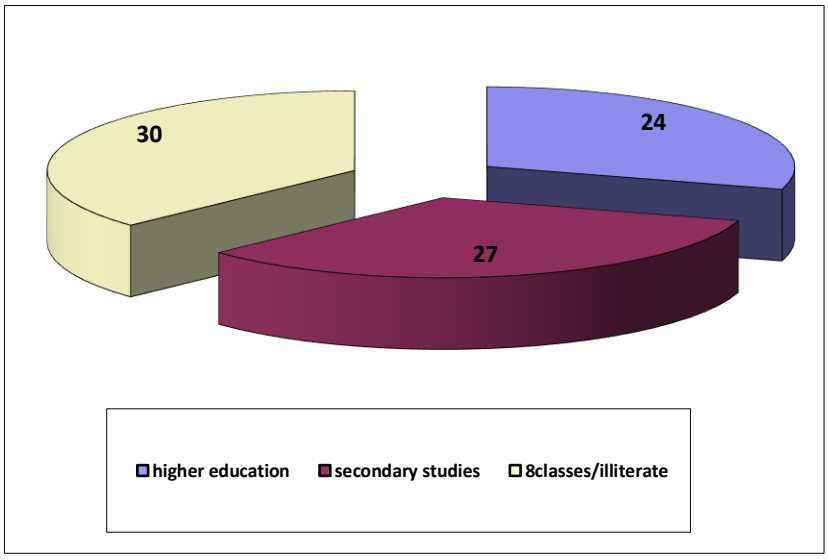

Figure 3

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Among those with c, about 33.3% (8 mothers) did not perform the daily cleaning ritual correctly, compared to 44.4% (12 mothers) with medium studies or 78.37% - 29 mothers from the group with more less than 8 classes and here unschooled ones. (Figure 4)

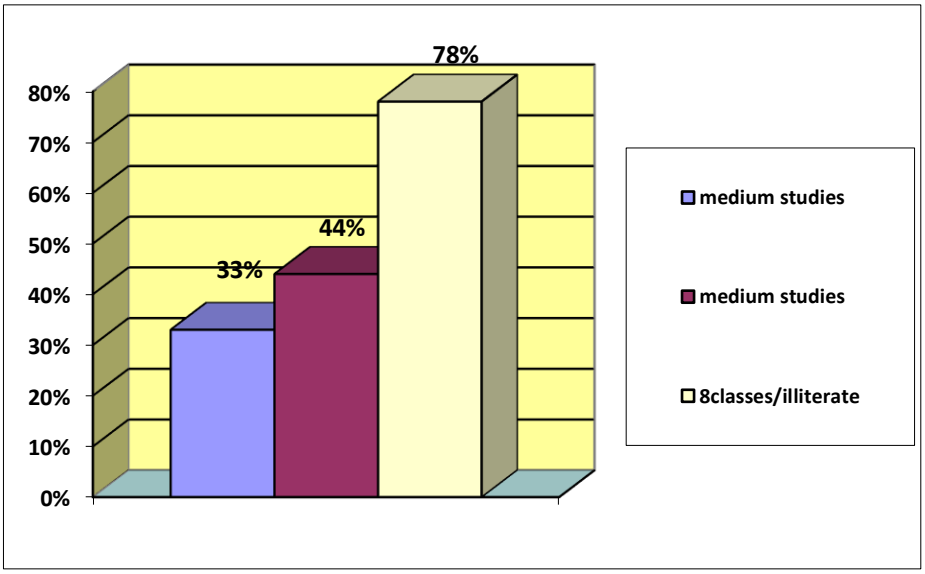

Figure 4

In the group of mothers with higher education, higher socio-economic level was found the "reverse of the medal": exaggerated concern for children's hygiene, excessive use of detergent products, 4 children out of the 24 (16,66%) developed initial contact dermatitis, 2 out of 4 later evolving with atopic dermatitis though atopia was not found in the family inheritance of the children.

DISCUSSIONS

Preferably, all dermatocosmetic products for sensitive skin of children are used, preserving the protective barrier and skin pH, are non-abrasive to avoid microlesiona that create the skin entry site, integrity of the skin, the largest organ to defend and protect the other structures of the human body must be carefully preserved.

In the case of atopy or patent allergies, hygiene products suitable for any skin problem will be used. The risk of atopy / allergy in a child is 12% if the parents were not allergic, rising to 20% if one is allergic, to 40% if they are both allergic and reaching 70% for the same type of allergy of the parents.

In recent years, there are studies in Europe that show an explosion of allergies, strictly related to industrial, technological development, excessive use of home care products or personal hygiene. The European Union has developed, through ECHA (European Chemical Agency), a list of restricted substances called REACH.

CONCLUSIONS

1. The correct hygiene of each child begins with that of his family
2. Various taboos: muffling the child, avoiding the daily bath for fear of cold etc. and lack of information are completely harmful to the beneficial evolution of the child
3. Continuity solutions (unhealed umbilical plaque) and incorrect hygiene create the premise of serious, even fatal potentials - septicemia of the newborn with the entrance gate skin and initial debut as omphalitis.
4. The degree of parental education is directly proportional with correct adherence to hygiene rules and with possible exaggerations and the appearance of possible cutaneous diseases (eczema, contact dermatitis or atopy)
5. All methods of body hardening ensure effective use, a harmonious development of the baby
6. Calling an education for correct hygiene through schools at young ages would have future generations of parents correct and assumed information.
7. The incidence and prevalence of direct transmitted diseases (contact, fecal-oral transmission, etc.) would decrease dramatically with the conscious application of the hygiene of the entire family, not just the child.
8. Antibiotic, harmful, small-age, could be avoided, in many cases, simply by applying proper hygiene.

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Medicine

UPDATING THE SIGNIFICANCE OF SCREENING AND TRIAGE METHODS OF CERVICAL LESIONS – REVIEW

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ABSTRACT:
THE IDENTIFICATION OF WOMEN HAVING OR BEING ABLE TO DEVELOP CERVICAL CANCER IS A MAJOR CONCERN WORLDWIDE EXPRESSED BY ENDEAVORS TO IMPLEMENT NEW METHODS OF SCREENING AND TRIAGE FOR PRECANCEROUS CERVICAL LESIONS (PCL). THE PRESENT WORK ENCOMPASSES RESULTS OF MANY TRIALS AND OBSERVATIONAL STUDIES PUBLISHED ON INTERNATIONAL DATABASES DURING LAST 10 YEARS. THE PURPOSE OF CURRENT STUDY IS TO HIGHLIGHT THE FEATURES OF ADVANCED BIOTECHNOLOGIES, CONCERNING THEIR CAPACITY OF LEADING TO AN EARLY RIGHT DIAGNOSIS SUPPORTING, TO COMPARE THE EFICACITY OF THESE TESTS SUCH SENSITIVITY AS SPECIFICITY, TO TRIAGE BETWEEN REGRESSIVE AND PROGRESSIVE PCL, TO HIGHLIGHT THE DIFFERENCE CONCERNING SCREENING METHODS ADOPTED BY THE NATIONAL PROGRAMMES WORLDWIDE. THE RESULTS SHOWED THAT PAP TEST IS AN EXPENSIVE METHOD, WITH HIGH LEVEL OF SUBJECTIVE ASSESSMENT AND WITHOUT POSSIBILITY OF DISCRIMINATION BETWEEN DIFERENT KINDS OF CYTOLOGIES. HUMAN PAPILLOMAVIRUS (HPV) GENOTYPE ALONE DOESN'T ALLOW TO DISTINGUISH TRANSIENT VERSUS PROGRESSIVE HPV INFECTION. IMMUNOSTAINING CYCLINE P16INK4A AND THE MORE PERFORMANT TEST, DUAL TEST P16INK4A/ KI 67 ALLOW THE TRIAGE OF CASES ABLE TO DEVELOP CANCER USED ALONE OR AS CO-TEST TO PRIMARY HPV SCREENING. PRIMARY CERVICAL SCREENING WITH HPV GENOTYPE ASSOCIATE WITH ONE OF CO-CIYTOLOGIC TEST IMPROVE THE ACCURACY OF AN EARLY DIAGNOSIS.

KEY WORDS: CERVICAL CANCER, PRECANCEROUS CERVICAL LESIONS, BIOTECHNOLOGIES, PAP TEST, HPV GENOTYPE, P16INK4A, P16INK4A/ KI 67

INTRODUCTION

Despite the sustained efforts to identify precancerous cervical lesions and to stop cervical neoplastic progression, the cervical cancer incidence attains high rate, being the fourth most common cancer diagnosed in women. Several screening tests for cervical cancer have been agreed over the years and the recording of the results formed a very large scientific database. This huge amount of information obtained following multiple randomized trials conducted in the last 30 years made possible the comparison between the test methods results in order to identify one method or an association of methods able to achieve the highest percentage of sensitivity in early identification of precancerous cervical lesions. Modern biotechnologies offer a variety of screening and triage tests for cervical lesions. In the current situation, it is necessary to have a better knowledge of the benefits and limitations provided by biotechnologies approved as an integral part of cervical cancer national screening programs. The identification of women who can develop or have cervical cancer is a major global concern materialized through sustained efforts to implement various cervical cancer screening methods (CC) and triage of precancerous cervical lesions (PCL). This study seeks to summarize the particular characteristics of advanced biotechnologies and their ability to establish correctly an early diagnosis able to enact adequate therapeutic approaches.

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DIAGNOSTIC AND SCREENING BIOTECHNOLOGIES IN CERVICAL CANCER - REVIEW

Our study aims to analyze the results of several trials and observational studies published in the international databases (PubMed, Medscape, Scopus) over the past 10 years. The adopted criteria consist in identification of implemented biotechnologies, comparing the effectiveness of the results of these determinations in terms of sensitivity in CC detection and PCL triage, specifying the duration of obtaining the test result, mentioning the screening interval recommend. The study also included criteria such as presenting opportunities for accessibility of women around the globe to test methods and the balance between cost and benefits of the screening and diagnosis assumed methods.

IDENTIFICATION OF DIAGNOSTIC AND SCREENING BIOTECHNOLOGIES IN CERVICAL CANCER

Babeş Papanicolaou cytological testing – PAP test

Pap cytological test is performed both in national screening programs, and in opportunistic screening situations related too cervical cancer. Pap cytological test is characterized by high specificity and low sensitivity. The Pap cytological test sensitivity regarding the detection of intraepithelial cervical neoplasia grade 2 or more (CIN2 +) is appreciated as being limited between 53%-70% [11]. Over the past 12 years, a series of comparisons have been made on the quality of the cytological testing results: dry cytology versus liquid-medium cytology [12], [13]. The testing results using dry cytology vs cytology in liquid environment remain identical without changes in specificity and sensitivity. Hospitex Diagnosis Report (2013) emphasizes the fact that single layered blades obtained from cells collected in liquid environment (CLE) are more secure and more powerful representative in comparison with the conventional screening procedure. The limitations of cytological testing consist in high cost due to investments in training for the cytologist and due to the necessity of periodic test repeating. Initially recommended to be annually repeated, currently the cytological test is recommended to be repeated between 3-5 years in case of negative testing for HPV-HR [14]. Among other Pap cytological testing limitations, an important one is the test inability to allow differentiation of AUC-US or L-SIL lesions that spontaneously

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resolve or are able to progress towards cancer. Statistical data underline that 10-15% of the cases with ASC-US and L-SIL cytology develops CIN3 [15].

Results of the ATHENA study, published in 2014 highlight the existence of a significant percentage, of 57% women aged between 25-29 years with negative cytology for intraepithelial lesions (NIEL) that have been shown to present at histopathological examination injuries ≥ CIN3. This evidence has dramatically changed the orientation regarding the priority of screening methods in cervical cancer. Currently Pap Test has acquired a special meaning in the national programs being recommended for co-testing, alongside with primary HPV testing, which allows an increased diagnosis accuracy [16].

Testing HPV infection

Nowdays, there is a statistically acknowledged tendency as regards the need to change the cervical cytological screening method with testing for the presence of infection with human papilloma virus (HPV), increased risk (HR). Therefore, the HPV testing is required as the first screening method in cervical cancer.

HPV is a non-enveloped, double-stranded DNA virus. The viral genome consists of 3 different regions: the long control region (LCR) or the URR (upstream regulatory region) - responsible for the translation and replication-, and two ORF regions (open reading frames) -early (E1-E-7) and late (L1 and L2) respectively)-. The HPV genome proteins accomplish different roles. The E1 and E2 proteins act in the cellular cycle adjustability, the E6 and E7 proteins are transforming; the late proteins are located in the L1 and L2 regions; the E4 protein function is not yet fully known and the E5 protein interferes with the cellular immortalization.

During the integration of the HPV -DNA in the host cell, the viral genome perturbs the reading hatches (ORFS) of the E1 and/or E2 levels. The loss of the E2 function causes the loss of cellular regulation control and the development of oncoproteins E6 and E7 [17].

There are three acknowledged methods of identifying the HPV types: nucleic acid-hybridization assays, signal-amplification assays, nucleic-acid amplification. These methods are used in many tests under commercial names.

The nucleic acid-hybridization assays methodology is found in the commercial tests Southem blot, In situ hybridization, Dot blot hybridization. This method detects the HPV infection in the cervical samples and provides substantial information regarding the HPV infection types. The disadvantages of the nucleic acid-hybridization assay are low sensitivity, requiring a bigger quantity of purified DNA, and taking a longer period of time for data processing.

The signal-amplification assays method is found in the commercial tests Hybrid Capture 2 -HC2- (Digene) și Cervista. The Hybrid Capture 2 -Hc2 (Digene) test approved in the US by the Food and Drug Administration (FDA) in 2003 detects 13 HPV - HR types (16, 18, 31, 33, 35, 39,

[15] Arbyn, M., F. Buntinx, M. V. Ranst, E. Paraskevaidis, P. Martin-Hirsch, and J. Dillner. 2004. "Virologic Versus Cytologic Triage Of Women With Equivocal Pap Smears: A Meta-Analysis Of The Accuracy To Detect High-Grade Intraepithelial Neoplasia". *JNCI Journal Of The National Cancer Institute* 96 (4): 280-293. doi:10.1093/jnci/djh037

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45, 51, 52, 56, 58, 59, 68) and 5 HPV - LR types (6, 11, 42, 43, 44). The test uses the non-radioactive amplification method of the specific antibodies signal and chemiluminescence paired with hybridising the HPV-DNA target and the RNA marked in the solution samples. The Hc2 test identifies the high risk cases of LCP infected with HPV-HR 16 and 18 in a 10-15% percentage, and other types of HR-HPV with percentage < 3% [18].

The Cervista test is approved in the US by the Food and Drug Administration (FDA) since 2009. The Cervista test detects 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68); it identifies the nucleic acid sequences by using the signal-amplification assay and fluorescence. The advantages of the Cervista method: 100% sensitivity in detecting CIN 3, 98% sensitivity in detecting CIN 2, high sensitivity and specificity in HPV 16 and 18, lower rates of false positive results, higher percentage of identifying the precancerous cervical lesions than the H2 test [19].

The methodology that uses the nucleic acid amplification is used in the following commercial HPV tests: Microarray analysis, PapilloCheck, PCR, PCF-RFLP, Real-Time-PCR, Abbott Real Time, Cobas 4800 HPV, Genome sequencing, CLART HPV, INNO-LIPA, The Linear Array, Clinical Arrays, Pre Tect Proofer, APTIMA. The clinical use of these tests is different.

Cobas 4880 HPV test was approved in the US by the Food and Drug Administration (FDA) since 2014. The test is used as a first choice screening method in the cervical cancer. The Cobas test uses an automatic method that uses the Real-Time PCR. The amplification and the viral detection take place in a single tube with genotyping for HPV 16, HPV 18 and other 12 types of HPV (HR 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 ,68) and with using the β-globin for controlling the process [20]. The advantages of the Cobas 4880 HPV are the short period of 4 hours testing and the accuracy of identifying the HPV HR types. The method removes subjectivity by using the automated reading. The Cobas 4880 HPV test is validated for cervical cancer screening as well as for ASC-US lesions triage [21].

The signal-amplification assays method is also found in The Linear Array HPV genotyping (Roche Molecular Diagnostics, USA). The advantages of this testing consists in identifying 36 HPV types and classifying them into risk groups (15 types of HPV HR-, 15 probably HPV HR-, 10 HPV- LR types and 9 HPV types with undetermined risk).

[18] Stănculescu, Ruxandra, Elvira Brătilă, Vasilica Baușic, Teodora Camelia Vlădescu, Florina Vasilescu, Alexandra Baușic and Costin Berceanu. 2017. "Review of the biotechnologies and test used for precancerous cervical lesions diagnosis". *Rom J Morphol Embryol*. 58(1):7-1, 2066-8279

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The same methodology is used in the Clinical Arrays HPV test (Genomic SAU, Madrid Spania) which detects 35 genotypes that are individually assigned to HR-HPV or LR-HPV. The method identifies the singular or multiple infection.

In comparison with the previous tests that only confirm the presence of the HPV infection, the HPV-mRNA test adds more information because it identifies a HPV transformed, progressive infection, that affects the cellular cycle after the intracellular HPV viral genome integration into the host DNA. This is proven by detecting the E6 and E7 proteins overexpression in HPV HR infections [22].

The commercial tests able to identify the E6/ E7 mRNA HPV –HR are the Pre Tect Proofer test (Norchip) -that allows the detection of the E6/ E7 mRNA in 5 HPV-HR types (16, 18, 31, 33, 45)-, and the APTIMA test(GenoProbe) – that has a higher availability of detecting the E6/ E7 mRNA in more HPV-HR types, that is 14 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).

The E6/ E7 mRNA detection is also possible by using the QIAGEN test (US), covering a range of 13 HPV-HR types (16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59, 66). This method is used in the areas without a water source and electricity. The devices occupy a small space (25cmx50cm). Another advantage is the short period of processing the data (just 2h 30 minutes in comparison with longer duration for Hc2).

Therefore, the testing methods used for HPV infections have benefits and limits as regards an accurate diagnosis and choosing the most adequate medical attitude. According to our study criteria, we will make a distinction between the benefits and the limits of HPV testing methods using the tests presented until now.

The conclusions of collected studies reveal that the Cervista HPV testing has high sensitivity and specificity in detecting the HPV 16/18 types. The Cervista assay has 100% sensitivity in detecting the HPV types capable of evolving into CIN3 lesions [23]. Detecting the HPV 16 and 18 allow the stratification of the HPV infection oncological risk, without a crisp distinction between HPV infections that resolve spontaneously and progressive HPV infections. The identification of the E6/E7 mRNA in HPV-HR confirms the existence of a HPV transformative infection .

The presence of a 1:1 ratio between E2 and E6/7 genes confirms the HPV integration by using the Real-Time PCR method. The precancerous lesions progression risk varies with the HPV HR type: 10-15% risk for HPV HR type 16/18 and < 3% for other HPV HR types [24].

Besides the technologies proving HPV infection, a special role is played by biotechnologies that can detect the viral load. They are used within the PCR, Fluorescence in situ hybridization and RealTime PCR tests.

Analyzing the data published in the literature leads to formulating the benefits and disadvantages of detecting the presence of an HPV infection.

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[24] Abreu, André L P, Raquel P Souza, Fabrícia Gimenes, and Marcia E L Consolaro. 2012. "A Review Of Methods For Detect Human Papillomavirus Infection". *Virology Journal* 9 (1): 262. doi:10.1186/1743-422x-9-262

It can be unequivocally affirmed that the HPV types inducing PCL or CC are different. Types 16 and 18 are the most commonly involved types in CC, HPV type 16 is dominant in CIN3 + lesions. Equally, it is known that the variation in PCL and CC risk is influenced by the type of HPV infection, viral load, vaginal microbiome, individual immunity and it is related to geographical areas.

It is also relevant that the viral integration is an early event that occurs before morphological changes progressing to cancer. Viral HPV DNA integration does not coincide with the immediate presence of a high grade lesion[25]. Triggering strategies according to Eurogin Roadmap 2017 take into account the fact that the detection of HPV infection does not distinguish between transient and progressive infection, HPV detection has a late prediction of the risk of precancerous lesions, a risk manifested over years. Practically, the HPV testing is able to identify women who may be at increased risk of cancer in the future without the presence of detectable cervical lesions.

The prevalence of the type of HPV infection is correlated both with the presence of PCL/CC and the presence of HPV in the healthy population [26]. The use of detecting the presence of the HPV infection and genotyping is extensive, and it offers extensive diagnostics. Currently, HPV DNA genotyping requires being the primary screening method in many national programs and is equally useful for co-testing together with the Pap test, and also for the triage of cases with equivocal cytology or low grade, surveillance of cases with abnormal cytological screening results with negative colposcopies and biopsies. Also, repeating the HPV testing is required for the prediction of the evolution after the treatment of CIN lesions. These recommendations are also joined by the need to check the persistence of HPV strains, identifying the prevalence of certain HPV strains to assess the overall impact of HPV vaccination.

Therefore, HPV genotyping is required as the primary screening method within national programs, but can be also significant in the triage methods of the progressive cervical lesions.

The benefits of primary HPV-HR screening consist in identification of cases assigned to risk groups, setup of time interval for new control, stating the diagnostic for lesions ≥ CIN2+ in a percentage significantly higher than that for Pap test. Another advantage is prolongation to 5 years for screening interval for tested cases with negative HPV-HR result.

The results of studies show that, by identifying the HPV-HR positive persistent cases with normal cytological result, the primary HPV-HR screening leads to increased number of colposcopy recommendations in order to get a presumptive colposcopic diagnostic.

Co-testing and triage of the ASCU-US and LSIL lesions

A delicate problem is the one related to the precancerous cervical lesions triage for the purpose of using methods to differentiate the lesions that spontaneously resolve from the progressive ones. This difficulty in diagnosis is exceeded by highlighting the immunocytological/immunohistological markers in cases with positive Pap Cytology for PCL. The benefits and limitations of these tests are revealed by the large studies results published

[25] Ho, Chih-Ming, Bor-Heng Lee, Shwu-Fen Chang, Tsai-Yen Chien, Shih-Hung Huang, Chiu-Cho Yan, and Weng-Fang Cheng. 2011. "Integration Of Human Papillomavirus Correlates With High Levels Of Viral Oncogene Transcripts In Cervical Carcinogenesis". *Virus Research* 161 (2): 124-130. doi:10.1016/j.virusres.2011.06.012.

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worldwide, as well as the following studies: ATHENA, HERMES, PALMS, KPNC, Compass Trail and Newsletter on Human Papillomavirus-HPV Today 2015, Study Pilot UK 2019. The first marker approved to be introduced in clinical practice for the identification and triage of PCL is represented by p16ink4a cyclin. This is a tumoral suppression protein that intervenes in adjusting the cellular cycle. Overexpression of the p16ink4a cyclin represents an indicator for the excessive presence of E7 viral oncoprotein, as well as other oncoproteins that inactivates pRB [27]. p16ink4a cyclin is a specific marker capable of identifying the dysplastic cervical epithelium, respectively CIN2+ lesions. The sensitivity and specificity of P16ink4a cyclin varies between 0.59 and 0.96, respectively 0.41-0.96 [28]. According to the diagnostic and supervision protocol algorithm of the PCL, positive testing for P16ink4A in ASCUS and LSIL cytology requires investigation completed by colonoscopy/biopsy [29]. Testing can be performed both on cytological sample and on histological sections. The benefit of immunoexpression identification of 16 ink4a cyclin consists in the fact that testing allows positive HPV cases identification. The New Technologies for Cervical Cancer Screening (**NTCC**) Trial, which compares the results of the HPV testing association with cytological testing versus singular cytological testing as primary screening method, concluded that women at age between 35-60 have a 6 time increased risk of developing CIN3+, respectively 4,7% vs 0,8% in cases with HPV positive/p16ink4a positive (HPV+/p16+) vs HPV positive/p16ink4a negative (HPV+/16-) [30]. Besides the mentioned benefits, P16ink4a testing has limitations related to the fact that individually morphological interpretation is necessary regarding the nucleus and cytoplasm appearance inside which the p16INK4a cyclin immunoexpression appears and also completing the Wentzensen score of quantification/intensity. So, interpreting the test involves subjectivity reported at cytologist's own experience of evaluating [31]. Among other limitations is the fact that p16INK4a test cannot select with clarity a real progress towards cancer, cannot distinct between high and low transformative risk HPV infections. Many studies conclusions revealed the fact that women that have been tested HPV+/p16+ require colposcopy immediately, meanwhile in cases with women tested HPV+/p16-

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second testing after 2 years is recommended[32]. P16ink4a cyclin testing limitations are exceeded by introducing in clinical practice 16INK4a/Ki 67 testing, (Dual test), statistically proven to have a better reproducibility and accuracy than P16ink4a testing.

Statistical data confirm that p16INK4a/Ki 67 testing has a greater sensitivity than Pap test in identifying cervical lesions associated with HPV-HR, while specificity remains identical to Pap test.

In the last years testing of HPV infection associated with cytological co-testing by dual test p16INK4a/Ki67 was established in clinical practice, thus enhancing the accuracy of the diagnosis. Double testing advantages consist in the fact that it allows triage of HPV positive cases, with identifying HPV positive progressive cases. Among other benefits of p16INK4a/Ki 67 double testing include automatic reading of the result, which cancels subjectivity in interpreting the results, as well as reducing the duration of cytologist training [33], [34]. Immunoexpression of the p16ink4a/Ki 67 dual test biomarker allows to identify high histological lesions of intraepithelial cervical neoplasia. Comparisons on the specificity of the p16ink4a/KI 67 test versus Pap test and HPV genotyping demonstrate that dual assay has specificity similar to PAP test but higher in relation to HPV DNA genotyping.

CONCLUSIONS

Primary screening in cervical cancer by HPV genotyping with Pap co-testing are endorsed by current biotechnologies so this becomes an adequate option, supported by statistical data.

Primary HPV screening associated with Pap co-testing increases the colposcopies number but mitigates inappropriate surgery.

The negative result of abovementioned tests justifies widening the screening time interval to 3-5 years

In the event that the primary screening was performed with Pap cytological test, the triage of ASC-US and L-SIL lesions is possible by conducting cell expression identification tests of p16ink4a immunomarkers or p16ink4a/Ki67 dual test.

A promising objective for research is to investigate which will be the effects of nonavalent HPV vaccine vs bivalent and quadrivalent HPV vaccines on the presence and progression of PCL.

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All authors report no potential conflict of interest.

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RESHAPING PURPOSE - USE OF METFORMIN IN ENDOMETRIOSIS AN APPROACH TO RECENT LITERATURE

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ABSTRACT:
ENDOMETRIOSIS CAN BE CLASSICALLY DEFINED AS THE PRESENCE OF A FUNCTIONAL ENDOMETRIAL LAYER WITH ENDOMETRIAL GLANDS OUTSIDE THE UTERINE CAVITY, INDUCING A CHRONIC, INFLAMMATORY REACTION, LINKED TO CHRONIC PELVIC PAIN AS WELL AS FERTILITY ISSUES. A LARGE AMOUNT OF LITERATURE SURVEYS AS WELL AS CURRENT STUDIES RECKON ENDOMETRIOSIS AS THE RESULT OF A DEFECTIVE IMMUNOSURVEILLANCE

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CASCADE IN FERTILE WOMEN, SUGGESTING THAT THE CONSTANT PERITONEAL INFLAMMATORY ENVIRONMENT STIMULATES PROGRESS AS WELL AS MAINTENANCE OF THE DISEASE. THEREFORE, NOVEL THERAPEUTICAL APPROACHES ARE NEEDED IN ORDER TO TARGET DIFFERENT PATHOGENIC SPECTRUMS. METFORMIN, A WIDELY USED BIGUANIDE IMPROVING INSULIN SENSITIVITY IN DIABETES, CAN ALSO PROVIDE BOTH ANTI-INFLAMMATORY PROPERTIES AS WELL AS A MODULATORY EFFECT ON OVARIAN STEROID PRODUCTION, TWO ACTIONS THAT HAVE BEEN SUGGESTED TO OFFER NEW PERSPECTIVES IN ENDOMETRIOSIS THERAPY. MANAGING ENDOMETRIOSIS REQUIRES TARGETING A COMPLEX NETWORK OF LOCALLY PRODUCED CYTOKINES MODULATING THE GROWTH AND INFLAMMATORY BEHAVIOR OF ECTOPIC ENDOMETRIAL IMPLANTS. THEREFORE, WHAT WE DEFINE AS THE MAIN OBJECTIVES OF THIS LITERATURE REVIEW BECOME AS FOLLOWING: TO ASSESS WHETHER METFORMIN MAY BE EFFECTIVE AS A NOVEL APPROACH FOR ENDOMETRIOSIS, DOSE-DEPENDENTLY OR NOT, AS WELL AS TO EVALUATE THE EFFECTS OF THIS AGENT ON INFLAMMATORY RESPONSES, ESTRADIOL PRODUCTION AND NEVERTHELESS ENDOMETRIOTIC STROMAL CELLS PROLIFERATION IN BOTH IN VITRO AND IN VIVO CONDITIONS.

KEY WORDS: ENDOMETRIOSIS, METFORMIN, IL-8, IL-6, IMMUNOSURVEILLANCE, VEGF

BACKGROUND:

It is a well acknowledged fact that endometriosis can be defined as the presence of functional endometrial glands and stroma within the pelvic peritoneum and other extrauterine sites, inducing a chronic inflammatory reaction linked to pelvic pain and infertility[10]. It is estimated that it affects 2–10% of women in the reproductive age group. Endometriosis is viewed to be a polygenically inherited disease of complex multifactorial etiology showing a wide range of immunological surveillance issues as well as a wide spectrum of cytokines to be involved[11]. Sampson's theory regarding the transplantation of endometrial tissue on the pelvic peritoneum via retrograde menstruation is the most widely accepted explanation for the development of pelvic endometriosis[12]. Since retrograde menstruation is observed in almost all reproductive-aged women undergoing menstrual bleeding, endometriosis is postulated to develop as a result of the co-existence of a defect in clearance of the menstrual efflux from pelvic peritoneal surfaces, raising questions of the immune system's integrity[13]. Alternatively, intrinsic molecular aberrations in

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pelvic endometriotic implants were proposed to significantly play a role in the development and progression of endometriosis, by initiating or sustaining inflammatory responses. Secretion of interleukin-6 (or even IL-8) from endometriotic cells has been proposed to be a driver of endometriosis progression[14]. Aberrant expression of aromatase, certain cytokines such as interleukin-6 present (IL-6) in the peritoneal fluid or even aberrant expressions of tissue metalloproteinases in addition to deficiency of 17-hydroxysteroid dehydrogenase (17- HSD) type 2 as well as resistance to the protective action of progesterone are just a few of the encountered molecular abnormalities[15]. Aromatase is the key enzyme for the conversion of precursor steroids to estrone and estradiol[16], whereas 17-HSD type 2 in endometrium metabolizes the biologically active estrogen termed estradiol to an inactive steroid termed estrone. Since endometriosis is an estradiol-dependent disorder[17], aberrant aromatase expression and 17-HSD type 2 deficiency in this tissue are of paramount importance in its pathophysiology[18].

The morphologic appearance of endometriosis is marked by proliferation, infiltration and severe adhesions around the surrounding tissues, explaining a wide range of clinical manifestations varying from pelvic pain to infertility[19]. Research into its pathogenesis has focused on anatomic, hormonal, immunologic and genetic factors[20], although the entire etiopathology has not been clearly explained yet. Medical treatments for endometriosis are usually aimed at reducing the endogenous steroid production. Medroxyprogesterone acetate, danazol, oral contraceptives and GnRH-a are all effective in the pain-associated symptoms of endometriosis[21] and are also effective in the regression[22] of the endometriotic lesions. However, their adverse effects limit their long-

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term use, and recurrence rates after cessation of therapy are high. Additionally, they have no benefit for endometriosis-associated infertility[23]. Therefore, new agents, which present synchronous fertility treatment with improved side-effect profiles, are needed. These treatments should also be as effective as hormonal treatments.

What needs to be taken into consideration are anti-inflammatory agents which may nurture greater therapeutic potential for the disease than actually expected[24].

THE METFORMIN EFFECT:

Metformin is one of the most widely used antidiabetic agents improving insulin sensitivity. In reproductive medicine, the drug has been successfully used for the treatment of polycystic ovary syndrome, known to be etiologically determined by insulin resistance[25]. In women with PCOS, metformin treatment restores the cyclic nature of menstruation and increases ovulation, fertilization and pregnancy rates[26]. The supposed mechanism of action of metformin in endometriosis is proposed to be a decrease in aromatase enzyme activity and inhibition of proliferation on endometrial glands[27]. Therefore, circulating estradiol levels are decreased in response to the increase in SHBG levels, thus reducing and affecting the dynamics of endometrial ectopic tissue[28].

Amongst other properties which may be eventually translated, metformin seems to reduce obesity-associated inflammatory status and other inflammatory responses, reducing serum C-reactive protein levels in women with polycystic ovary syndrome[29]. In addition, it has direct effects on steroidogenesis in ovarian granulosa cells and thecal cells.

An antiproliferative effect of metformin has also been demonstrated in leptin-stimulated vascular smooth muscle cells[30]. Abundant aromatase expression and elevated local estrogen levels

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suggesting local estradiol production by the aromatase enzyme have been demonstrated in endometriotic tissues[31], however lacking from disease free endometrium. On the other hand, metformin has been shown to inhibit FSH, insulin-stimulated progesterone and estradiol production in granulosa cells[32]. Thus, metformin may inhibit endometriosis through suppression of both ovarian and local production of estrogens.

ENDOMETRIOSIS PATHOGENESIS - A CLASSIC:

Endometriosis is characterized by inflammation, estrogen dependency, and proliferation of endometriotic cells[33]. The main targets we are going to focus on throughout our discussion, of paramount importance shall be steroidogenesis and inflammation.

A. STEROIDOGENESIS – TARGETING THE AROMATASE ACTIVITY

Among estrogen-responsive pelvic disorders, aromatase expression was studied in greatest detail in endometriosis. Firstly, extremely high levels of aromatase RNA[34] were found in extraovarian endometriotic implants and endometriomas. Secondly, endometriosis-derived stromal cells in culture incubated with a cAMP analog displayed extraordinarily high levels of aromatase activity comparable to that in placental syncytiotrophoblast[35].

Mullerian tissues are known targets of estrogen action[36]. Until recently, estrogen action has been classically viewed to occur only via an "endocrine" mechanism: in other words, it was thought that only circulating estradiol, whether secreted by the ovary or formed in the adipose tissue, could exert an estrogenic effect after delivery to target tissues via the bloodstream[37]. Several communication manners have been proven to function among aromatase pathways of expression[38].

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Studies on aromatase expression in breast cancer demonstrated that paracrine mechanisms play an important role in estrogen action in this tissue[39]. Estrogen produced by aromatase activity in breast adipose tissue fibroblasts was demonstrated to promote the growth of adjacent malignant breast epithelial cells[40]. There has also been demonstrated an "intracrine" effect of estrogen in uterine leiomyomas and endometriosis. Disease-free endometrium and myometrium, on the other hand, lack of aromatase expression[41].

Endometriosis should be accepted as an estrogen-dependent condition because it is seen during the reproductive years and generally disappears after menopause[42]. Medical therapy is often aimed at lowering the estrogenic and inflammatory status of endometriosis since they seem to be the main promoters of disease progress.

B. PROLIFERATION AND INFLAMMATION

Endometriosis is an estrogen-dependent disorder defined as the presence of endometrial tissue outside of the uterine cavity[43]. Although the pathogenesis of endometriosis still remains unclear, the most accepted theory assumes that the endometriotic implants originate by the migration of eutopic endometrial cells through retrograde trans tubal flow into the peritoneal cavity of menstrual debris, with future implantation and growth on an ectopic site[44]. Oxidative stress has been proposed as a potential factor involved in the pathophysiology and ectopic tissue attachment process.

Many factors are important for the degradation of extracellular matrix and the implantation of endometrial tissue in ectopic sites - notably, matrix-metallo-proteinases (MMPs)[45]. It has been shown that blocking MMP activity inhibits the formation of ectopic lesions in experimental models. Vascular endothelial growth factor (VEGF) has been implicated as inducer of attachment,

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proliferation, and neovascularization[46]. Moreover, antiangiogenic agents inhibited the growth of ectopic tissue by disrupting the vascular supply.

Uterine movement is suggested to play roles in various events related to endometriosis[47]. In view of the current concept underscoring the biological implications of mechanical stretch, we speculated that the mechanical stretch exerted by uterine movement might stimulate the production of biochemical mediators like IL-8 in endometrial cells and contribute to inflammation-associating processes, such as menstruation and endometriosis[48].

Endometriosis has a direct effect on adhesion formation. The inflammation associated with endometriosis, through increased levels of peritoneal fluid VEGF, may promote angiogenesis for progressive growth of endometriosis[49].

IL-1β-induced secretion of IL-8 from endometriotic cells has been proposed to be a driver of endometriosis progression. IL-8 levels are increased in the peritoneal fluid of women with endometriosis[50].

The basis for medical treatments of endometriosis is to reduce the endogenous steroid production[51]. However, long-term use of drugs such as progestogens[52] (oral or intrauterine device), danazole, GnRH-analogs, and aromatase inhibitors, is limited by their adverse effects, cost, limited treatment duration, delayed conception, and the high recurrence rates after cessation of therapy. Also, they have no benefit for endometriosis-associated infertility[53].

HOW DOES METFORMIN ACT AROUND THESE PROCESSES

Metformin is one of the oldest and most widely used oral agents in the treatment of type 2 diabetic subjects, with no effect on insulin secretion[54]. Metformin has not only been shown to reduce the risk for vascular complications but also has protective effects largely independent of its

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well-known antihyperglycemic action[55]. Besides showing antioxidant properties it shows beneficial effects on VEGF and MMPs[56].

IL-1β-induced secretion of IL-8 from endometriotic cells has been proposed to be a driver of endometriosis progression, being increased in the peritoneal fluid of women with endometriosis[57]. Interestingly, metformin has suppressed IL-8 release from human adipose tissue in vitro, and a recent report demonstrated that metformin inhibited IL-1β-induced release of IL-6 and IL-8 in human vascular wall cells[58]. Although we show here that metformin can inhibit IL-1β-induced secretion of IL-8 from ESCs, at the same doses, metformin did not inhibit secretion of IL-8 from eutopic endometrial stromal cells. Thus, metformin seems to exert its anti-inflammatory role by reducing proinflammatory cytokine secretion in specific cell types.

When it comes to endometriosis associated infertility, anti-endometriotic drugs usage may be beneficial for ovulation induction in these cases[59]. Aromatase inhibitors are effective in treating infertility, however displaying hypoestrogenic effects[60]. Metformin therapy for endometriosis may be more beneficial due to the lack of serious side effects reported with the other drugs[61]. Combined with the anti-inflammatory and anti-estrogenic effects of metformin in specific cell types, the direct anti-proliferative effects on stroma cells support its therapeutic potential for endometriosis[62].

The mechanism of action of metformin on endometriosis was proposed to be a decrease in aromatase enzyme activity in the endometriotic implants and suppression of ovarian

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steroidogenesis[63]. Interestingly, metformin has inhibited FSH and insulin-stimulated progesterone and estradiol production in granulosa cells. Metformin may inhibit endometriosis through suppression of both ovarian and local products of estrogens[64].

RESULTS &PARTICULARITIES OF METFORMIN USE IN ENDOMETRIOSIS

1. ISOLATION OF ENDOMETRIAL STROMAL CELLS IN CULTURES

In an in vitro study[65], it was shown that metformin, a biguanide insulin sensitizer, suppresses the inflammatory response, the activation of aromatase enzyme and the proliferation in endometriotic stromal cells after culture in a sterile medium.

a. Isolation and culture of human endometriotic stromal cells (ESCs)[66] has been done in order to obtain a specific measurement of IL-8 needed to assess metformin's effects on proinflammatory status. Preincubation with metformin significantly decreased IL-1β-induced IL-8 production in ESCs in a dose-dependent manner compared with controls. The maximal effect was observed at 1000 μM, but significant decreases were seen at 10 μM[67].
b. In the same culture, aromatase mRNA expression was assessed by real-time quantitative PCR[68]. Metformin decreased cAMP-induced aromatase mRNA levels in ESCs in a dose-dependent manner. The effect of metformin on aromatase activity was evaluated by measuring estrone levels in conditioned media of ESC cultured with androstenedione[69].
c. The effect of metformin on the proliferation of ESCs was examined by measuring incorporation of BrdU into DNA[70]. Measurement of lactate dehydrogenase (LDH) release and trypan blue exclusion test were conducted to examine the effect of metformin treatment on cell viability, as markers of cell proliferation inhibition.

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The present study of Takemura[71] et al demonstrated that metformin suppressed the production of IL-1β-induced IL-8, the activation of aromatase, and the proliferation of ESCs. These effects of metformin support its therapeutical potential in limiting the development of endometriosis.

2. HIGHLIGHTING ENDOMETRIOSIS - METFORMIN DYNAMICS IN RAT MODELS

In a rat model with surgically induced endometriosis, Oner et al[72] found that metformin and letrozole caused a statistically significant regression of endometriotic implants impacting levels of SOD, VEGF, TIMP-2, and MMP-9[73], findings which support that metformin treatment reduces the size of endometriotic implants in rats.

3. METFORMIN DYNAMICS IN HUMAN MODELS

The prospective study started by including 69 cases subdivided into 34 cases as a control group and 35 cases as a metformin treated group[74]. The patients were divided into the following groups:

1. Group-1 (treated endometriosis group)[75]: consisted of 35 infertile patients diagnosed by diagnostic laparoscopy to have stages 1–2 (minimal-mild, MM) endometriosis. These patients were complaining of one or more symptoms such as dysmenorrhea, pelvic pain, dyspareunia or menorrhagia. The severity of the disease was graded during previous diagnostic laparoscopy according to the revised four-stage scoring system of American Society of Reproductive Medicine (ASRM)[76]. At least 3 months following diagnostic

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laparoscopy, participants were instructed to take oral metformin 500 mg three times daily for 6 months starting with their next menses plus a multivitamin once daily[77].

2. Group-2 (non-treated endometriosis group): consisted of 34 cases with stages1–2 (minimal-mild, MM) endometriosis diagnosed by diagnostic laparoscopy and were complaining of one or more symptoms such as pelvic pain, dyspareunia or menorrhagia. These cases were enrolled in the study at least 3 months after the diagnostic laparoscopy, and received as placebo a multivitamin once per day during the 6 months follow up period[78].

The plasma levels of VEGF, IL-6, and IL-8 were analyzed by using ELISA kits performed according to the methods recommended by the manufacturer, samples were obtained for an estimation of the cytokines levels at the start of the study, in the morning from the fasting patients during the follicular phase (days 5–10) and also at the follow up visits after 3 and 6 months. There was a significant decrease in incidence of symptoms associated with endometriosis such as dysmenorrhea, pelvic pain and dyspareunia[79]. Moreover, and unique to metformin, there seems to be no impedance to ovulation, contrary to other current treatment options do not offer a chance at conception. In the present study, the % of pregnancy after 6 months of metformin therapy was 25.71%, and this was statistically significant ($P < 0.001$) when compared with the % after 3 months therapy, and with the % in the control group after 6 months[80]. The results of our study showed that the levels of IL-6, IL-8 & VEGF were significantly decreased after 6 months of metformin therapy ($P < 0.05$) when compared with the 3 months level[81]. This can be explained by the anti-inflammatory effects of metformin therapy to decrease the inflammatory process associated with the endometriotic implants.

DISCUSSIONS

Metformin therapy in patients with endometriosis resulted in a significant reduction in the symptomatic cases, increased chance of pregnancy, and a decrease in the levels of serum cytokines, suggesting that it may have a therapeutic potential as an anti-endometriotic drug.

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However, when it comes to other insulin sensitizers such as rosiglitazone and pioglitazone were found in vitro to decrease chemokine and cytokine expression in endometriotic stromal cells, and modulate angiogenesis[82], and also to induce regression of endometriotic lesions[83].

Moravek et al[84] provided a preliminary data about the effectiveness of rosiglitazone, a

insulin sensitizer, in treating endometriosis-related pain in six patients and concluded that it was effective and promising for usage in endometriosis patients desiring the chance to conceive.

CONCLUSIONS

What we wanted to emphasize throughout this review is that metformin, an easily available drug, facile to use orally, and of minimal cost proves to be a well-tolerated treatment for endometriosis that relieves pain, reduces menstrual disorders, and improves fecundity[85]. The findings of the cited study combined with the data gathered from experiments and endometriosis induction in rats and nonhuman primates, show promise for the potential of using metformin in endometriosis patients desiring the chance to conceive[86]. However, what would need to be thoroughly done in order to properly apply the results we currently have would be to reevaluate and manipulate large numbers of cases supported by diagnostic laparoscopies in the end[87].

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All authors report no potential conflict of interest.

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HPV AND BUSCHKE-LOWENSTEIN DISEASE

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ABSTRACT:
INTRODUCTION. HUMAN PAPILLOMAVIRUS (HPV) IS A VIRAL INFECTION WHICH GENERATES EPITHELIAL TUMOURS OF THE MUCOUS MEMBRANES AND SKIN ESPECIALLY WARTS. THE DIMENSIONS OF THE WARTS COULD VARY AMONG INDIVIDUALS FROM A FEW MILLIMETRES TO A CONSIDERABLE SIZE BEING CALLED GIANT CONDYLOMA ACUMINATUM (GCA). THIS GIANT CONDYLOMA ACUMINATUM WAS FIRST DESCRIBED BY BUSCHKE AND LÖWENSTEIN IN 1925 ON PENILE FORESKIN, NOWADAYS KNOWN AS BUSCHKE – LOWENSTEIN TUMOUR (BLT).
MATERIAL AND METHODS. THE PURPOSE OF THIS PAPER IS TO REVIEW THE AVAILABLE DATA REGARDING THE ETIOPATHOGENY OF BLT DIAGNOSIS AND NEW LINES OF TREATMENT. WE USED PUBMED AND SCOPUS DATABASES FOR REVIEWS AND FULL ARTICLES.
CONCLUSIONS. BUSCHKE – LOWENSTEIN IS A RARE DISEASE CAUSED BY INFECTION WITH HPV, ESPECIALLY ASSOCIATED WITH "SEMI – MALIGNANT " VERRUCOUS CARCINOMA OR SQUAMOUS CELL CARCINOMA. COINFECTION WITH HIV ENHANCE THE ONCOGENIC ABILITY OF HPV, BUT THE ONCOGENIC PROCESS IS SLOW, SO AN EARLY REMOVAL OF THE LESION WHEN NOTICED BY THE PATIENT IS USEFUL TO PREVENT A GCA. PRE-EXPOSURE AND POST-EXPOSURE VACCINES ARE VERY IMPORTANT IN PREVENTING BLT AND REDUCING THE VIRAL LOAD IN THE INFECTED.

Keywords: HPV, BUSCHKE-LOWENSTEIN, RARE DISEASE.

INTRODUCTION

Human papillomavirus (HPV) is a viral infection which generates epithelial tumors of the mucous membranes and skin. HPV is a nonenveloped, icosahedral virus, with a genome coresponding to all HPVs of approximately 8-kilobase pair molecule of circular, double stranded DNA[10]. In Romania, the main cancer screening measure is HPV vaccination, supported by the Public Health Ministry, but unfortunately parents are still reluctant to vaccinate their young girls[11]. The actual classification system for HPV is based on resemblance in genomic sequences and usually associated with the three major clinical categories:

- Anogenital or mucosal.
- Epidermodysplasiaverruciformis.
- Nongenital cutaneous.

The anogenital warts are a painless outgrowth on the skin surface, in most cases associated with human papillomavirus (HPV) infection subtypes 6 and 11[12]. Almost 80% of genital warts are usually correlated with low-risk HPV subtypes 6 and 11 but the high-risk HPV subtypes 16, 18, 52, and 56 are also implicated, coinfection occurring frequently, causing important morbidity in

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general population[13]. The Pap smear is a trustful screening test even during pregnancy, with abnormalities in 5-8% of the results[14]. Generally, the majority of warts are benign andregress after 18 months, but the dynamics between the virus and the immunologic response of the host can transform a long-standing wart in a malignant one[15]. The dimensions of the warts could vary among individuals, from a few millimetres to a considerable size, being called giant condylomataacuminatum (GCA). The giant condyloma acuminatum was first described by Buschke and Löwenstein in 1925 on penile foreskin[16]. GCA is rare, having a 0.1% incidence rate in general population, but on the other hand it has almost 50% recurrence rate[17]. There are also environment factors that increase the risk of GCA like: sex (male), smokers, beginningof sexual life at young age, poor hygiene, uncircumcised men.

ETIOPATHOGENY

Different therories are present in the literature regarding the malignant potential of this disease. One of them states that this is a disease with high risk of evolving in agressive squamous cell carcinoma (SCC). Georgi Tchernev et al concluded that the period of malignant transformation ranges between 3 to 5 years. After malignant transformation, it usually turns into more aggressive disease, which frequently metastasizes to the pelvic organs and regional lymph nodes. This category of patients has generally a bad prognosis in comparison with the patients that treat the disease in the early stages[18].

The second theory about this disease consider it a 'semi-malignant' verrucous carcinoma, due to their invasive local evolution[19]. Some authors describe Buschke-Lowenstein tumors (BLT) as a variant of verrucous carcinoma, while others authors consider it as a differentdisease[20]. Chu et

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al evaluated almost 40 cases of BLT in the literature and reviewed their management and behaviour. The tumour was reported to have a significant rate of recurrence and a risk of malignant transformation to squamous cell carcinoma (SCC), with no distant metastases. Trombetta et al noticed the presence of neoplastic foci in as many as 50% of the 52 reports of patients undergoing surgery for BLT[21].

Also, studies showed that the early stages of Buschke-Löwenstein tumours and genital warts have similar histopathological and clinical features. In advanced stages of BLT, ithas a specific clinical feature like: uneven surface, cauliflower like shape, white to yellowish colour. From the histopathologicalpoint of view, the tumour is characterized by: papillomatous formations, with acanthosis and hyperplastic epithelium, consisting in cells with hyperchromatic nuclei and pale cytoplasm, maintaining the basal membrane. In the literature, studies concluded that HPV 16 and 18 are frequently connected with thetransformation of the tumour to squamous cell carcinoma, while HPV 6 and 11 most often transform inverrucouscarcinoma[22]. Also, the routine methods of investigation, such as PCR or immunohistochemistry testing for HPV, are justifiedand very important for morphological confirmation of Buschke-Löwenstein tumours and especially for choosing the correct treatment option of these patients. These investigation methodsare of key importancefor starting the exact treatment for the less favorable, poorly differentiated variants of squamous cell carcinoma with potential for lymph node metastases.

PATHOLOGY

Sexually transmitted infection with HPV is characterized by the invasion the genital epidermal basal layer cells as a consequence of micro-abrasions. Human Papilloma Virus finalize its cycle outside the genital epithelial basement membrane, and the E7 gene of HPV decrease the capacity of the antigen presenting cells in the skin, facilitating the virus to stay hidden for extended periods of time sothe virusavoid the host immune response.In conclusion, E6 and E7 oncogenes damage the host cells to induce telomerase resulting in cellular immortalization of the infected cellsandcause chronic oxidative stress increasing the susceptibility of damaging the DNA and starting the way for carcinogenesis[23]. Studies concluded that coinfection with HIV/HSV-1 boost the oncogenesis and the patient positive for HSV-1 IgG beingvulnerable for carcinomatous transition[24]. Squamous cell clusters with keratinization among the condyloma stroma and increased mitotic activity in histopathological examination indicates nests of carcinomatous transformation. SCC of the anogenital area is a slow growing tumor, but associated with hypercalcemia suggests an aggressive phenotypic change and a poor prognosis.

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TREATMENT

The first choice of treatment for giant Buschke – Löwensteintumour is considered wide excision of the lesion. Surgery alone has good results - a disease-free status in 45% of patients[25]. The treatmentoption is influenced by several factors, including the size and thickness of the lesions, anatomic location of the lesions, quantity, HPV classification, immune-competentor immune-compromisedstatus but, no current treatment acquires complete HPV eradication[26].

More than that, oral and topical chemotherapy is used with success as adjuvants to surgery or as treatment for recurrences. Exemple of topical therapy alone, such as 5-fluorouracil or Interferon (IFN), are not sufficient to prevent progression or control the disease[27].

Topical cidofovir is also used as gel 1.5%, with results even in cases with no response to conventional treatment. A case reported in literature noted that bleomycin injected intralesional in the wound was effective, with visible results and with no recurrence at 2 years after treatment[28].

The theory that postulated the viral origin of these tumors made IFN a chimiotherapy agent for this etiology. A reported case of genital CGA argues that IFN 2-alfa can induce the apparent complete resolution after 6 months of treatment[29]. Intralesional administration was effective, with complete responses in 47-62% of cases. Recurrence is frequent, with a rate of 40%. Large lesions represent an indication for systemic administration of IFN.

An infiltrating condylomaacuminatum with giant dimensionssuffered a major response after 9 months of continuous treatment with IFN administration twice weekly, although changes in dimensions were not observed in the first several months.

Imiquimod, a topically-applied aminoquinoline that is an immune modulator that induces interferon production,was considerdeffective in combination with carbon dioxide laser ablation in unstable patients, who were unable to tolerate surgery, whose tumor was positive for HPV-6[30].

There are some reported cases that described the use of traditional systemic antitumor agents, such as bleomycin, cisplatin, leucovorin and methotrexate for patients with recurrence disease after multiple surgeries for CGA[31]. There were no evidences of active disese 1 year later. Use of mitomycin-C and 5-fluorouracil determined a tumor shrinkage in combination with

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radiotherapy. For vaginal CGA, use of Etretinat, a synthetic oral retinoid and photodynamic therapy represented a success.

Radiation therapy remains a controversial method of treatment. Few studies presented evidence of an aggressive behavior after radiation therapy, but more than a few cases in literature document resolution of small tumors after this type of therapy[32]. In some cases, of a patient with poor surgical risk, if it is necessary, a large dose of radiation minimizes the chances of mutation and may be effective. As an example, the literature describes a case ofa patient who remained disease-free when he was reevaluated at 20 months after therapy with radiation at 4500 cGy in 25 fractions[33].

Bulky tumors which have exophytic growth and tumor size more than 10 cm in their greatest diameter, have been shrunk with more types of therapy; preoperative hemoradiation, radical surgery[34], sometimes followed by reconstructive surgery, when necessary[35].

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A complete response was obtained after mitomycin C and 5-fluorouracil, administered simultaneously with radiation therapy 50.4 Gy on the tumor bed, and prophylactic radiation of the regional lymph nodes. For recurrent, initial bulky condyloma acuminata, autologous vaccines were prepared with condyloma cells. It was well tolerated and produced a good clinical response.

For all these series, after 1 year, the recurrence rates with various treatments were 50% for surgical excisions alone, 85% for IFN alfa, and 4.6% after excision and vaccination with autologous vaccines[36].

Despite cryotherapy, imiquimod, and surgical debulking, refractory and recurrence disease was successfully treated with combinations of systemic interleukin-2 and topical cidofovir[37].Another treatment combination consists of retinoid administered orally and intramuscular IFN-gamma. The result is a complete clearance in 3 months of a GCA in a 16-year-old girl[38].

CONCLUSIONS

In the literature, there are different theories about the potentiality of Buschke-Löwenstein tumours to be malignant. Sexually transmitted infection with HPV is characterized by the invasion the genital epidermal basal layer cells, as a consequence of micro-abrasions. The HPV oncogenes damage the host cells to induce telomerase, resulting in cellular immortalization of the infected cells, and cause chronic oxidative stress, increasing the susceptibility of damaging the DNA and starting the way for carcinogenesis. The first choice of treatment for giant Buschke - Löwenstein tumour is considered the wide excision of the lesion. Surgery alone have good results - a disease-free status in 45% of patients. The treatment option is influenced by several factors, including the size and thickness of the lesions, anatomic location of the lesions, quantity, HPV classification, immunocompetent or immunocompromised status, but no current treatment acquires complete HPV eradication.

Buschke–Lowenstein tumour is a rare disease caused by infection with HPV, especially associated with ”semi–malignant” verrucous carcinoma or scuamous cell carcinoma. Coinfection with HIV enhances the oncogenic ability of HPV, but the oncogenic process is slow, so an early removal of the lesion when noticed by the patient is useful to prevent a GCA. Pre-exposure and post-exposure vaccines are very important in preventing BLT and reducing the viral load in the infected patients.

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All authors report no potential conflict of interest.

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UTI TO INFANT - DIAGNOSTIC TRAPS

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ABSTRACT:
UTI IS ONE OF THE MOST DIFFICULT DIAGNOSES IN INFANCY, DUE TO CLINICAL MANIFESTATIONS FROM ATTENUATED TO POLYMORPHS.
THE CORRECT DIAGNOSIS RAISES A NUMBER OF PROBLEMS, STARTING WITH THE TECHNICAL ONES, THE CORRECT COLLECTION OF URINE CULTURE AND ENDING WITH THE ALGORITHM FOR DIAGNOSIS AND PREVENTION OF RENAL SCARS. THE STUDY CONDUCTED ON INFANTS SHOWS AN IMPORTANT WEIGHT OF UTIS FROM INFANTS' HOSPITALIZED INFANTS.
PROPER ANTIBIOTIC TREATMENT, AFTER THE RESULTS OF THE ANTIBIOGRAM, ENSURES HEALING, SUBSEQUENT SUCCESSIVE CONTROLS SHOULD BE INCLUDED IN THE FOLLOW-UP PROTOCOL AND RECURRENCE PREVENTION.

KEYWORDS: UTI, SUGAR, PREVENTION, ANTIBIOTIC.

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UTI assumes colonization with microorganisms in the urinary tract and association of clinical symptomatology. It described after location and clinical manifestations several categories; pyelonephritis, cystitis, asymptomatic bacteriuria (detection of bacteriuria in an insignificant titre and no associated clinical manifestations) or UTI with undetermined localization. By convention, a value of more than 10^5 bacteria / mm^3 was considered significant in order to establish the diagnosis of UTI[9].

Evolutionally, the described forms are with different terminologies - acute UTI (clinical manifestations and significant bacteriuria), recurrent UTI (repeated episodes with negative urocultures) and persistent UTI (positive uroculture even in the absence of symptomatology)[10].

All studies have revealed only a "predisposition" of the UTI according to gender: the female is more commonly affected, the explanations being inclusive of anatomical - short urethra, adjacent to the rectum. Certain physiological conditions (pregnancy involving compression in the ureters or bladder, relaxation and stasis in the pyelocaliceal system) or pathological (constipation with compressive mechanical effect or bacterial transparietal migration between the rectum and the bladder, create the premises for the appearance or recurrence of UTI[11].

In infants, the literature appreciates a double prevalence in girls versus boys (6.5% vs. 3.5%), much higher at 1-2 years (8.1% in girls vs. 1.9% in boys). In boys circumcised, the UTI frequency is low (approximately 10 times) less than uncircumcised boys.

Age of infant is an age that raises many traps toward correct diagnosis, treatment, evolutionary follow-up, clinical picture[12].

Symptomatology is nonspecific and polymorphic: fever, loss of appetite, agitation, vomiting, diarrhea, static or downward weight curve. Practically, UTI diagnosis can be diagnosis of exclusion, the illness mimicking other diseases (digestive, neuropsychic, flu itself), and less suggesting a kidney disorder. Another age specificity, linked to the anatomical aspect - a short, horizontal ureter, with an imperfect functional antireflux mechanism, predisposes the infant to the appearance of high UTI (pyelonephritis) with a high risk of kidney damage and scarring, vesicoureteral reflux being on the other hand the most common and most severe in this age.

Any flora apparently without a clinical cause in the infant leads to investigations for the UTI, the more the baby is younger (<2 months) and the symptoms associate dehydration and toxic condition[13].

There is, as an example of the first intention, LE test is a rapid and indicative test (leukocyte esterase-nitrite test), the confirmatory paraclinical exam is the urine culture that have to be properly aseptically collected, in compliance with the local hygiene rules.

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Again, infant age imposes limitations on urine culture collecting techniques, the lack of predictability of the mictional act, the reduced bladder capacity, the imprecise temporal location of the first urine "in the morning" makes it quite improbable to use the midstream clean-catch method, possible and reliable technique for older children and adults.

Other collector possibilities are: sterile collector (plastic bag attached to the perineum), collecting pads (Euron, urial), bladder catheterization or suprapubic bladder puncture. There are limitations and inconveniences of each procedure: sterile bags must be handled by specialized personnel, attachment to the perineum is done after hygiene and rigorous disinfection of the area immediately after collected the sterile container with the pathological product sample must be sent to the sowing laboratory, or improper handling of the container results in a very high risk of contamination and, consequently, false positives, collector swabs present the same high risk of contamination, iar cateterismul vezical si punctia vezicala suprapubiana sunt masuri invazive, primite cu reticenta de catre parinti, dar din punct de vedere logic, prin accesarea directa a vezicii urinare, par cele mai fiabile metode de recoltare sterila a urinii. Disinfection of the skin, the puncture guided by a trocar placed suprapubian assumes a small degree of urine retention, the incidents can accidentally affect the intestine and can trigger a peritoneal sowing under low immunity conditions characteristic of the young age.

Bladder catheterization is usually performed in urology; the catheter insertion is associated with increased risk of infection.

There is no infallible collection method to ensure this "gold standard" indispensable to the UTI diagnosis – urine culture. Although the invasive measures appear to be more reliable and less affected by false positive results, rarely resort to these (psychological background of the parent "injury" of the child, necessity of maneuvers in specialized services, etc.)[14].

The most commonly used methods in the order of use are: sterile urine bags or urine collector for the "midstream clean-catch" method. The risk of contamination of the sample at the time of collecting is still high and the number of false positives is increased with the inevitable consequences - antibiotic abuse, some with nephro / ototoxic potential, intestinal microbial alteration, prolonged diarrheal episodes, stagnation or weight loss, impairment of status nutritional and biological somatic of the infant's immature organism[15].

A 12-month study was performed in infants hospitalized in the Pediatric Clinic with unclear symptomology, inconclusive for UTI (fever, vomiting, anorexia, growth failure, diarrhea, etc.), by performing urine cultures by the perineal adhesive bag method.

Of the 106 confirmed cases, out of a total of 430 cases of infants hospitalized in one year, at the repeat of urine cultures, after 24 hours, by the same technique, 82 cases were found to have significant bacteriuria (> 100,000 cfu/ml), the remaining 24 cases being counted as false positives, the percentage being significant.(Figure 1)

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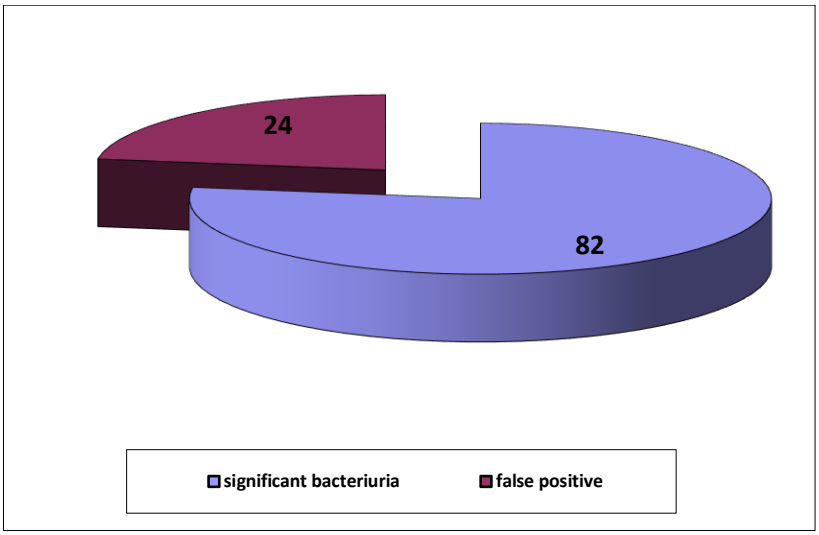

Figure 1

In all 82 cases of UTI, antibiogram were performed and the etiology was varied: 56 cases presented E. coli, 10 cases Klebsiella, 9 Proteus, 6 Pseudomonas, 2 Enterococcus, 3 Staphylococcus aureus, 1 group B Streptococcus. B. (Figure 2)

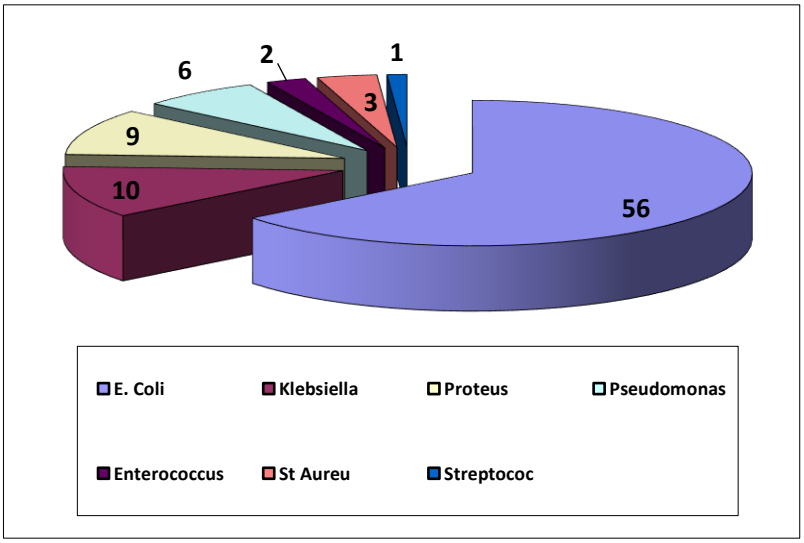

Figure 2

Antibiotic treatment started with monotherapy, in 9 cases it was necessary to supplement the initial schedule with a second antibiotic, usually an aminoglycoside. According to the antibiogram, the antibiotics used were Ampicillin (8 cases), Cefort (35 cases), Sulcef (24 cases), Cefuroxime (7 cases), Gentamicin (4 cases) and Amikozit (17 cases), including dual therapy. (Figure 3)

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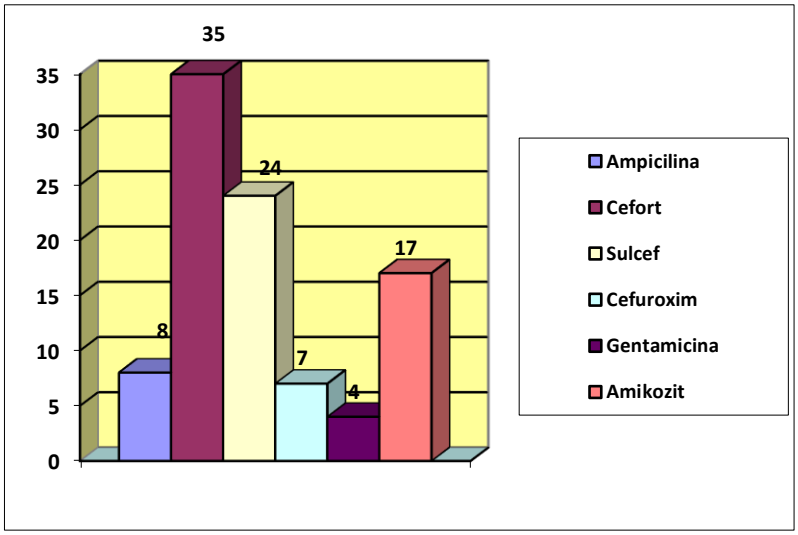


Figure 3

Follow-up urine cultures on the 2nd to 3rd day of treatment demonstrated the absence of significant bacteriuria in 38 infants (46.34%), at 7 days of treatment all urine cultures were negatively.

The recurrence was recorded in 21 cases in the first 6 months, 7 of which complementary imaging methods (mictional cystography, renal ultrasound) revealed malformative changes (hydronephrosis, vesicoureteral reflux). (Figure 4)

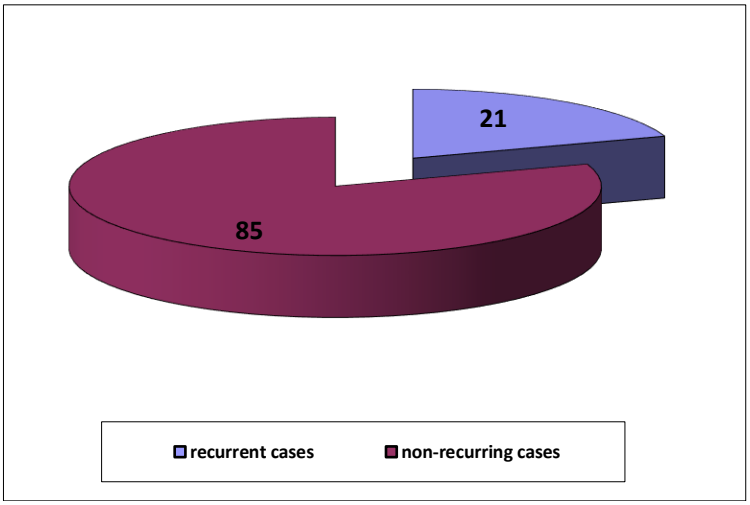


Figure 4

DISCUSSIONS

The UTI diagnosis, especially in the case of recurrence, should be complemented by imaging exploration with the aim of discovering, localizing and choosing the optimal therapeutic method for the resolution of urinary tract malformations.

Ultrasound is used, which can highlight hydronephrosis and megaureth or massive ureteral reflux.

Ureterocele, changes in renal ecogeneity or renal parenchymatous index

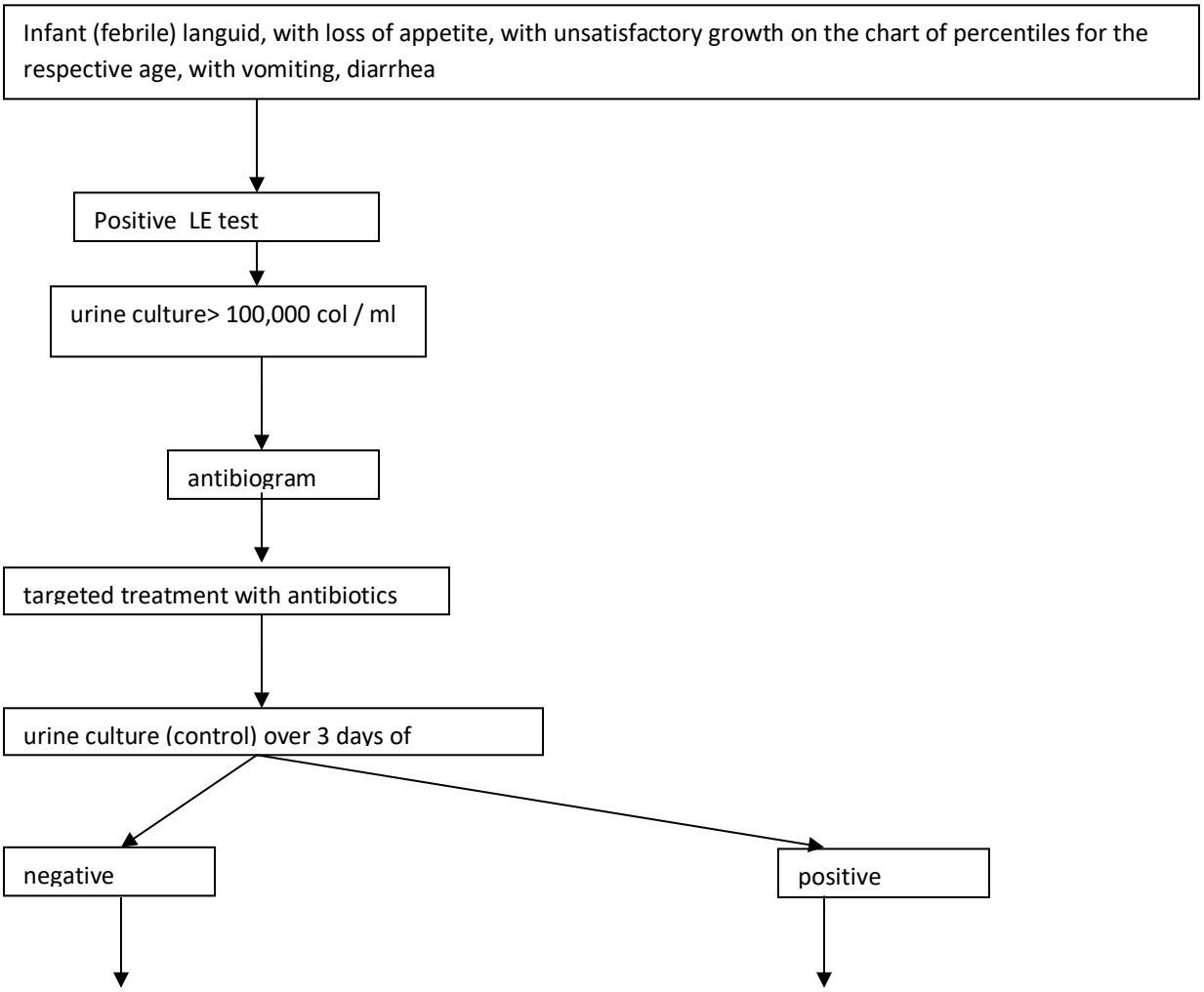
Mictional ureterocistography is the method of choice for vesicoureteral reflux, anatomic particularities cause over-50% of infants a low-grade vesicoureteral reflux, children with significant reflux have a 10-fold greater risk than non-reflux renal scars. The imaging method is performed with a contrast substance to increase the accuracy of the diagnosis, with a child in afebrility and having sterile urine culture to not advance to the kidneys.

Renal scintigraphy with dimercaptosuccinic acid indicates alterations of renal parenchyma and is recommended for recurrent UTI in infants 4 months after the acute episode.

Urography iv is not a recommended method in infant imaging investigations.

But in situations where dilation of the ureter or basin is evident, the ureterocystography does not specify reflux, urography may indicate megaurether by obstruction at the ureteral bladder junction or dilated basin through the ureteropelvic junction syndrome.

A diagnostic algorithm for UTI in infants could be:



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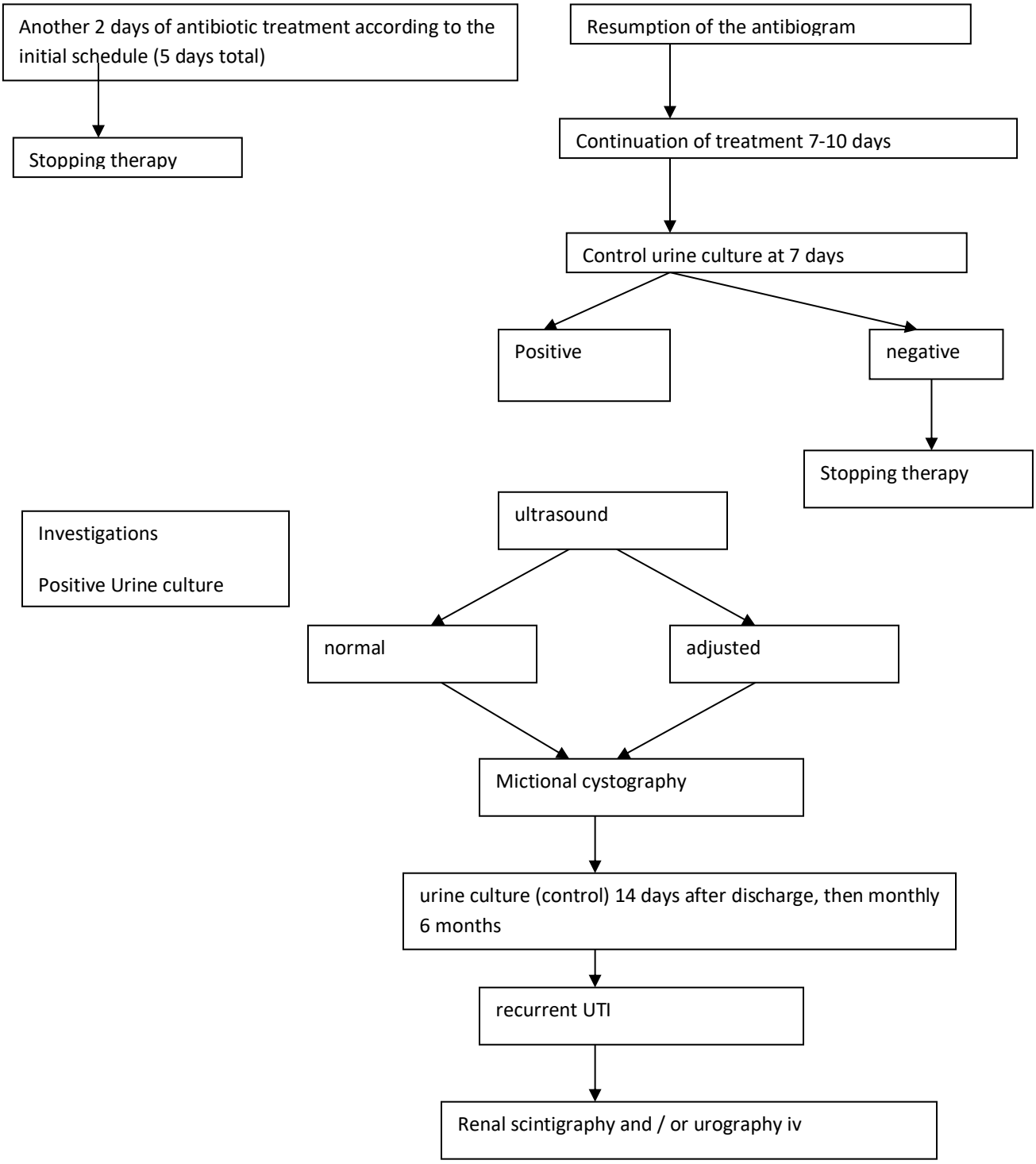

This algorithm could greatly restrict recurrences, implicitly repeated pyelonephritis with a high risk of definitive renal scarring. In case of malformative injuries, it would place the correct indication and operator time.

CONCLUSIONS

1. UTI in the baby is a difficult diagnosis, often established after exclusion of another pathology, a baby with fever and / or growth failure will necessarily have to be investigated for a possible UTI.
2. Uroculture remains the "gold standard" test for diagnosis provided it is performed correctly, respecting the aseptic rules.
3. Clinical study shows an important percentage of UTI in total infant hospitalization
4. Negative urine cultures after 2-3 days of antibiotic treatment can be interpreted as false positive initial results, transient or asymptomatic bacteria.
5. Always antibiogram dictates correct treatment, poor or absent response to an antibiotic where the germ is susceptible to the antibiotic shows "in vivo" in vitro variability response and indicates initial antibiotic review or association with a second antibiotic.
6. Complementary imaging methods are indispensable for complex diagnosis and evidence of a underlying UTI malformation
7. Simulating a simple diagnostic algorithm reduces recurrence and therefore reduces the risk of scarring.
8. Complete re-establishment of the biological and nutritional status of a baby with UTI is achieved over a minimum of 2-4 weeks of antibiotic cure, with a vicious circle of infection-catabolism.

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ALTERNATIVE TREATMENT IN ENDOMETRIOSIS

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ABSTRACT:
ENDOMETRIOSIS IS A CHRONIC DISORDER CAUSING REDUCED QUALITY OF LIFE MAINLY DUE TO CHRONIC PELVIC PAIN. MEDICAL AND SURGICAL TREATMENT OPTIONS ARE NON-CURATIVE AND SOMETIMES HAVE ADVERSE EFFECTS. IN RECENT YEARS ALTERNATIVE TREATMENT HAS BECOME MORE AND MORE POPULAR DUE TO ITS FEWER SIDE EFFECTS. FRUIT AND VEGETABLE CONSUMPTION, ESPECIALLY ORGANIC, RED MEAT AND OMEGA 3 FATTY ACIDS HAVE SHOWN IMPROVEMENT IN THE PROGRESSION OF THE DISEASE. NATURAL SUPPLEMENTS SUCH AS SILYMARIN, MELATONIN, EVENING PRIMROSE OIL AND CERTAIN VITAMINS ARE ASSOCIATED WITH DECREASED LEVELS OF CHRONIC PELVIC PAIN. REGULAR PHYSICAL ACTIVITY AND ACUPUNTURE REPRESENT A FEASIBLE APPROACH IN THE LONG RUN FOR THE ALLEVIATION OF CHRONIC PELVIC PAIN.

KEYWORDS: ENDOMETRIOSIS, DIET, VITAMINS, PHYSICAL EXERCISE, ACUPUNCTURE

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INTRODUCTION

Endometriosis is a chronic gynecologic disorder affecting nearly 10-15% of child-bearing age women. It is strongly associated with a reduced quality of life due to its sometimes debilitating symptoms such as dysmenorrhea, dyspareunia, chronic pelvic pain, irregular bleeding and infertility. The most frequent locations for endometriotic lesions are the ovaries and pelvic peritoneum, while deep infiltrating endometriosis alters the anatomy of the uterosacral ligaments, pouch of Douglas, rectum and lower urinary tract. However, rare locations have been described in case reaports, such as cerebral, pulmonary and even umbilical, as a primary location[5].

Optimal evaluation of endometriotic lesions is crucial for choosing the appropiate treatment plan. Clinical examination and transvaginal ultrasound are readily available and suitable tools for diagnosis. Endometriomas are easily evaluated with transvaginal sonography, but it has lower sensibility and specificity when it come to deep infiltrating endometriosis. Several studies have pointed out the higher accuracy in evaluating the posterior pelvic compartment of the sonovaginography with ultrasound gel. Colorectal endometriosis cannot be detected at physical examination, especially when the lesions are extented above the sigmoid. But recently the evaluation of the small and large bowel endometriosis is possible with the use of magnetic resonance imaging in combination with computed tomography-based virtual colonoscopy[6].

Treatment options available today, both medical and surgical, focus mainly on chronic pelvic pain and infertility, but are non-curative and often lead to recurrence after cessation or adverse reactions. The long term hormonal treatment often prescribed is progesterone, which is used only in order to avoid relapses. Several studies have revealed the importance of the immunohistochemical profile of the endometriotic lesion in order to assess the pathogenesis, therapeutic intervention and probability of relapse. Once again it is stated the importance of treatment individualization in endometriosis[7]. Current guidelines do not offer precise

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recommendations regarding the best treatment. Studies in the last decade are focusing upon lifestyle changes such as diet, vitamin and antioxidant supplementation and even acupuncture.

THE INFLUENCE OF DIET

Diet is considered an important risk factor for the development and progression of many chronic diseases such as hypertension, diabetes, cancer, but in recent studies it was also connected with endometriosis. Nutrient deficient diet interferes with DNA methylation, oxidative stress and result in epigenetic abnormalities, overexpression of estrogen receptor β and steroidogenic factor 1 leading to increased estradiol and prostaglandin levels, inflammations and cell growth[8].

One of the path studied was the relationship between fruit and vegetable consumption and the risk of endometriosis. These are not only a source of antioxidants, but they also promote estrogen excretion. A large prospective ongoing cohort study, the Nurses' Health Study II, began in 1989 and included 116429 registered nurses aged 25-42 years who completed a questionnaire regarding food intake among other lifestyle, demographic and environmental information. Overall a greater intake of fruits and vegetables, especially >5 portions/day was associated with 18% lower risk for developing endometriosis in comparison with an intake of <2 portions/day. Even more, women eating up to 3 portions of fruits/ day had a 14 % lower disease risk than those consuming <1 portion/day. This was particularly observed with citrus fruits, especially oranges – 22% lower risk. In terms of vegetable consumption, there was no correlation with the risk of endometriosis. However, more than 1 portion/ day of cruciferous vegetables (cabbage, broccoli, cauliflower) lead to a 13% higher risk of endometriosis[9]. However fruits and vegetables rich in fiber and nutrients contribute to estrogen excretion, leading to hormonal regulation[10].

The same aspect was pointed out in a study by Parrazini et al in 2004. This case-control study of 504 women aged 20-65 years revealed a lower risk of developing endometriosis in women consuming large amounts of fruits and green vegetables[11]. In 2011 a study by Trabert reached a

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different conclusion: >2 portions/day of fruits was associated with a higher disease risk. This opposite result was explained by the presence of pesticides in fruits which are responsible for an increased level of oxygen reactive species, diminishing their antioxidant effect. However the consumption of fruits should not be discouraged, more than that, the organic fruit consumption should be preferred[12].

The same cohort from the Nurses' Health Study was analyzed to study the relationship between dietary fat consumption and endometriosis. Palmitic acid, trans-unsaturated fatty acids (fried foods, margarine, crackers) and diets rich in animal fat were associated with a greater risk, specifically a risk of 80% with palmitic acid intake. But long term intake of Omega 3 resulted in a lower risk of laparoscopically diagnosed endometriosis[13]. Omega 3 fatty acids reduce inflammation through prostaglandin E3 and E3α thereby improving dysmenorrhea, while Omega 6 fatty acids promote inflammation through prostaglandins E2 and F2α. A study from 2009 revealed less pain and inflammation after surgical management of endometriosis in women taking Omega 3 supplements[14].

Alcohol seems to be a risk factor while caffeine consumption has not been clearly demonstrated. A study showed that caffeine increases estrogen and sex hormone binding globuline levels[15].

A beneficial effect was linked with the consumption of low fat dairy products. A study from 2013 revealed that women consuming >3 portions/day had a 18% lower risk of developing endometriosis than those consuming 2 portions/day. This is also in connection with vitamin D plasma levels, which are inversely related with endometriosis occurrence[16].

The influence of red meat, poultry, fish and seafood was investigated in several studies. Red meat was found to be have a major role in the development of endometriosis. It seems that the effect of red meat is independent of animal fat. The study conducted by Yamamoto found an increased risk in women eating >2portions/ day and a lower risk with 2-4 portions/ week , while replacement of red meat with fish or seafood led to a lower risk. One possible explanation for the detrimental effect of red meat is the heme iron content, which causes DNA damage and increases oxidative stress. Secondly, an intriguing fact is that women consuming larger amounts of red meat

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are expected to be overweight or obese because of the higher caloric intake which is contradictory to the literature which states that lower BMI is a risk factor for endometriosis[17]. Thirdly, red meat consumption increases estrogen levels and promotes inflammation through arachidonic acid. This omega 6 polyunsaturated fatty acid stimulates the synthesis of prostaglandins and leukotrienes[18].

NUTRITIONAL SUPPLEMENTS

Vitamins A, C, D and E provide antioxidant effect leading to reduced inflammation and antiproliferative action. Analyzing the population from the Nurses' Health Study it appears that only food rich in these antioxidants (not supplements) lead to a lower risk of endometriosis. The same conclusion was reached by Mier-Cabrera who found an improvement in antioxidant markers after a four-month antioxidant rich diet[19].

The consumption of vitamin B1, B9, C and E solely from food sources is inversely related to endometriosis risk. Vitamin C and E lower free radical and reactive oxygen species levels, which are involved in the proliferation and adhesion of endometrial cells in the peritoneal cavity. Vitamin B6 intensifies the estrogen metabolism into an inactive form and converts the linoleic acid into gamma linolenic acid, thereby increasing the level of anti-inflammatory prostaglandins[20].

Vitamin D suppresses COX-2 expression in the endometrium, thereby reducing IL-6 and prostaglandin levels, as well as prostaglandin receptor expression. A dose of 300,000 UI of vitamin D administered before the menstrual cycle led to a reduction in pain and nonsteroidal anti-inflammatory use in women with dysmenorrhea during a two month study[21].

Vitamin C is also known for its antioxidant, anti-inflammatory and angiogenic effects. In an animal model study from 2013, administration of intravenous vitamin C was associated with a significant reduction in endometriotic implant volume compared with the placebo group. The same beneficial effect was observed upon endometriotic cysts. Oral supplements with high doses of vitamin C led to a significant reduction in endometriotic cyst volume and weight[22]. In another

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study the vitamin C effect was greater among smokers, than non-smokers. Controversly, mokings found to have a protective effect upon endometriosis through the reduction of estrogen levels[23].

A randomized placebo-controlled study conducted in the USA evaluated the effects of vitamin E (1200UI) and vitamin C (1000mg) compared with placebo during 8 weeks. The results showed a reduction in chronic pelvic pain, dysmenorrhea and dyspareunia (43%, 37% and 24% respectively) compared with the placebo group. There was also a reduction in inflammatory markers in the peritoneal fluid at the end of the treatment[24].

Magnesium and phosphorus intake have also been studied in relation with endometriosis. Magnesium has a beneficial effect upon retrograde menstruation by reducing the smooth muscle contractility of the fallopian tubes. Low magnesium levels were found in women with history of miscarriages and with premenstrual syndrome. But high magnesium and phosphorus intake have inverse relationship with endometriosis risk and are associated with lower levels of inflammatory markers in the peritoneal fluid, such as IL-6 and TNFα[25].

Evening primrose oil is a well known natural remedy for breast pain, premenstrual syndrome, but also for endometriosis and irritable bowel syndrome. Its effect is based upon an omega-6 fatty acid, gamma-linolenic acid which is converted into anti-inflammatory prostaglandins. So evening primrose oil relieves menstrual cramps, reduces breast tenderness associated with premenstrual syndrome, irritable bowel flare-ups and controls endometriosis-associated inflammation. However there can be minor side effects, such as headaches and nausea[26].

Silymarin is an extract from the dried fruits of milk thirstle, is well known for its antioxidant effect and is used mainly in case of toxic liver damage or as an adjunct therapy in chronic hepatitis and cirrhosis. In animal model studies it has proven to be a cell membrane stabilizer, thereby inhibiting cells' damage. In a recent article from 2018 published in the Taiwanese Journal of Obstetrics and Gynecology the effect of silymarin, cabergoline and letrozole was studied on induced endometriosis in a rat model. There were four study groups divided upon their medication, the fourth group being the control one. After 3 weeks the results revealed that the mean volume of the implants and the histopathological score decreased significantly in the silymarin, letrozole and cabergoline group, respectively, compared to the control group. The total antioxidant activity was also measured in the serum and peritoneal fluid and was found to be higher in the silymarin group[27].

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The effects of silymarin have also been studied in the field of infertility. Beneficial evidence was revealed with regard to early granulosa cell apoptosis[28] as well as protective effects on ovarian reserve and sperm motility[29]. There are no side effects associated with the use of silymarin.

Melatonin is particularly used a sleep supplement and is naturally produced by the pineal gland at night. Recently, melatonin is also used for analgesic, antioxidant and anti-inflammatory properties. In a study of 40 women with chronic pelvic pain, aged 18-45 years, the administration of 10mg of melatonin daily for 8 weeks produced remarkable results: overall 80% reduction in the need for analgesics, reduced chronic pelvic pain by 39.80%, reduced dysmenorrhea by 38.01%, reduced dysuria and dyschezia and, of course, improved sleep quality[30].

A study published in the Journal of Reproductive Medicine revealed the beneficial effect of pycnogenol, a French maritime pine bark extract, as a natural remedy for endometriosis. 58 women surgically diagnosed with endometriosis were followed at 4, 12 24 and 48 weeks after conservative surgery. 32 women were included in the pycnogenol and took 60 mg/ day for 48 weeks and 26 women were administered gonadotropin-releasing agonist. Results showed that women taking pine bark extract experienced a 33% reduction in pain, including severe pain, which persisted without relapse, unlike the gonadotropin-releasing group[31].

PHYSICAL ACTIVITY

Regular physical activity is known to have a protective effect in the progression of diseases which have an inflammatory component such as type 2 diabetes, inflammatory bowel disease, colon and breast cancer and so is the case of endometriosis. Physical exercise performed on a regular basis has numerous valuable effects: increases the level of cytokines with anti-inflammatory and antioxidant properties, increases sex-hormone binding globuline level, thereby decreasing estrogen's bioavailability, reduces insulin resistance and hyperinsulinemia, reduces menstrual flow[32].

Although studies available regarding the beneficial effect of physical activity as a risk factor or as a part of treatment plan are scarce a few conclusions have been drawn: the positive effect is limited to women starting physical exercise before 26 years for minimum 2 hours/week,

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and as a prevention method, intense physical exercise should not be performed in the menstrual period. Of particular interest for women taking gonadotropin-releasing agonist treatment, physical activity is an additional therapy in the recovery of bone density[33].

Yoga, defined by the World Health Organization, as a mind and body practice is able to reduce stress, strengthen musculature and alleviate symptoms of numerous chronic diseases through a combination of contemplative techniques, breathing and meditation exercises and specific postures. A study which included 15 women with symptomatic endometriosis who attended yoga practice for 8 weeks reported an overall positive effect upon pelvic pain, in addition to improved self-awareness and self-esteem[34].

ACUPUNCTURE

Acupuncture implies the insertion of fine metallic needles into the skin at specific sites and the Chinese traditional medicine promotes it as a technique for balancing the Yin and Yang, strengthening body's resistance to disease. It has the ability to interfere with pain mechanisms, inducing changes in the central nervous system leading to a reduction of perceived pain level. One of the mechanisms is the release of endogenous opioids in brain stem, subcortical and limbic structures leading to obstruction of brain structures involved in the transmission of pain and activation of endogenous descending pain inhibitory pathways. And secondly, acupuncture prompts production of cortisol and adenocorticotrop hormone from the pituitary gland leading to an anti-inflammatory effect[35].

Although it is still a controversial technique, is it more and more used as a long term pain management option with no adverse effects when performed by a skilled therapist. A meta analysis published in the Journal of Pain Research included 99 women diagnosed with endometriosis who underwent acupuncture. The number of treatment varied from 9 to 16, the needle retention time was 15-20 minutes and the acupuncture points were the lower pelvic region and back, lower abdominal area, hands and feet. After treatment, all women reported lower pain score levels, increased health-related quality of life and decreased analgesic intake and noticeable stress level[36].

PSYCHOLOGICAL AND SEXUAL INTERVENTIONS

Endometriosis has a long-term negative effect upon women's psychological health and quality of life. The multidisciplinary team involved in the treatment of these patients should also include a psychologist and a sexual therapist. Due to its chronic status, numerous and complex therapeutic options, uncertain prognosis and threat of infertility, women with endometriosis

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develop anxiety and depression which could negatively interfere with the course of treatment. Mindfulness psychological interventions, individual or group session, counseling, patient education taking into account all aspects of female sexuality, all lead to an overall long-lasting improvement on health-related quality of life[37].

CONCLUSION

Endometriosis is a chronic condition with a multifactorial etiology which benefits from a complex treatment, both medical and surgical. More and more attention is drawn nowadays to lifestyle and dietary interventions. The consumption of fruit and vegetables, especially organic, has a beneficial impact on the progression of the disease. Also, natural supplements, dairy products rich in calcium and vitamin D and omega 3 fatty acids decrease the risk of endometriosis.

Therapies aiming at diminishing pain such as yoga and acupuncture show promising results, but more randomized studies are necessary to evaluate different treatment strategies in order to maximize the benefits of these alternative medical treatments.

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All authors report no potential conflict of interest.

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Medicine

MICROBIOME - REALITY OR MYTH?

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ABSTRACT:
THE HUMAN MICROBIOME, AS A WHOLE, AND DUE TO THE COMPLEXITY OF THE GENE, IS THE ONE THAT MAKES THE MAJOR DIFFERENCE BETWEEN INDIVIDUALS OF A SPECIES, OUR VARIABILITY, AS REPORTED ONLY ON ITS OWN GENOME, WAS ESTIMATED TO BE 0.1%.
THE STUDY OF THE INFANT GROUP OF PROBIOTICS IN THE CONTEXT OF AN EPISODE OF DIARRHEAL DIARRHEA INDIRECTLY DEMONSTRATES THE IMPORTANCE OF HEALTHY MICROFLORA BY: RAPID NUTRITIONAL RECOVERY, REDUCING THE DURATION OF HOSPITALIZATION, REDUCING THE RISK OF SUBSEQUENT RECURRENCE.
BUCCOPHARYNGEAL RECOLONIZATION WITH SAPROPHYTIC BACTERIA SALIVARIUS STREET HAD A POSITIVE ASPECT IN RELIEVING SYMPTOMS, RESTORING THE NORMAL PHARYNGEAL MACROSCOPIC APPEARANCE, AVOIDING ANTIBIOTIC THERAPY.

KEY WORD: MICROBIOME, VARIABILITY, PROBIOTIC STR. SALIVARIUS.

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The notion of microbiome was introduced by one of the pioneers of molecular biology Joshua Lederberg, in 1958 to name the entirety of microbial microorganisms, their genetic elements and their interactions in an environment.

Practically, the human body carries 10 times more microbial cells than its own constitutive cells, and the number of microbial genes is 150 times larger than the genes of the human body. Our symbiosis with our own microbe gives birth to a unique hybrid organism[10].

With the revolution of the new theories, a greater importance was given to epigenetics, considering our inherited genetic baggage is just a cornerstone, interacting with environmental factors, activating gene behavior and modifying our structure and adaptation to the environment[11].

There is an abundance of studies in recent years that demonstrate the early epigenetic intervention in the preconceptional stage and microbial modeling ever since birth. Maternal hygiene, eating habits, preconception pathology, diet or stress during pregnancy, influences the component of its own microflora.

The act of birth is decisive for the "choice" of the initial bacterial baggage. Naturally born children colonize the existing bacteria at the level of the mother's vagina, while the majority flora is represented by different species of lactobacillus (sp and harbor, L. crispatus, L. inners, L. jensenii, L. gasseri) which develops under strict anaerobic conditions[12].

There are racial variations with regard to a certain bacterial preponderance: the white race and the Asians have a higher predominance of lactobacilli than the Hispanic or Blacks. Lactobacillus sp. predominates in Europeans and L. inners in African and Hispanic.

Even the pH varies depending on ethnic differences: 4,7-5 to the Hispanic and black race and 4,2-4,4 to the Asian and the White race.

The appearance of vaginal dismicrosms, vaginosis or vaginitis involves local microbial colonization, with the breakdown of the local defense barrier. By the act of birth the newborn can colonize digestive and tegumentary with specific flora (Gardnerella vaginalis, Sneathia, Eggerthella, Peptoniphilus, Prevotella, Anaerococcus, Atopobium, Mobiluncus, Finegoldia) or even E. coli and Neisseria gonorrhae. Cesarean-born babies colonize with bacteria from the mother's skin. Normal skin is colonized by Propionibacterium (anaerobes in hair follicles), Pitysporium and aerobic coci (Staphylococcus, Micrococus, S. hominis, S. epidermitis, S capitis, S colonies, S haemolyticus, S saprophyticus, A warnerii, Corynebacterium spp. bovis, C minutissimum, C xerosus, C hofmani, Brevibacterium spp, Propionibacterium acnes, P granulosum, P avidum)[13].

There are also racial differences here. S aureus is more common in white children than in blacks.

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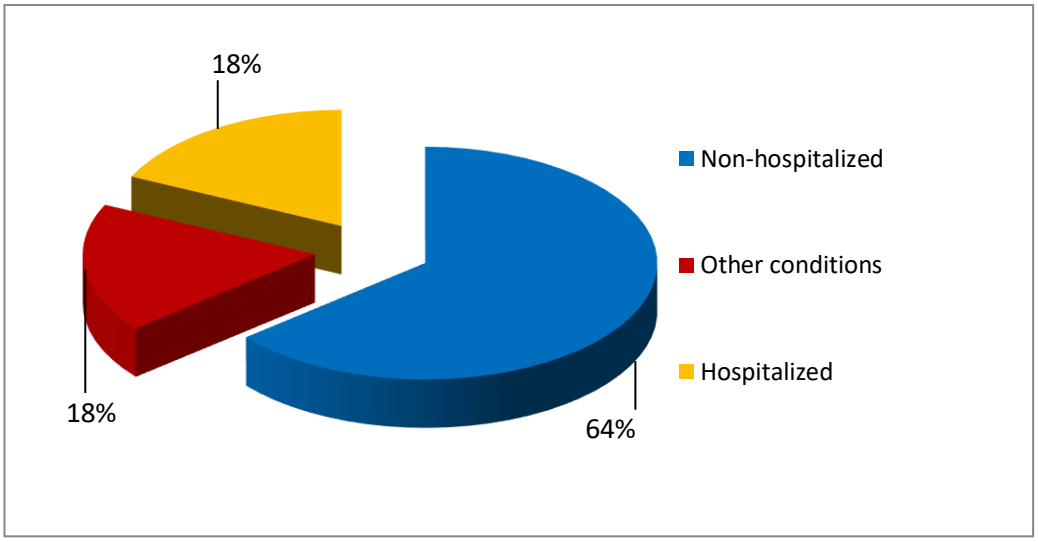
Tegumentary application of antiseptics that inhibits gram positive cocci causes secondary growth of gram negative ones.

Prospective studies have been conducted. One included 50 naturally-fed infants and only probiotics for rotavirus infection. Another, 50 children who were treated for pharyngitis and pharingoamigdalitis have received local treatment based on str Salivarius.

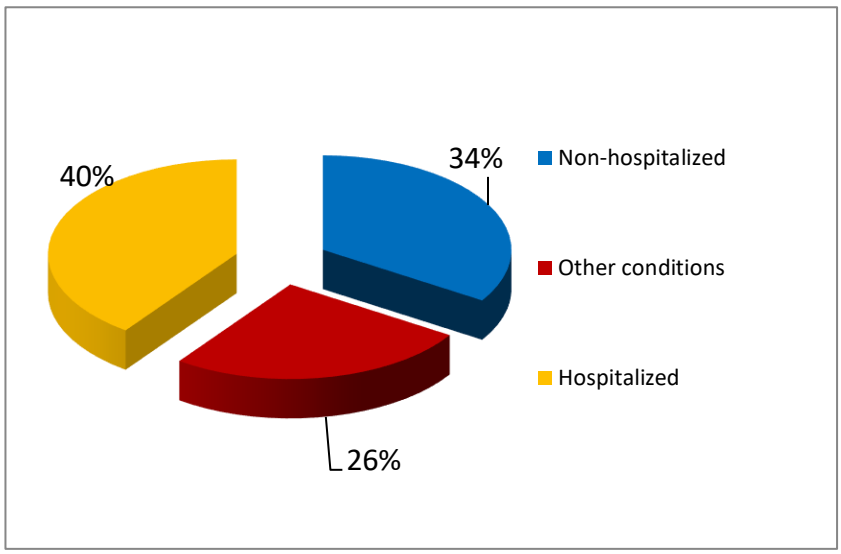
In the first study, the results were reported in a control group of artificially fed infants prior to rotavirus disease, and antibiotic therapy was introduced at the start of treatment throughout hospitalization. The pathophysiology of the rotavirus viral digestive infection is known to cause the fixation to the intestinal cells brush border, with their destruction following the invasion. The lactase that helps to digest lactose is destroyed, causing intolerance (transient) to lactose that explains diarrheal disease.

In naturally-fed infants, the duration of hospitalization was on average 28-34 hours shorter than the control group. Supplementing the diet with probiotics resulted in a quicker nutritional recovery (on average 40-42 hours) than the control group. Antibiotherapy administered prolonged diarrhea duration up to 3-5 days after discharge and weighing or descending weight curve. The median duration of hospitalization was 3 ± 1 days for infants treated with probiotics and 5 ± 2 days for those who received the antibiotic. In the antibiotic-treated group, the return to the milk preparation used before the illness was achieved after 6 ± 2 days after discharge, the children who were naturally fed did not change the dairy preparation during or after the disease.

In a 6-month post-illness period, 64% of babies breast-feeding naturally and treated with probiotics were no longer hospitalized for digestive conditions, 18% had other conditions and 18% had hospitalized digestive episodes.



In the control group, 40% were hospitalized for digestive affections, 26% for other conditions, the remaining 34% were hospitalized.



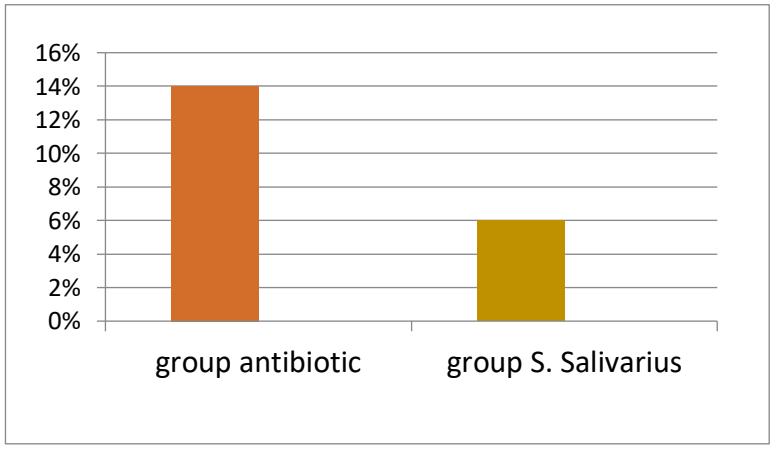
Study 2. Pharyngitis and pharyngitis in str Salivarius treatment - 50 cases versus 50 cases treated with antibiotic (on own initiative or medical indication.) Pharyngeal exudate was negative or found Staph.

The disappearance of symptomatology (dysphagia) occurred after approximately 72 ± 7 hours in the group treated with Salivarius S strains and at 48h ± 7 hours in those treated with antibiotics. It is possible to interfere with the "placebo" effect or psychological impact of "serious" antibiotic therapy at the expense of re-colonization treatment with healthy microflora.

Normalization of the clinical appearance at the pharyngeal inspection did not show any noticeable differences in the two therapies and occurred in about 4th-5th day of treatment.

In 26% of patients treated with antibiotic, it was necessary to change the antibiotic after 3 days of initial treatment with the antibiogram.

In those treated with Str salivarius, was added bucopharyngeal antiseptics up to 18%. The percentage of colonization with Candida albicans spores was 14% in patients treated with antibiotics and in the S salivarius treatment group was 6%.



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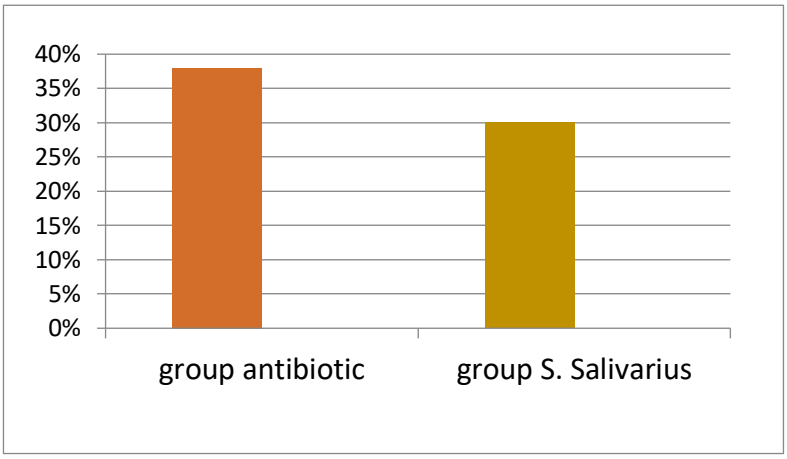
Recurrence of pharyngitis at 6 months after antibiotic treatment and 6 months from the first 10-day cure with Str salivarius (were 3 total cures, 10 days / cure) was 38% in the antibiotic treated group and 30% at the group treated with Str salivarius.



DISCUSSIONS

The mechanism of action of probiotics seems to be complex: it occupies competitively the mucosal sites for which pathogenic bacteria or viruses colonize, alter and maintain a local pH unsuitable for disease conditions, compete with pathogens or neutralize toxic compounds produced by them (radicals free cell-cytotoxic effect).

CONCLUSIONS

Both studies suggest that the exogenous intake of "healthy" bacteria potentiates local flora in the mucous membranes (digestive and respiratory) to play antimicrobial defense.

Future therapies tend to become more and more personalized, according to the personal microbe, according to some studies, may have the "weight" of the blood groups, appreciating with some accuracy the potential type of affections that an individual might suffer.

In well-documented situations (viral infections with mucosal tropism viruses), probiotic therapy acts as a shield, preventing bacterial or candid colonization and replacing antibiotic therapy that is unnecessary or even harmful.

Both selected groups of children were fed naturally, providing an equal "start", knowing the protective role (via the "j" secretion synthesized in the galactosporal channels of the mammary gland that binds 2 secreting IgA molecules, of breast milk).

The costs of inappropriate antibiotic treatment far outweigh those of a probiotic treatment, the side effects of the first one being redoubtable - secondary candidiasis, a collateral effect that eventually amplifies the costs of therapy.

Ensuring a normal microflora with mucosal protective function seems to reduce the risk of recurrent infections.

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