Opis

JBUON 2017; 22(4): 844-852
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

REVIEW ARTICLE

Current strategies and future perspectives in fertility preservation for cancer patients

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Summary

Nowadays, cancer is being detected at younger ages. Health care providers should consider cancer patients' desire towards fertility preservation before the initiation of possibly sterilizing treatments. The aim of the current review was to register the current state of fertility preservation procedures available for male and female cancer patients.

Key words: *cancer patients, fertility preservation*

Introduction

There is an ongoing trend to detect cancer at earlier stages and in younger patients. Therefore, lately, preservation of fertility in this population has become a matter of great interest.

Furthermore, we are witnessing an increased pressure on health care providers to offer fertility preservation to cancer survivors which are at reproductive age [1,2].

Almost half of cancer patients are able to bear children or want to do so at the time of diagnosis [3]. Additionally, health care providers should not presume that older men may not be as interested in fathering children, as some may desire children later in life or with a different spouse. However, survivors have lower pregnancy rates than the general population. The impact on fertility depends on many factors, with survivors of leukemia showing lowest rates for pregnancy, and testicular cancer and Hodgkin lymphoma showing increased rates over time [4].

The aim of the current review was to establish the current state of fertility preservation procedures. A review of the literature was completed using advanced search on PubMed with the following terms "ovarian tissue cryopreservation", "embryo cryopreservation", "oocyte cryopreservation", "ovarian transposition", "live birth", "pregnancy", "male infertility", and "cancer". We included all relevant articles from 2004-2016.

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Received: 22/11/2016; Accepted: 15/12/2016

Fertility preservation in female patients

Chemotherapeutic agents affect severely the reproductive and endocrine function of the ovaries, and most women developing amenorrhea and never regaining menstrual cycles [5]. Studies show that premature ovarian failure increases with age and varies with regimen, duration and total cumulative dose of chemotherapy [6]. It has been estimated that 60-80% of women who are treated with cyclophosphamide, methotrexate and 5-fluorouracil will develop premature ovarian failure (POF) [7,8]. Several studies have observed that a significant number of younger patients who did regain ovarian function after chemotherapy were at risk of undergoing premature menopause a number of years after treatment. The beneficial effects of adjuvant chemotherapy for breast cancer may result, in part, from suppression of the ovarian function [9,10].

Radiation therapy also has severe adverse impact on endocrine and reproductive function depending on patient age, administered dose and the irradiation field [11]. POF can be induced directly or by affecting the hypothalamic-pituitary axis [12,13].

The choice of fertility preservation method in a practical manner depends on patient age, possibility to delay chemotherapy or radiation, presence of male partner or willingness to use a sperm donor and whether patient malignancy permits ovarian stimulation. According to Chian et al. strategy, we present an update of the current fertility preservation methods in female patients [14].

Embryo and oocyte cryopreservation

For reproductive age women with available male partner (or a willing to use a sperm donor), and for whom health care providers (oncologist, fertility specialist) decide that treatment can be delayed to perform ovarian stimulation; embryo cryopreservation is the current option. Oocyte conservation is suitable for women without a partner, or for those who do not accept embryo freezing [14].

Both options require ovarian stimulation, transvaginal oocyte retrieval, thus making both techniques available only for post pubescent girls who have sufficient time to undergo ovarian stimulation, before starting oncological treatment.

The conventional ovarian stimulation protocols can increase estrogen levels up to twenty times, raising concerns about the estrogen sensitive tumors growth. To avoid the undesirable effects of estrogen, new protocols that use Letrozole and FSH have been developed. Some authors who used the Letrozole and FSH protocol in patients with estrogen sensitive breast cancer reported no significant increase risk in short-term recurrence and number of embryos obtained comparable with other ovarian stimulation protocols. [15-17].

Embryos and oocytes can be cryopreserved using: slow freezing and vitrification. Until recently, efforts to freeze oocytes with the slow freezing protocol remained one of the biggest weaknesses of assisted reproduction, as survival rates were much lower than those of cryopreserved-thawed embryos. Nowadays, the vitrification technique has revolutionized cryopreservation techniques and has now become the standard procedure for embryo and oocyte conservation with high survival and pregnancy rates. A study published by Levi-Setti et al., conducted over a period of 5 years showed that cryopreservation of oocyte by vitrification had a higher survival rate than cryopreservation of oocytes by slow freezing. Pregnancy chances were significantly higher when using fresh or cryopreserved embryos compared to using embryos obtained from cryopreserved oocytes [18].

In case of patients for whom delaying treatment is not an option or for whom ovarian stimulation is not indicated by health care providers, immature oocyte retrieval followed by *in vitro* maturation could be considered. Immature oocytes are extracted from the antral follicles and matured *in vitro*, in order to produce embryos that increase the likelihood of conceiving for these patients. *In vitro* maturation (IVM) can be performed regardless of the patients' menstrual cycle phase, without affecting oocyte quality. Maturation and fertilization rates are comparable after luteal phase and follicular phase retrieval [19].

In 2014 Prasath et al. reported the first case of live birth after IVM in a patient with bilateral borderline serous carcinoma of the ovary [20].

There is an ongoing concern about the timing of cryopreservation of the immature oocytes, since performing it at the germinal vesicle stage may cause certain damage in its quality [21].

IVM can be combined with ovarian tissue cryopreservation (OTC). According to a study published by Hourvitz et al. in which 255 cancer patients were included in fertility conservation programs, employing a combination of OTC, oocyte aspiration and *in vitro* maturation (AIVM), and with oocyte retrieval from ovarian tissue (OTIVM) resulted in more oocytes ($p<0.001$), more metaphase II oocytes ($p<0.001$), better maturation rate ($p<0.01$) and more cryopreserved oocytes ($p<0.05$) than by employing just OTIVM or OTC. Also, the same study found that compared to using just ovarian tissue oocyte cryopreservation,

more oocytes with better maturation rate are obtained if oocyte aspiration is performed right before ovarian tissue cryopreservation [22]. Ovarian tissue cryopreservation

Ovarian cortex biobanking, as a method of preserving fertility, is considered as an option for women, in whom need of chemotherapy is immediate or for pre pubertal girls for whom ovarian stimulation and *in vitro* fertilization (IVF) can't be applied [23].

The main advantage of this method applied for ovarian tissue resides in the fact that the ovarian cortex is the source of primordial and primary follicles [24], assuring a high amount of female gametes. Also, retrieval can be performed without delay in a minimally invasive manner. While the structure of the tissue can remain unaltered, its function can be destroyed irreversibly [25]. The method needs the use of cryoprotective agents because of the risk of ice crystal formation. Toxicity of these agents is another problem limiting the technique's success [26] and it depends on the chemical properties of each agent, duration of exposure, and temperature [27]. Several studies suggest that primordial follicles are more resistant to cryoinjury and to cryoprotectants due to their dormant metabolic state [28].

The strategy underlying this procedure is to harvest and store the ovarian tissue fragments until the patient is ready for transplantation, aiming at the restoration of endocrine and reproductive functions, otherwise destroyed by chemotherapy. The ovarian tissue grafts can be transplanted to the pelvis, near the original ovary sites blood supply (orthotopic transplantation) or to other sites such as abdomen or forearm (heterotopic transplantation). Orthotopic transplantation is the most used method of transplantation and with this technique several pregnancies were obtained; meanwhile, heterotopic transplantation offers a series of advantages regarding its monitoring, but there have been no pregnancies reported yet using this method [29].

The endocrine function duration after transplantation varies between 9 months and 3 years. An important amount of follicles are lost right after transplantation due to local ischemia leading to repeated grafting procedures [30,31].

OTC is an invasive procedure and still considered experimental for young patients, although in some countries, such as Israel, efforts were made to reconsider this [32]. The American Society for Reproduction Medicine guidelines classifies OTC as experimental and recommends applying it on carefully selected patients [33]. The American Society of Clinical Oncology Practice Update also labels OTC as experimental and recommends this procedure only in experienced centers [34]. Both publications raise the theoretical problem concerning transplantation of cancer cells with the graft.

In 2004 Donnez et al. reported the first live-birth after cryopreservation and orthotopic transplantation of ovarian tissue in a woman with stage IV Hodgkin lymphoma. Five months after transplantation, hormone levels and ultrasound findings were consistent with ovulatory cycles and after 11 months pregnancy was confirmed by ultrasound. The patient delivered at term. Concerning the theoretical aspect, regarding the transplantation of malignant cells, histological assessment was completed showing no such findings [35]. Several pregnancies followed this success (Table 1).

Table 1. Ovarian tissue cryopreservation outcomes (2004-2016)

Year of publication/ authors [Ref]	*Orthotopic / heterotopic transplantation*	*Endocrine function restoration (months)*	*Pregnancy / live birth*
2004, *Donez et al* [35]	+/-	5	1/1
2005, *Meirow et al* [36]	+	8	1/1
2007, *Demeestere et al* [37]	+/+	5	1/0
2010, *Demestere et al* [38]	+/+	33	1/1
2008, *Andersen et al* [39]	+/-	4	2/2
2010, *Ernst et al* [40]	+/-	4	1/2
2011, *Donez et al* [41]	+/-	2-5-6	13/13
2010, *Roux et al* [42]	+/-	4	1/1
2010, *Sanchez-Serrano et al* [43]	+/-	2	1/2
2012, *Muller et al* [44]	+/-	3	1/1
2014, *Macklon et al* [45]	+/-	1	1!
2015, *Tanbo et al* [46]	+/-	?	2/2
2016, *Dunlop et al* [47]	+/-	3,5	1
2016, *Meirow et al* [32]	+/-	1-6	16/10

In 2006 a cooperation network to aid fertility preservation for oncologic patients (both women and men) was founded in Germany. Since then, it has extended to more than 100 institutions across the country and in Switzerland and Austria. In 2016, the largest case series published by Fertiprotekt network reports 21 pregnancies and 17 live-births after orthotopic ovarian cortex transplantation [35]. For the purpose of improving some aspects of the procedure Oktay et al. performed ovarian tissue transplantation using a human extracellular tissue matrix scaffold in two patients diagnosed 12 and 7 years respectively before, with hemophagocytic lymphohistiocytosis and non-Hodgkin lymphoma. The transplantation performed was minimally invasive. One pregnancy was ongoing at the time of publishing and one patient delivered a healthy baby [36]. Even though official peers consider OTC as an experimental method of preserving fertility, efforts should be made to offer this for whom other options are not available.

Ovarian transposition

Ovarian transposition is a surgical procedure that should be considered in order to preserve fertility in female patients with genital (cervical cancer, vaginal cancer), urinary (rhabdomyosarcoma of the bladder) and hematologic (Hodgkin's disease) malignancies as well as sarcomas of the pelvic region (Ewing's sarcoma), anorectal cancer or neurologic malignancies that are treated with pelvic radiation. Oophoropexy reduces ovarian exposure to only 5-10% [37-40].

Pelvic radiotherapy may cause ovarian and uterine damage. Radiation tolerance of the uterus and the ovaries depends on the total radiation dose, the fractionation schedule, the volume of the tissue which is irradiated and the patient's age. The more younger the patient, the higher the chance she has to preserve residual ovarian function. The dose of irradiation at which ovarian failure occurs in 97.5% (ESD – the effective sterilizing dose) of patients after treatment is 20.3Gy at birth, 18.4Gy at 10 years, 16.5Gy at 20 years and 14.3Gy at 30 years. Fractionated doses of radiation are less toxic than a single dose [41,42].

Radiation damages the DNA of the ovarian follicle which might lead to decreased follicular reserve. Mature follicles are more radiosensitive than primordial follicles. To destroy half of the follicular reserve, less than 2Gy is needed. Ovarian failure is produced by a dose of irradiation of 24Gy, if it is applied conventionally [40,43]. Ovarian transposition, also known as oophoropexy is a procedure in which one or both ovaries are moved from the irradiation field. Ovaries can be moved to the parabolic gutters, above the pelvic brim, in line with the iliac crests or anterior the psoas muscle, depending on the radiation field, by open surgery, laparoscopy or robotic surgery. Metal clips are placed in order to identify and confirm that the ovary is out of the irradiation field. The procedure should be done as close as possible to the beginning of radiotherapy, and remains the standard of care for patients treated with pelvic radiation. It may be combined with oocyte, embryo or ovarian tissue cryopreservation. Sometimes, to achieve pregnancy, re-transposition is necessary. A recent approach is to transpose one ovary and remove the other one for cryopreservation [37,41,44].

Complications regarding this procedure are relatively rare, but sometimes chronic pelvic pain, adhesions, fallopian tube infarction, ovarian migration and metastasis to the transposed ovaries can occur. The hormonal function is preserved in 70-93% with ovarian transposition before radiotherapy in patients < 40 years. Thibaud et al. reported the first results of 18 children born after ovarian transposition – 2 of them became amenorreheic, 16 had menstruated and 2 pregnancies occurred on a follow-up of 8.6 years [45].

Terenziani et al. reported a number of 14 pregnancies – 12 live births (1 twin) and 3 miscarriages after ovarian transposition in 11 patients with Hodgkin's lymphoma, after a follow-up of 14 years. None of these patients needed artificial insemination or ovarian de-transposition [46].

Ovarian transposition in pediatric patients is still inadequately studied, but the success rate seems to be 60-83%. In adults' long-term outcome studies, only a few pregnancies have been reported – 5 pregnancies in 10 patients with Hodgkin's lymphoma [47] – 3 pregnancies in 107 cervical cancer patients [48] and 3 pregnancies in 12 patients (9 Hodgkin's lymphoma, 3 rectal cancer) [49], but the ovarian hormonal function was well preserved [38].

A surrogate pregnancy may be a valid option for women with cervical cancer treated with radical hysterectomy, lymphadenectomy and oophoropexy, followed by ovarian stimulation, oocyte retrieval from the genetic mother, IVF and embryo transfer to the surrogate mother. Legislation in many countries forbids this approach [50].

Köhler et al. described in a study published in 2016 a successful delivery after ovarian transposition and uterus fixation in a patient with anal cancer followed by chemo-radiation and recto-anal resection [51].

Fertility preservation in male patients

Cancer, treatment and fertility

Cancer itself may influence spermatogenesis, though the mechanisms are not well understood. Preexisting poor quality of germ cells, systemic effects of cancer, endocrinological or immunological effects probably exert some effect [52,53]. All cancer therapies - radiotherapy, chemotherapy, stem cell transplantation, surgery - can impact fertility, either directly affecting spermatogenesis or hormone production. It is important to explain their different risks and benefits, as these may influence patients' treatment decision [54].

Unfortunately, there are no available options for the protection of the gonadal epithelium. Prepubertal age is not a protective factor from gonadotoxic injury, as the cytotoxic treatment directly affects the early germ cells that undergo spontaneous degeneration before the haploid stage [55].

Radiotherapy

Radiotherapy has been utilized in the treatment of prostate, bladder, penile, testicular and rectal cancer. The initial modalities for radiation delivery have evolved from conventional external beam radiotherapy to fractionated intensity modulated radiotherapy. However, it may still have irreversible detrimental effects on fertility and spermatogenesis. The gonadal epithelium is very sensitive to radiation because of its rapid division rate. While Leydig cells can resist to doses up to 20Gy in prepubescent males (and 30Gy in adult males), immature spermatogonia are more sensitive, with doses of 0.1Gy able to influence their shape and number. Radiation doses under 0.8Gy lead to oligozoospermia, while doses up to 2Gy can lead to temporary azoospermia. Higher doses can lead to permanent sterility [56].

The radiation to the testis may result either from direct exposure or scattered radiation, with some authors mentioning that 18.7% of radiation administered in pelvic cancers is received by the testis [57]. It may take 10 to 24 months for the sperm to return to pretreatment levels. The impact of radiation therapy on sperm DNA integrity is not yet known [58].

Chemotherapy

Similarly to radiotherapy, chemotherapy may alter the function of Leydig cells and cause hypogonadism. The amount of damage is also dependent on the type of regimen, the age of the patient and the total dose administered. However, it has been proved that hormonal therapies do not lead to faster recovery of spermatogenesis, nor do gonadotropin releasing hormone antagonists prevent long term infertility when high doses of chemotherapy are used [59].

Combination chemotherapies have been developed to reduce the negative effects and potentiate the efficacy of the agents used, and have become the golden standard in the treatment of several cancers. Although this synergy is desirable against cancer cells, it also has a detrimental impact on fertility, with the possibility of incurring permanent azoospermia. For instance MOPP (mechlorethamine, oncovin, procarbazine and prednisone) used for Hodgkin's lymphoma can cause azoospermia in 90% of men for up to 4 years after treatment. The combination of bleomycin, etoposide and cisplatin used in some testicular cancers has been associated with increased sperm DNA abnormalities [60]. A recent animal study has described a protective effect of the humanin analogue against spermatotoxic effects during chemotherapy, but no human studies are available [61].

Surgery

Surgery may also affect fertility, either directly by affecting the integrity of the genitourinary tract (for instance in bladder or prostate surgery) or its function (for instance, causing retrograde or anejaculation by affecting the lumbar sympathetic plexus or hypogastric plexus during retroperitoneal lymphadenectomy for testicular cancer). Many pelvic operations may also affect the erectile function. As such, even though sperm quality may not be changed, there is a possible permanent loss of fertility, which the patient should be aware of. Currently, there is an effort to develop and deploy nerve sparing techniques whenever possible to maintain erectile and ejaculatory function, with good results.

Surgical approaches to fertility preservation

Several surgical methods for preserving fertility have been described - testis sparing surgery, testis transposition, sperm retrieval, testicular stem cell transplantation.

Although rare, synchronous and metachronous bilateral germ cell tumors occur in 2-5% of the patients, and bilateral orchiectomy will lead to permanent infertility as well as cardiovascular, metabolic and psychological problems. Thus, in extremely selected cases, there is an option for organ sparing surgery, provided the tumor is limited to the testis and of small size (less than 2 cm) [62]. Because of an increased risk of local recur-

rence or adjacent testicular intraepithelial neoplasia, close follow-up is required in all patients undergoing enucleation. Ideally, semen collection is performed prior to surgery, or at least before definitive radiotherapy. Organ sparing surgery may also be an option in the rare cases of Leydig cell tumors, which account for 0.8-3% of all testicular tumors [63].

Testicular transposition is a rarely used technique, that may allow for fertility preservation in patients requiring radiotherapy in the pelvic region (for instance, for rhabdomyosarcoma of the bladder, prostate, or paratesticular tumors). The testicle to be preserved is transposed to the thigh or anterior abdominal wall, and later replaced in the scrotum following completion of local irradiation [64,65]. Due to the rarity of the procedure, its role in normal practice has yet to be defined.

Sperm extraction and preservation

In a study on adolescent patients, Bahadur et al. reported that adequate semen samples were obtainable in the majority of patients regardless of cancer type (86.1%) [66]. If there is no ejaculation or the patient has a history of retrograde ejaculation, a urine analysis following masturbation should be performed to assess the presence of sperm in urine. If positive, alpha agonists may be tried to direct the flow of sperm forward. Failing that, a trial of urine alkalization, collection post ejaculation and isolation of viable sperm may be performed. If no ejaculation can be achieved, vibrostimulation or electroejaculation can be performed, usually under general anesthesia [67]. In azoospermic men surgical means of sperm extraction may be required, such as microsurgical sperm extraction (microTESE), testicular sperm extraction or microsurgical epididymal sperm aspiration (MESA). In men with azoospermia prior to chemotherapy due to their cancer pathophysiology, oncological testicular sperm extraction may be performed, as described by Schrader et al., which uses microsurgical dissection and extraction of seminiferous tubules during the initial gonadal surgery [68].

For prepubertal males there are limited options for fertility preservation, focusing on *in vitro* generation of sperm from harvested spermatogonial stem cells or preservation of immature testicular tissue [69]. Once collected, cryopreservation allows the sperm to remain in a suspended animation state and able to be stored for up to 15 years [70]. Current assisted reproduction techniques - ARTs (*in vitro* fertilization - IVF - and intracytoplasmic sperm injection - ICSI) allows for conception using testicular and epididymal sperm, or sperm with suboptimal motility or morphology.

Fertilization methods

After treatment, many men may need artificial reproductive techniques to procreate as IVF/ICSI. Of these, 15% will require the use of cryopreserved semen due to persistent azoospermia. No studies have so far proven any increase in the rate of malignancy or congenital abnormalities in children born fathered by cancer survivors, but close follow up is recommended. In a large cohort study published by Boice et al. the incidence of anomalies in children of cancer survivors was the same as in the general population. This held true for patients who fathered children after undergoing radiation therapy or alkylating agent therapy [71]. A recent Swedish and Danish study has shown a modest but statistically significant increase in the risk of congenital abnormalities in children of males who had undergone cancer treatment, irrespective of means of conception (natural or ART). The study involved the analysis of a cohort of 8670 children with paternal history of cancer treatment. Of these, 508 children were conceived using ARTs. The children of male cancer survivors were more likely to have major congenital defects than the control group, (RR = 1.17, 95% CI=1.05-1.31, p=0.0043, 3.7 vs 3.2%). Interestingly, the data available allowed the comparison of incidence of congenital abnormalities in children born from semen preserved pre-treatment versus post-treatment, and proved to be equal in both groups (4.4%), indicating that factors other than anticancer therapies may be causing this trend [58].

ART in cancer survivors seems to be as effective as in the general population. Garcia et al. reported that the use of cryopreserved semen is about 10% and the success rate for live births is comparable to that of non-cancer patients [72].

Conclusions

Embryo and sperm cryopreservation are established methods of fertility preservation. Other options, such as ovarian or immature testicular tissue cryopreservation are still in their infancy, but regarded with high hope. More research and funding are needed to embrace the need of reinstating and maintaining cancer patients' fertility.

Conflict of interests

The authors declare no confict of interests.

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Review

Med Ultrason 2017, Vol. 19, no. 4, 430-437
DOI: 10.11152/mu-1248

Abdominal wall endometriosis: an update in clinical, imagistic features, and management options

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Abstract

Abdominal wall endometriosis (AWE) is a rare condition defined by the presence of endometrial tissue in the subcutaneous fatty layer and the muscles of the abdominal wall. It is usually caused by the dissemination of endometrial tissue in the wound at the time of obstetrical and gynecological surgeries. AWE is rare and difficult to diagnose. The most frequent clinical presentation is that of a palpable subcutaneous mass near surgical scars associated with cyclic pain and swelling during menses. AWE may be an underreported pathology partly because it has scarcely received attention in the radiologic literature. Its frequency is expected to rise along with the increasing rate of cesarean deliveries; thus, it is important that physicians or sonographers are familiar with this pathology. The purpose of our review is to present the latest data regarding risk factors, clinical and imagistic findings, and management of AWE.

Keywords: abdominal wall; endometriomas; endometriosis; magnetic resonance imaging; ultrasound.

Introduction

Endometriosis is a condition characterized by the presence of uterine mucosal tissue outside the uterus. It affects 6%-10% of women of reproductive age being usually located in the pelvis [1]. Extra pelvic endometriosis occurs less frequently, being described almost everywhere in the body: lung, liver, gallbladder, gastrointestinal tract, perineum, central nervous system, umbilicus, inguinal hernias, or the abdominal wall. The latter may occur after surgical procedures that involve the uterine cavity, such as a cesarean delivery, allowing endometrial tissue to be transplanted. Meyer reported the first case of abdominal endometriosis in 1903 [2]. Since then, several case reports or mini-series have been reported in the literature. Abdominal wall endometriosis (AWE) may be an underreported pathology, partly because it has scarcely received attention in the radiologic literature. Dissemination of information regarding AWE outside the field of obstetrics and gynecology may be useful for guiding other physicians regarding the correct therapeutic approach to adopt, thus preventing recurrence and malignant disease [3].

The purpose of our review is to present the latest data regarding risk factors, clinical findings, imagistic findings, and management of abdominal wall endometriosis.

Received 12.09.2017 Accepted 07.10.2017
Med Ultrason
2017, Vol. 19, No 4, 430-437
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Methods

We performed a systematic literature search of PubMed/MEDLINE, Google Scholar, and Ovid for all research articles using the terms "abdominal wall endometriosis," "abdominal wall endometriomas," "abdominal wall mass," and rectus abdominis scar" published up until May 2017. The references of those articles were then reviewed and additional publications were evaluated.

Prevalence

It is difficult to accurately determine the prevalence of the disease in the general population. The prevalence of AWE is generally estimated to be between 0.03% and 1% [4,5]. Leite et al estimate that AWE will appear in 0.03 to 3.5% of cases after obstetric procedures [3]. It is expected that its frequency will rise along with the increasing rate of cesarean deliveries being performed.

Pathogenesis

The exact etiopathogenesis of endometriosis remains controversial and several theories have been proposed including coelomic metaplasia, implantation or retrograde menstruation, vascular and lymphatic metastasis, dissemination, direct transplantation, and aerosolization [6].

Two theories have been suggested for the occurrence of AWE [7]. One theory suggests that endometrial cells may be transported to ectopic sites (particularly during surgical procedures that open the uterus). This is the most accepted theory and can explain why many patients with scar endometriosis do not have signs or a history of peritoneal endometriosis. The other theory postulates that, under the right circumstances, primitive pluripotential mesenchymal cells may undergo specialized differentiation to form endometriomas. This theory can explain the few cases of AWE reported in the literature in patients without a surgical history.

Risk factors

Almost all cases reported in the literature occur after surgery involving the uterine cavity. Very few cases of AWE reported in the literature appear without any surgery [8-11].

The majority of AWE reported has been described as being adjacent to cesarean-delivery scars [12-15]. In evaluating the obstetric history of 81 patients with endometrioma, Wicherek et al stated that cesarean delivery performed before spontaneous onset of labor may substantially increase the risk of occurrence of scar endometriomas [16]. In a recent study of a series of 34 patients with histologically proved AWE after cesarean delivery, Khan et al noted a higher BMI in women with abdominal wall endometriosis compared with controls. They hypothesized that suboptimal closure of either the uterine incision or the pelvic and abdominal layers due to the surgical difficulties encountered in obese patients may explain and support the implantation theory [17]. Sumathy et al consider that the popularisation of single-layer closure of the uterus and nonclosure of parietal and visceral peritoneum could be risk factors [18].

Although cesarean delivery and abdominal hysterectomy scars are the most common predisposing factors, AWE has also been reported in laparoscopic trocar tracts or amniocentesis needle tracts [19]. The first case of AWE after laparoscopy was reported by Healey in 1995, and since then, 15 similar cases have been reported [20-22].

UAE is reported to be associated with pelvic endometriosis is reported to be between 5.3% in a study by Ding and Zhu to 14.2–26% of cases [18,23].

Pathology

AWE may be confined to the superficial layers of the abdominal or pelvic wall, but it often infiltrates the deeper layers, commonly the rectus muscle. Based on the position near the abdominal wall layers, AWE can be superficial (in subcutaneous tissue only, above fascia), intermediate (infiltrating the abdominal rectus muscle fascia), and deep (in the abdominal rectus muscle, below fascia).

On gross specimen, the endometriotic tissue appears as a pinkish mass of firm consistency, and sometimes microcysts with brown material can be clearly distinguished from the surrounding yellowish subcutaneous fat [18]. When present in the muscle, AWE can also be easily distinguished due to its irregular appearance and hard consistency [18]. The literature reviewed reported that the average lesion size ranged from 2.3 to 3.2 cm, whereas the largest lesion measured 12 cm [24,25].

A definitive diagnosis is made only at histopathologic analysis. Histopathologically, endometriosis can be diagnosed by the presence of endometrial glands, stroma, or hemosiderin pigment. AWE is associated with cytogenic chorion and hyperplasia of smooth muscle tissue within the soft tissue and musculature of the anterior abdominal and pelvic wall, inflammatory cells, and surrounding fibrosis [26].

Diagnosis

Typical patients with AWE present with a triad consisting of a history of cesarean delivery, cyclic pain associated with menses, and nodules near a surgical scar [27]. Physical examination is essential for an accurate diagnosis. The nodule is usually found by palpation of the subcutaneous cellular tissue, around a scar. It is located cephalad and/or lateral to the Pfannenstiel skin incision. The cephalad location occurs because the fascial incision is generally not directly below the skin incision. This

effect is often more pronounced in patients with higher BMIs [28].

Apart from a cesarean scar, other documented locations for the tumor include 'Villar's nodule' (endometriosis in umbilical trocar scars), sterilization scars, inguinal scars, appendectomy scars, and upper abdominal scars [7,29,30]. Increase in the size of the lump, bleeding, and skin discoloration with cyclical changes of menstruation are not characteristically seen in all cases, but are pathognomic of scar endometriosis. Some reports consider the cyclical nature of the complaint as an important factor that predicts abdominal wall disease [31], whereas others do not consider it a universal characteristic of the disease [32,33].

It is estimated that a correct diagnosis of scar endometriosis is preoperatively made in approximately 20%-50% of patients [31,33]. One pitfall in diagnosing AWE is that endometriosis may occur years after an operation or the nodule may not always be palpable. In some cases, the clinical aspect could be atypical, with no cyclic pain or modification of the nodule, and this might explain why its clinical recognition occurs late. In these cases, AWE can be confused with other surgical conditions. Often a general surgeon consults on AWE cases, and it is important that not only gynecologists but also general surgeons are familiar with this entity [34].

Sometimes, scar endometriosis may be incidentally discovered in women who undergo imagistic examination for other reasons.

Imaging ultrasound

Ultrasonography usually represents the first step in the evaluation of soft tissue masses. The role of ultrasound is to confirm the presence of a lesion, even if small in size, and to provide useful information regarding its size, location, margins, and structure. It is very easy with ultrasound to differentiate cystic from solid masses. However, when the mass is solid, ultrasound lacks specificity and additional diagnostic methods are necessary. When AWE is compared with other forms of endometriosis, we can say that AWE has features similar to those of deep infiltrating pelvic endometriosis [35].

Regarding the scanning technique, proper magnification is recommended of the image to reduce the field of view to 3-5 cm in depth [36]. It is important first to identify on a transverse or longitudinal section the normal abdominal layers of the abdominal wall far from the site where the AWE is suspected. The following layers should be identified: the hyperechoic subcutaneous tissue and the hypoechoic muscle layer covered by the thin hyperechoic abdominal fascia. The peritoneum can be identified as a thin hyperechoic line located above the intestine. The peritoneal fat located underneath the muscular layer can help in identifying the peritoneum. Then, sliding the probe while exerting slight pressure will help to locate the AWE, as a result of the pain induced [36]. Usually AWE appears on two-dimensional ultrasound as a nonhomogeneous hypoechoic mass with echogenic spots or thick echogenic strands that represent a fibrotic component in the abdominal wall (fig 1a) [24].

The margins are ill defined and there are blurred outer borders. The mass infiltrates the surrounding tissue due to an inflammatory reaction triggered by the monthly hemorrhage in this tissue [37]. A hyperechoic ring can be observed at the periphery of the nodule, and on histology, it represents edematous adipose tissue filled with cells of inflammatory origin (histiocytes and granulocytes) [36]. The echogenic patterns are dependent on the haemorrhagic and fibrous components of the lesions and on the phase of the patient's menstrual cycle [38]. Some authors consider however that the echo pattern is not always correlated with the menstrual pattern [33]. In some published cases, the ultrasound pattern is described as cystic or multicystic (fig 1b) [39]. Occasionally, a lesion appears with only punctate hypoechoic cavities in the abdominal scar [26].

The irregular shapes and borders of the endometriotic nodule appear clearer on the coronal plane of 3D ultrasound (fig 2) [40]. Three-dimensional ultrasound has proved to be very useful in recent years both in gynecology and obstetrics [41,42]. The 3D coronal plane provides a more precise analysis of the surrounding tissue and the depth of infiltration can be easily seen. 3D plays an important role in preoperative assessment by measuring the mass in all three planes and assessing whether the AWE invades the abdominal fascia. This is important in pre-

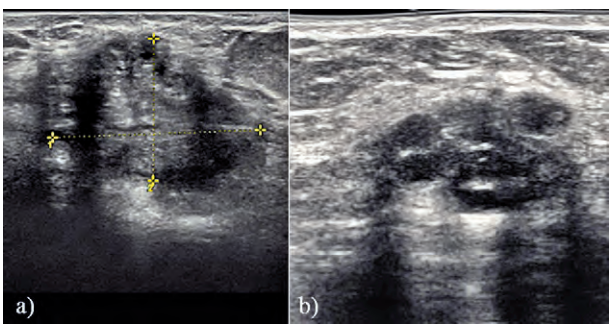

Fig 1. a) Transverse sonogram of the abdominal wall showing a 33/22-mm abdominal wall endometriosis nodule with hypoechoic content and blurred outer margins (calipers). The nodule is enclosed in the subcutaneous fatty tissue above the muscular fascia, along the scar of a previous Cesarean section; b) Transverse sonogram of the abdominal wall showing a nodule with hypoechoic content and cystic images.

operative evaluation of the mass, especially if no other imagistic tools like CT or MRI are used. In the case of a large nodule, mesh may be needed to repair the fascia, and these procedures require adequate counselling and precise surgical planning. Therefore, exact knowledge of the AWE location is helpful in planning the surgery, choosing the reference hospital and surgical team, predicting the time of surgery, and securing additional material, such as mesh [43].

The vascular pattern can be different, from small and scanty vascularized lesions to large lesions with several vascular pedicles entering the mass from different points to abundant central vascularization (fig 3). Usually the small lesions are located in hypovascular subcutaneous fat, thus their growth is limited [44]. Doppler velocimetry can show intralesional vascularization if the tumor size is greater than 15 mm [44]. The presence of central vascularity is a highly determinative finding of malignancy for soft tissue lesions, according to Fleischer et al [45]. Both clinicians and radiologists should consider, in the presence of a rapidly growing, painless mass and the absence of previous surgery, malignant soft tissue masses in the abdominal wall as a differential diagnosis.

Elastography is a novel ultrasound-based imaging modality that assesses the elasticity of visualized tissues. One of the many elastography methods is strain elastography, based on the phenomenon that after applying pressure with the probe the strain of hard tissues is lower than that of soft tissues. Gradient values of strain are visualized on a color map, representing the stiffness of the examined area [43]. Wozniak et al showed that elastography improves the performance of B mode ultrasound in the preoperative assessment of AWE location. Moreover, the accuracy of alpha-blend elastography in the preoperative assessment of AWE location is not decreased in overweight and obese patients [43].

All the described ultrasound findings are nonspecific, and a wide spectrum of disorders that may result in a mass in the abdominal wall should be considered in the imaging differential diagnosis. This includes primary or metastatic tumor, desmoid tumor, lymphoma, and non-neoplastic causes, such as suture granuloma, ventral hernia, hematoma, abscess, or retained surgical material [8,46]. Sonography may exclude granuloma, hernia, hematoma, abscess, or seroma in view of the solid appearance and vascular nature of AWE [24].

Acute hematomas are typically highly echogenic whereas seromas and resolving hematomas are hypoechoic or anechoic. Abscess demonstrates an air-fluid level. Correlation with clinical history is important, because both hematomas and abscesses manifest in the

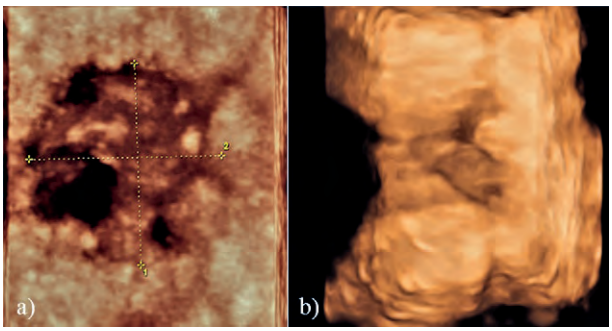

Fig 2. a) The reconstructed coronal plane with 3D ultrasound shows the heterogeneous mass with irregular and speculated margins; b) The reconstructed coronal plane with 3D ultrasound shows the heterogeneous mass with irregular and speculated margins and cystic images inside of the tumor.

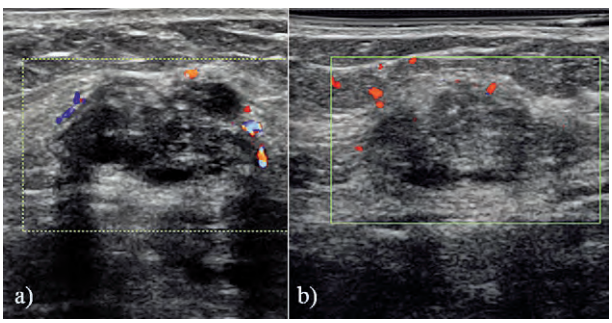

Fig 3. Color Doppler US images showing peripheral flow within the mass (a and b).

setting of a recent surgery or procedure [47]**.** Lymphoceles are usually anechoic and have septa. A granuloma on ultrasound may appear as irregular and inhomogeneous, partly hyper- and partly hypoechoic and is difficult to characterize at imaging alone and may require biopsy [47].

Difficulties can appear in the differential diagnosis with desmoid tumours. Abdominal wall desmoid tumours are rare, slow-growing, benign, muscular-aponeurotic fibrous tumours with the tendency to locally invade and recur. Desmoid tumours lack a capsule and are infiltrative. Therefore, although histologically benign, they are locally aggressive. Typically, desmoid tumours do not manifest with cyclical pain imaging, and clinical findings must be closely correlated because the ultrasound appearance of a desmoid tumour may closely resemble that of scar endometriosis. On ultrasound, desmoid tumours are usually small and hypoechoic with well-defined margins (fig 4) [48].

Metastases usually present on ultrasound as poorly defined hypoechoic masses with increased vascularity. In lymphoma, ultrasound findings are often nonspecific, and the appearances are variable, including a large mass,

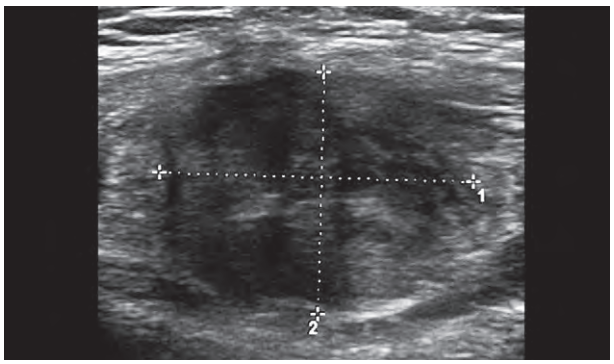

Fig 4. Abdominal desmoid tumor – hypoecoic nodule with regular contour.

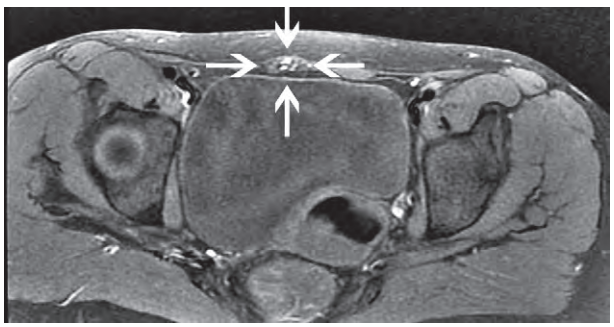

Fig 5. MRI- Axial T1- enhanced T1-weighted fat-sup¬pressed showing a mass (white arrows) within the anterior abdominal wall abutting the right rectus abdominis muscle with foci of hyperintensity suggesting haemorrhage.

nodal or confluent nodal structures, small nodules (<1 cm), and disseminated myositis and panniculitis [49].

Magnetic Resonance Imaging

MRI is typically considered the best method for describing the anatomy of a soft tissue mass and its surrounding structures. MRI imaging is preferred in younger patients, because of its improved tissue characterization and lack of ionizing radiation. It can detect even very small lesions and has the advantage of a clearer depiction of the delineation between muscles and abdominal subcutaneous tissues and infiltration of abdominal and pelvic wall structures [26]. MRI can determine the location and depth of infiltration in the surrounding tissues, information useful in choosing the best method for closing the fascia defect during surgery.

AWE appears as a hyperintense heterogeneous nodule associated with a surgical scar on both T1- (with and without fat suppression) and T2-weighted images, a result of subacute hemorrhage within the endometriotic crypts although this finding may vary [26,38,50] (fig 5). MRI imaging is capable of showing the presence of hemorrhage in the abdominal wall mass and can provide information on the chronicity of a hematoma associated with scar endometriosis or other processes [47]. A feeding vessel also occasionally can be seen. Alternatively, scar endometriosis may be isointense relative to muscle on T1-weighted images and may be difficult to identify [33]. In cases with chronic scar endometriosis, lesions may have spiculated margins and low signal intensity on T2-weighted images, because of both dominant fibrotic and hemosiderotic components [26,47].

However in AWE, the signal characteristics and contrast enhancement pattern of AWE are not specific, and the true histological nature of soft-tissue masses often cannot be made, with a few exceptions (eg, lipoma or haemorrhage) [46,51].

Computerized tomography

Computerized tomography (CT) involves irradiation and requires an intravenous contrast agent. On CT AWE typically appears as a solid soft-tissue mass directly associated with an area of surgical scarring [47]. The findings depend on the phase of the menstrual cycle, the proportion of stromal and glandular elements, the amount of bleeding, and the degree of inflammatory and fibrotic response. It may be hyper attenuating compared with muscle, although its attenuation may vary [37,52.]. Usually, mild to moderate enhancement is seen after administration of intravenous contrast material [24,47]. Feeding vessels may be seen within or adjacent to the mass. However, it may be difficult to distinguish scar endometriosis from bland scarring and other processes at unenhanced and contrast-enhanced CT; thus, correlation of clinical and imaging findings is important [26].

The different imaging modalities (ultrasound, MRI, CT) are nonspecific but useful in determining the extent of disease and assist in the planning of operative resection, especially in recurrent and large lesions [53].

Fine needle aspiration

Ultrasound-guided fine needle aspiration **(**FNA) is an accurate, inexpensive diagnostic procedure in women with abdominal wall masses, showing the difference between benign and malignant conditions. It has been reported to diagnose endometrioma in isolated cases [54-58]. FNA is easy to perform because the nodule is firm [59]. With FNA, clusters can be identified of epithelial endometrial-like cells, endometrial-like stromal cells, and haemosiderin-laden macrophages in the lesion. Sometimes because of the limited amount of sample material as well as the presence of fibrotic tissue in the old

lesions of endometriosis, the diagnosis on FNA may be inconclusive and an additional histologic biopsy may be considered [11].

However, FNA use is controversial because of the risk of causing new implants at the puncture sites [27]. To avoid the spread of endometriosis after these procedures, it is advisable to include the site of aspiration in the field of operative resection [53].

Risk of malignization

Malignant change in AWE is rare and is reported in only about 1% of cases [24]. The principal risk factors of malignant transformation of endometriosis include advanced age of the patient, if they are postmenopausal, and if the tumor diameter of an endometriotic lesion is 9 cm [60]. Malignancy of AWE may occur from just a few months until up to 18 years after surgery [24]. Clear cell carcinoma is the most common histological subtype, and the 20-month survival rate is only 57% [61]. Several other types have been sporadically reported in the literature, including carcinosarcoma, cystadenocarcinoma, and serous papillary carcinoma [62-64]. The main prevention method is represented by wide excision of AWE with clear margins.

Management

Some studies have reported the use of medical treatment (oral contraceptives, gonadotropin-releasing hormones, danazol, and progesterone) though with a low rate of success and often followed by recurrence after cessation of the drug [31].

Surgery represents the treatment of choice for AWE because it offers the best chance for both definitive diagnosis and treatment. It is important to remove the nodule and the adjacent fascia completely; otherwise, AWE can reoccur or new lesions can form if the neighbouring parenchyma is inoculated during the operation [31]. In cases of small nodules, which are present in most cases, superficially located surgery can be relatively easy. In several cases, the excision may be technically difficult and the surgical mass excised may need to be enlarged if the lesion extends to deeper tissues, such as aponeurosis, muscle, or more rarely, peritoneum. In these cases, sometimes a mesh or aponeurotic muscle flap for covering the defect is needed [65]. Surgical resection leads to healing in 95% of cases, and relapses occur in the rest of the cases [8]. Usually a high rate of recurrence (9.1%) is related to a previous inadequate resection of the endometriotic lesion [31]. The margins should be at least 1 cm [18].

Although all authors recommend adequate surgical excision for favourable outcomes, no studies have addressed whether the size of the surgical margin affects the recurrence rate.

Prevention

Because scar endometrioma mainly originates during cesarean delivery, it is important to reevaluate the best practices and care related to this procedure. Several methods are recommended to avoid the incidence of AWE, including handling uterine tissue very gently, ensuring meticulous control of bleeding, washing of the intraabdominal cavities before closure of the abdomen, and avoiding subcutaneous dead spaces [66]. Sumathy et al recommend exteriorisation of the uterus for suturing, leaving alone the endometrium while suturing, strict irrigation of the abdominopelvic cavity, approximating the visceral and parietal peritoneum, and using separate needles for uterine and abdominal wall suturing [18]. Up to now, no data suggest that these strategies can prevent the occurrence of scar endometriosis. New studies are required to determine which prophylactic measures are most efficient.

Conclusion

AWE is a condition that generally, but not exclusively, occurs in patients who have a palpable mass associated with pain and a previous pelvic surgery. Combination of 2D, 3D, and Doppler sonography in correlation with clinical data and patient history (particularly the occurrence of previous surgery adjacent to the lesion) may aid considerably in making the correct diagnosis of AWE. The different imaging modalities (ultrasound, MRI, CT) are nonspecific but useful in determining the extent of disease and assist in the planning of operative resection, especially in recurrent and large lesions.

Although rare, it is important that physicians or sonographers are familiar with this pathology. Considering the increasing rate of caesarean deliveries, it is expected that this pathology will be encountered more frequently in daily practice.

Conflict of interest: none

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Review

Med Ultrason 2018, Vol. 20, no. 1, 88-94
DOI: 10.11152/mu-1350

The role of 4D US in evaluation of fetal movements and facial expressions and their relationship with fetal neurobehaviour

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Abstract

The introduction of four-dimensional (4D) ultrasonography (US) allows the study of fetal movements and facial expressions in real time. The possibility of evaluating fetal movements has led to the study of fetal neurobehaviour, which has been for a long period of time a mystery for physicians. The study of fetal activity in utero could differentiate between normal and abnormal behavioural patterns, thus making possible the early recognition of fetal brain impairment. Facial expressions observed with 4D US represent a marker for neurobehaviour and at the same time could enhance fetal-maternal bonding. The present review represents an update of the literature on fetal movements, facial expressions, and their relationship with fetal neurobehaviour.

Keywords: fetal neurobehaviour; facial expressions; four-dimensional US; KANET test

Introduction

It is well accepted that the human brain represents the most complex and difficult organ to explore and the difficulties are even greater in the fetal and neonatal life. Despite this difficulty, it is important that children at risk of neurological impairment should be examined as early as possible (newborns and infants), for the assessment of their neurological development and early detection of central nervous system disorders [1].

Understanding the structure and function of the fetal nervous system has been both a desire and challenge for physicians over many centuries [2]. While brain structure studies started many years ago by post-abortion fetus examination, the study of fetal brain function remained for a long time a mystery for physicians. The latest development in the field of ultrasound (US) has made possible the study of fetal brain function early in fetal life. From the beginning, three-dimensional and four-dimensional (4D) ultrasonography (US) proved to be valuable tools for the diagnosis of several malformations of the fetus starting with the first trimester of pregnancy [3,4]. Further development of these technologies has permitted not only the study of the anatomy but also of the behaviour of the fetus. 4D US has the great advantage over 2D US because it enables simultaneous spatial imaging of the entire fetus and its movements [5,6]. It also initiated the development of new research directions, such as "fetal neurology", "fetal psychology" and "fetal neurobehaviour". Therefore, knowledge on fetal neurobehavior and neurodevelopment will be advanced through fetal behavioral research using this technique [7].

Cerebral palsy (CP) represents a group of disorders of movements and postural control caused by a defect or lesion of the brain and it is the most common chronic motor disability in childhood [8]. Identification of children with developmental disorder, such as CP, at early age is

Received 31.10.2017 Accepted 24.11.2017
Med Ultrason
2018, Vol. 20, No 1, 88-94
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a difficult task for physicians [9]. Usually the diagnosis of CP is made six months postnatal when the child is already severely affected. Traditionally, it was accepted that brain damage appears during birth or early neonatal period. Actually this concept has changed and now is considered that antenatal factors are among the most important etiologic factors [10,11]. According to this theory many cases have the origin in prenatal period, therefore, constant attempts to diagnose neurological impairment in fetal life have been made in the last years [12].

Several studies on neonatal behavior showed that the assessment of behavior is a better predictor of neurodevelopmental disability than neurological examination [1,2]. An evolving challenge for the medical profession was and still remains to better define normal and abnormal fetal neurological function in utero, in order to better predict which fetuses are at risk for adverse neurological outcomes antenatal, regardless of intrapartum management [1,13]. Related to this purpose, fetal behavior is defined by fetal movement felt by the mother or observed by a more objective method, such as US [14].

The aim of this review is to present the current status of 4D US on fetal movement and fetal facial expressions as important markers for fetal brain development and function.

Fetal movements

Numerous studies have employed conventional two-dimensional (2D) US or 4D US for the assessment of fetal behaviour as an indicator of fetal brain function and development [15-18]. Fetal movements were first assessed only by conventional 2D US on different periods of the pregnancy. While several studies of fetal movements were performed in the first trimester of pregnancy, others were focused on the second and third trimester [19,20].

In 2005 Kurjak et al [13] started to analyse and to establish the standards of normal fetal behavioural patterns after 30 minutes of recording the fetus by 4D US. The authors showed that some types of movements are present through all trimesters while others are specific for different periods. For example, startle and stretch are specific for the first trimester, while some hand movement patterns or facial expressions, such as sucking and swallowing, are better observed in the second part of the pregnancy [13]. The main categories of fetal movements are synthetically described in table I.

Other studies have shown that the first trimester of pregnancy is characterized by a high frequency of fetal movement patterns, progressively increasing with gestational age. The only exception is represented by the startle movement, which has the same pattern during early gestation [21]. In the second trimester of pregnancy the incidence of body movements increases considerably alternating with longer periods of quiescence. The most frequent fetal movement involves the arms, whereas the least active is mouth movement. Among facial expressions, two types could be easily differentiated: smiling and scowling [14]. At the end of pregnancy, due to cerebral maturation process, the number of generalized movements decreases and, at the same time, an increase in facial movements (opening/closing of the jaw, swallowing and chewing) appear [14].

KANET scoring system

Kurjak et al developed the first scoring system for fetal neurobehavior based on prenatal assessment by 3D/4D

Table I. Categories of fetal movements according to Kurjak et al [13].

	Type of movement	Movement speed	Gestational age (weeks)
Whole-body movements	General movements	slow/rapid	>8
	Sideways bending	rapid	7 – 8
	Stretch	slow	>9
	Fetal rotation	rapid	>10
Head movements	Isolated head retroflexion	slow/fast, jerky	>10
	Isolated head anteflexion	slow	>10
	Isolated head rotation	slow	>10
	Jaw movements	slow/rapid	>11
Limb movements	Isolated arm/leg movement	slow/rapid	>10
	Startle	rapid	>8
	Clonic movements	very rapid	>10
	Twitches	rapid	>9
	Hand-head contact	slow	>10
Functional movements	Breathing – paradoxical breathing sequences	slow	>10
	Sucking and swallowing	slow/rapid	>9
	Hiccup	rapid	>9
	Yawning	slow	>11

sonography – Kurjak antenatal neurodevelopmental test (KANET) [22]. The KANET scoring system combines parameters from other prenatal tests used previously (such as general movements assessment) and postnatal Amiel-Tison neurological assessment at term (ATNAT) [23]. KANET is a method that has been applied for the past 10 years and studies show that it is a strong diagnostic tool and can be introduced into everyday clinical practice [24].

The KANET test should be performed in the 3rd trimester from 28th to 38th week of gestation for a period of 15-20 minutes. The fetus should be awake and if is sleeping, the exam should be postponed. Ten parameters are evaluated: assessment of cranial sutures, isolated head anteflexion, isolated hand, leg, hand to face and finger movements, isolated eye blinking, facial alterations, and mouth opening. These parameters were selected based on neurological development and on the theory of central pattern generators of general movements emergence [14,24]. The score has a three-point scale for several fetal movements (isolated head anteflexion, isolated hand, leg, hand to face, and finger movements) and a two-point scale for assessment of cranial sutures, isolated eye blinking, facial alterations and mouth opening. When the obtained score is abnormal or borderline the test should be repeated every two weeks till delivery [25]. KANET was used in several studies to compare fetal behavior and neurodevelopment of the fetus between low and high-risk pregnancies. All of the studies showed a score significantly higher in the low risk group compared to the high-risk group [26-28]. Antsaklis et al studied the differences between the fetus of a normal pregnant women compared with pregnant women with diabetes under treatment with insulin. The results showed higher scores in the non-diabetic group, suggesting that there are differences in the fetal behaviour between diabetic and non-diabetic fetuses [29].

KANET score was also used to study the fetal behaviours in twin pregnancies and two types of activities were observed: spontaneous and reactive. Twins showed different behavioural pattern than singletons with less activity and a part of the overall motility was due to inter-twin contacts [30]. The different behavioural pattern of the twins does not translate into brain pathology but maybe into a different reaction to a different environment than singletons.

In cases of fetuses from high-risk pregnancies with KANET score modified or borderline, it is important to continue the follow-up after delivery. Postnatal evaluation should be done with ATNAT test and a brain US in the early neonatal period and every 2 weeks afterward till discharge. If the infants are affected by grade 3 and above intraventricular hemorrhage or if there is a suspicion of hypoxic ischemic brain damage, MRI should be recommended [25]. Children with CP should be reassessed at the age of 6 years.

In order to increase the reproducibility of the score, a standardization of the KANET test was recently proposed [25]. New modified KANET test should be used with eight instead of 10 parameters: facial and mouth movements were combined in one category and isolated hand movements together with hand to face movements were combined in one category [25].

Facial expressions

In daily life, our face presents a reflection of our reaction to different internal or external stimuli. Fetal response to different stimuli is observed by studying fetal face with 4D US. Facial expressions could represent the evidence of fetal brain development and may mirror the development of fetal consciousness [31] (fig 1).

The development of 4D US enabled to observe and study several fetal facial expressions such as: blinking, yawning, sucking, mouthing, tongue expulsion, scowling, and smiling from the early second trimester of pregnancy [16]. The fetal face expressions can reflect the fetal brain development and function during different stages of the fetus in utero [31].

The full range of facial movements can be studied by 4D US starting with 20 weeks of gestation because at this time both the facial and trigeminal nerves and facial muscles are formed [32]. They are easier to be studied after 24 weeks of gestation because of the deposition of adipose tissue starting this period [33]. The main facial expressions that can be observed are:

Fetal mouthing is considered to be the most frequent facial expression observed with 4D US [21,32,34-37].

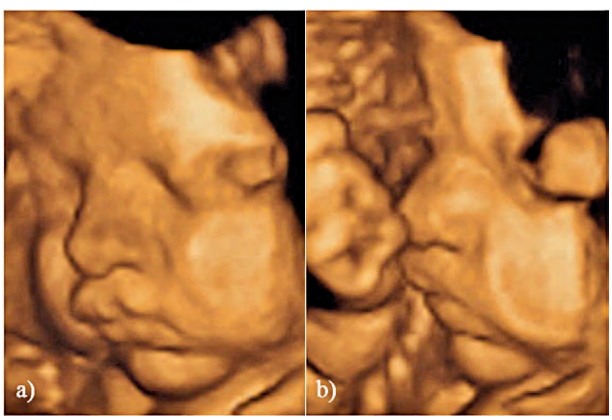

Fig 1. 4D US image of fetal face at 32 weeks of gestation: a) image of the nose, mouth and cheeks are clearly observed; b) the fetus is touching his nose with the hand

Reissland et al described different patterns in fetal mouthing movements. They are represented by neutral mouth movements with a decreasing frequency with advancing gestation and lateralized mouth movements, which increase in frequency with advancing gestation [38] (fig 2a).

Blinking represents a reflex response and is an important parameter for fetal brain functional development, easily observed with 4D US [31]. It is regulated by the dopamine system and the increased rate of eye blinking along the pregnancy might be a parameter of the central dopamine system [39-42] (fig 2b). During pregnancy the rate increases until term when usually a slight decrease is observed [21,34-37].

Yawning – usually yawning during pregnancy experiences a change in frequency that indicates maturation of the brain [43] (fig 3). The frequency of yawning decreases after 28 weeks of gestation [44].

Suckling represents an essential process in the postnatal life. Several authors suggested that a fetus might prepare for feeding after birth in the second half of pregnancy [36]. The movement of fetal suckling is the indirect proof of normal brain development in utero (fig 4a).

Tongue expulsion outside the mouth requires both intact muscles with their innervations and higher brain centers with normal development in order to be performed. This facial movement could be another indirect sign of normal brain development, but there is a need for further research in order to establish the exact relationship between fetal tongue expulsion and the brain function [31].

Scowling – this facial expression could represent a sign of pain or stress of the fetus. The frequency of fetal scowling increases with advancing gestation. This observation suggests that fetal scowling is present while

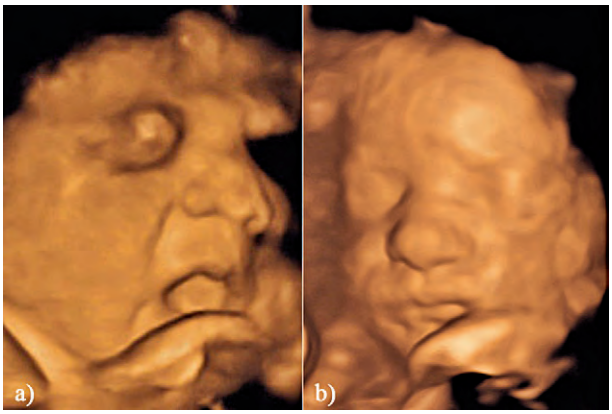

Fig 2. 4D US image of the fetus at 33 weeks of gestation: a) the mouthing movement is observed; b) the same fetus observed while blinking.

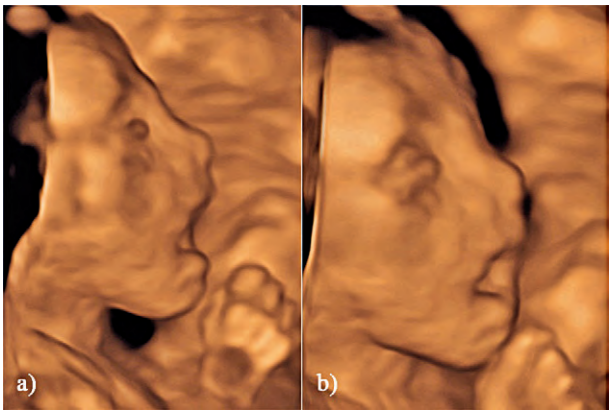

Fig 3. 4D US image a fetus at 29 weeks of gestation: a) the profile view of the fetus while yawning; b) the same fetus with the hand near the face while yawning.

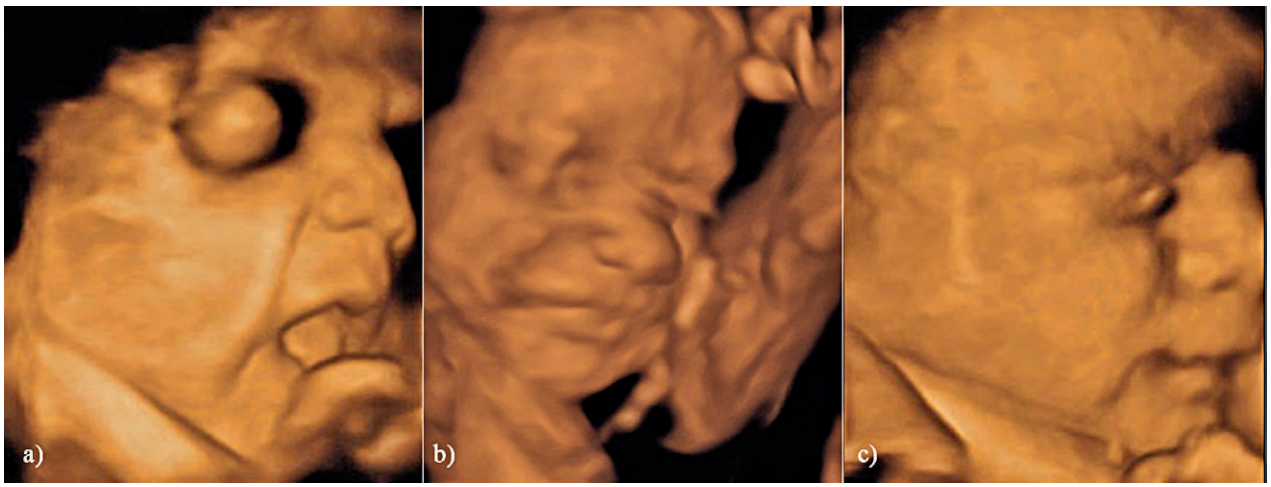

Fig 4. 4D US image a fetus at 29 weeks of gestation: a) the profile view of the fetus while yawning; b) the same fetus with the hand near the face while yawning.

the fetal brain matures with advancing pregnancy [45] (fig 4b).

Smiling. There are different patterns of fetal smiling ranging from a simple facial expression to complex facial movements involving bilateral elevation of the mouth angles, or even to form laughter gestalt [33]. This facial expression is very impressive for the parents and could represent an indicator of fetal brain development [31] (fig 4c). At the same time it is considered that a fetal smile is spontaneous and does not result from social effects [46].

Several studies about fetal facial expressions have been published. Reissland et al compared facial expression of fetus from smokers versus non-smokers women. It appears that fetuses of mothers who smoked displayed higher rates of mouthing movements compared with those of nonsmokers [47]. Fetuses suffering from stress display higher frequencies of facial expressions compared with non-stressed fetuses [31].

Lopes-Teijnn et al evaluated the effects of music on fetus and observed a fetal response to intravaginal music in pregnant women between 14-39 weeks of gestation. The increased frequencies of tongue expulsion with advancing gestation observed in this study could indicate the developing of the auditory system with its neural pathways starting at 16 weeks of gestation [48].

Ferrari et al showed that fetuses seem to respond to specific maternal stimuli represented by a specific song with increased mouth opening. This response could represent a liaison that can contribute to harmony between mother and child in postnatal life [49]. Also when the pregnant women touched her abdomen the fetus showed mouthing movements according [50]. It was observed that fetuses in the third trimester showed an increased regulatory (yawning) response to the stimuli when compared with those in the second trimester [50].

Nakamura et al observed that fetuses in primiparas showed a higher rate of eye blinking than those in multiparas. Because, according to these authors primiparas had a higher state of relaxation than multiparas this study of facial expressions suggests that that relaxation might promote fetal brain maturation, especially the central dopamine system, which regulates blinking [51].

The observation of facial expressions is important not only for studying fetal neurobehaviour but also for fetal-maternal bonding. Evidence suggests that enhancing fetal-maternal bonding contributes to positive health behavior [52]. It was hypothesized that 3D US, by making possible to better visualize the baby, may have the potential benefit of increasing the bonding of mothers to their expected newborns and strengthening the support system for their families [53].

Conclusions

Human brain being the most complex structure of the human body represents a challenge for understanding and diagnosing its impairments from the early stages of life. The introduction of 4D US allowed the study of fetal movements and facial expressions in real time. 4D represents an important tool in the evaluation of both fetal brain development and function. The study of fetal activity in utero could differentiate between normal and abnormal behavioural patterns, and thus make possible the early recognition of fetal brain impairment. The fetal neurobehaviour evaluation by KANET score appears to be a feasible technique in monitoring fetuses of high-risk pregnancies.

Conflict of interest: none

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Review

Med Ultrason 2018, Vol. 20, no. 2, 221-227
DOI: 10.11152/mu-1325

Ultrasound and magnetic resonance imaging in the prenatal diagnosis of open spina bifida

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Abstract

Open spina bifida, also known as spina bifida aperta is a neural tube defect involving the lack of closure of vertebral arches and associated meninges and/or spinal cord abnormalities. Ultrasound examination is the gold standard for the diagnosis of spina bifida aperta. It represents the main imaging tool used to ascertain this diagnosis early in gestation. Three-dimensional ultrasound is necessary to detect the level and the size of the defect. Magnetic resonance imaging (MRI) represents a more sensitive tool, giving specific information of the defect and associated anomalies, playing an important role in ruling out differential diagnosis. Due to the advent of MRI use, it is possible today to achieve *in utero* treatment of fetuses with this pathology. The aim of the current review is to provide an update of literature regarding the role of ultrasound and MRI in the prenatal diagnosis of spina bifida aperta.

Keywords: spina bifida; magnetic resonance imaging; Chiari II; ultrasound; prenatal diagnosis.

Introduction

Spina bifida is a congenital malformation belonging to the larger category of neural tube defects with a reported incidence of 1 to 1000 births [1,2]. It is the most common central nervous system (CNS) malformation compatible with life, represented by the lack of dorsal closure of the vertebrae which lack neural arches and may be located at any level along the spine. These defects can be classified into *closed* when the spinal defect is skin covered (spina bifida occulta) or *open* (spina bifida aperta) when the lesion site is not covered by skin and involves abnormalities of the meninges and/ or the spinal cord (meningocele/myelomenigocele). Spina bifida aperta is frequently associated with Chiari type II malformation, which is an abnormality of the posterior fossa characterized by the herniation of the cerebellum and brainstem through the foramen magnum [3]. Also, due to mechanical obstruction, it is often associated with ventriculomegaly [4,5].

Prenatal ultrasonography (US) represents the gold standard in fetal neural defects evaluation and diagnosis, whereas fetal magnetic resonance imaging (MRI) is used to refine the diagnosis. The role of prenatal diagnosis is to provide information that may help medical staff in counseling future parents regarding the pathology of their offspring, the therapeutic methods available in utero/postpartum, and the expected short and long-term outcome [6,7].

The aim of this review is to provide a literature update regarding the contribution of prenatal US and fetal MRI evaluation in the prenatal diagnosis of spina bifida aperta.

Received 28.09.2017 Accepted 05.11.2017
Med Ultrason
2018, Vol. 20, No 2, 221-227
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2D ultrasonography in spina bifida

The main method used when screening for fetal anomalies is represented by two-dimensional (2D) US. The growing interest in performing anatomical assess-

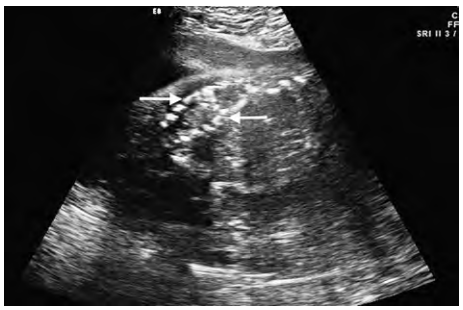

Fig 1. 2D ultrasound of myelomeningocele of the lumbar spine cord at 25 weeks of gestation (between arrows).

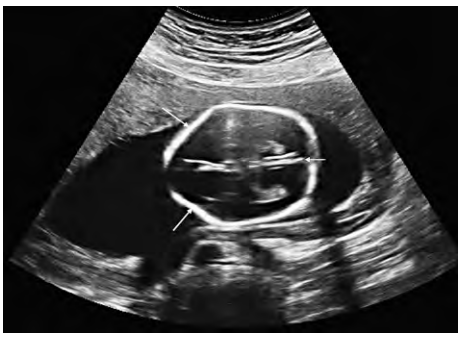

Fig 2. Lemon and banana sign (arrows) – 19 weeks of gestation

ment for Down syndrome in the first trimester of pregnancy has led to an improvement in 2D US resolution [8,9]. Several studies show a high sensitivity and specificity of 2D US in terms of evaluating bone anomalies, noting an increase in the detection of skeletal defects [8,10-14]. The technical specifications of the US machine, such as a high-resolution and high frame rate, are essential to obtain quality images [6,15,16]. It is recommendable to visualize structures in at least two planes to lower the risk of artifacts. The diagnosis of spina bifida aperta was usually made at the time of the second trimester scan, between 18-24 weeks of gestation [1,5,6,14] by visualization the spinal cord herniation (fig 1)

Nowadays the trend is to detect the malformation in the first trimester or early in the second trimester of pregnancy. Fetal CNS evaluation requires examination in the transthalamic, transcerebellar, and transventricular planes [17]. These planes allow the visualization of skull contour, falx cerebri, cavum septum pellucidum, cerebellum, cisterna magna, thalamus, posterior and lateral horns of the ventricles [6,18-21]. At this gestational age, examination of the entire length of the spinal cord may be difficult, depending also on the fetal position, thus the diagnosis of spina bifida aperta is often based on intracranial indirect findings as the fetal head is easier to examine [1,8,16,22]. These indirect signs of myelomeningocele are the ,,lemon sign'' – biconcave frontal bones and ,,banana sign'' – an abnormal, curved and thin form of the cerebellum (fig 2) [1,6,8]. Ventriculomegaly is also, a common finding in the Chiari II malformation but is nonspecific for this kind of pathology.

In fact, when the antero-posterior diameter of the cisterna magna and the shape of the cerebellum are normal, a type II Chiari malformation can be excluded [22].

Another useful 2D US parameter is the clivus-supraocciput angle which is represented by the angle obtained on the mid sagittal section by drawing two lines between the clivus and the supraocciput. The value of the angle is a specific parameter for detecting the Chiari II abnormality and may be used in the differentiation of other causes of ventriculomegaly [23]. D'Addario et al published a comparative study on 310 pregnant women with normal fetuses and 44 fetuses diagnosed with ventriculomegaly due to different pathologies [16]. Results showed that the value of the clivus-supraocciput angle was below the 5th percentile on the nomogram in fetuses with Chiari II malformation, suggesting a smaller size of the posterior fossa.

3D ultrasonography in spina bifida

Three-dimensional (3D) US has evolved very fast over the past few years due to high performance software in engineering, architecture and design [24]. The advantage of this technique is that it allows the acquisition of volumetric data which can be processed by the operator even after the completion of the examination [10,24,25]. It also allows multiple views of surfaces and structures as well as of certain tissues such as bones to be extracted from the volumetric data set and also the reconstruction of the fetal bones while focusing the examination on the area of interest [8,24]. Recent data from the literature advocates for 3D US in assessing skeletal anomalies and phenotypic features of the fetuses [8,24-26]. There is an ongoing debate about the contribution of 3D US over 2D US in the diagnosis and management of spina bifida aperta. Several publications support the 3D US advantages over 2D US, due to the possibility of showing a continuous structure image of the spinal cord rather than an independent one characteristic to 2D views (fig 3) [6,27,28].

In order to facilitate the clinicians' diagnosis of the Chiari II malformation, Leibovitz et al aimed to elaborate nomograms for the dimensions of the posterior fossa (PF) applicable in the 2nd and 3rd trimester of pregnancy, by assessing the mid-sagittal cranial plane with three-dimensional multiplanar imaging [22]. In addition, the

study compared the PF measurements between normal fetuses and those with neural tube defects. The nomograms were created using scans performed on 378 normal fetuses with a gestational age between 15-35 weeks. The area of the posterior fossa (PFA) and the perimeter of the posterior fossa (PFP) were included as parameters in the growth graphs.

The authors delimited the PF according to the following landmarks: from clivum to tentorium (CTD) and from the level of the upper mesencephalic edge to the occipital bone (TOD). The results showed a liniar relationship between the gestational age and PFA, PFP, TOD and CTD in normal developed fetuses. The study identified 39 fetuses with PF anomalies (including 11 cases of Chiari II malformation) which were analyzed by calculating z-scores for PFA, PFP, TOD, and CTD and compared to the developed nomograms. In all 11 cases with Chiari II malformation, highly contrasting z-scores for the PF size parameters were demonstrated. Moreover, PFA, showed the greatest deviation in z-scores, which ranged between −2.66 and −7.08 (mean, –5.31) meaning the size of posterior fossa was significantly smaller than nomograms. Another relevant study conducted by Scheier et al [6] on 11-13 weeks foetuses with spina bifida found important differences in the size of the posterior fossa in Chiari II malformation cases, supporting, previous data reported, even in earlier gestational age.

The largest study including 1030 fetuses among which 30 were diagnosed with spina bifida aperta measured the changes occurring in the first trimester of pregnancy in the PF. In all fetuses the brain stem and brain stem to occipital bone diameter (BSOB) were measured in the mid-sagittal view. In the control group, the brain stem to BSOB ratio decreased and the brain stem and BSOB diameter increased significantly with crown-rump length. In 96.7% of the cases with spina bifida aperta, the brain stem diameter was above the 95th percentile compared to the control group. The brain stem to BSOB ratio was above the 95th percentile in all cases and the BSOB diameter was below the 5th percentile in 86.7% of cases, all of these findings suggesting a smaller size of PF already from the first trimester of pregnancy [29].

These results are one of the first reported and of high importance as the current trend is to establish the diagnosis of spina bifida diagnosis at the end of the 1st trimester or early in the 2nd trimester of pregnancy [30-32]. For an accurate US evaluation, it is important that the examiner should be familiar with spine and intracranial structures anatomy, according to the gestational age.

Different attempts to use other parameters were described. Several published papers analyzed the possibility of diagnosing spina bifida at the end of the first trimester of pregnancy by 2D and 3D US by evaluating other US markers such as: the anteroposterior diameter of the cisterna magna and the fourth ventricle (intracranial translucency), the brainstem diameter, the brainstem-occipital bone distance, the diameter of the roof of the third ventricle and the diameter of the aqueduct of Sylvius, in axial or mid-sagittal planes [33-36]. From all these measurements the antero-posterior decrease of the intracranial translucence (fourth ventricle) and the cisterna magna obliteration were proven to be the most common markers associated with spina bifida aperta in the first trimester of pregnancy [7,15,29,32-37].

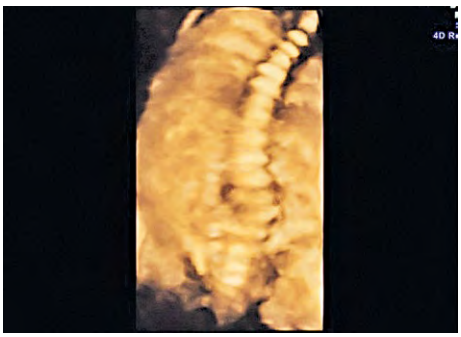

Fig 3. 3D ultrasound of myelomeningocele at 25 weeks of gestation

Loureiro et al published a study seeking changes in the cerebral ventricular system in fetuses with spina bifida aperta with gestational ages between 11-13 weeks [28]. Four hundred and two fetuses were included in the study, among them 10 fetuses with spina bifida aperta. They were evaluated by transvaginal US with the acquisition of 3D volumes of the brain. Lateral ventricular measurements, third ventricle roof diameter, aqueduct of Sylvius diameter and fourth ventricle diameter were performed. Unlike normal fetuses, in fetuses with spina bifida aperta the diameter of third and fourth ventricle, aqueduct of Sylvius, and lateral ventricular area were significantly smaller. These results are concordant with findings that the amount of cerebrospinal fluid is reduced in fetuses with spina bifida aperta.

Knowing the exact level of the spinal defect is an important factor when choosing therapeutical methods. Therefore, its precise identification is mandatory. In this regard, Buyukkurt et al aimed to predict antepartum the level of the defect in fetuses with spina bifida by using 3D imaging. Afterwards, postpartum, the exact level of the spinal aperture was confirmed by radiography or by autopsy (in the case of antepartum fetal demise).The detection rate of the defect's level using this technique was 79%, suggesting that the method is a useful tool for prenatal determination of lesion level in spina bifida [23].

US vs MRI in spina bifida

US examination is constantly evolving thus enabling the evaluation of fine structures, being the main imaging tool used to ascertain the diagnosis of spina bifida aperta early in gestation. US is less expensive, more widely accessible than MRI and is the main method of detecting fetal malformations in the first trimester of pregnancy. Moreover, it has an important role in deciding when to use more complex investigation methods, such as fetal MRI, and is certainly the basis for *in utero* surgical treatment of spina bifida aperta. Even though it is considered the gold standard for the diagnosis is has several disadvantages especially in case of obese women where the degree of penetration of the US beam is low or in case of oligoamnios when visualisation and diagnosis could be difficult. Low penetration (amplified by the increase of the gestational age) through fetal bone tissue, makes US unable to fully evaluate the fetal skeleton.

Another limit of the US examination is found in cases of cerebellum or cerebral trunk herniation, where it cannot establish the severity of this anomaly. In all these cases MRI proofs as a superior method confirming the diagnosis and also revealing associated fetal abnormalities. MRI represents an important imaging tool, neurosurgeons can rely on, in order to counsel future parents regarding the type of intervention that the fetus might need. It is also the main method for monitoring the postoperative outcome of the fetuses [38].

Early studies on fetal MRI began in the 1980's. Initially, MRI was only used for CNS evaluation. Due to active fetal movements the examination was impaired and that represented a major drawback. With the development of fast-moving sequences that take less than 20 seconds, this limit has been eliminated and fetal malformations can be detected much more easily and accurately [38,39]. Due to the high resolution of the soft tissues, a MRI examination reveals a clear distinction between maternal and fetal organs, and has the advantage of acquiring more data in comparison to US [40-42]. MRI images may be obtained in sagittal, transverse, and coronary sections. Being a non radiant method it is safe to use during pregnancy. The use of contrast media is not recommended due to its transplacental passage [43-45].

MRI allows a more accurate prenatal diagnosis than US, due to the detailed description of the cerebellum and the posterior fossa and can give a full assessment of the spinal lesion or other complications in Chiari II malformation (fig 4a) [42,46-49].

Some specific features of in utero Chiari II malformation, such as cerebellum herniation and possible changes in brain parenchyma signal can be better evaluated by fetal MRI than US (fig 4b) [50].

Being the investigation that establishes the definitive diagnosis as well the site of the spinal lesion (fig 4c) [1], and because it is less dependent of the fetal position [51], MRI brings important information when it comes to the *in utero* or *postpartum* surgical treatment options of fetuses with spina bifida aperta [2].

Recent studies discuss this issue and emphasize the advantages and disadvantages of such procedures in Chiari type II malformation [52]. Beghly et al showed that the low antero posterior diameter of the PF associated with hydrocephalus is one of the characteristic MRI images of Chiari II malformation [53]. The confirmation of cerebellum herniation, which can only be performed by fetal MRI, is one of the main criteria for *in utero* surgery of myeolomeningocele [54].

In the last years, fetal surgery has evolved, providing therapeutic options for treating congenital malformations. In utero surgery for myelomeningocele is not indicated before 18 weeks of gestation due to insufficient

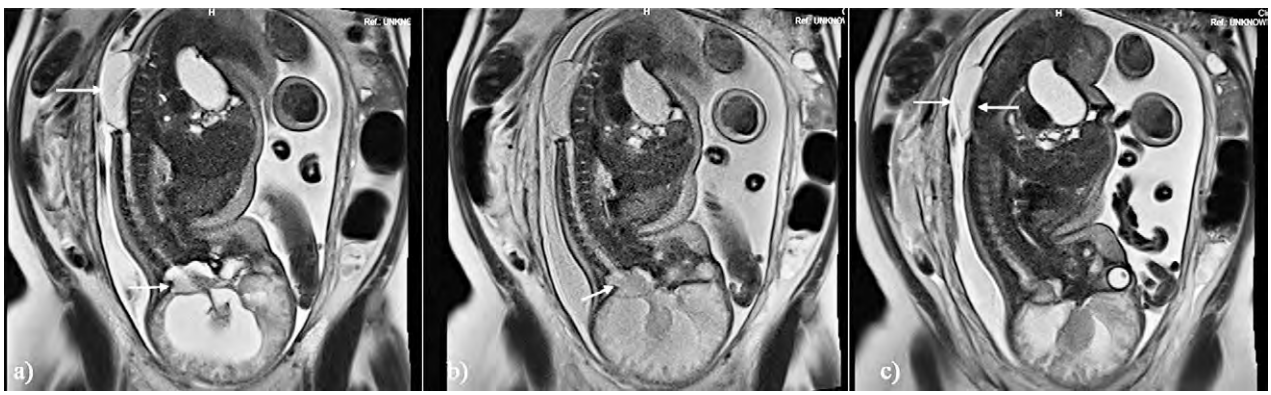

Fig 4. MRI examination in a case with Chiari II malformation at 28 weeks of gestation: a) MRI T2 – weighted sagittal view. The cerebellum herniation (superior arrow) and the spinal cord defect (inferior arrow; b) MRI T2 – weighted sagittal view,cerebellum herniation (arrow); c) MRI T2 weighted sagittal view – lumbar lesion (between arrows).

fetal growth and increased tissue fragility [55]. After 32 weeks of gestation, the risk of preterm birth is far too high compared to the benefits of surgery so, intrauterine repair of myelomeningocele is usually performed between 19-25 weeks of gestation [56,57].

A study regarding the surgical treatment of myelomeningocele named Management of Myelomeningocele (MOMS), has clearly shown the advantages and risks of intrauterine surgery [58]. The purpose of the study was to evaluate whether results obtained from *in utero* surgery performed between 19-25 weeks of gestation are superior to those obtained from postpartum surgery. The MOMS study showed that at the age of one year only 42% of the prenatal surgery group needed ventriculoperitoneal (VP) shunting as compared to 82% of the postnatal surgery group. The study also revealed a significant improvement in child neuromotor function at two and a half years. They found that 42% of the children from the prenatal surgery group were walking without support while only 21% of the children from the postnatal surgery group were walking without help. Compared to the postnatal surgery group, hindbrain herniation was significantly reversed in the fetal surgery group. The MOMS study also highlighted some of the possible complications of in utero surgery such as increased risk of preterm birth, premature rupture of membranes and oligoamnios.

The repair of myelomeningocele in utero, with all the benefits it offers, is dependent on the MRI examination which establishes whether the criteria for the surgery are met.

Conclusions

Early detection of open spina bifida is mandatory for the option of intrauterine surgery which has better results in comparison to postpartum surgery in terms of offspring outcome. Prenatal US represents at this point the gold standard for the detection of spina bifida aperta in early gestation. Furthermore, 3D US is employed to offer supplementary information in the determination of the level and the size of the spinal injury. MRI, on the other hand, represents an additional high resolution imaging tool providing more specific information and playing an important role in the differential diagnosis of fetal central nervous system malformations. US and MRI are complementary examinations which assist clinicians in providing adequate parental counselling, choosing the appropriate therapeutic strategy and to predict fetal outcome.

Conflict of interest: none

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Seminars in Arthritis and Rheumatism ▮ (2018) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Seminars in Arthritis and Rheumatism

journal homepage: www.elsevier.com/locate/semarthrit

Paternal exposure to antirheumatic drugs—What physicians should know: Review of the literature

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ARTICLE INFO

Keywords:
Paternal exposure
Antirheumatic drugs
Sperm parameters
Fertility
Offspring outcome
Pregnancy outcome
Biologic therapy
DMARDs

ABSTRACT

Reproduction capacity and long-term preserved hormonal function are important aspects with big impacts on patients' quality of life. Updated information on the interaction between drug therapy and reproductive function is essential when discussing family planning with patients. Currently, limited data is published regarding paternal exposure to different medications. Thus, it may be a challenge for the practitioner to choose the right therapy for a young male patient. Therefore we reviewed the literature, for effects of antirheumatic drugs on male gonadal function with a focus on spermatogenesis and offspring.

Key messages

1. Practitioners should be aware of the fact that many of the currently implemented restrictions for drug prescription are due to lack of robust data regarding paternal exposure and not because harmful effects on spermatogenesis or pregnancy outcome were proven.
2. Many antirheumatic medications can be continued in men who want to father a child.
3. Regular pre-conceptional counseling should be given to male patients of fertile age as an integral part of family planning.

Introduction

Although overall less affected by chronic rheumatic, inflammatory and autoimmune diseases compared to females, men develop some pathologies with a higher prevalence than women, e.g., spondyloarthritides (SpA), Behçet's vasculitis and gout. Furthermore, it is known that active, longstanding, multi-articular or systemic rheumatic diseases and their therapies may have a detrimental effect on reproduction [1–11]. Exposure to different environmental and/or medical agents may impair spermatogenesis or lead to abnormal pregnancy/offspring outcomes [12–14]. Humans can continue to have intercourse during pregnancy, and the possibility exists that teratogenic agents in the seminal fluid lead to local exposure of the conceptus or to systemic maternal exposure due to mucosal female reproductive tract absorption. Due to the blood-testis barrier, however, most drugs will be present in very low levels in seminal fluid and are thus of little concern in humans [12,15].

Evaluation of male reproductive function includes semen analysis, urologic clinical examination, testicular ultrasound, hormone profile and anti-sperm antibodies detection [14,16,17]. The rate of infertility in the general population is 8–17%, with male infertility as the single cause in about 26% of infertile couples [15]. Abnormal semen production was found to be responsible for approximately 50% of all male factor infertilities [18].

The definition of normal semen parameters is made by reference to a threshold that must be exceeded to achieve conception. The analysis of sperm count, motility, and morphology relies on the comparison to current WHO definitions, but these have been repeatedly questioned since studies performed in healthy fertile men (with pregnant partners) as well as in healthy military recruits showed lower parameters than the threshold being compatible with reproduction [14,19,20].

The large fluctuation in results is amplified by reported intra-sample variability due to a different number of days of abstinence prior to semen analysis, different age groups, presence or absence of stress, and other factors like individual sperm glucose levels and seasonal variation [21–27].

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https://doi.org/10.1016/j.semarthrit.2018.01.006
0049-0172/

In fact, fertility is not so much depending on the absolute number of spermatozoa but on their functional ability. The process of spermatogenesis in men lasts between 70 and 90 days, a time frame that includes the phase for germ cells to develop from spermatogonia to spermatozoa and the passage through the epididymis. A daily spermatozoa production may reach up to 200–300 millions sperm cells but only approximately 100 millions will remain competent (Fig.) [28].

Counseling on family planning should be part of the consultation not only in women but also men with rheumatic disease, particularly before prescribing immunmodulating drugs. The following systematic review summarizes the literature on effects of antirheumatic drugs on male gonadal function according to the guidelines of the PRISMA statement [29]. The methods are described in the supplementary data.

Spermatogenesis and effects on offspring are presented for drugs where at least some human data have been published.

Drugs and male gonadal function

NSAIDs

Mechanism of action

NSAIDs reduce pain and inflammation due to several mechanisms of action—prostaglandin (PG) and non-PG mediated. The primary effect of NSAIDs is to inhibit cycloxygenase 1 and 2 (COX 1, COX 2) and subsequently to impair the transformation of arachidonic acid to PG, prostacyclin and tromboxanes. The extent of the two isoforms enzyme inhibition varies among the different NSAIDs and defines the 2 main types of NSAIDs: nonselective (inhibition of both COX 1 and COX 2 to a significant degree) and selective COX 2 inhibitors. Indeed, the degree of their COX inhibition capacity may define their activity and potential toxicity [30].

Effect on spermatogenesis

Whether NSAIDs can impair fertility in men with rheumatic disease has not been fully investigated [31]. In a prospective longitudinal controlled study performed in men with active ankylosing spondylitis (AS), 20 patients with daily exposure to different NSAIDs were on a maximal dose for at least 6 months, 12 patients were exposed only to NSAIDs, 7 had additional therapy with Sulfasalazine (SSZ) and 4 a combination of SSZ and Methotrexate (MTX). Sperm analysis showed comparable results in patients and healthy age matched controls [32]. Likewise another study of 20 AS patients treated with NSAIDs found sperm parameters normal [16]. By contrast, an observational study included 1376 men who attended an infertility clinic and were using non-prescription NSAIDs (mainly Aspirin, >6 months) for unknown reasons. The subjects were split in different groups according to the NSAIDs intake as follows: the control group (no NSAIDs); group A, 1–4 NSAID pills/month; group B, 5–9 NSAID pills/month; group C, 10–20 NSAID pills/month; group D, 1 or more NSAID pills/day. A dose-dependent decrease in seminal volume, sperm concentration, quality and motility was observed; reversibility was not studied [33].

Effect on offspring

A population based study, identified 183 men with pre-conceptional exposure to Indometacin (≥1 pregnancy/couple; exact number of pregnancies not specified) and found no link between paternal drug exposure and adverse pregnancy outcomes [34]. Records of 1198 fathers exposed to different medications out of which 723 had exposure to NSAIDs (≥1 pregnancy/couple; exact number of pregnancies not specified), showed an overall odds ratios (OR) for malformations of 1.19 and for major malformations 1.26, respectively, when compared to the general population [35].

Comment: Small studies of chronic use of NSAIDs in patients give no sign of impairment of spermatogenesis. The study of men attending an infertility clinic is biased by the unknown indication, the predominant use of aspirin and the lack of studying reversibility. There is no evidence for harmful effects of NSAIDs on offspring [36].

Glucocorticoids (CS)

Mechanism of action

Corticosteroids are potent anti-inflammatory hormones used to treat inflammatory, immunological and allergic disorders. In supra-physiologic dosage, they produce effects on gene transcription (inhibition of the synthesis of all known inflammatory cytokines, blocking promoter sites of pro-inflammatory genes, stimulating the production of anti-inflammatory cytokines), effects on posttranslational events (inhibit the secretion of inflammatory cytokines, inflammatory eicosanoids and induced COX-2) [30].

Corticosteroids (CS), synthetic and endogenous, may induce an inhibitory effect on the male hypothalamic–pituitary–gonadal axis through direct and indirect action on the synthesis and release of gonadotropin-releasing hormone (GnRH), luteinizing hormone

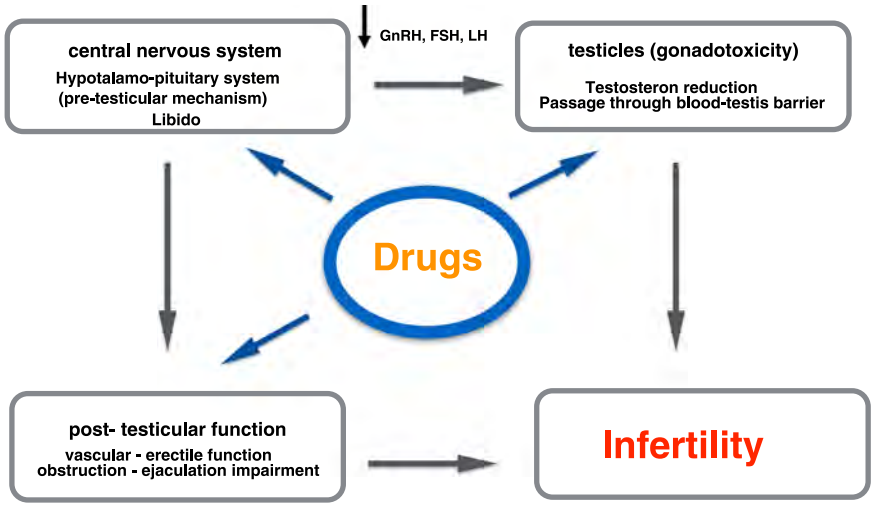

Fig. Summarizes modes by which drugs can influence male gonadal function.

(LH) and follicle stimulating hormone (FSH), and through direct influence on the steroidogenesis and/or gametogenesis [17,37].

Effect on spermatogenesis

Use of low/moderate doses of CS alone or in combination with azathioprine (AZA) in inflammatory bowel disease (IBD) showed no correlation with infertility in men [2,15,38–40].

Effect on offspring

Two registry based Norwegian studies show no increase of adverse pregnancy outcomes (spontaneous abortions, preterm births, small for gestational age newborn children and perinatal death) in 1477 men exposed to prednisolone [34] nor an increased OR for major malformations in 633 men exposed to CS [35]. In both studies, ≥1 pregnancy/couple was achieved but the exact number of pregnancies was not specified.

Comment: In spite of the frequent co-administration of CS with other immunosuppressive drugs investigations on their separate effect on male reproduction are sparse. Thus far no sign of CS induced harmful effects have emerged for low (< 10 mg prednisone/day) doses.

Drugs with teratogenic potential

Metotrexate (MTX)

Mechanism of action

MTX is a structural analogue of folic acid that can competitively inhibit the binding of dihydrofolic acid to the enzyme dihydrofolate reductase responsible for reducing dihydrofolic acid to its active metabolite (folinic acid). Subsequently, decreased levels of folinic acid will negatively interfere with the cellular metabolism (purine and pyrimidine metabolism, synthesis of aminoacids). Secondary mechanisms of action described are regulation of proinflammatory and antiinflammatory cytokines, suppression of the clonal growth of lymphocytes T and B (LT and LB) [30].

Effects on spermatogenesis

Previous reports based on animal studies showed alterations in sperm DNA after MTX exposure [14]. Case reports in humans are conflicting: a transient adverse effect on sperm quality vs no harmful effects [15,41–44].

Effects on offspring

Recent data from a prospective observational study including 113 pregnancies [45], 49 pregnancies from the Norwegian Medical Birth Registry [46] and 127 live births from the Danish Medical Birth Registry [47] show that continuous treatment with MTX (≤30 mg/week) is not associated with a higher risk of congenital malformations nor with adverse pregnancy outcomes in case of paternal exposure prior to conception and during pregnancy, when compared to unexposed fathers [45–47]. A retrospective study of 42 pregnancies fathered by MTX exposed men found no increase in the miscarriage or congenital malformation rate [48]. One single study identified two oro-facial congenital malformations in a group of 50 newborn children after paternal exposure to MTX [35].

Comment: Since controlled studies have not shown increased malformation rates, most experts plead for the maintenance of MTX therapy in men throughout the disease course to avoid unnecessary flares that may contribute to infertility [45–47,49]. Other experts are in favor of stopping MTX 3 months prior to conception considering the risk of a direct toxic effect on the conceptus by exposure to contaminated seminal fluid during intercourse in pregnancy [50].

Cylophosphamide (CYC)

Mechanism of action

CYC is an alkylating agent, one of the most potent immunosuppressive therapies available. It is converted to its active form mainly in the liver. It has been used extensively to treat different neoplastic and severe manifestations of autoimmune and inflammatory diseases [30].

Effects on spermatogenesis

One of the most common long-term side effects of cytotoxic agents in men is gonadal dysfunction. Spermatogenesis is disrupted because the germinal epithelium is very sensitive to damage. The level of damage is depending on the stage of testis maturation, postpubertal testis being more susceptible [51]. The impact on sperm production is drug specific and dose dependent [52–60]. In prepubertal males, the minimum total gonadotoxic dose of CYC is around 200–300 mg/kg vs 100 mg/kg for adult males [61]. A high rate of oligospermia and azoospermia was found in 14 postpubertal males exposed to < 200 mg/kg of intravenous CYC [62].

The incidence of gonadal dysfunction was 80% in 116 men when the cumulative dose of CYC was higher than 300 mg/kg [51]. Sperm analysis of children exposed to CYC for different oncologic disorders after equivalent cumulative dose (CED) exposure to less than 4000 mg/ m^2 CYC was normal in 89% of the patients. CED between 4000 and 8480 mg/m^2 produced oligospermia and CED 10,830 mg/m^2 produced azoospermia [59,60]. In the adult population, a total dose of 6–10 g/m^2 may result in irreversible azoospermia as shown in several oncologic studies [63,64]. From pretreatment levels that were similar to those of control subjects, sperm production declined to azoospermia within 4 months of treatment.

Lower doses may allow recovery of normal sperm parameters after drug cessation in 40–70% of the patients by 5 years after treatment [63,64].

Sperm abnormalities have been reported after IV CYC therapy in patients with JIA, SLE (juvenile and adult), secondary amyloidosis and Behcet disease [65–68]. Recovery of sperm function in adolescents with long-term CYC treatment (1–6 years) after bone marrow transplantation was possible [69,70]. However, no safe threshold of the cumulative dose of CYC is known, and it is not possible to predict which patients will become infertile [65,70,71].

Effects on offspring

Animal experiments have shown a dose dependent prevalence of pre and post-implantation embryo loss, malformations and growth-retarded fetuses, after paternal exposure, with more important effects during CYC treatment and shortly thereafter [72–75].

No robust data on human paternal pre-conceptional exposure to CYC and offspring outcome is available. Previous epidemiological studies failed to demonstrate any increase of teratogenic or carcinogenic effects in children of men who survived cancer treatment but termination of medication took place long before conception [76,77].

Comment: CYC treatment is not compatible with pre-conception or pregnancy paternal exposure [13,15,49]. Men should receive counseling on fertility preservation by sperm or testicular biopsy cryopreservation for future assisted reproduction techniques (ART) prior to CYC treatment [13,78].

Mycophenolate mofetil (MMF)

Mechanism of action

Mycophenolate mofetil (MMF) a powerful inhibitor of LB and LT proliferation, has indication for the prevention of acute allograft rejection, and as glucocorticoid sparing agent in different rheumatic diseases, mainly SLE [30].

Effects on spermatogenesis

MMF showed no effect on fertility in male rats at doses 4–9 fold higher than the standard clinical dose. There are no human studies reporting effects on spermatogenesis or drug levels in seminal fluid for monotherapy [79–81]. In triple therapy regimens with AZA, Cs and MMF, no sperm parameters alteration was detected 2 years after renal transplantation [82,83].

Effects on offspring

Initially using condoms during treatment and for at least 90 days after drug cessation both in reproductive competent and vasectomized men was recommended for sexually active men to avoid the risk of drug transfer into seminal fluid. In addition, their female partners were advised to use highly effective contraception during treatment and for a total of 90 days after the last dose of MMF [79–81].

Recently, three registry based studies of pregnancies after paternal exposure to mycophenolate derivatives did not identify an increased incidence of congenital malformations compared to disease matched controls and the general population [84–86]. In 152 male transplant recipients (fathering 205 pregnancies/194 live births) pregnancy outcomes were: 10.8% prematurity rate, 14 spontaneous abortions and no therapeutic abortions or stillbirths. Among the live births, 6 malformations were reported, an incidence of 3.1%. No pattern of malformations was identified [86].

Morken et al. compared 492 infants (474 deliveries) fathered by males after transplantation, receiving triple regimen with combinations of CS, calcineurin inhibitors (cyclosporine A or tacrolimus) and AZA or MMF with a large number of infants fathered by males before their solid organ transplantation as well as deliveries in the general population. No significant difference in congenital malformations was found [85]. The findings were confirmed by an analysis of 155 pregnancies fathered by male transplant recipients treated with MMF compared to 195 non-exposed pregnancies. No increase in the malformation rate was found [84].

Comment: Based on these data, most experts regard mycophenolate derivates compatible with paternal exposure [49,84–86].

Other DMARDs

Sulfasalazine

Mechanism of action

Salicylazosulfapyridine (sulfasalazine, SSZ) is a sulfonamide transformed in the colon into sulfapyridine and 5-aminosalicylic acid (5-ASA). Sulfapyridine is absorbed and 5-ASA is excreted in the feces. Sulfapyridine represents the active moiety in RA and shows disease modifying properties. In contrast, 5-ASA is the active metabolite in inflammatory bowel disease (IBD) [30].

Effects on spermatogenesis

SSZ can produce temporary oligospermia, reduced mobility and increase of pathologic sperm morphology. In patients with IBD treated with SSZ up to 60% developed infertility [2,16,87–95]. However, a prospective controlled study by Micu et al. showed no alteration of sperm parameters during longstanding therapy with SSZ in 11 patients with active AS (3 g/daily for 2 years) [32].

Transient infertility is induced by the sulfapyridine metabolite (animal studies), is dose dependent, and not influenced by folic acid supplementation. Sperm parameters normalize after 2–3 months of medication cessation, in some cases followed by successful conception [91,92,96–98].

Effects on offspring

Two Norwegian population based studies, identified 74 pregnancies after paternal exposure to SSZ, compared to a healthy control group no increase in congenital malformations were reported in the children [35,46].

A higher risk of congenital malformation was suggested in a single study of children born to couples with SSZ exposed men [94]. By contrast, a recent meta-analysis found no significant risk for congenital malformations, stillbirths, spontaneous abortions, preterm deliveries and low birth weight after paternal exposure to SSZ [97].

Comment: Male patients who wish to father a child should stop SSZ 3 months before conception [49] to allow for recovery of sperm quality though it has not been proven that this would enhance attempted conception. Restoration of semen quality and fertility was reported in several studies of men with IBD after SSZ cessation and substitution with Mesalazine (5-ASA) or other 5-ASA derivates [93,98].

Azathioprine and 6-mercaptopurine

Mechanism of action

Azathioprine is derivative of thioguanine, a purine mimic antimetabolite. After gastrointestinal absorbtion, approximately 50% will be transformed into its principal active metabolite-6 MP. Two enzymes—thiopurine S-methyltransferase (TPMT), and hypoxanthine phosphoribosyl transferase are responsible of further metabolization. The toxicity of AZA and 6-MP is predominantly related to the activity of TPMT; 11% of the population have low levels of TPMT [30].

Effects on spermatogenesis

Dejaco et al. studied 18 men with IBD and AZA treatment. No impairment of the sperm parameters was noticed and 6 men conceived during the study [40].

Effects on offspring

In a prospective study of 115 pregnancies after paternal exposure to AZA and 6-MP no increase in congenital malformations was found in the exposed versus the large control group (3.0% vs 2.2%). No specific pattern of birth defects and no indication of chromosomal aberrations was present in the exposed group. Elective terminations of pregnancy were more frequent in the exposed group and spontaneous abortions slightly increased [99].

A retrospective study of 154 pregnancies from 76 male patients analyzed 44 pregnancies after 6-MP cessation, 3 months prior to conception, 37 pregnancies from men with ongoing therapy at conception and 73 pregnancies from men not exposed to 6-MP. No difference in pregnancy outcomes were detected in the 3 groups [100]. Another registry based study identified 124 pregnancies of AZA exposed fathers. The malformation rate was similar to that present in the non-exposed population [35].

A registry based retrospective controlled study identified 54 pregnancies with prescription of AZA or 6-MP to the father, 1–121 months before conception. In contrast to the control group, the mothers were older. Six children with congenital abnormalities (polysyndactylia, oesophagus atresia, hidronephrosis, megalourether, and ventricular septal defect) were reported fathered by men

treated with AZA or 6-MP, 9–38 months before conception. When limiting male exposure to within 3 months before conception, no congenital abnormalities were detected [101].

A retrospective interview study showed a higher incidence of pregnancy-related complications (2 spontaneous abortions and 2 congenital anomalies: a missing thumb and acrania with multiple digital and limb abnormalities) when fathers were exposed to AZA pre-conceptionally [102].

Other retrospective controlled studies in male renal transplant recipients found no obvious effect of long-term treatment with small-doses of immunosuppressants on male fertility nor significant differences to controls regarding spontaneous abortions, ectopic pregnancies, anembryonic pregnancies, fetal deaths, preterm births, low birth weight or congenital malformations [103–105].

Comments: Most controlled studies have not detected harmful effects of AZA and 6-MP on male reproduction. A meta-analysis stated that there is no increased risk of congenital abnormalities in offspring of fathers treated with thiopurines and that male fertility does not appear to be affected [106].

Cyclosporin

Mechanism of action

Cyclosporine selectively inhibits calcineurin, thereby impairing the transcription of interleukin IL-2 and several other cytokines in LT. It is mainly used in solid organ transplantation but occasionally also in autoimmune diseases [30].

Effects on spermatogenesis

Fertility measured by semen analysis and hormonal profile in 9 renal transplant recipients treated with Cyclosporine were normal in 8 patients. Three out of 4 patients did achieve conception [107]. Similarly 19 men receiving a triple regimen with cyclosporine, AZA and CS or cyclosporine, MMF and CS showed a normal spermatogram 2 years after renal transplantation [83].

Impregnation capacity was evaluated in 26 male renal transplant recipients. Patients were divided into 3 groups according to the dosage of Cyclosporine and time frame after the renal transplant (<6 months, 4.1–6 mg/kg/d, 6–24 months, 2.1–4 mg/kg/d and >24 months, 1.3–2 mg/kg/d). Significant differences were found in sperm motility and sperm head deformity at higher exposure dose and more recent surgical history compared to the group with lower dosage and longer time from the transplant. The authors concluded that a 2 years' interval after transplantation is best for impregnation [104].

Effects on offspring

A multicenter, controlled study identified 212 patients with renal transplant who fathered 216 children. 148 men received a combination of cyclosporine (1.2 mg/kg/d), MMF and CS. Premature delivery was significantly increased when fertilization took place <2 years from transplant surgery but all neonates were healthy. The fertility capacity index was similar to the healthy control group [82]. The results support registry based data of male transplant recipients treated with a triple regimen with combinations of CS, calcineurin inhibitors and AZA or MMF [84]. No increase in congenital malformations or other adverse child outcomes were detected in exposed pregnancies compared to infants fathered by males before their solid organ transplantation and deliveries in the general population.

Comment: In doses below 2 mg/kg/day Cyclosporine has no negative impact on male fertility nor does paternal exposure harm child outcomes [49,82,83,85,103–106].

Colchicine

Mechanism of action

Colchicine acts by inhibiting β-tubulin polymerization into microtubules. It has also a powerful antimitotic effect, therefore a negative impact on semen parameters in infertile patients has been discussed [108].

Effects on spermatogenesis

Reports have focused on patients with Familial Mediterranean Fever (FMF) because of life-long therapy with colchicine and because of previous reports showing a high percentage of oligospermia (37%) [109]. More recent studies point out that secondary amyloidosis may have biased some studies [110].

Testicular function and spermatograms were found normal in six healthy males [111] treated with standard doses of colchicine for four to six months, and in six patients with FMF receiving long-term therapy with colchicine [112]. Ben-Chetrit et al. studied sperm motility at different Colchicine concentrations and noticed that sperm motility was inhibited significantly only at a dosage 3000 fold the therapeutic concentration and after a long exposure time (18 hours) [113]. Therefore it seems unlikely that colchicine would inhibit sperm motility in vivo [110,112]. Semen parameters and hormone levels were in the normal range in men receiving long term colchicine therapy [111]. A review paper concluded that colchicine has no significant impact on semen parameters and hormonal levels. Fertility in patients with FMF, in both genders, was better preserved when chronic treatment with Colchicine was given [114].

Effects on offspring

Fifty-three exposed men fathering 222 pregnancies were compared to a control group of 230 healthy couples. Seven percent of the pregnancies ended with spontaneous abortion, 3 newborns had congenital malformations. In the control group, the authors detected 16% abortions and 6 children with congenital malformations. The rate of second trimester abortions in both groups was comparable [115].

Comment: Colchicine has no significant impact on semen parameters. The results indicate that neither FMF nor colchicine increase the rate of abortions or congenital malformations. Therefore, colchicine does not need to be discontinued in men with FMF before conception [49,111–115].

Leflunomide

Mechanism of action

Leflunomide (LEF) is an isoxazole derivative rapidly absorbed from the gastrointestinal tract and converted to its active metabolite Teriflunomide which inhibits the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH) and subsequently the synthesis of pyrimidine [30].

Effects on spermatogenesis

There are no robust data on a mutagenic action of Leflunomide or its metabolites in humans. Brent et al. highlight the minimal risk of Leflunomide therapy on reproductive capacity in men. Administration of a therapeutic dosage of Leflunomide showed low blood concentrations (8.1 ±3 ng/ml) of 4-trifluoromethylaniline (the metabolite mutagenic in high concentrations in some but not all animal tests) in 75% of the exposed men and was not detected in 25%. The author states "men inseminating their spouse while taking leflunomide are not at increased risk parenting a child with birth defects or genetic disease since

Table 1
Summary of drugs compatible with paternal exposure in regard to male fertility and pregnancy outcome

	Spermatogenesis			Pregnancy and child outcome			
Drug	Type of study	Dose	Results	Type of study	Congenital malformations	Other adverse outcomes	Comments
NSAIDs (selective and non-selective COX inhibitors)	**Human**, controlled (3-6 months exposure)	Standard	Normal sperm parameters (40 AS patients) [16,32]	**Human**, registry (Indometacine, 183 men/≥1 pregnancy/ couple; number of pregnancies not known) [35]	OR, $m = 1.19$	No adverse pregnancy outcome	Small studies of chronic use of NSAIDs in patients give no sign of impairment of spermatogenesis.
(Aspirin, Indometacine)	**Human** (6 months exposure)	Non-prescription use, self-reported dosing	Dose dependent reduction of sperm volume, concentration, quality, motility (1376 men) , [33]	**Human**, registry (1198 men/≥1 pregnancy/couple; number of pregnancies not known)) [35]	OR major malf = 1.26 (compared to controls)		No evidence for harmful effects of NSAIDs on offspring. Experts regard NSAIDs as compatible with paternal exposure [36]
Corticoids	**Human**, controlled	< 30 mg/day	Normal sperm parameters (10 IBD patients, CS + AZA) [38] and fecundity (168 IBD patients) [39]	**Human**, registries (1477 men, Prednisolone) [34] and (633 men) [35]/≥ 1 pregnancy/ couple; number of pregnancies not known)	OR, $m = 1.06$ [33] OR, $m = 1.26$ [33]	No adverse pregnancy outcome	No evidence for harmful effects on sperm parameters and offspring for low (< 10 mg prednisone/day) doses. Experts regard CS as compatible with paternal exposure [49]
MTX	**Animal** studies, [14]	5–40 mg/kg, 5-10 weeks	Gonadal toxicity	**Human**, prospective observational study and registry data (113 +49+127 pregnancies) [45–47]	No increased risk for malformations compared to controls	No adverse pregnancy outcome compared to controls	Controlled studies have not shown increased malformation rates. Most experts plead for the maintenance of MTX therapy in men throughout the disease course [45–47,49]. Other experts are in favor of stopping MTX 3 months prior to conception, out of theoretical concerns
	Human, case report	< 25 mg/week	Reversible oligospermia or azoospermia (5 men with PsA, malignancies) [41]	**Human**, retrospective study (42 pregnancies) [48]	No increased risk for malformations compared to controls	No adverse pregnancy outcome	
	Human, case series	< 25 mg/week	No impaired sperm quality [42]	**Human**, registry data (50 newborn) [35]	2 malformations; no increased risk compared to controls	No adverse pregnancy outcome	
	Human, cases	< 25 mg/week	No impaired sperm quality (4 SA patients, concomitant SSZ and NSAIDs) [32]				
Cyclophospha-mide	**Human**	6–10 g/m^2 cumulative doses	Oligospermia and azoospermia with irreversible testicular damage at high doses [51,57–63]	**Animal** studies, [72–75]	High prevalence of pre and post-implantation embryo loss, malformations and growth- retarded fetuses [72–75]		CYC treatment is not compatible with pre-conception or pregnancy paternal exposure [13,15,49]. Sperm cryopreservation is recommended.
				Human	No robust data at present.	No robust data at present.	
Mycophenolate Mofetil	**Animal** [79–81] **Human**	4–9 X human dose No data for monotherapy	No impairment of fertility [79–81] Triple regimens mycophenolate mofetil, Azathioprine, CS show no sperm parameters alterations [82,83]	**Human**, registry data (152 men/ 250 pregnancies; 492 children; 111 men/155 children) [84–86]	No increased risk for malformations, compared to controls [84–86]	In one study—10.8% prematurity rate, 14 spontaneous abortions; no therapeutic abortions or stillbirths [86]	Experts regard mycophenolate derivates compatible with paternal exposure [49,86]

Sulfasalazine	**Human**		Qualitative and quantitative sperm parameters alterations and transient infertility and recovery of spermatogenesis 2–3 months after drug cessation in 60% of the patients (IBD patients) [91,92,96–98]	**Human**, registry data—57 pregnancies [35], 17 exposed men [46]	No increased risk for malformations, compared to controls [35,46]	No adverse pregnancy outcome [35,46]	Experts consider that men taking SSZ may have reduced fertility. There is no evidence, however, that conception is enhanced by stopping SSZ for 3 months prior to conception [49]
Azathioprine 6Mercaptopurine	**Human**, prospective, controlled with Azathioprine	50-150 mg/day, >3 months	Normal sperm parameters, [40]	**Human**, retrospective and prospective studies with Azathioprine, 6 Mercaptopurine (IBD patients, transplant patients, >500 pregnancies) [35,99–101,103–105]	No increased risk for malformations compared to controls	No adverse pregnancy outcome	No evidence for harmful effects on sperm parameters and offspring [37]. Experts regard thiopurines compatible with paternal exposure [35,49,99–106]
Cyclosporine	**Human**	Cyclosporine 1, 2–3 mg/kg/day, >3 months	Normal sperm parameters (8/9 transplant recipients, [107]	**Human**, controlled (212 men/216 children) [82]	No increased risk for malformations compared to controls [82]	Premature delivery when conception was <2 years from transplant, [99].	No evidence for harmful effects on sperm parameters and offspring. Experts regard Cyclosporine compatible with paternal exposure [49,82,83,85,104,106]
	Human	Cyclosporine 1, 2–3 mg/kg/day,	Normal sperm parameters after 2 years from transplantation in 19 men [83]	**Human**, registry data (474 deliveries [85]	No increased risk for malformations compared to controls [85]	No adverse pregnancy outcome [80]	
	Human	Cyclosporine 2–4 mg/kg/day, 4–6 mg/kg/day	Dose-dependent impact on sperm parameters. Normal fertility after 2 years from transplantation (26 men) [104]				
Colchicine	**Human**	1–2[85]mg/day, 4–6 months	Normal sperm parameters in 6 healthy men [111]	**Human** (53 men/222 pregnancies) [115]	No increased risk for malformations compared to controls [115]	No adverse pregnancy outcome, [111]	No evidence for harmful effects on sperm parameters and offspring. Experts regard Colchicine as compatible with paternal exposure [49,111–115]
	Human	1–2 mg/day, long term	Normal sperm parameters in 6 FMF patients [110]				
	Human, in vitro		Sperm motility was inhibited significantly only at a dosage 3000 X standard [111]				
TNF i	**Human**, case reports	Standard dose of Infliximab, Adalimumab	Oligospermia, [120,121]	**Human**, prospective, cross sectional/longitudinal controlled studies , national databases (57 + 51 + 13 + 372 + 27 children) [46,125–130]	No increased risk for malformations compared to controls [46,125–130]	No adverse pregnancy outcome compared to controls [46,125–130]	No evidence for harmful effects on sperm parameters and offspring. Experts regard TNFi as compatible with paternal exposure [32,49,122–130]
	Human, prospective , controlled, cross sectional and longitudinal	Standard dose of TNFi (Infliximab, Etanercept, Adalimumab), >3 months	Sperm quality comparable to controls (40 SpA patients) [32,122]				
	Human	Standard dose of TNFi (Adalimumab)	Asthenozoospermia improved after TNFi treatment (10 SpA patients) [123]				
	Human	Standard dose of TNFi (Etanercept, Adalimumab)	No relevant negative effects on sperm parameters compared to baseline (27 men with PsA, 85% with sperm abnormalities) [124]				
	Human	Certolizumab 400 mg, single dose vs placebo	No negative effects on sperm parameters compared to placebo (20 healthy men) [79]				

TNFi—TNF alpha inhibitors, SpA—spondylarthritis, AS—ankylosing spondylitis, PsA—psoriatic arthritis, IBD—inflammatory bowel disease, CS—corticosteroids,OR—odds ratio.

leflunomide and its metabolites are not mutagenic or cytotoxic". Despite this statement, the author suggests the wash-out procedure and the cessation of the medication 3 months prior to conception also in men [116].

Effects on offspring

Only one healthy neonate with a father exposed 6 months prior to conception is published in the literature [117], and two other cases from a registry [36,44,46].

Comment: At present there is no conclusive data. A wash-out procedure and 3 months pre-conceptional drug cessation are advised by the manufacturer. Most experts regard it as compatible with paternal exposure [49].

Biologic therapies

IgG concentrations in human semen are low, between 1% and 10% of the plasma IgG level [118]. Therefore, levels of monoclonal antibodies targeting cytokines may be low. A diversity of cytokines is present in seminal plasma with an indication that high levels of pro-inflammatory cytokines like TNF alpha and IL-6 impair spermatogenesis [119] (Table 1).

Except for TNF alpha inhibitors (TNFi) data regarding paternal exposure to biologics are limited or completely absent (Table 2).

TNF alpha blockers

Five inhibitors of tumor necrosis factor (TNF)-alpha (TNFi) are available for clinical use: Infliximab, Etanercept, Adalimumab, Golimumab, and Certolizumab pegol.

Mechanisms of action

Infliximab inhibits binding of TNFα to its receptors. Etanercept is a fusion protein fusing the TNF receptor to the constant region of the IgG1 antibody. Adalimumab inhibits binding of TNFα to both of its receptors and lyses cells that bear TNFα on their surfaces. Golimumab binds to both soluble and transmembrane forms of TNFα. Certolizumab pegol binds and neutralizes both soluble and transmembrane TNFα and inhibits signaling through both TNFα receptors in vitro [30].

Data on paternal exposure are available for Infliximab, Etanercept, Adalimumab and Certolizumab, but not for Golimumab.

Effects on spermatogenesis

Earlier case reports and studies without control groups reported sometimes negative effects on spermatogenesis when men were exposed to Infliximab or Adalimumab [120,121]. New data are reassuring in regard to safety of paternal exposure to TNFi.

Villiger et al. published a prospective, cross sectional study analyzing sperm parameters in 20 SpA patients exposed to TNF inhibitors (exposure: 16 patients to Infliximab, 3 to Etanercept and 1 to Adalimumab) and compared sperm parameters with both a control group of 11 not exposed Spa patients and the healthy population. Semen analysis in the 3 groups showed no significant differences. Moreover, the TNFi exposed SpA patients showed a higher degree of sperm motility in comparison to the other 2 groups [122].

Another prospective, longitudinal, controlled study conducted by Micu et al. investigated semen parameters in 20 AS patients exposed for 3–6 months or 12 months (6 patients) to standard doses of TNFi. Four patients were exposed to Infliximab, two to Etanercept and fourteen patients were exposed to Adalimumab [32]. Comparison of sperm parameters in 10 patients vs 20 controls showed that baseline progressive and nonprogressive sperm motility was significantly lower in the patient group before TNFi treatment in parallel with higher FSH, LH levels and lower Testosterone. Astenozoospermia improved after twelve months exposure to TNFi. Only patients with persistent high disease activity remained asthenozoospermic [123]. A prospective study of 101 semen samples from 27 men with psoriasis found sperm or seminal plasma abnormalities in 85% before start of TNFi treatment. Therapy with TNF i (Etanercept, Adalimumab) did not have any negative effects on relevant sperm parameters [124]. In a clinical trial assessing the effect of certolizumab pegol on semen quality parameters, 20 healthy male subjects were randomized to receive a single subcutaneous dose of 400 mg of certolizumab pegol or placebo. During the 14-week follow-up, no treatment effects of certolizumab pegol were seen on semen quality parameters compared to placebo [79–81].

No human data for Golimumab is availlable in the literature.

Effects on offspring

Pre-conceptional paternal exposure to Infliximab showed a normal pregnancy evolution in three different studies including (10 + 41 + 4 men and 4 + 41 + 6 children) [125–127].

A registry based study confirmed the absence of negative effects of TNFi on pregnancies fathered by 57 patients/116 deliveries with pre-conception exposure to TNFi (type not specified, monotherapy or in combination with MTX) compared to 2 reference groups of 600.000 deliveries from patients without preconceptional exposure and the general population. Three birth defects were identified: one neonate with pes equinovarus (combined MTX and TNFi exposure in the father), and 2 newborns with unspecified abdominal atresia (TNFi exposures) [46].

Normal fertility and pregnancy outcome was shown in a prospective controlled study in 13 male patients with AS exposed at least 12 months (12–72 months) to standard doses of different TNFi (Infliximab, Etanercept, Adalimumab) who fathered 13 healthy children [128].

Larsen et al. analyzed pregnancy outcomes (congenital malformations, preterm birth, and small for gestational age) from a nationwide cohort of pre-conceptionally exposed men to different DMARDs (2007–2013) and compared data to non-exposed patients. A total of 372 children were fathered by men treated at least once with TNFi (all categories of TNFi, unknown dose and administration frequency), within 3 months before conception. Hundred fifty-five patients were exposed to Infliximab, 136 to Adalimumab, 69 with Etanercept, 11 with Golimumab and one with Certolizumab. One hundred thirty-eight children were born to fathers with IBD, and 253 children to fathers with rheumatologic or dermatologic disease. Regardless of the paternal disease and the type of combination therapy of a TNFi and a cDMARD administered to the father no statistical difference in pregnancy outcome was identified between exposed and non-exposed pregnancies [129]. Among the 33 paternal exposures to Certolizumab 27 healthy live births were reported along with 4 miscarriages, 1 induced abortion and 1 stillbirth [130].

Comment: TNFi appear not to influence male fertility nor to harm their offspring. There is no robust data supporting differences between different TNFi when judging their impact on spermatogenesis as well as on offspring. TNFi should not be stopped when planning a pregnancy due to the high risk of disease relapse [32,46,49,125–130].

Other conventional synthetic and biologic DMARDs

A summary of drugs with no or limited data regarding male fertility and pregnancy outcome after paternal drug exposure are presented in Table 2. Even in the absence of robust data, no restriction of use in male patients has been claimed for

Table 2
Summary of drugs with no or limited data regarding male fertility and pregnancy outcome after paternal drug exposure

	Spermatogenesis			Pregnancy and child outcome			
Drug	Type of study	Dose	Results	Type of study	Congenital malformations	Other adverse outcomes	Comments
Hydroxycloro-quine	No data		Influence on human spermatogenesis not known	Registry based, **human**, few born children (< 6) [46]	Similar to general population; pregnancies not studied	Not studied	No conclusive data. Experts regard it as compatible with paternal exposure [49]
Leflunomide	**Human**	20 mg/day	Low drug levels in seminal fluid [114]	**Human**, 3 born children [36,46,116]	One healthy child recorded [113]	Not studied	No conclusive data. Wash-out procedure and 3 months pre-conceptional drug cessation advised by manufacturer. Experts regard it as compatible with paternal exposure, [49]
Tacrolimus	**Animal** **Human**		Reduced sperm counts and sperm motility [79–81] Volume and sperm motility decreased [81]	**Human** (20 males, dose 0.1 mg/kg/day) [137]	20 males achieved fatherhood [137].		No conclusive data. Experts regard it as compatible with paternal exposure, [49]
Abatacept	No data		No data	**Human**, 10 pregnancies [136]	No malformations [136]	No adverse outcomes [136]	No conclusive data. Experts regard it as compatible with paternal exposure [49]
Tocilizumab	**Animal** [79–81]	> 10× human dose	Normal fertility	**Animal** (> 10× human dose)	No data	Not studied	No conclusive data. Experts regard it as compatible with paternal exposure [49]
	Human		No data	**Human**; 13 pregnancies [131]	No malformations [131]	No adverse outcomes [131]	
Rituximab	**Animal**		No deleterious effect on reproductive organs in males [79–81] Influence on human spermatogenesis not known	**Human**, 22 pregnancies; 11 documented [132]	No malformations, 7 healthy children; 2 ongoing pregnancies [132]	2 spontaneous abortions [132]	Experts regard it as compatible with paternal exposure [49]
Anakinra	**Animal** [79–81]	> 100× human dose	Normal fertility	No data	No data	Not studied	No conclusive data. Experts regard it as compatible with paternal exposure [49]
Ustekinumab	**Animal** [79–81]	> 45× human dose	Normal fertility	**Human**, 97 pregnancies, 63 live births; 24 pregnancies are not documented [79–81]	No malformations in 63 documented pregnancies; 8 live births with congenital or adverse effect [79–81]	8 spontaneous abortions, 2 elective terminations; 8 live births with congenital or adverse effect [79–81]	No conclusive data. Experts regard it as compatible with paternal exposure [49]
Sekukinumab	**Human** [133–135]		Low IL-17 levels correlate with lower sperm mortality rates. low drug levels in seminal fluid [135]	No data	No data	Not studied	No conclusive data. Experts regard it as compatible with paternal exposure [49]
Apremilast	**Animal** [79–81]	> 3× human dose	Normal fertility [79–81]	No data	No data	Not studied	Data not conclusive
Tofacitinib	**Animal** [79–81]	133× human dose	Normal sperm parameters, normal fertility [79–81]	No data	No data	Not studied	Data not conclusive

Hydroxychloroquine (antimalarial drug containing the 4-aminoquinoline radical), Leflunomide, Tacrolimus (calcineurine inhibitor), Rituximab (monoclonal antibody to CD20 causing CD20+B-cell depletion), Abatacept (inhibits T-cell activation by blocking interactions between antigen-presenting cells and T cells via binding to CD80/CD86 on antigen-presenting cells), Tocilizumab (monoclonal antibody directed against interleukin-6 receptors blocking downstream signaling) and Secukinumab (fully human Ig G1 antibody that selectively binds to and neutralizes IL-17A) [44,46,49,79–81,131–137]. No conclusive data are available for: Anakinra (recombinant interleukin-1 receptor antagonist), Ustekinumab (human monoclonal antibody that binds to and interferes with IL-12 and IL-23 actions), Apremilast (inhibits phosphodiesterase 4 which results in increased intracellular cAMP levels and regulation of inflammatory mediators), and Tofacitinib (a JAK3 inhibitor) [79–81]. The product information sheets of the four latter drugs, approved by FDA and EMA, do not recommend restrictions for male patients based on reassuring animal data.

Conclusions

The effect of medications on male reproduction has been a neglected area. Data are particularly sparse for effects on spermatogenesis both for classic conventional synthetic DMARDs (csDMARDs) as well as for newly introduced biologic drugs targeting cytokines and immune cells. The quality of available studies is often weak and statistically underpowered which exclude making firm statements. Recommendations on restrictions of use of certain drugs in men are often extrapolated from pregnancy experience or based on theoretical concerns arising from pharmacologic properties of a given drug. The resulting product information accessible on the internet may create anxiety among couples and cause termination of pregnancy. Colchicine is an example. Practitioners should know that many of the currently implemented restrictions for drug prescription are due to lack of robust data regarding paternal exposure and not because harmful effects on spermatogenesis or pregnancy outcome were proven. When counseling male patients, the potential of untreated, severe rheumatic disease must be balanced against possible negative effects of medications on reproduction.

When immunosuppressive treatment for active autoimmune disease is considered, physicians explain the risk of infections and answer patient concerns on the risk of cancer development, but rarely do they proactively discuss the potential effect of cs DMARDs and biologic agents on reproduction [138]. Many antirheumatic medications can be continued in men who want to father a child. Regular pre-conceptional counseling should be given to male patients of fertile age as an integral part of family planning.

Appendices. Search strategies: according to PRISMA guidelines

An extensive systematic literature review was conducted, according to the guidelines proposed at the PRISMA statement. PubMed, EMBASE and Cochrane databases platforms were searched for articles (human model, animal model) published in peer-reviewed journals the last search run on September 2017 and published in English language. The authors selected mainly articles focused on human model exposure. Papers showing data about animal paternal exposure were selected to be cited for medications were very little or no human data was published. In addition, online available information was collected from FDA, EMA and pharma companies original site.

PubMed/Embase/Cochrane databases and medicines.org.uk/fda.com/ema.europa.eu website search strategy was focused on identifying data related to: male gender, the type of disease, drugs, sperm parameters, pregnancy and offspring. Mesh terms and individual words and combinations were searched in http://www.pubmed.gov, https://www.elsevier.com/promo/rd-solutions/embase, http://onlinelibrary.wiley.com/cochranelibrary/search, https://www.medicines.org.uk, http://fda.com, http://ema.europa.eu

Male gender: “male/males”, “men”, “father”, “fathered”, “expectant father”, “paternal exposure”, “pre-conceptional paternal exposure”, “animal model”, “human model”, “controls”

Disease: “Rheumatoid arthritis”, “Spondylarthritis”, “Spondylarthropaties”, “Ankylosing Spondylitis”, “Psoriatic Arthritis”, “Juvenile idiopatic arthritis”, “ Lupus”, “SLE”, “Vasculitis”, “Behcet Disease”, “Inflammatory Bowel Diseases”, “Crohn Disease”, “Ulcerative Collitis”, “Gout”, “Mediteraneean Fever”, “FMF”, “Oncologic Diseases”, “ Organ Transplant”, “Cancer”, “Cancer survivors”, “Childhood cancer”

Drugs: Groups and individual drug names were used- “antirheumatic drugs”, “NSAIDs”, “antimalarial drugs”, “steroids”, “TNF alpha blockers”, “TNF alpha inhibitors”, “biologic DMARDs”, “Biologics”, “synthetic DMARDs”, “Cytotoxic therapies”, “ Teratogenic agents”, “Teratogenic drugs”, “Calcineurin Inhibitors”, “Thiopurine”, “CS”, “Immunosupressants”, “Drug security profile”, “Aspirin”, “Indometacine”, “Cox 2 inhibitors”, “Diclofenac”, “Prednisone”, “Prednisolone”, “Metotrexate”, “Cyclophosphamide”, “Mycophenolate Mofetil”, “MMF”, “Sulfasalazine”, “Mesalazine”, “Sulfapyridine”, “Azathioprine”, “6- Mercaptopurine”,

“Cyclosporin”, “Tacrolimus”, “Colchicine”, “Leflunomide”, “Etanercept”, “Infliximab”, “Adalimumab”, “Golimumab”, “Certolizumab pegol”, “Hydroxycloroquine”, “Rituximab”, “Abatacept”, “Tocilizumab”, “Anakinra”, “Ustekinumab”, “Secukinumab”, “Apremilast”, “Tofacitinib”.

Sperm parameters: “sperm parameters”, “spermiogram”, “normospermia”, “oligospermia”, “azoospermia”, “asthenozoospermia”, “asthenooligozoospermia”, “sperm motility”, “sperm concentration”, “sperm alteration”, “fertility”, “infertility”

Pregnancy: “pregnant partners”, “pregnant spouses”, “pregnancy in couples”, “abortion”, “stillbirths”, “fertility”, “infertility”

Offspring: “offspring”, “live children”, “malformations”, “Major malformations”, “premature delivery”, “small for gestational age”, “neonatal death”

Conclusions based on data extracted from papers: safety evaluation on sperm parameters and pregnancy outcome/offspring after drug of interest exposure, recommendations for practitioners according to current available literature data.

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Cancer Management and Research

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REVIEW

Fertility preservation in Hodgkin's lymphoma patients that undergo targeted molecular therapies: an important step forward from the chemotherapy era

This article was published in the following Dove Press journal:
Cancer Management and Research

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Abstract: In total, 80%–90% of Hodgkin's lymphoma (HL) patients are curable with combination chemoradiotherapy. Due to improvements in therapeutic strategies, 50% of all relapsed/refractory patients may undergo complete clinical responses and have long-term survival. Treatment options for HL are effective, but may have a negative impact on post-chemotherapy fertility. Thus, cryopreservation of semen prior to treatment is recommended for male patients. For female patients, assisted reproductive techniques (ART) consult and fertility preservation should be offered as a therapeutical option. In the last years, new targeted molecules have been available for HL treatment. These new drugs showed a high rate of overall responses in the setting of heavily pretreated patients, most of them in relapse after autologous stem cell transplantation, a group previously considered very poor risk. Up to 50% of patients have a complete response and an improved overall survival. Future studies will address the usefulness of novel molecules as a frontline therapy. Considering the high response and survival rates with monoclonal antibody-based therapeutics, fertility has become a concerning issue for long-term HL survivors. As progress has been made regarding ART, with the rigorous steps planned for HL patients, more survivors will become parents.

Keywords: Hodgkin's lymphoma, infertility, pregnancy, fertility preservation

Introduction

Due to modern combined chemoradiotherapy strategies, Hodgkin's lymphoma (HL) is now considered to be a malignant disease with a high curability rate and a 5-year progression free survival of 87%.[1] Patients diagnosed with early stage HL are generally treated with short courses of chemotherapy plus consolidation radiotherapy. Patients with an advanced stage disease are treated with combination chemotherapy.[2–4] The progress made in the last few years with intensive chemotherapy, autologous stem cell transplantation (ASCT), and novel targeted molecules has improved the response and survival rates, even for advanced stage of relapsed/refractory HL.[5–9] Patients with progressive disease after salvage therapy were considered, a decade ago, to be a very poor prognosis group. Eligible patients are now being offered the chance of undergoing an allogeneic stem cell transplantation, in association with an increased risk of therapy related-mortality.[10–12] Targeted molecules show unprecedented response rates in present-day chemotherapy.[13–17] Still, large cohort studies have yet to address the concerning issue of long-term complications. One such complication is infertility in both men and women. In women, infertility may

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http://dx.doi.org/10.2147/CMAR.S154819

be due to chemotherapy-induced diminished ovarian reserve (chDOR) or to premature ovarian failure (POF). chDOR manifests as secondary amenorrhea and persisting high levels of gonadotropins.[18] POF is defined as the loss of ovarian function of peripheral origin before the age of 40. In men, chemotherapy may be complicated by temporary or definitive azoospermia. Even more, there is the issue of subfertility, a known complication of HL that affects both sexes, predominantly men. A possible explanation would be the disturbed cellular immunity.[19–21]

In the current paper, we aim to review the current know-how on fertility complications induced by chemotherapy and radiotherapy, as well as by novel drugs approved for HL therapy. Other possible treatment options, such as assisted reproductive techniques (ART) for HL patients with fertility problems and long-term survivors, are discussed, with the purpose of designing an algorithm with distinct steps on HL diagnosis, treatment, and tissue/sperm preservation.

Methods

The analysis was made following an extensive search of the National Library of Medicine's MEDLINE database using PubMed and Google Scholar, as previously described.[22] Papers included in the analysis were limited to English, German, Romanian, and French language publications, but were not limited to any geographical region, from January 1976 to July 2017. Only papers published between 1976 and 2017 were considered in order to avoid any inconsistencies in diagnostic criteria and also cover the period of publications on HL. The search strategy was based on the combination of the keywords "Hodgkin's lymphoma", "infertility", "pregnancy", "fertility preservation". Subsequently, an additional manual search of the citations of the previously selected papers was performed.

We identified 268 candidate papers, of which nine papers were excluded as they were related to pediatric oncology. Nine other papers related to other types of lymphoma and solid cancers, being excluded, raised the number of analyzed papers to 250.

Current knowledge on fertility and HL chemotherapy

The risk of infertility depends on patient's age, type, and dose of chemotherapy.[23–28] The risk of infertility is higher after the age of 30, as the follicle reserve diminishes with age.[29–33] A large Norwegian study enrolled women diagnosed with and treated for HL, and reported that patients aged under 25 years had the same risk of infertility as the patients older than 30 years, but with delayed complications (15 vs 2 years).[34]

In men, spermatogonia is constant throughout life.[35] In a recent study by the European Organization for Research and Treatment of Cancer Lymphoma Group (EORTC) and Groupe d'Etude des Lymphomes de l'Adultes (GELA), risk factors associated with infertility in men treated for HL were considered to be the general symptoms at diagnosis, especially fever and night sweats, and an increased erythrocyte sedimentation rate (ESR), probably due to proinflammatory cytokines.[36]

Early stage HL is treated with the ABVD (doxorubicine, bleomycin, vinblastine, dacarbazine) protocol as the standard-of-care.[37,38] Hodgson et al,[39] as well as Brusamolino et al,[40] have shown that ABVD chemotherapy is safe and birth rates are comparable with the general population. Escalated BEACOPP (bleomycine, etoposide, doxorubicine, cyclophosphamide, vincristine, procarbazine, prednisone) protocol is used for advanced stage HL, which, according to a multivariate analysis of a German cohort, is positively associated with the development of consequent infertility.[41] Similar results were later reported by Decanter et al,[42] a group that correlated a decrease in the serum levels of anti-Müllerian hormone (AMH) in patients treated with both ABVD and alkylating agents-containing regimens, but the recovery was complete after 1 year in the ABVD group.[42] The alkylating agent cyclophosphamide is known to have a high risk of gonadotoxicity.[43] Cyclophosphamide alters the ovarian reserve in a dose-, duration-, and age-dependent manner; 40% of female patients under the age of 40 years have developed chDOR after cyclophosphamide-based chemotherapy regimens. Alkylating agents induce a risk of infertility of 3.98 when compared to the general population, with the risk increasing due to the cumulative dose.[44,45]

For men treated with the ABVD protocol, the recovery of spermatogenesis is similar with the recovery of ovarian function in women, 6–18 months following the end of therapy. van der Kaaij et al[36] report a recovery time for the follicle-stimulating hormone (FSH) of 18 months for 82% of male patients treated without alkylating agents vs 27 months for 30% of patients treated with alkylating agent-containing regimens.[36] As in female patients, alkylating agents and platinum-based regimens are the most important risk factor for infertility in men, with the risk being dose-dependent. The decline in sperm count was reported after 2–3 months of chemotherapy. The corticosteroids included in the escalated BEACOPP regimen also have an inhibitor effect on the hypothalamic–pituitary–gonadal axis of male patients.[46,47]

An additional risk factor for therapy-related infertility is pelvic radiotherapy. For male patients, recent progress in ART could lead to an improved rate of fatherhood following treatment, even in oligozoospermic patients. Radiotherapy is used in combination with chemotherapy for early stage HL, as well as for bulky metastatic lymph nodes in advanced stage HL. A dose of 2.5–5 Gy is associated with infertility in 30%–40% of women aged 15–40 years and in 90% of women older than 40 years. Radiation is toxic, both for active and dormant follicles. For men, irradiation with 1–2 Gy is associated with a consequent risk of sterility.[48] A 7.5 Gy radiation to the testis induces the highest risk of sterility.[47] The data regarding the stage of the disease is controversial. Some studies report no association between disease stage and fertility, but probably the risk associated with disease stage is related to the therapy used.

Second-line therapies include DHAP (dexamethasone, high-dose cytarabine, cisplatin), ICE (ifosfamide, carboplatin, etoposide), IGEV (ifosfamide, gemcitabine, etoposide, vinorelbine), GDP (gemcitabine, dexamethasone, cisplatin), GVD (gemcitabine, vinorelbine, dexamethasone), MINE (mesna, ifosfamide, novantrone, etoposide) chemotherapy regimens, none of which have yet been evaluated for the risk of infertility. The infertility risk associated with an ASCT was not prospectively evaluated so far, but few case reports of pregnancies after BEAM (BCNU, etoposide, cytarabine, melphalan) conditioning chemotherapy have been published.[49,50] Several studies and reports of the European Blood and Marrow Transplantation (EBMT) have shown a high risk of infertility associated with allogeneic transplantation, especially when total body irradiation (TBI) was used as part of the conditioning regimen.[49] However, up to 20%–25% of patients recover their fertility several years after myeloablative allogeneic transplantation.[47]

Overall, on a Dutch cohort, de Bruin et al[51] have shown that 97 out of 518 patients treated with chemotherapy for HL developed a premature menopause. The largest study that evaluated the risk of infertility in patients treated for HL was published by Swerdlow et al.[50] The study evaluated 2,127 female patients treated from 1960 to 2004, with the evaluation being made from 2003 to 2012. Among these patients, 1,292 developed early or premature menopause. The group showed a cumulative risk of infertility associated with age, at least six cycles of alkylating agent therapy, BEAM chemotherapy, or 5 Gy pelvic radiotherapy. There was an increased risk for older patients, but there was also evidence of cumulative incidence years after treatment.

Targeted new therapies and fertility

In the last 2 years, important progress has been made in the treatment of HL with monoclonal antibody-based drugs. These novel targeted molecules have shown unprecedented overall response rates for heavily pretreated patients, most of whom are in relapse after multiple lines of chemotherapy and ASCT.[52,53] It is too early to have fertility studies considering the limited experience, but specialists and patients should be aware of safety information regarding pregnancies. Some information is available for rituximab, in use since 1997 for different Non-Hodgkin's lymphoma (NHL) subtypes, and rituximab appears to be safe.[54,55] There is limited data on fertility issues, but the available animal studies show embryofetal abnormalities correlated to targeted therapy. The anti PD-1 molecules are IgG4 that crossed the placental barrier, and monomethyl auristatin E (MMAE) has proven testicular toxicity.

Brentuximab vedotin is an anti-CD30 antibody drug conjugate, covalently linked to a antimicrotubule agent monomethyl auristatin E (MMAE), with proven efficacy in CD30 lymphoproliferative diseases such as HL, anaplastic large cell lymphoma (ALCL), and other types of non-Hodgkin's lymphoma (NHL).[56–59] This drug is approved for relapsed/refractory HL after ASCT or after two prior lines of chemotherapy, brentuximab vedotin showed an overall response rate of 75% and a complete response rate of 34%, in phase 2 trials as a single agent.[60,61] Recent results of the AETHERA study group suggest a role of brentuximab vedotin treatment as consolidation after ASCT for high risk patients.[62] CD30 is not expressed in physiological conditions, with some exceptions such as decidual cells in the uterus and endometrium during pregnancy.[63,64]

Up to this point, no studies have been published regarding the use of brentuximab in pregnant women, but preclinical studies on animal models have shown significantly decreased embryo viability and fetal malformations.[61] Thus, both HL and ALCL patients are advised not to become pregnant during brentuximab therapy and 6 months after the last dose. For men treated with brentuximab, the same rules as for chemotherapy should be applied regarding sperm collection, since non-clinical studies have revealed testicular toxicity. MMAE has aneugenic properties leading to testicular atrophy and degeneration, that are partially reversible. Male patients should use contraception methods for at least 6 months after the last dose. There is no information on breastfeeding, but it is possible that a very low quantity of brentuximab could be found in milk, since it is a large protein.

Brentuximb is an important acquisition for the HL treatment, but, regarding conception, the same rules as for chemotherapy should be applied.

Nivolumab is a fully human anti PD-1-monoclonal antibody, used in clinical trials for the treatment of HL in the setting of relapsed/refractory disease after ASCT followed by brentuximab vedotin therapy.[65] In a recent large Phase II clinical trial, 80 patients who failed both ASCT and brentuximab vedotin have received nivolumab. The overall response rate was 66%, with a complete response of 8.8% and partial response of 57.5% of patients. The 6-month overall survival was 99%.[66–68] So far, there are no studies on fertility in men or women treated with nivolumab. There is no data on pregnancy outcome under nivolumab treatment, but animal studies show embryofetal toxicity.[69] The only published literature is from various regulatory agencies recommendations for contraception during nivolumab therapy and 5 months after the last dose.

Pembrolizumab is another anti PD-1 monoclonal antibody tested in patients with HL.[70] Recently, the results from the Keynote-013 Phase I/II clinical trial have been reported with impressive outcome.[71] Among the 31 patients treated with pembrolizumab, all of them after relapse from ASCT, the overall response rate was 65%, with 16% complete response and 48% partial response rates.[72] Still, there is no available clinical data on fertility under pembrolizumab therapy, but animal studies have revealed no negative effects. As with nivolumab, there are no data on the use of pembrolizumab in pregnant women, but animal studies reveal fetal harm and fetal loss. So far, recommendations treatment with pembrolizumab is not to be used in pregnancies.

Still, both anti PD-1 antibodies are IgG4, known to cross the placental barrier.[73,74] PD-1 blockade with nivolumab or pembrolizumab is safe and effective. Treatment is usually administered for 3–6 months, up to 2 years. Retreatment is also allowed in the case of initial response, with most frequent adverse events being the immune reactions. It is important to emphasize that patients treated with anti PD-1 monoclonal antibodies are heavily pretreated with chemotherapy and ASCT, both therapies known to impair fertility in men and women. Current studies evaluate the role of anti PD-1 monoclonal antibodies as first-line therapy. We hope for new data on fertility complications due to these targeted molecules.

Rituximab is a chimeric mouse/human IgG1k monoclonal antibody targeting the B cell surface antigen CD20.[75–77] Rituximab is used in the clinic for treating diffuse large B cell NHL, follicular NHL, as well as for nodular lymphocyte predominant HL. The median half-life is 18–22 days, but the drug can be detected in blood up to 24 weeks after administration. The B cell depletion induced by rituximab can last for 6 months to years in some patients. Chakravarty et al[78] have shown that most of the 231 pregnancies included in the study, with preconceptional and antepartum exposure to rituximab, resulted in uncomplicated live births. In this study, there was no pattern of congenital abnormalities identified and associated with rituximab. There was also no pattern of neonatal infections, but cytopenias were detected in seven of the eleven reports. This observation has led to the recommendation of blood count for all newborns exposed to rituximab, especially shortly before or during gestation. Given the prolonged B-cell depletion after rituximab administration, regular check-ups for both the mother and the newborn should be performed. No clear conclusion can be drawn from current reports regarding male exposure to rituximab, and data are still insufficient for the evaluation of gonadal toxicity in men.[78]

Gonadal function evaluation and fertility preservation options

There is no consensus on ideal parameters of fertility.[79] FSH is elevated in the case of impaired ovarian function, and it has been used as the most important ovarian function parameter, but it's high intercycle variability makes this serum analysis unreliable. Currently, AMH is being used as the best tool for assessment of ovarian function, as it demonstrated high sensitivity and stability.[80] Another evaluation assay is the determination of inhibin B hormone, secreted by the follicles recruited during the ovarian cycle and involved in the negative regulation of FSH. Nevertheless, this type of analysis is not available in all laboratories, especially in developing economies.[81] The transvaginal ultrasound performed at day 3 of the menstrual cycle provides the number of follicles between 2 and 10 cm, that correlate with the ovarian reserve.[82] Unfortunately, in the case of amenorrhea, the ultrasound has not proven to be useful. Most specialized centers in reproductive techniques use both AMH and/or FSH serum levels and ovarian ultrasound.[83,84]

For male patients, the best available assessment is semen analysis, which provides data on sperm count, as well as vitality and mobility of the spermatozoids. The test should be performed at least 3 months after chemotherapy, as spermatogenesis takes ~74 days. FSH and inhibin B serum levels could also be used.

The most important step on fertility preservation for patients treated for HL is the multidisciplinary collaboration between hematologist, ART specialist, and gynecologist. The

use of gonadotropin-releasing hormone analogs (GnRH-a) during chemotherapy is still controversial.[85,86] Several randomized trials suggest the efficacy of GnRH-a in the reduction of chDOR risk for female patients undergoing chemotherapy, based on a chemical-induced menopause which can protect the ovary from the cytotoxic effect. A German trial has evaluated the use of oral contraceptives in comparison to GnRH-a for female patients treated with escalated BEACOPP, and found no efficacy of GnRH-a. Currently, there is no evidence that GnRH-a administration during chemotherapy could increase the rate of pregnancies, with no clear recommendation regarding their use. Still, important advantages for the clinician are the good control of the menstrual cycles and the reduction of irregular bleeding.[87–91]

Currently, three preservation methods are available for female patients: oocyte cryopreservation, in vitro fertilization for embryo cryopreservation, and cryopreservation of ovarian tissue. Recently, a new technique of retrieval of cumulus oocyte complexes, followed by in vitro maturation and vitrification, in combination with ovarian tissue cryopreservation, was reported in France.[92,93] With regard to oocyte cryopreservation, ovarian stimulation for 4–6 weeks is still needed, and the success rate is only 3%.[94,95] Fertilization of the oocyte by intra-cytoplasmic sperm injection and subsequent vitrification could increase the success rate to 6.8%.[96] The in vitro fertilization followed by embryo cryopreservation is a feasible option for patients with a stable partner, where a delay in chemotherapy is not contraindicated, and where an ovarian stimulation of 9–14 days, but sometimes 4–6 weeks, is possible. The success rate has been reported to be 18%.[20]

The cryopreservation of the ovarian tissue is a new promising procedure, but it's results need further confirmation, since only a few pregnancies have been reported so far, with a risk of 50% loss of ovarian reserve and an additional risk of malignant cell reimplantation.[97]

For male patients that undergo chemotherapy, semen collection and preservation must be proposed to the patient prior to therapy. There is a known risk of subfertility in HL patients,[19,20] and normal sperm is essential for embryo development.[98,99] It is of utmost importance to perform quality control assays prior to preservation. There is no consensus on the ideal method of quality measurement and there are several available methods such as the SCSA (sperm chromatin structure assay;[100] the detection of single and double DNA breaks by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)[101] the comet assay,[102] or flow cytometry using monobromobimane for reactive SH groups in protamines and using chromomycin A3 for DNA compaction.[103] O'Flaherty et al[19] have reported increased DNA damage in newly diagnosed HL patients when compared with controls, using two control methods: comet assay and flow cytometry with monobromobimane and chromomycin. Semen collection may be possible after initiation of chemotherapy, but the risk of genetic defects in the offspring is unknown.[19] Some groups report success with cryopreservation and subsequent transplantation of spermatogonial stem cells, but these options are still experimental and are offered only to patients in whom semen cryopreservation is not possible.[104] As in female patients, there is limited data regarding the efficacy of hormone suppression in reducing the risk of infertility during chemotherapy.[105,106] All patients should be offered the possibility of sperm preservation, with the best local available quality control assay.

The American Society of Clinical Oncology published in 2013 updated recommendations on fertility issues in patients with cancer. For men, sperm cryopreservation is the recommended method as the only proven fertility preservation method. For women, embryo and oocyte cryopreservation are established fertility preservation methods. The authors suggest ovarian transposition in the case of pelvic radiotherapy. Nor for men or women, there is no recommendation for hormonal suppression, since there is insufficient data on effectiveness.[107]

Figures 1 and 2 present the algorithms for fertility preservation in HL patients, as a proof-of-concept.

Conclusion

Treatment of young patients diagnosed with HL is multidisciplinary and involves a team of hematologists, gynecologists, and fertility specialists, who should all keep in mind that increased disease activity could be associated with adverse pregnancy outcome.[108–113] The risk of chemotherapy-induced infertility should be discussed with all newly diagnosed HL patients. A thorough evaluation of ovarian function and semen should be performed in all young HL patients. Even if the infertility risk associated with ABVD regimen is known to be low, fertility issues and preservation methods should be discussed with all patients under the age of 40 diagnosed with early stage HL before the beginning of therapy. Patients diagnosed with advanced stage HL, treated with combination chemotherapy, should also be offered fertility preservation methods prior to therapy, as well as counseling, which must be offered to all patients regarding the risks of pregnancy during treatment. Other

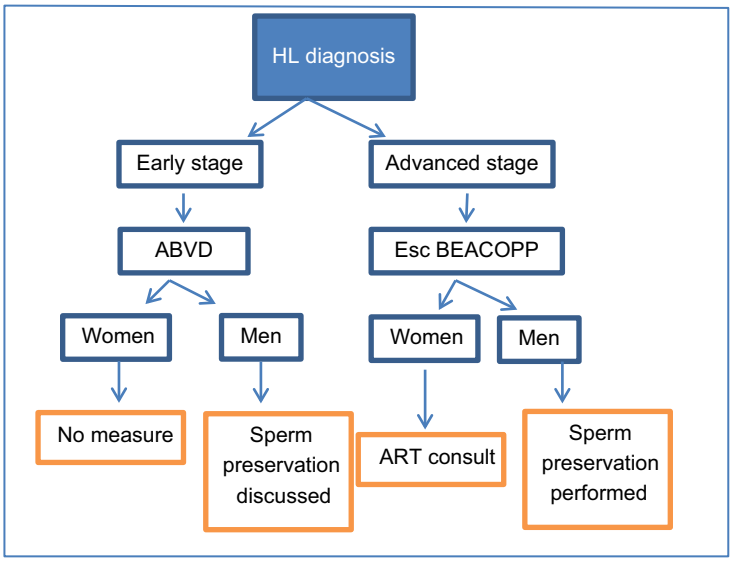

Figure 1 Algorithm for fertility preservation in HL patients, at diagnosis.
Abbreviations: HL, Hodgkin's lymphoma; ABVD, doxorubicine, bleomycin, vinblastine, dacarbazine; Esc BEACOPP, escalated bleomycine, etoposide, doxorubicine, cyclophosphamide, vincristine, procarbazine, prednisone; ART, assisted reproductive techniques.

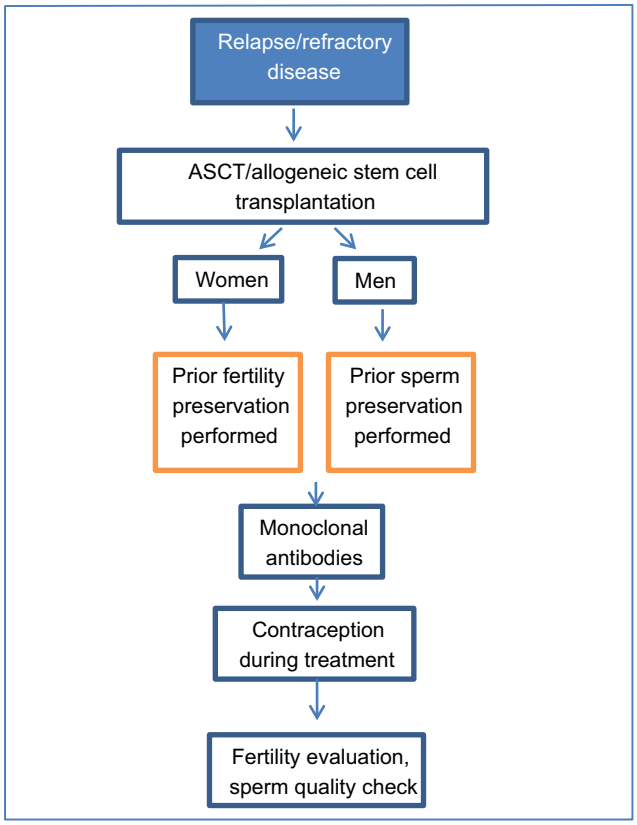

Figure 2 Algorithm for fertility preservation in relapsed/refractory HL patients.
Abbreviations: HL, Hodgkin's lymphoma; ASCT, autologous stem cell transplantation.

long-term complications of chemoradiotherapy, such as secondary acute leukemia or breast cancer or thyroid dysfunction, should be kept in mind when deciding in favor of fertility preservation at the initial HL diagnosis, considering the timeframe for next chemotherapy and the possible contraindication for ovarian stimulation.

Current recommendations suggest a planned pregnancy after 6–24 months following chemotherapy, considering the approximate 6 months interval for follicullar maturation and the relapse risk which is highest during the first 2 years. For the anti-CD20 monoclonal antibody rituximab, the recommendations are for contraception during treatment and no less than 12 months after the last dose. For anti-PD-1 monoclonal antibodies, strong recommendations are in favor of contraception. Still, most available data on fertility issues and pregnancy rate and pregnancy outcome are from voluntary reports, from clinical trials, or from registries, thus making clear interpretation of data difficult. Long-term follow-ups of pregnancies during chemo-, immunotherapy are scarce, and reports on pregnancies and their outcomes should be encouraged, in order to have better guidelines.

With the impressive results obtained with targeted molecules, even in the setting of relapsed/refractory disease, long-term survivors of HL will be seen. Future studies will assess the best approach regarding the use of monoclonal antibodies in frontline or relapsed settings, with single agents or combination therapy. Due to the important progress made with the addition of monoclonal antibodies, fertility issues need to be carefully studied in future trials for responding patients.

Acknowledgment

All authors have read and approved the final version of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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Pregnancy Outcomes in Couples with Males Exposed to Longterm Anti–tumor Necrosis Factor–α Inhibitor Therapies: A Prospective Study

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ABSTRACT. ***Objective.*** To examine the pregnancy achievement and outcomes in couples in which men with spondyloarthritis (SpA) were exposed to tumor necrosis factor inhibitors (TNFi).

Methods. Information about pregnancies involving fathers with SpA was prospectively collected by 6 Romanian rheumatology centers.

Results. Twenty-seven patients achieved 33 pregnancies and fathered 30 healthy children. Three elective abortions (personal reasons) and no spontaneous abortions, preeclampsia/eclampsia, stillbirths, congenital malformations, or pathologies in the children were recorded. Five patients showed normospermia before and after longterm TNFi treatment.

Conclusion. Pregnancy and child outcomes in male patients with SpA exposed to longterm TNFi therapy were reassuring. (First Release April 15 2019; J Rheumatol 2019;46:1084–8; doi:10.3899/jrheum.180588)

Key Indexing Terms:
FERTILITY MEN PREGNANCY OFFSPRING TNF INHIBITORS

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Accepted for publication December 18, 2018.

A large proportion of patients with spondyloarthritis (SpA) are affected during peak reproductive years, rendering therapy with biologic disease-modifying antirheumatic drugs (bDMARD) necessary to control active disease[1]. Recent recommendations have offered strategies for preconception counseling and treatment with bDMARD during pregnancy[2,3,4,5]. By contrast, there are still limited data available on the safety of preconception use of bDMARD in men.

Previous studies evaluating fertility, pregnancy, and offspring outcomes in smaller groups and large male cohorts exposed to bDMARD reviewed by Micu, *et al*[6] indicate no impairment of spermatogenesis by tumor necrosis factor–α inhibitors (TNFi). Outcomes of pregnancy and offspring compared to nonexposed patients or the general population have been found to be normal. Drug effects on male fertility require sperm analysis and often include a limited number of individuals[7,8,9,10,11,12]. Pregnancy and offspring outcome after male preconception exposure can be studied based on administrative registries but analysis is made mostly in a retrospective manner; data on disease activity and length of drug exposure are seldom available and pregnancy course in

partners is often not recorded[13,14,15]. Prospective data eliminate these shortcomings. The aim of our study was to examine prospectively pregnancy achievement and outcomes in men with SpA exposed to > 12 months of therapy with TNFi including the preconception period.

MATERIALS AND METHODS

Male patients with an established diagnosis of SpA[16,17] fathering children were prospectively included in the study, at 6 Romanian rheumatology centers. The study was performed between 2012 and 2017 and was approved by the ethics committee (N683/20.12.2012 and N2394/3.04.2017); informed written consent was obtained from patients.

Cases were fathers with TNFi continuous exposure > 12 months including the preconception period (defined as TNFi exposure according to standard protocols within 3 months before conception). The prospective data collection comprised demographic data, disease-related variables, pregnancy outcome in female partners, and offspring outcome. For comparison of pregnancy outcome with the general population, data were extracted from the ATLAS platform (surveillance software linking diagnosis, investigations, and medication, mainly for economic reasons) of the 1st Gynecology Clinic, Cluj-Napoca, Romania, between 2012 and 2017.

A standardized dataset was completed for each patient/couple (in case a pregnancy was identified and followed) in all participating centers. All patients had a monthly visit with the doctor for the receipt of TNFi prescription. Information about fertility treatments and pregnancy occurrence in the couple was obtained at each visit. Analysis of sperm variables before and after longterm TNFi exposure was available in 5 patients (in 3 of them, evaluation was made during a previous study[10]).

Pregnancy and offspring outcome variables were collected according to the standard protocols of the obstetrics/neonatology/pediatric units of the hospital.

Statistical analysis. The assessment of the normality of data was performed using the Shapiro-Wilk test. Descriptive statistics were performed for the continuous and categorical variables and results were expressed as mean ± SD or number of cases and percentages.

RESULTS

In the 6 centers, 202 male patients with SpA who were exposed to TNFi were identified. Their mean age was 30 (range 18–71) years. Among these, 27 men with ankylosing spondylitis (AS; positive radiographic criteria) exposed to continuous, longterm (range 12–129 mos) monotherapy with TNFi were involved in 33 pregnancies. Thirty healthy children were born and 3 elective abortions (personal reasons) were recorded.

Table 1 presents the demographic and disease-related variables in the case couples. Table 2 shows the outcome of 33 pregnancies fathered by patients compared to 12,142 pregnancies of the general population. No increase in pregnancy complications or congenital malformations occurred in cases. All children were born healthy with a weight ≥ 2500 g (range 2800–4400 g). A trend for a higher percentage of live births, cesarean delivery, and prematurity was detected in the case group. The 6 premature children were born at Week 36 of gestation (5 boys and 1 girl) with a weight range of 3300–3800 g.

One patient switched owing to loss of efficacy from longterm therapy with adalimumab (ADA) to etanercept (ETN) 2 months before conception; he reached remission again at conception time. Two men changed preconception TNFi exposure in subsequent pregnancies: 1 from ADA to ETN and 1 from infliximab (IFX) to ETN. One patient stopped IFX 2 months prior to conception; all other patients followed a continuous TNFi regimen. Five patients presented normospermia both before TNFi therapy initiation and after 12 months of treatment with standard doses of ADA (Table 3). In this subgroup, 7 pregnancies were achieved, with 5 children and 2 elective abortions (personal decision; not because of malformations).

DISCUSSION

To our knowledge, our study is the first real-life prospective study in fathers exposed to TNFi demonstrating no negative effect on pregnancy and child outcomes when TNFi were administered over the long term, including the 3 months prior to conception.

Sperm analysis of 5 patients before and after longterm exposure to ADA showed normospermia in all patients who fathered 5 healthy children. This confirms previous studies in patients with AS and psoriatic arthritis in which no impairment of spermatogenesis was found after short-term and longterm exposure to IFX, ETN, and ADA[9,10,11,12]. Several of these studies showed impaired spermatogenesis before initiation of a TNFi and normalization of the spermatogram during treatment[9,11,12].

The absence of sperm alterations under TNFi therapy is reassuring; however, it neither confirms fecundity nor the absence of chromosome alterations in germ cells. Normal fecundity needs to be confirmed by pregnancy achievement in the couple, and this depends on both male and female factors. Twenty-six couples included in our study achieved 32 pregnancies within 1 year of the intention of reproduction, indicating normal fecundity. Normal fecundity during TNFi therapy has also been recorded in retrospective case series[7,8].

Three recent registry-based studies investigated pregnancy outcomes fathered by men with rheumatic, gastrointestinal (inflammatory bowel disease), and dermatologic diseases exposed to TNFi. They found no increased adverse pregnancy or child outcomes compared to nonexposed, disease-matched, or nondiseased controls[13,14,15].

A nationwide study identified 372 children fathered by men treated at least once with TNFi (all types, unknown dose and administration frequency, monotherapy or combined with conventional DMARD). Regardless of the paternal disease and the type of combination therapy administered to the father, no statistical difference was identified between exposed and nonexposed pregnancies for congenital abnormalities, preterm birth, and small for gestational age[15].

Prospectively collected registry data evaluating clinical safety outcomes in patients with Crohn disease (biannual records) identified 59 pregnancies (42 with gestational and 17 with pregestational exposure) in partners after paternal exposure to at least 1 infusion of IFX (median: 3 infusions

Table 1. Demographics and disease-related variables in patients and partners.

Variables	Fathers	Mothers
Race, white	27	27
Age at conception, yrs (min;max)	34.6 ± 5.5 (24;47)	29.5 ± 3.2 (20;35)
Diagnosis		
Axial involvement in AS	19 (70.3)	0
Axial and peripheral involvement in AS	8 (29.6)	0
PsA^	0	1 (0.4)
Disease duration at conception, yrs	10.6 ± 5.7	4 (1 mother)
Abnormality at laboratory screening or imaging at conception	0	0
In remission at conception (BASDAI)	26 (96.3)	NA
TNFi dosage and exposure		
ADA, 40 mg/2 weeks	12 (36.4)	0
ETN, 50 mg/1 week	14 (42.4)	0
IFX, 5 mg/kg/8 weeks	7 (21.2)	0
TNFi therapy duration, mos	42.6 ± 26.0	0
Other therapies		
NSAID*	14 (43.8)	1 (0.4)
SSZ (+ NSAID)	0	0
MTX	0	0
Fertility treatments	0	0
Exposure to smoking, illicit drugs, toxic agents, drinking habits		
Smoking	3 (11.1)	0
Drinking habits, occasional	27 (100)	0
Illicit drugs exposure	0	0
Toxic agent exposure	0	0
Medical history/comorbidities		
History of genital tract infections	0	0
Epidemic parotitis involving testis in teen years/adulthood	0	–
Cystitis during pregnancy§	–	6 (22)
Varicocele	0	–
Other comorbidities	0	0
Desire to conceive	26 (96.3)	NA

Values are mean ± SD or n (%) unless otherwise specified. ^ Onset of PsA in 1 mother preconception. *Very rare exposure to NSAID (1–2 times/mo). § The partners of male patients were monitored 3 times during pregnancy. AS: ankylosing spondylitis; PsA: psoriatic arthritis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; TNFi: tumor necrosis factor inhibitors; ADA: adalimumab; ETN: etanercept; IFX: infliximab; NSAID: nonsteroidal antiinflammatory drugs; SSZ: sulfasalazine; MTX: methotrexate; NA: not available.

for gestational and 1 for pregestational exposure). Regardless of the disease activity at conception, no significant differences of pregnancy and child outcomes were observed compared with a nonexposed disease-matched group for the proportion of live births, spontaneous or elective abortions, preterm birth, healthy infants, or congenital abnormalities, or extended hospitalization of newborns. The majority of the partners' outcomes resulted in live births with healthy children across the exposure groups[14]. The data are in line with the results of our study analyzing reproduction after longterm exposure to TNFi in male patients.

Registry data are derived from electronically reported administrative health records or surveillance registries. The strength of registries is collection of large amounts of data on exposure and outcome and generation of a large nondiseased comparator group. Registries often include patients with different diagnoses and pathologies that could influence pregnancy outcomes. Other shortcomings of retrospective data collection are that possible confounders such as disease activity, lifestyle factors, comorbidities, duration of drug exposure, and drug class may not be included, as well as a precise record of all medications (often only prescription data are given). By contrast, prospective clinical studies provide detailed demographic and disease- and therapy-related data but are limited by sample size.

Our study has several limitations. The number of TNFi-exposed patients and the number of pregnancies is small compared to registries, and the pregnancy outcome data could be chance findings. Enrollment of male patients into a prospective study is time-consuming because inflammatory rheumatic diseases have a lower prevalence among men compared to women[1,14]. We did not analyze a disease-matched control group. Patients with milder disease are generally less compliant with medical visits. The comparison group from the general population lacked data on fecundity and demographic data of fathers and mothers. The strength of our study is the prospective design and that the group of fathers was homogeneous regarding diagnosis, and all

Table 2. Pregnancy and offspring outcomes in patients compared to the general population^.

Pregnancy Evolution and Outcomes	Cases	General population
No. pregnancies	33	12.142
TTP < 12 mos	32 (96.9)	NA
Live births	30 (91)	9667 (79.6)
Male sex	13 (43.3)	NA
Stillbirths*	0	107 (0.9)
Gestational age at delivery, weeks	37.57 ± 1.01	NA
≥ 37	24 (80.0)	8593 (88.9)
< 37	6 (20.0)	1074 (11.1)
Type of delivery for live births		
Vaginal	28 (93.3)	9459 (97.8)
Cesarean	2 (6.6)	208 (2.2)
APGAR score	9.6 ± 0.7	NA
Weight of live newborn, g	3390.7 ± 342.6	NA
Weight > 2500 g∞	30 (100)	9566 (98.9)
Small for gestational age∞	0	101 (1.0)
Spontaneous abortion¶	0	1135 (9.4)
Elective abortion (weeks 8–9)¶	3 (9.0)	1233 (10.2)
Preeclampsia/eclampsia	0	110 (1.1)
Congenital malformations	0	140 (1.4)
Other neonatal diseases that require prolonged stay in neonatal intensive care unit	0	18 (0.2)

Values are mean ± SD or n (%) unless otherwise specified. ^ Data from ATLAS platform (surveillance software linking diagnosis, investigations, and medication). * *In utero* fetal death after 20 weeks of gestation. ∞ Normal weight was defined as > 2500 g at term; small for gestational age fetuses were those with a weight < 2 SD adapted for gestational age and sex of the child. ¶ Spontaneous abortions were defined as clinically recognized pregnancy losses before 20 weeks of gestation; elective abortions were defined as pregnancies that were terminated on personal request for nonmedical reasons, up to 12 weeks of gestation. TTP: time to pregnancy achievement. APGAR: Appearance, Pulse, Grimace, Activity, Respiration evaluation in the newborn; NA: not available.

Table 3. Sperm variables in 5 patients before and after longterm TNFi exposure.

Sperm Analysis	P1	P1′	P2	P2′	P3	P3′	P4	P4′	P5	P5′
Volume, ml	3	2	3.5	2	3	2	1.5	3	4	6
pH	7.5	7.5	7.8	7.4	7.8	8	8	8	8	8
Liquefaction, min	10	10	5	15	10	10	10	20	20	10
Agglutination¶	–	–	–	–	–	–	–	–	–	–
Concentration (10^6/ml)	50	55	45	30	45	65	43	100	66	40
Sperm cell motility, %										
Rapid progressive	40	50	37	50	50	50	30	30	50	60
Slow progressive	20	20	30	0	15	20	35	30	10	0
Nonprogressive	0	10	0	0	10	10	0	0	15	0
Immobile	40	20	33	50	25	20	35	40	25	40
Leukocytes	0	0	0	0	0	0	0	0	0	0
Morphology										
Normal forms, %	65	60	70	60	63	55	53	70	63	50
Atypical forms (head + midpiece + tail), %	35	40	30	40	37	45	47	30	37	50
Results*	N	N	N	N	N	N	N	N	N	N

P1–5: evaluation of patient sperm variables before TNFi exposure. P1′–5′: evaluation of patient sperm variables after 12 months TNFi exposure. ¶ Agglutination is present (+) or absent (–). * Diagnosis was based on being within reference values of the World Health Organization[18]. TNFi: tumor necrosis factor inhibitors; N: normozoospermia.

received > 12 months monotherapy with a TNFi. Indeed, the followup of 5 patients with longitudinal sperm variables analysis resulting in pregnancy achievement and positive outcome strengthens the study conclusion, showing that normal sperm variables and fertility/fecundity preservation is possible in patients with SpA after longterm TNFi exposure.

The prospective analysis of the pregnancy and offspring outcomes in patients exposed to longterm TNFi therapy,

including the preconception period, is reassuring regarding reproduction capacity and the health status of the offspring. Larger prospective controlled studies are needed to confirm these findings.

ACKNOWLEDGMENT

We thank biologists Stela Surd and Marinela Gîrlovanu (Assisted Reproduction Department, 1st Gynecology Clinic, Cluj-Napoca, Romania) for their valuable contribution to this study.

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Review

Med Ultrason 2020, Vol. 22, no. 4, 469-475
DOI: 10.11152/mu-2583

Logistic models and artificial intelligence in the sonographic assessment of adnexal masses – a systematic review of the literature

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Abstract:

Adnexal masses are common, yet challenging, in gynecological practice. Making the differential diagnosis between their benign and malignant condition is essential for optimal surgical management, but reliable pre-surgical differentiation is sometimes difficult using clinical features, ultrasound examination, or tumor markers alone. A possible way to improve the diagnosis is using artificial intelligence (AI) or logistic models developed based on compiling and processing clinical, ultrasound, and tumor marker data together. Ample research has already been conducted in this regard that medical practitioners could benefit from. In this systematic review, we present logistic models and methods using AI, chosen based on their demonstrated high performance in clinical practice. Although some external validation of these models has been performed, further prospective studies are needed in order to select the best model or to create a new, more efficient, one for the pre-surgical evaluation of ovarian masses.

Keywords: artificial intelligence; deep learning; diagnosis; image interpretation; computer-assisted

Introduction

Ovarian tumors commonly present diagnostic challenges in gynecological practice. For instance, it is important to make the difference between a benign and a malignant ovarian tumor before surgery, especially in young patients who desire fertility. While benign masses can be treated conservatively [1] or by surgical removal thorough minimally invasive surgery [2], masses suspected of being malignant should be referred to a tertiary care center which may already be dealing with high numbers of ovarian cancer cases [3]. Therefore, accurate diagnosis is essential for planning appropriate patient management [4].

The diagnostic performance is in direct correlation with the experience of the physician [5]. The first imagistic step in evaluating an adnexal mass is ultrasonography (US), but US reports are sometimes misleading and confusing for the clinician [6]. To distinguish between benign and malignant tumors by means of US examination is not always easy because, for example, the imagistic features of borderline tumors overlap significantly with those of invasive epithelial cancers. In order to overcome such a difficulty, simple rules [7] or scoring systems were introduced with reportedly good results [8]. However, their application is not superior to the subjective impression of an experienced examiner [9], so further alternatives to improve imagistic diagnosis are still required.

Received 01.04.2020 Accepted 22.05.2020
Med Ultrason
2020, Vol. 22, No 4, 469-475
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One possible way to improve the accuracy of US diagnoses of ovarian tumors is to use logistic models and artificial intelligence (AI) [10]. In the literature, there is ample research with regard to the development of algorithms or AI technologies.

In recent years, imagistic assessment practices have been enhanced by the introduction of new software based on automated identification (computer-aided detection) and characterization (computed-aided diagnosis -CAD) with the aim to assist physicians in the diagnostic process [11,12].

Logistic models apply mathematical methods to reported incidence of events in order to create an algorithm, which, may be used to predict further occurrences. Logistic models are the most common method used to predict dichotomous outcomes, e.g. benignity versus malignancy.

Artificial neural networks (ANN) are computing systems based on a collection of connected units or nodes called artificial neurons inspired by the biological neural networks that constitute animal brains [13,14].

Artificial intelligence (AI) is represented by several algorithm-based applications that can solve problems by simulating human mental processes and intellectual activity such as learning from the data and experience [10]. Several subset technologies have been developed recently under the umbrella of AI: machine learning (ML) and deep-learning (DL). They both can be applied to solve human problems in many different ways. ***Machine learning*** as one form of artificial intelligence uses algorithms that learn from given data and then teach themselves to adapt to new circumstances and perform certain tasks. In this way, computers can adapt and handle new scenarios by analysis, self-training, observation, and experience. Machine-learning artificial neural networks adapt and learn best when large amounts of data are available [15]. ***Deep-learning*** is a subfield of machine learning that uses algorithms inspired by the structure and function of the brain, called artificial neural networks. This technology is modeled after the human brain, and so, each time new data is added, it increases the capabilities of the system. Because it has the capacity to analyze massive amounts of data, these algorithms are able to quickly find correlations that the human mind cannot [16].

A glossary of all these terms is presented in Table I.

The aim of this review was to perform a systematic review of the literature and to provide up-to-date information regarding the application of logistic models and AI in the pre-surgical differentiation of adnexal tumors. With this review, we hope to increase the awareness and comprehension of clinical practitioners regarding the usefulness of logistic models and AI-related clinical practices in this particular field of imagistic assessment.

Table I. Glossary of terms used in artificial intelligence

Term	Definition
Artificial intelligence	Programs with the ability to learn and reason like humans
Machine learning	Algorithms with the ability to learn without being explicitly programmed
Deeplearning	Subset of machinelearning in which artificial neural networks adapt and learn from a large amount of data
ANN	Artificial neural networks inspired by biological neural networks.

Material and methods

We systematically searched articles published between January 1990 and January 2020 in the following databases: Scopus, PubMed, Web of Science and Cochrane Library, and we also screened for relevant publications in the grey literature. We used the following search words and phrases: artificial intelligence, deep learning, diagnostic imaging, logistic regression analyses, logistic models, ovarian tumors and adnexal masses.

Results

Following the systematic search process,153 citations were retrieved from the mentioned databases. Of these, 117 articles were excluded based on analyzing their titles and abstracts, resulting in 36 articles to be appraised in detail. We excluded general reviews and papers not related to our aim and, finally, 17 studies made the subject of this review.

For proposed logistic models, we analyzed 9 articles summarized in Table II.

For AI, deep and machine learning we analyzed 9 articles summarized in Table III.

Discussion

Logistic models and AI applications are being introduced in many medical fields and are marked demonstrable, valuable contributions in early detections, disease diagnoses, and therapeutic developments [10]. Several authors have proposed and published various logistic models to support less experienced sonographers predict adnexal malignancy. One of the first scoring systems, proposed in 1990 by Jacob et al, was the Risk of Malignancy Index (RMI) based on CA 125, ultrasound and menopausal status. The authors reported sensitivity for cancer of 85% and a specificity of 97%. Although MRI

Table II. Studies of logistic models in ovarian tumors

Author, year	No of patients	Variables analyzed	Performance
Jacobs, 1990 [17]	143	CA 125, ultrasound findings, menopausal status	Sn 85% Sp 97% for cut-off level of 200
Prompeler, 1997 [18]	754	Ascites, solid areas without acoustic shadows, masses with at least 30% solid area, tumor diameter, multilocular structures, surface of the cyst	For premenopausal women: Sn 86.5% Sp 92.6% For postmenopausal patients: Sn 93% Sp 82.7% Cut-off level of 10%
Tailor, 1997 [19]	67	Age, maximum tumor diameter, tumor volume, unilocularity, papillary projections, random echogenicity, highest peak systolic velocity, time-averaged maximum velocity, pulsatility index, resistance index	Sp 90.4% Sn 93.3% Cut-off value of 25 for malignancy
Alcazar, 1998 [20]	79	Menopausal status, color Doppler findings, ultrasound morphology	Sn 84.6% (95% CI, 59 to 98%); Sp100% (95% CI, 92 to 100%); PPV 100% (95% CI, 92 to 100%); NPV 95.7% (95% CI, 85.5 to 99.5%)
Timmerman, 1999 [21]	191	Menopausal status, CA 125 level, the papillary growth (>3 mm in length), color score indicative of tumor vascularity blood flow	Sn 95.9% Sp 87.1%
Alcazar, 2001 [23]	377	Menopausal status, tumor blood flow location, papillary projections, CA 125, Lowest RI	Sn 96.9 (83.8–99.9) Sp 94.2 (87.7–93.8) PPV 83.8 (67.9–93.8) NPV 98.9 (94.4–99.9)
Maret, 2002 [24]	130	Gray scale, RI < 0.53, flow location	Sn 83% Sp 93%,
Timmerman, 2005 [7]	754	LR1 - 12 predictors:personal history of ovarian cancer, current hormonal therapy, age of the patient, maximum diameter of the lesion, pain during examination, ascites, blood flow within a solid papillary projection, a purely solid tumor, the maximum diameter of the solid component, irregular internal cyst walls, acoustic shadows, color score LR1 - 6 predictors:age (years), presence of ascites, presence of blood flow within a papillary projection, maximum diameter of the solid component, irregular internal cyst walls, presence of acoustic shadow	LR1 Sn 93% Sp 77% Cut-off 10% LR2 Sn 92% Sp 75% Cut-off 10%
Meys, 2017 [22]	851	Age, serum CA125 level, type of center (oncology center/ other hospital), diameter of the lesion (mm), proportion of solid tissue, papillary projections more than 10 cyst, locules, acoustic shadow, ascites	Sn 0.98 (95% CI, 0.93-1.00) Sp 0.62 (95% CI, 0.55-0.68) Cut-off of ≥10%

Sp - specificity, Sn - Sensitivity, PPV - positive predictive value, NPN - negative predictive value

was recommended by many national guidelines, the external validation of this model yielded poor performance results [34].

Several years later, Tailor et al [19], Alcazar et al [20] and Timmerman et al [21] developed and put forth new logistic models to classify adnexal masses based on different ultrasound parameters, demographic data and markers (Table II). Unfortunately, most of these logistic regression models, when subjected to external validation, did not retain their original performance [35]. The key weakness of all these logistic models was the number of cases used to obtain the results. Hsieh FY et al. stated that a logistic model should be developed on the basis of several hundreds of cases and none of the mentioned studies fulfilled this requirement [36].

The International Ovarian TumorAnalysis (IOTA) study was established to develop robust rules and prediction models that can be used by different examiners in various clinical settings. The IOTA authors developed simple ultrasound-based rules ('simple rules') and IOTA Logistic Regression models (LR) for ovarian tumors. The simple rules consist of five ultrasound features of

Table III. Studies with AI algorithms used in differentiating ovarian pathology

Authorship	No of patients	Methods used	Performance
Timmerman, 1999 [21]	191	ANN	Sp 93.5% Sn 95.9%
Biagiotii, 1999 [25]	226	Three-layer back-propagation networks	Sn 96% Sp 97.7%
Acharya et al., 2012 [26]	20	CAD technique	ACC of 95.1%, Sn of 92.5% Sp of 97.7%.
Acharya, 2012 [27]	20	SVMclassifier	ACC of 99.9%
Acharya, 2014 [28]	20	Automatic CAD	ACC of 99.8%
Khazendar, 2015 [29,30]	177	SVM	Classification decisions of high, medium and low confidence, respectively, provided ACC of 90%, 81% and 69%.
Lu., 2003 [31]	425	Bayesian MLPs, Bayesian LS-SVMs, RVMs	Sp85% Sn 81.48%
Aramendia-Vidaurreta, 2015 [32]	145	CAD technique	ACC 98.78%, Sn 98.50%, Sp of 98.90% ACU of 0.997
Martınez-Mas, 2019 [33]	187	CAD systemsand ML techniques: KNN, LD, SVM, ELM	KNN classifier- ACC 60% LD, SVM and ELM classifier-ACC 85%

Sn - Sensitivity, Sp - specificity, AUC - area under the curve, ACC - Accuracy, SVM - Support vector machine, CAD - Computer-Aided Diagnostic, MLPs - multi-layer perceptrons, LS-SVMs - least squares support vector machines, RVMs - relevance vector machines, KNN - K-Nearest Neighbors, LD - Linear Discriminant, ELM - Extreme Learning Machine

malignancy (M-features) and five ultrasound features suggestive of a benign mass (B-features). A mass is classified as malignant if at least one M-feature and none of the B-features are present, and vice versa. If no B- or M-features are present, or if both B- and M-features are present, then the rules are considered inconclusive (unclassifiable mass) and a different diagnostic method should be used [37].

In the case of inconclusive results using the IOTA 'simple rules', two logistic models may present a viable alternative. Logistic Regression model 1(LR1) uses 12 demographic and ultrasound variables, while Logistic Regression model 2(LR2) uses six demographic and ultrasound variables. Both regression models showed excellent diagnostic performance.For LR 1 at cut-off level of 10%, the sensitivity and specificity of this model reached 92% and 75%, respectively. For LR2, at cut-off level of 10%, the sensitivity and specificity of this model reached 92% and 75%, respectively.The results of these models match those of experienced sonographers.

Another model with good reported performance is the Assessment of Different Neoplasias in the Adnexa (ADNEX) [38]. This model is based on serum CA125 levels, two clinical parameters, and six ultrasound parameters (Table II). This method can predict the probability of malignancy in five main categories (benign, borderline, stage I malignant disease, stage II–IV malignant disease and metastases), enabling clinicians to optimize the surgical treatment.

However, logistic models are not useful in all cases. About 7% of adnexal masses that are considered appropriate for surgical removal cannot be accurately classified even by the more experienced ultrasound examiners using subjective assessment. Valentin et al showed that logistic regression models to estimate the risk of malignancy, CA 125 measurements and the RMI are not helpful in these cases [39].

Nowadays, AI technology promised to provide a viable alternative to logistic regression. According to Tu et al,it has several advantages over logistic models. ANN require less formal statistical training, less ability to implicitly detect complex nonlinear relationships between dependent and independent variables, and the possibility to detect all interactions between variables [40]. In 1999, Timmerman et al introduced the first variant of ANN (ANN1) that used the following parameters: papillary projections, blood flow, CA125 level and the menopausal status. According to calculations using sophisticated mathematical models, the probability of malignancy >45% revealed a sensitivity and specificity of 87.5% and 92.7%, respectively. Thereafter, a second version was created (ANN2) based on papillary projections, smooth

surface, unilocular cyst, ascites, bilateral lesions, tumor marker CA125 and the menopausal status. The probability of malignancy>60% resulted in the sensitivity and specificity of 93.8% and 95.1%, respectively [41].

In 2012, Acharya et al developed an adjunct CAD technique that uses both images of the ovary acquired by 3D sonography and data mining algorithms to differentiate accurately benign from malignant tumors. They used 1,000 benign and 1,000 malignant images obtained from 10 patients with benign and 10 with malignant disease, respectively, and based on these images they developed a decision tree classifier. This yielded a sensitivity of 92.5% and specificity of 97.7%. However, the small number of patients whose imagistic findings were used represents a notable weakness of this study [26].

In the same year, Acharya et al reported using a support vector machine (SVM) classifier together with a radial basis function to automatically classify benign and malignant ovarian tumor images. They obtained high performance (accuracy of 99.9%) due to the combination of the 16 texture features that quantify the subtle changes in the images belonging to both classes. Moreover, in order to help physicians with their diagnoses, the team developed a novel integrated index called the Ovarian Cancer Index, essentially a combination of the texture features.However, this study was also restricted to 20 patients. Also, the system is not completely automatic and depends on the operator's experience, since a gynecologist andradiologist still need to delineate a region of interest to enclose the suspicious portion of the image [27].

Two years later, Achraya et al developed an automatic CAD system for ovarian tumor classification. In order to improve lower heterogeneity from the previous algorithms, they used 3-D color Doppler images. This probabilistic neural network classifier obtained an accuracy of 99.8% on a database of 2600 images on 20 patients [28].

Khazendar et al proposed a new method using a support vector machine (SVM) for automatic ovarian tumor classification, based on two different types of features extracted from ultrasound images of the ovary: the histogram and local binary pattern. The system was implemented on 187 ultrasound images from 177 patients. Based on classification decisions of high, medium and low confidence, respectively, the method provided accuracies of 90%, 81% and 69% [29,30]. Although this model was developed using a relatively large cohort of patients, numerous images were considered ineligible for inclusion in the high-confidence range [32].

Lu et al developed and evaluated several SVM on 425 patients with adnexal masses and they achieved an accuracy of 84.38% [31]. Their results showed that Bayesian models have the potential to provide a reliable preoperative distinction between malignant and benign ovarian tumors, and to assist the clinician in making a correct diagnosis [31].

Aramendia-Vidaurreta et al described a new method for the automatic distinction of adnexal masses based on a neural networks approach. Their method combines the patient age with several features extracted from ultrasound images of the ovary (local binary pattern, fractal dimension, entropy, invariant moments, gray level co-occurrence matrix, law texture energy and Gabor wavelet). The performance of the method is very good, its accuracy being as high as 98.78%, with 98.50% sensitivity, 98.90% specificity, and an area under the curve of 0.997 [32].

Martinez-Mas et al used four machine-learning techniques (K-Nearest Neighbors (KNN), Linear Discriminant (LD), SVM and Extreme Learning Machine (ELM) in order to provide automatic classification of the ovarian tumors with a high rate of accuracy. According to their results, the KNN classifier provides inaccurate predictions (less than 60% of accuracy) independently of the size of the local approximation, whereas the classifiers based on LD, SVM and ELM are robust in this biomedical classification (more than 85% of accuracy) [33].

One question that arises is "which is better: logistic models or artificial intelligence?" Several studies in the literature compared the performance of logistic models versus AI. Biagiotii et al compared the efficiency of ANN with that of multiple logistic regression (MLR) models on 226 patients with ovarian tumors (51 malignant and 175 benign cases). They developed a three-layer backpropagation network using the following variables: age, papillary projections, random echogenicity, peak systolic velocity, and resistance index. They showed that ANN had significantly higher sensitivity than MLR (96% vs 84%; McNemar test, P 5 .04) and could thus potentially help physicians differentiatebetween adnexal masses [25].

Holsbecke et al performed a comparison between logistic models and ANN models using data from 1,066 patients with ovarian tumors (800 patients with benign tumors and 266 patients with malignant tumors). Their results showed that the performance of the risk of malignancy index was similar to that of most logistic regression and artificial neural network models. The best result was obtained with a relevance vector machine with radial basis function kernel [42].

Obviously, there is a need for more prospective studies to compare and appraise the benefits of using logistic models and AI. Both methods show promising results in making the difference between benign and malignant ovarian masses. The integration of these techniques in

daily practice will allow timely and effective diagnosis, as well as proper management. An AI-assisted diagnosis could even allow non-specialists to confidently make decisions, which would normally require specialist input, such as, in emergency settings. Complete dependence on this technology is unlikely, but medical practitioners will eventually need to accept and to adapt to AI, changing the experience of clinical practice by contributing valuable opportunities for effective patient data analysis and informed decision making [43,44].

Conclusion

Both logistic models and AI can be efficiently used to distinguish between benign and malignant adnexal tumors. Both methods are suitable for analyzing massive amounts of data well in excess of what the human mind could. Both approaches have proved capable of providing automatic classification with a high rate of accuracy and, as such, they are expected to play an important role in future diagnostic procedures. These technologies, however, should not be viewed as a replacement, but rather as complementary tools for improved clinical practice.

Conflict of interest: none

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Review

Can Anorectal Atresia Be Diagnosed in the First Trimester of Pregnancy? A Systematic Literature Review

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Received: 27 September 2020; Accepted: 28 October 2020; Published: 30 October 2020

Abstract: Anorectal atresia (ARA) is a common congenital anomaly, but prenatal diagnosis is difficult, late, and unspecific. Utilizing a case of a 46 year old primipara with an egg donation In Vitro Fertilization (IVF) pregnancy, diagnosed at the first trimester scan with an anechoic isolated structure, which indicates anal atresia, we performed a systematic literature review in order to evaluate early prenatal ARA diagnosis. A total of 16 cases were reported as first trimester ARA suspicion, and only three had no associated anomalies. The most frequent ultrasound (US) sign was the presence of a cystic, anechoic pelvic structure of mainly tubular shape, or a plain abdominal cyst. In the majority of cases, structures were thin-walled and delimitated from the bladder. The presence of hyperechoic spots signifying enterolithiasis and peristaltic movements were helpful in order to establish the bowel origin of the lesion. Considering the high eventuality that the lesion is transitory, meaning later in pregnancy the fetus looks normal, early detection of such a sign should prompt further structural detailed evaluation, karyotyping, and appropriate pregnancy and postnatal counselling.

Keywords: anorectal atresia; congenital anomaly; early prenatal diagnosis; ultrasound

1. Introduction

An imperforate anus can be part of more complex congenital abnormal conditions that include anal atresia and anorectal atresia (ARA). The prevalence is high, ranging from 1/1500 to 1/5000 newborns [1]. The mechanism behind ARA is an impaired development of the urogenital septum that prevents the distal rectal pouch reaching the perineum, and also involves abnormal musculature development of the region (internal sphincter, external sphincter, and puborectalis muscle perianal muscular complex (PAMC)) [2].

ARA is associated in the literature with other malformations or chromosomal anomalies, at rates as high as 70% [3]. The most frequent associations are with trisomy 21, vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities (VACTERL) syndrome, caudal regression syndrome, complex genitourinary malformations, and omphalocele-exstrophy-imperforate anus-spinal defects syndrome (OEIS) [1,3,4].

Prenatal diagnosis of ARA is difficult, and the signs are unspecific and indirect, including bowel dilatation and abnormal intestinal calcification [5]. There have been few cases of isolated ARA reported

in the first trimester, and no systematic evaluation of the ultrasound (US) signs that should not be overlooked at this stage. The diagnosis is often made late in pregnancy or even after birth [6], although considering the morbidity, pathologic associations and impaired outcome, these babies could benefit from early recognition and proper counselling. More accurate examination and looking for the proper ultrasound signs (as described below) can improve the diagnosis rate in the first trimester). Among the most frequent complications of significant life-quality impact are fecal incontinence, bowel dysfunction, recurrent urinary infections, and sexual dysfunction [7].

The study was prompted by a case of a 46 year old primipara, with an egg donation In Vitro Fertilization (IVF), pregnancy presented for first trimester scan at 11 + 3 weeks. Other than an anechoic tubular structure in the fetal abdominal left quadrant, no other anomalies were noticed. The karyotype was performed and the result arr (1–22) x2, (XY) x1 was considered a normal variant. ARA was diagnosed and considered isolated since further detailed examination performed in the second and third trimester found no other anomalies. The baby was born at 36 weeks and low anorectal atresia with perineal fistula was diagnosed. Surgery was scheduled for three months of life.

The aims of this systematic literature review were to assess the performance of the first trimester scan in ARA diagnosis and to indicate the most specific ultrasound signs of malformation, mainly when it is isolated, in the actual context of the first trimester ultrasound scan, thus expanding its capability of detailed analysis of the fetal anatomy.

2. Materials and Methods

The analysis was performed according to the guidelines provided by Moher in 2009, indicated in "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" as a PRISMA statement. No ethical committee approval was needed, given the type of study [8].

Search and Information Sources

We searched the main databases, PubMed, Embase.com and Cochrane, electronically and with the following combination of key words and terms: "first trimester", "early prenatal diagnosis", "anal atresia", "ano-rectal anomaly", "ultrasound", and "abdominal cystic anomalies". All types of studies (retrospective, prospective, case control, systematic review) on ARA prenatal diagnosis were considered. The period included all papers published since 1990 and until 2020.

Inclusion criteria were: (1) papers describing ultrasound anomalies consistent with ARA suspicion/diagnosis, (2) cases reported in the first trimester of pregnancy (no further than 14 weeks), and (3) ARA diagnosis confirmed post-termination or postpartum. No language restriction was applied.

The search and title evaluation were performed by two authors (LP and RMS) and assessed for the relevance of content. If the abstracts were relevant for the inclusion criteria, the full papers were extracted from the original publication. Differences of opinion were resolved by discussion with RC and RM. We excluded titles without full-text and images, and those where the diagnosis was performed after 15 weeks. After collecting the data, we excluded duplicated studies, and the remaining titles were analyzed based on the inclusion criteria. There was no need to contact the authors.

Statistical analysis was not applicable considering the qualitative evaluation and low number of reported cases.

The database search provided 198 items, from which 76 were excluded as duplicates. After abstract assessment, 21 studies were retained and considered relevant for early prenatal diagnosis of ARA. Finally, seven papers were excluded for not reporting outcomes or not fulfilling the required criteria, and 16 cases of ARA diagnosed in the first trimester and confirmed postpartum or post pregnancy termination were extracted from 14 papers (two of them reporting two cases of early diagnosis) (Figure 1). To these we added our case, diagnosed at 11 + 6 weeks. The flow chart of the search is illustrated bellow. The main parameters that were analyzed are described in Table 3.

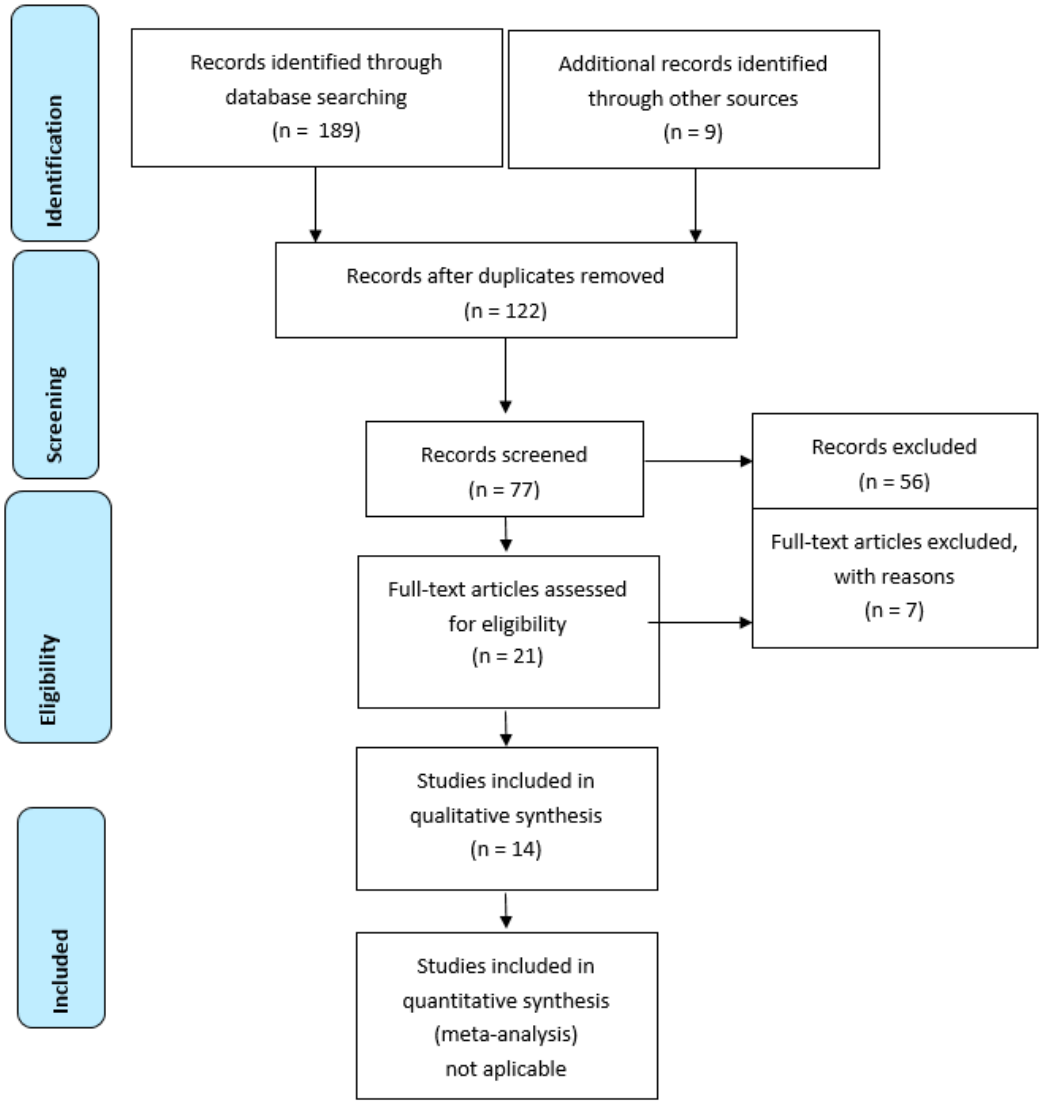

Figure 1. Flow diagram.

Table 1. Study characteristics.

Study, Author Type Year	Number of Reported Patients	Gestational Age at Diagnosis (Weeks)	Ultrasound Findings at Diagnosis	NT (mm)	Associated Anomalies	Fetal Gender	Genetic Assessment	Follow-Up Image Findings	Pregnancy Outcome	Neonatal/Post Termination Diagnosis
Bronshtein M CS 2017 [9]	2	13 + 4 14 + 0	transient, distended, and right-sided sigmoid colon transient, distended, and right-sided sigmoid colon	NR NR	Ambiguous genitalia, single echogenic kidney, frontal bossing, skull demineralization, hemivertebra No	NR NR	NP NP	NP Spontaneous resolution of cystic mass at 19 weeks.	TOP first trim Live born	Anal atresia confirmed, VACTERL Anal atresia confirmed
Carroll SG CR, 1996 [10]	1	12	Abdominal multiple cystic structure	NR	No	F	Normal 46 XX	Megacystis, hyper echogenic bowel, ascites, oligohydramnios.	TOP Sec trim	Female pseudo hermaphroditism with agenesis of the urethra, vagina, and rectum.
Chen M 2009 CR [11]	1	12	Multiple dilated bowel loops within the lower abdomen	1.9	NR—poor visibility due to maternal obesity	F	N 46 XX	NR—poor visibility due to maternal obesity	Spontaneous miscarriage sec trim	Atrogryposis multiples, CoA, univentriculr heart
Correia P CR, 2017 [12]	1	12	Hypoechoic tubular-shaped cyst was observed, on a retrovesical location, at the left lower abdomen.	NR	Abnormal male genitalia	M	N46XY	Dilated sigmoid showed intraluminal hyperechogenic foci suggestive for vesicorectal fistula	TOP sec trim	Anorectal agenesis, vesicorectal fistula, hypospadias
Dhombres F POS [13]	1	12 + 2	Hyperechogenic pelvic structure	1.9	No	NR	NP	Resolution of image at 17 weeks	Live born	Isolated imperforate anus
Gilbert A CR 2009 [14]	1	12 + 6	Cystic structure with a distal tapered appearance within the abdomen and pelvis of the fetus with an echogenic focus that did not exhibit echogenic shadowing	N	No	M	N	No anomaly at 24 weeks.	Emergency CS 29 weeks. –IUGR and fetal distress	Imperforate anus horseshoe kidney and low termination of the spinal cord at the third lumbar vertebral body.

Table 2. Study characteristics.

Study, Author Type Year	Number of Reported Patients	Gestational Age at Diagnosis (Weeks)	Ultrasound Findings at Diagnosis	NT (mm)	Associated Anomalies	Fetal Gender	Genetic Assessment	Follow-Up Image Findings	Pregnancy Outcome	Neonatal/Post Termination Diagnosis
Girz 2008 CR [15]	1	12 (?)	large cystic structure on the anterior fetal abdomen (identified as omphalocel).		Thoracic kyphoscoliosis	F	N46XX	Persistent anomaly 20 weeks consistent with OEIS	TOP 20 weeks	OEIS
Lam YH CR 2002 [16]	1	12	sausage shaped cystic mass (11 × 6 × 6 mm) in the right lower anterior abdominal cavity	1.4	No	M	N 46XY	No progression of the cystic mass, oligoanhidramnios	TOP Sec trim	anal atresia, malrotation of the gut, dilated sigmoid colon and rectum and a perimembranous ventricular septal defect
Liberty G SLR&CR 2018 [17]	1	13 + 1	cystic structure measuring 7 × 8 × 4 mm was identified in the right lower abdomen which tapered toward its distal part in the pelvis	1.7	No	M	N CGarray 46XY	16 weeks., cystic mass replaced by tubular shaped echogenic structure 21 weeks. absence of target sign (anal sphincter) prominent midline skin bridge in the fetal perineum	TOP sec trim	Absence of anal sphincter, high type ARA.
Mallman, M.R 2014 CR [4]	1	14 + 1	41 × 34 mm large cystic structure in the lower abdomen with	NR	Bladder extrophy, omphalocel OEIS	M	NP	NP	TOP 17 weeks	OEIS confirmed
Novikova I, CR 2011 [18]	2	11 + 2 11 + 3	dilated bowel within the lower abdominal cavity (10.0 mm × 2.0 mm) 8.4 mm anechogenic tubular structure in the lower abdomen (misdiagnosed as megacystis)	2.5 1.05	No No	F M	N 46 XX T21 47XY	16 weeks.–anhidramnios, no urinary bladder, no kidneys NP	TOP sec trim TOP early sec trim	Imperforate anus, rectal atresia, multiple anomalies compatible with Fraser syndrome Inconsistent due to tissue destruction during D&C (rectum dilatation?)

Table 3. Study characteristics.

Study, Author Type Year	Number of Reported Patients	Gestational Age at Diagnosis (Weeks)	Ultrasound Findings at Diagnosis	NT (mm)	Associated Anomalies	Fetal Gender	Genetic Assessment	Follow-Up Image Findings	Pregnancy Outcome	Neonatal/Post Termination Diagnosis
Santos J2013 Cr [19]	1	13	abdominal cystic formation	NR	caudal dysplasia with hypoplastic lower limbs	M	N 46XY	NP	TOP first trim	Multiple anomalies suggestive for VACTERL syndrome
Taipale P CR 2005 [20]	1	12	hypoechogenic cystic mass (14 × 7 × 8 mm) in the lower abdomen	1.1	No	M	NP	Similar findings as in first trim	Living birth	Anal atresia with fistula
Wax 2008 CR [21]	1	13	large multilocular cystic ventral wall mass measuring 4.7 3 4.0 3 3.5 cm	Cystic Hygroma	Thoracic hemyvertebrae	M	N46XY	16 weeks omphalocel splayed lumbosacral vertebrae and bilateral clubbed—OEIS	TOP 20 weeks	OEIS confirmed bifid phallus, symphyseal diastasis, imperforate anus, and no clear buttocks cleft

NT: nuchal translucency; F: feminine; M: masculine; N: normal; NP: not performed; NR: not reported; OEIS: omphalocele, exstrophy of the fetal bladder, imperforate anus, spinal anomalies; VACTERL syndrome: vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities; TOP: Termination of pregnancy; sec: second; CoA: Coarctation of Aorta; IUGR: Intrauterine Growth Restriction; ARA: Anorectal atresia; D&C: Dilatation and curretage.

3. Results

The retained titles yielded a small number of prenatal ARA cases diagnosed in the first trimester; 16 cases, to which we added the case we diagnosed at 11 + 6 weeks. The maternal and paternal ages could not be statistically evaluated for correlation with the anomaly since not all papers reported them. Gestation age at diagnosis of ARA ranged between 11 + 2 weeks to 14 + 1 weeks.

Fetal gender was not reported by all authors (three cases), but from the rest we noted a predominance of male fetuses (Male/Female (M/F) ratio 2.5/1). No title that reported signs of ARA referred to a twin pregnancy. Karyotyping was not performed in five cases, but did reveal a case of Trisomy 21 (T21), and in 11 cases was normal, including the case we reported, with arr (1–22) x2, (XY) x1 considered a normal variant.

Although, according to the inclusion criteria, the scans must be performed in the first trimester, in seven cases the nuchal translucency was not reported, and in the remaining cases, only one fetus had increased Nuchal Translucency (NT) reported as cystic hygroma. The other nine fetuses had NTs in the normal range.

Ultrasound signs of ARA in the first trimester were described mainly as cystic structures: uni or multilocular; hypoechoic round, tubular, ovalar structures; hyperechoic structures in one case; located in the fetal abdomen/pelvis; delimitated from the urinary bladder.

In four cases, the lesions disappeared later in the pregnancy, and in five cases, the second trimester scan was not performed. In the remaining seven cases, the lesions remained stable, progressed, or changed shape and aspect. Associated anomalies were reported in only 35% of cases, and consisted of ambiguous genitalia, spine anomalies, cloaca anomalies, kidney anomalies, and OEIS syndrome.

The pregnancy outcome was reported as live born in four cases, among which one was an emergency cesarean section (CS) for fetal distress at 29 weeks. One showed a true isolated ARA-associated perineal fistula, but with a very thin membrane covering the rectal pouch. The rest of the cases finished as termination of pregnancy (TOP) in the first trimester (two cases) or second trimester (nine cases). One was a spontaneous miscarriage.

4. Discussion

ARA is difficult to identify in the first trimester, when diagnosis relies on indirect signs. In assessing the first trimester detection on non-chromosomal anomalies, we found few reports on ARA, as Table 3 illustrates. The prenatal diagnosis rate of ARA detection is low (16%), even when considering diagnosis throughout the whole pregnancy period [6].

In an extensive study on the detection rate of different anomalies in 45,191 fetuses, Syngelaki reported no ano-rectal anomalies were detected [22]. This can be explained by the late first trimester development of the PAMC, so the absence of the anal sphincter could not be identified at the first trimester anomaly scan [2].

More recently, Rohrer evaluated the prenatal diagnosis of the ARA by ultrasound vs. magnetic resonance investigation (MRI) in 56 children over 10 years but did not report a diagnosis made below 20 weeks of gestation [23]. They concluded that, depending on the level of obstruction, the main sign of ARA is bowel dilatation and associated anomalies, and MRI is useful for correct identification of the fluid-filled pelvic organ (rectum, vagina, bladder, cloaca). Despite this important contribution to diagnosis, MRI is not indicated in the first trimester of pregnancy.

In 14 cases, the diagnosis was suspected due to finding cystic structures in the fetal abdomen and pelvis. These structures can take many forms but are mainly tubular shaped, and the aspect is determined by the associated anomalies (megacystis, rectovesical fistula, cloaca complex anomalies). A well-delineated, tubular shaped structure was indicative in our case. Additionally, the presence of hyperechoic structures inside the cysts can indicate bowel conditions, due to enterolithiasis [2]. In one case, the appearance was a hyperechoic pelvic structure, and in two cases of OEIS, the cystic structure was located at the level of the anterior abdominal wall and was diagnosed as omphalocel [4,13,15].

In our case, the hyperechoic structure (enterolithiasis) had odd movement that we interpreted as being produced by intestinal obstructed peristalsis.

Anechoic cystic structures are due to dilated bowels filled with liquid of uncertain origin. As a mechanism of obstructed bowel filling, it was proposed that fetal amniotic liquid is swallowed and, in some cases, contributes to the constitution of a rectovesical fistula. The accumulation of liquid can be explained since the rectum ends in a pouch, and the intestinal mucosa is not capable of absorption in early gestation [17].

Obviously, a cystic mass in the fetal abdomen prompts differential diagnosis with conditions other than ARA. Khalil et al., in a cohort retrospective study on 14 fetuses in which cystic structures were found at the first trimester anomaly scan, did not report any ARA but concluded that, considering the high probability that obstruction and gut malrotation produce bowel dilatation, this diagnosis should be included in differential diagnosis algorithms [24].

The literature reports that cystic images can be transitory in pregnancy, with spontaneous resolution in the second and third trimester mainly if the right colon is affected. The pathophysiology of right colon dilatation is not completely understood, and malrotation of the sigma or of the whole large bowel can be involved [9].

The presence of the fistulous trajectory reported in three cases can be explained by embryologic development. During cloaca and uroseptum formation, a crucial event is the recanalization of the "plug" that occludes the anorectal canal by apoptosis, a process that occurs in late embryologic development [25]. According to this theory, failure of recanalization or an improper process leading to abnormal orifices (fistula) can occur in early embryonic stages, while other ARAs with a normally placed anal orifice are a consequence of an injury and occur in a later stage. In our case, considering that the intestinal distension was observed early in the first trimester and disappeared later, and a perineal fistula was observed, we can suppose that the apoptotic process of anal recanalization occurred very early, and a second trimester evaluation only would have missed the anomaly. The decompression of the distended colon through a perineal or rectovesical fistula has also been proposed by other studies on cystic anomaly resolution in late gestation. The authors suggested that this transitory sign can be present in 37% of ARA cases, and it is useful to predict the condition from the first trimester [17].

The appearance of anomalies at the subsequent examination were reported not only as transitory findings, but also as the changing of echogenicity from anechoic to hyperechoic structures in two cases. There are two mechanisms that have been proposed for this hyperechogenic aspect of the obstructed bowel, some authors suggest that digestive enzymes accumulate in the colon after the first trimester and modify the composition of the intestinal contents. In normal fetuses, those enzymes are excreted in the amniotic fluid through a normal anal orifice [26]. Urinary content of oxalate and phosphate salts can also be responsible for the hyperechogenic aspect of dilated bowel contents in ARA associated with rectovesical fistula [27]. In our case, the anechoic initial cyst in the abdomen resolved in the second trimester, but the slightly echogenic rimmed rectum cannot be classified as a hyperechogenic structure, as reported by other papers. The possible explanation for this is that only a small amount of digestive enzymes accumulated after the fistula constitution, and this almost normal appearance of the lower fetal abdomen could have been misleading if no scan was performed in the first trimester.

Direct signs of ARA are not available in the first trimester due to the late development and difficult evaluation of the PAMC. The indirect signs of cystic pelvis structures cannot identify the level of obstruction but can allow an early diagnosis by contrary PAMC systematic evaluation, as Lee performed in a recent study. This could orientate the level of obstruction but had a sensitivity of only 74% [28]. On a large low risk pregnancies cohort (63,101 cases), Su evaluated the size and aspect of the anal canal and rectum and reported a high rate of prenatal isolated ARA diagnosis of 87.5%, but only after 20 weeks [29].

The etiology of ARA seems to be multifactorial, including some familial cases with autosomal dominant inheritance. Chromosomal abnormalities were only reported in one case of T21 in the case series (Table 3). Gene mutations involved in this condition include *SHH*, *EN2*, and *HLXB9*, which

can be responsible for Pallister–Hall syndrome, Currarino syndrome, and Townes–Brocks syndrome, but are very rare [30]. In our case, the isolated finding led to a supposition that the anomaly was related to advanced paternal age (52 years) and the assisted reproductive procedure.

There is no consistent proof that, considering the minor gene polymorphism, the baby was not a carrier of a monogenic pathogenetic variant in our case (arr (1–22) x2, (XY) x1) that can be related to this anomaly. On the other side, the IVF procedures can be related to some genetic de novo anomalies and structural anomalies but so far there is no consensus in the literature on that issue [31].

The literature is scarce providing data for genetic causes of ARA. The studies include small samples and are mostly suspected to be gene approached, referring to *SHH*, *WNT*, and Fibroblast Growth Factor (FGF) signaling pathways as they are involved in multiple embryonic developmental processes. Multiple factors, such as genetic and environmental, are involved and genetic counseling is difficult since the risk of recurrence in unknown for non-syndromic ARA [32].

The cases reported in the literature found anomalies at the time of diagnosis in only six cases (ambiguous genitalia, single echogenic kidney, frontal bossing, skull demineralization, hemivertebra consistent with VACTREL, and caudal dysplasia). The postnatal or postmortem diagnosis indicated more syndrome cases (nine out of 12 cases), involving mainly the urogenital and cloacae-derived structures.

Considering that urogenital anomalies are a frequent association of ARA, Perlman evaluated 245 fetuses referred to a tertiary center for Congenital anomalies of the kidney and urinary tract (CAKUT) and found four cases with anal atresia, but only after 20 weeks. The detection rate was improved due to PAMC examination, and the authors highlighted that some of the cases could be overlooked because of the transitory signs of bowel dilatation after the first trimester [33].

The first anal atresia case identified in the first trimester by a US scan was reported by Girz as a part of OEIS, and the appearance was described as a large cystic structure on the anterior fetal abdomen (identified as omphalocel) [15]. In this case, the diagnosis was made after TOP triggered by spine anomalies. Other studies have reported other OEIS cases, but only three were picked up in the first trimester due to other associated anomalies [4,21].

Only five cases reported in the literature (including our case) were isolated anomalies, and this fact emphasizes the importance of first trimester diagnosis, which can possibly make the offer of genetic testing and close follow-up in pregnancy in order to correctly counsel and manage the situation [9,13,17,20].

An isolated anomaly, as in our case, and adequate fetal growth can allow pregnancy continuation until near term, and careful pregnancy evaluation is needed in order to assess the fetal wellbeing and to establish the appropriate birth modality.

There are some issues that further evaluation of ARA anomalies must address. First, there is a need to unify the terminology in order achieve consistent and reproducible reporting of cases (there are many synonyms, such as anal atresia, ano-rectal malformation, anal imperforation, etc.). Second, it is necessary to report maternal age, fetal sex, conception method, and other personal conditions such as obesity and diabetes, which could then be orientated towards the risk factors associated with ARA and prompt a detailed first trimester check.

5. Conclusions

A first trimester scan for detecting various pregnancy risks should be able to identify many fetal structural anomalies due to the high-resolution equipment and proximity of the fetal structures to the US transvaginal probe. Although isolated ARA is not a fatal disease, the physician should be aware that first trimester cystic or hypoechoic fetal pelvic structures trigger the diagnosis. Considering the transient character of those signs in the second trimester, when this condition can be overlooked, detailed ultrasound examination must be performed in order to exclude associated conditions and syndromes with an unfavorable outcome. Prenatal early diagnosis of isolated ARA does not change the prognosis of the pregnancy but offers the possibility of proper surgical counsel and immediate

postpartum referral. The most sensitive issue in the couple's counselling can be the context of advanced maternal age and Assisted Reproduction Techniques (ART) pregnancy, with a high likelihood of other pathological associations. In such a situation, precocity of the diagnosis makes further investigation possible and early identification of serious fetal anomalies informs TOP indication or confident pregnancy continuation.

Author Contributions: Conceptualization, L.P. and R.M.; methodology, A.N.; software, A.N.; validation, M.-O.P., and R.M.S.; formal analysis, M.-O.P.; investigation, R.C. and R.M.S.; resources, R.C. and R.M.S., L.P.; data curation, A.N.; writing—original draft preparation, L.P.; writing—review and editing, R.M.S.; visualization, R.M.; supervision, R.M.; project administration, L.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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The added value of three-dimensional ultrasonography in uterine pathology

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Received May 31, 2021; Accepted June 30, 2021

DOI: 10.3892/etm.2021.10696

Abstract. The rapid development achieved over the last decades in volume rendering of ultrasound data, known as three-dimensional (3D) ultrasound technique, leads to new opportunities for refining the diagnosis in many gynaecologic conditions. The aim of the present study was to evaluate the advantages of 3D ultrasound over two-dimensional (2D) ultrasound in uterine pathology and to establish the optimal time point during the menstrual cycle to perform 3D ultrasound examination in order to achieve the maximum of useful information. A cross-sectional study on 200 patients who underwent gynaecologic 2D and 3D ultrasound examinations was performed. The addition of 3D examination to 2D ultrasound in uterine pathology provided the most useful information concerning: Congenital uterine anomalies, intrauterine devices (IUDs), adenomyosis, and submucous myomas. The findings showed that the 3D ultrasound scan is a useful tool in gynaecology, especially in cases with congenital uterine anomalies, myoma, and IUD. Although initially it was used for research purposes only, recent findings suggest its usefulness in routine ultrasound scan and the possibility of witnessing its introduction as a recommended examination procedure in the foreseeable future. Further research should be conducted in order to establish the sensitivity of 3D ultrasound in the detection of minor endometrial conditions, by correlating the imaging findings with the hysteroscopic results.

Introduction

In the last few decades, significant progress has been made in the quality of imaging techniques in gynaecology that has consistently contributed to the improvement and refining of diagnoses. Despite competition from magnetic resonance imaging (MRI) and computer tomography (CT), ultrasound remains the first-line modality for gynaecologic applications. The introduction of three-dimensional (3D) ultrasound in the early 1990s was an important step, and numerous studies have been conducted to determine the impact of 3D ultrasound (1). Currently, 3D is widely accepted in the diagnosis of foetal abnormalities and in the assessment of the main complications of pregnancy (2,3). Few studies have been published in the field of gynaecologic applications of 3D ultrasound, rendering the present study a vital one.

The aim of the present study was to evaluate the advantages of 3D ultrasound over two-dimensional (2D) ultrasound in uterine pathology and to establish the optimal time point, with regards to the menstrual cycle, to perform 3D ultrasound examination in order to attain the maximum of useful information.

Patients and methods

Patient details. A cross-sectional study was performed in which 200 patients [age range, 19-76 years; mean, 40.38 years; inclusion and exclusion criteria provided in Table I] underwent gynaecologic and ultrasound examinations at Medis Medical Centre Iasi, Romania, between November 2019 and October 2020, comprising standard 2D followed by 3D ultrasound. For both examinations, a 3D endo-vaginal ultrasound probe [4-8 megahertz (MHz), Voluson E8; GE Healthcare] was used. The ultrasound examination began with a conventional 2D scan in order to establish the uterus position and its dimensions (longitudinal and antero-posterior diameters and the width of the corpus). Endometrial thickness was measured in the longitudinal plane, including the two layers at the level of its maximum thickness. Any pathological findings were recorded. The adnexal region was then scanned in order to identify the ovaries, the fallopian tube and a possible adnexal pathology. After the 2D ultrasound examination and Doppler assessment a diagnosis was established. The same sonographer, longitudinally along the length of the uterus, obtained the 3D volume. The coronal plane was obtained and then volume contrast imaging was gently applied (4). Both 2D images and

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Key words: three-dimensional ultrasonography, congenital uterine anomalies, coronal plane, intrauterine device, myoma

3D volumes were saved on the ultrasound machine's hard drive. The findings observed by 2D and 3D ultrasound were analysed by sonographers and compared. In cases with endometrial pathology, in order to confirm the diagnosis, a hysteroscopy (considered gold standard) was performed.

A comparison was made between the patients who benefitted from the reconstructed coronal view of the uterus vs. those who did not, based on the indication for scanning, and sonographic findings on the 2D ultrasonography.

Ethics. Each patient was properly informed regarding the conditions of the study and provided informed consent. Ethics approval was obtained from the Institutional Board of the 'Medis' Medical Centre (Iasi, Romania).

Statistical analysis. Data were analysed using the statistical package SPSS 26 (IBM Corp.) and JASP 0.13 (University of Amsterdam). The statistical tests used were: Chi Square, Shapiro-Wilk, Kruskal-Wallis, Levene and binomial test. The cut-off value of $P<0.05$ was considered to indicate a statistically significant difference. The confidence interval was designated at 95%.

Results

The age of the patients ranged between 19 and 76 years with a mean of 40.38 years (SD, 9.133). The indications for scanning for the 200 patients are presented in Table I.

In 71 cases (35.5%) (CI 28.9-42.6%) the ultrasound scan (both, 2D and 3D) revealed no uterine abnormalities. In 129 cases, different conditions were encountered. The results are presented in Table II.

The 3D ultrasound changed the diagnosis established by 2D ultrasound in 6 cases (3.0%) (CI 1.1-6.7%): Four cases with uterine congenital anomalies and two cases with IUD malposition in the uterine cavity.

In 53 cases (26.5%) (CI 20.5-33.2%), although the diagnosis was not changed it was completed by adding 3D and coronal plane examination. In cases with uterine myoma, the 3D ultrasound proved to be useful when myoma was situated intracavitary (total or partial) by showing the type of myoma (G0, 1 or 2 type), (Fig. 1A-C).

Uterine anomalies, classified as per the ESHRE/ESGE criteria (5) suspected by 2D, but completely diagnosed by 3D ultrasound were as follows: Arcuate uterus, 3 cases; septate uterus, 4 cases (Fig. 2A-C); bicorn uterus, 1 case and unicorn uterus, 1 case.

In two cases with IUD, the diagnosis of misplaced or incongruence between IUD and uterine cavity was missed by 2D ultrasound (Fig. 3A-C).

High level of agreement was found between 2D and 3D in cases of uterine myoma and adenomyoss, a κ value of 0.8 and 0.74, respectively. A moderate level of agreement was observed in cases of polyps (κ -0.67) and a low one in IUD (κ -0.44).

In order to establish the optimal time point for performing a 3D scan with regards to menstrual cycle, results were compared in relation to the endometrial thickness. The quality of 3D images was favorable in 149 cases (74.5%) (CI, 67.9-80.4%) and inadequate in 51 cases (25.5%) (CI: 19.6-32.1%). The quality of the image was quantified by the possibility of obtaining a clear picture of the uterine cavity and endometro-myometrial junction (EMJ). The mean endometrial thickness was 7.86 mm (SD, 2.903) with values between 2 and 19 mm. A significant statistical relationship existed between the quality of the image obtained and the thickness of the endometrium (P=0.622). A thin endometrium was associated with a poor 3D image. The present study showed a high probability to achieve a good image if the endometrium was ≥7.38 mm (value calculated using the Kruskal-Wallis non-parametric test).

Discussion

Ultrasonography is the most frequently used imaging technique in the assessment of the female genital tract. Usually the uterus and ovaries are evaluated using a 2D endovaginal ultrasonography. The main disadvantage of the 2D ultrasound scan consists in the difficulty of obtaining the coronal plane of the uterus. Adding a 3D ultrasound scan to the conventional examination may be beneficial as the coronal plane of the uterus can be obtained easily using the reconstruction 3D technique.

The present study revealed that from a total number of 200 examinations, 3D examination changed the diagnosis in 6 cases (3.0%) (CI: 1.1- 6.7%) and added useful information or reinforced the diagnosis in 53 cases (26.5%) (CI: 20.5- 33.2%).

The most useful information was obtained in patients referred to for ultrasonic scan for the following indications: Infertility, uterine haemorrhage and IUD placement follow-up. Concerning the accuracy of the final diagnosis, the most useful information was obtained in patients with uterine pathology: Uterine congenital anomalies, IUD misplacement, adenomyosis, and submucous myomas. Neither 3D nor volume contrast imaging (VCI) were useful in cases with normal uterus.

Although conventional 2D ultrasound has a good accuracy in diagnosing congenital uterine anomalies, it is highly dependent on the expertise of the examiner and is limited by the difficulty in obtaining the coronal plane of the uterus. Several studies have demonstrated the advantages of 3D ultrasound in diagnosing uterine anomalies (6-8). Comparing 3D ultrasound to laparoscopy and hysteroscopy, Mohamed *et al* recorded a sensitivity of 97%, specificity of 96%, positive predictive value of 92% and negative predictive value of 99% in the diagnosis of uterine anomalies (9). Ghi *et al* reported both a sensitivity and a specificity of 100% in the diagnosis of uterine malformations and 96% concordance between ultrasound and endoscopy with respect to the type of anomaly diagnosed (10). In this study, 3D was mandatory for the final diagnosis in all cases with uterine congenital anomalies. As the coronal plane enables the visualization of not only the endometrial cavity, but also of the uterine fundus, 3D scan was necessary for the differential diagnosis between bicornuate and septate uterus. Moreover, in these cases, 3D proved to be superior to hysteroscopy. Hysteroscopy is able to confirm an anomaly of the uterine cavity but is not able to provide any information regarding the external contour of the uterus. In addition, in the coronal plane of the uterus obtained with 3D ultrasound it is possible to measure the size of the septum and classify the anomaly according to the ESHRE/ESGE classification system of female genital anomalies (5). Although MRI is considered the gold standard for congenital uterine anomalies,

Table I. Indications for the ultrasound scan (N=200).

	Data	
Indication	No. of patients	Percentage
On demand at a routine gynaecological consult	51	25.5
IUD control	10	5.0
Abdominal pain, dysmenorrhea	38	19.0
Menorrhagia	33	16.5
Infertility	39	19.5
Menstrual disorders	13	6.5
Myoma, cyst	16	8.0
Total	200	100

IUD- intrauterine device.

Table II. The results provided by the ultrasound scan (N=200).

	Data	
Diagnosis	No. of patients	Percentage
No uterine abnormalities	71	35.5
Uterine myoma/myomas	52	26
Uterine congenital anomalies	10	5.0
IUD displacement - Cooper IUD	1	0.5
IUD displacement - levonorgestrel IUD	2	1
Endometrial polyps	17	8.5
Isthmocele	15	7.5
Adenomyosiys	22	11
Endometrial hyperplasia	10	5
Total	200	100

IUD, intrauterine device.

3D can provide the same type of information (11). However, in doubtful or complex cases, MRI should be performed, particularly for the assessment of the cervix and vagina (12). Thus, 3D ultrasound may be associated with 2D ultrasound in the diagnosis of uterine malformations.

Analysing the IUD cases from the current study, 3D was useful for both types: Copper IUD and levonorgestrel (LNG IUD). VCI proved to be particularly useful especially in cases with LNG IUD. Observations of this study confirm those of several studies performed in order to assess the role of 3D ultrasound in patients with IUD (13-16). These studies agree that the coronal view shows the entire device and its position within the uterus, in this way helping to identify the cause of pelvic pain and (or) bleeding in patients with an embedded IUD (14). Hösli *et al* demonstrated that 3D ultrasound offers the advantage of a better visualisation of LNG IUD and at the same time the assessment of uterine anomalies (13). The coronal plane is useful not only to detect the exact position of the IUD, but also to measure the uterine cavity and thus to establish the proper size of the IUD to be inserted in the uterus (17).

One interesting issue in gynaecology is the assessment of submucous myomas. It is important when diagnosing a myoma to know its level of extension in the uterine cavity. Moreover, before planning a hysteroscopic resection of a submucous myoma, it is necessary to know the type of myoma (G0, 1 or 2 type). Salim *et al* demonstrated that three-dimensional sonohysterography (3D-SIS) had a similar accuracy as hysteroscopy in classifying submucous fibroids (18). Lee *et al* demonstrated that 3D-SIS is reproducible among different observers for quantification of the percentage of a submucous fibroid protruding into the uterine cavity (19). Mavrelos *et al* demonstrated that 3D-SIS may be useful in predicting complete hysteroscopic resection of submucous myomas (20). Although findings of the present study show a high level of agreement between 2D and 3D ultrasound in the diagnosis of myoma (κ=0.8), reconstructed coronal plane added useful information for planning the surgical management of the case.

Regarding adenomyosis, 2D ultrasound was useful in establishing the diagnosis by revealing the classical ultrasound signs including: Anechoic foci, heterogeneous myometrium,

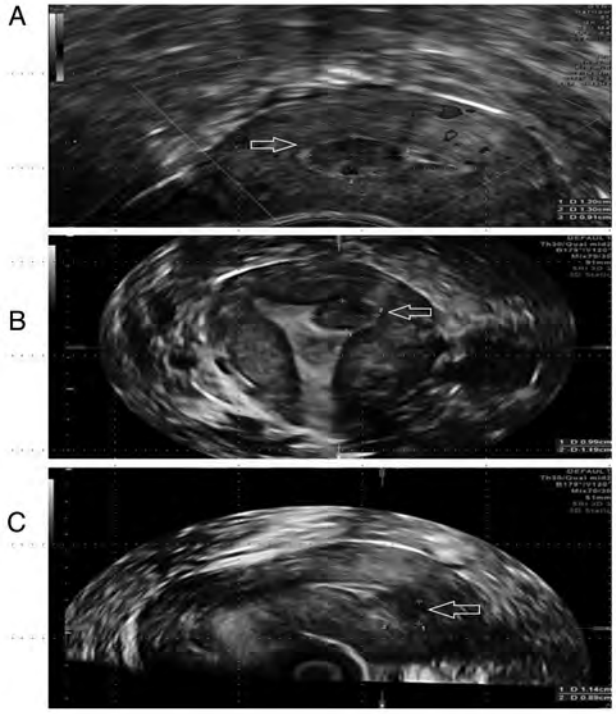

Figure 1. Submucous myoma (arrow). (A) 2D transversal plane of the uterus. (B) 2D ultrasound longitudinal plane of the uterus. (C) 3D ultrasound, coronal plane of the uterus. 2D, two dimensional; 3D, three dimensional.

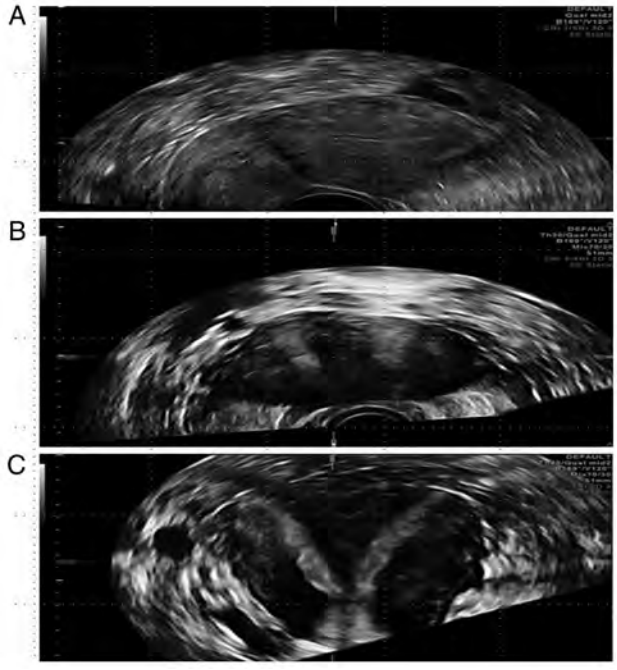

Figure 2. Septate uterus. (A) 2D ultrasound longitudinal plane of the uterus. (B) 2D transversal plane of the uterus. (C) 3D ultrasound, coronal plane of the uterus. 2D, two dimensional; 3D, three dimensional.

asymmetrical uterine wall, uterine enlargement and hyperechogenic striations (21). However, 2D ultrasound cannot provide information regarding the endometro-myometrial junction (EMJ). The changes in EMJ are considered a key element in diagnosing adenomyosis and traditionally the assessment of the EMJ has been part of the MRI evaluation

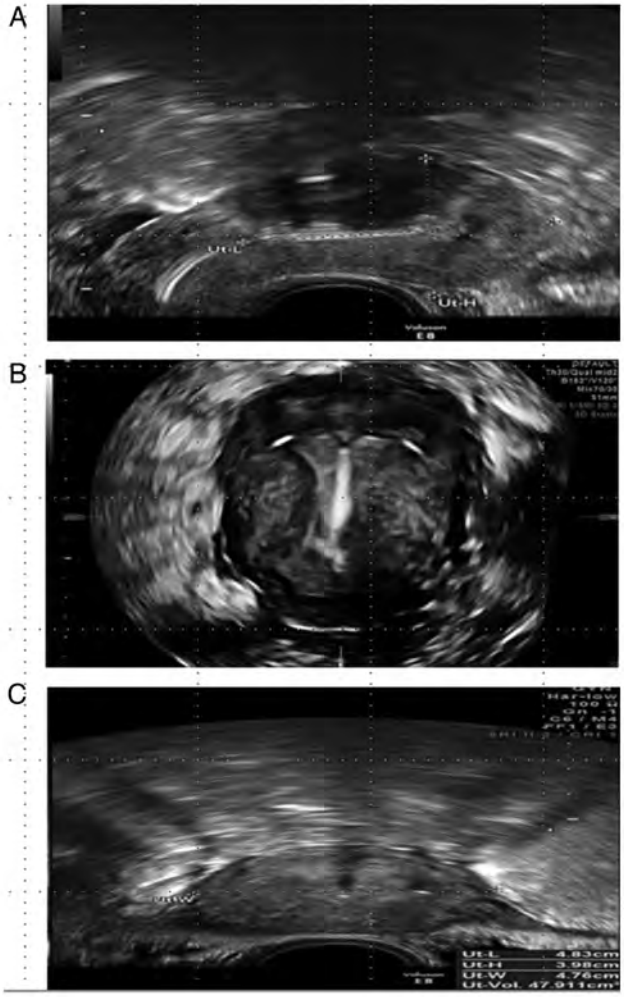

Figure 3. Intracavitary IUD. (A and B) 2D ultrasound longitudinal plane and transversal plane showing a normal position of the IUD in the cavity. (C) 3D ultrasound with coronal plane showing the IUD embedded in the myometrium. 2D, two dimensional; 3D, three dimensional; IUD, intrauterine device.

of the uterus (21). 3D US enables visualisation of the coronal plane of the uterus and consequently provides a clear image of the EMJ. Due to the possibility of visualising the EMJ, 3D ultrasound opens up new horizons in the diagnosis of adenomyosis. Adding VCI to the coronal plane provides a clearer view of the EMJ. 3D ultrasound allows visualisation of the lateral and fundal EMJ, which is almost impossible to observe only with 2D imaging. Thanks to the clearer view provided by the VCI, this procedure is considered as the best in analysing and measuring the junctional zone (22). In our cases, 3D and VCI identified changes in EMJ as different size of the EMJ in different parts of the cavity and the penetration of the endometrium into the myometrium. There is some supposition that adenomyosis began with EMJ alteration and therefore 3D and VCI would be useful for an early diagnosis of the disease (23).

In the present study, the 2D ultrasound scan diagnosed endometrial polyps in 17 cases (8.5%). In all cases the diagnosis was suspected by 2D ultrasound and confirmed by 3D and VCI. During the 2D ultrasound scan, a search for the pedicle artery sign was conducted in order to detect the

position of the stalk of the polyp (24). 3D and VCI proved to be useful in 2 of 17 cases by providing a clearer image regarding the topography of the polyps. At the same time, the coronal plane was useful in the patient's understanding of the disease. Identical size of the polyps using 2D and 3D scans was obtained. Based on longitudinal and coronal plane and using Doppler mode we localised the base of the polyps and the place of its stalk. In all cases of polyps, hysteroscopy confirmed the diagnosis. 3D scan seems not to be superior to 2D in diagnosing endometrial polyps, but it helps in reinforcing the diagnosis and is very useful for the patient's understanding of the disease.

Another important issue concerns the question if there is a specific time frame regarding the endometrial cycle to perform a 3D ultrasound scan for obtaining the ideal image of the coronal plane. In relation to the size of the endometrium, the coronal plane was difficult to obtain if the size was <7.38 mm. Difficulties in obtaining a proper image of the uterine cavity have been noted in cases with endometrium thickness <7.38 mm or in cases with multiple uterine myomas or adenomyosis. Benaceraff *et al* reported in a study on 66 patients that the coronal view is helpful in patients with an endometrium greater than or equal to 5 mm (14). Therefore, we recommend, in order to obtain a good 3D reconstructed image, that the sonography should be carried late in the follicular or in the secretory phase. In menopausal women, because the endometrial thickness is usually <5 mm, 3D is not helpful.

Another advantage of 3D ultrasound that should be considered is the capability of storing volumes, ensuring a further process of the information. In this way, it is possible to follow-up the patients for a long period of time and if a uterine pathology will appear in the future, a reassessment of the previously rendered volumes would possibly bring useful information regarding the pathogenesis of the disease.

The main limitation of this study is represented by the fact that hysteroscopy was not performed in cases where 2D and 3D findings were normal; thus, it was possible to overlook some minor pathologic findings.

As a conclusion, we can state that the 3D ultrasound scan is an extremely useful tool in gynaecology, especially in cases with congenital uterine anomalies, submucous myomas and the assessment of IUD placement. Although initially it was used for research purposes only, currently it has been proven to be useful in the routine ultrasound scan and there is a possibility of witnessing its introduction as a mandatory examination procedure in the foreseeable future.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

MG, RP, LMH and RM designed the study. MG, LMH, ISS and RP collected, analyzed and interpreted the patient data. MG, RM, MO, BFT, and AMG were responsible for discussion and interpretation of the data. BFT, AMG and ISS had major contributors in the writing of the manuscript. MG, RM, LMH and MO supervised and vizualised the final form. All authors have read and agreed to the published version of the manuscript. MG and RP confirms the authenticity of all raw data assesed in the manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the World Medical Association Declaration of Helsinki and was approved by the Institutional Board of the 'Medis' Medical Centre Iasi, Romania (approval no. 12/18.09/2019). Informed consent was obtained from the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

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