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REPRODUCTIVE HEALTH

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REPRODUCTIVE HEALTH

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Reproductive Health

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EMJ GORELY NEW MEDIA

European Medical Journal
EMJ Repro. Health Vol 1.1
August 2015

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Welcome

Hello and a warm welcome to the very first edition of *EMJ Reproductive Health*, a brand new resource bringing you the latest developments and innovations from the field. Inside you will find a broad selection of content, including a wealth of scientific papers and a full review of the recent meeting of the European Society of Human Reproduction and Embryology (ESHRE), which took place in Lisbon, Portugal, on 14th-17th June 2015.

Our team has been working tirelessly to compile a selection of the most engaging and insightful articles in order to make this inaugural edition a truly memorable one. Notable contributions come from Rosália Sá and Mário Sousa, who provide a fascinating review of the influence of sperm aneuploidy and DNA integrity on male fertility, and Ernesto González-Mesa et al. who describe the challenges associated with multiple births. Also included are an overview of the cellular and physiological factors that govern sperm motility and viability, provided by Leyla Sati and Gabor Huszar, and an update on the current status of surgical techniques for the treatment of varicocele, co-authored by Oktay Üçer and Bilal Gümüş.

As if all that was not enough, our exhaustive coverage of ESHRE 2015 is also a must-read. We have included a wide array of content from the event, including reviews of some of the most exciting presentations and abstracts that cover a range of topics from this most diverse discipline of medicine, as well as interviews with a number of prominent researchers and physicians who share their views on the current state of reproductive health. This is a truly exhaustive and vital companion piece to the event, both for those lucky enough to attend and for those who were unable to, and we very much hope that you enjoy reading it!

With the launch of *EMJ Reproductive Health*, it is our hope that this new eJournal will give you, our readers, a true snapshot of the field of reproductive health in 2015, and an insight into where it is headed. Newly developed technical innovations and clinical protocols for the treatment of infertility are transforming the lives of many prospective families across Europe and the rest of the world, and it is our hope that, in some small way, the information included in our publication can contribute to this crucial process. Finally, we would like to thank you for reading and wish you all the best in your future endeavours.

Spencer Gore

Team Principal, European Medical Journal

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Foreword

Prof Joep Geraedts

Emeritus Professor and Former Head of the Department of Genetics and Cell Biology, Maastricht University, Netherlands

Dear Colleagues,

I would like to welcome you to this new issue of *European Medical Journal Reproductive Health* 2015. Just a few weeks ago the European Society of Human Reproduction and Embryology's (ESHRE) 31st Annual Meeting was held in Lisbon; this year's scientific programme was of a high quality once again. One of the most exciting presentations of the congress for attendees was the Human Reproduction keynote lecture "*Preconception stress increases the risk of infertility: results from a couple-based prospective cohort study, the LIFE study*" by Courtney D. Lynch, USA. This lecture was held during the Robert G. Edwards' memorial keynote session on the morning of the first day and was followed by more than 2,500 participants who listened while Dr Lynch recounted her discovery that higher levels of stress as measured by salivary alpha-amylase are associated with a longer time-to-pregnancy and an increased risk of infertility. However, no association between salivary cortisol and fecundability was found. This was by no means the only highlight of a memorable congress however, and all of the main events have been analysed in this edition of *EMJ Reproductive Health.*

> "I am glad for our patients that we are witnessing such exciting times in the field of reproductive health."

This issue is also devoted to a number of aspects of male infertility. It contains reviews of sperm aneuploidy and DNA integrity, as well as sperm motility and viability — an overview of the cellular and physiological aspects that support these functions. A more clinical area comes in the form of a stimulating review on varicocele. In addition to these major advances in male infertility, there are also topics that have been discussed on the female side. An insight into bone metabolism and skeletal mass in polycystic ovary syndrome is provided, and fertility-related issues of cancer, including operative therapy and current role of laparoscopy in cervical carcinoma and fertility preservation in women with endometrial cancer are also covered. All of these topics will remain important issues in the years ahead.

I am glad for our patients that we are witnessing such exciting times in the field of reproductive health.

Yours sincerely,

Joep Geraedts

Emeritus Professor and Former Head of the Department of Genetics and Cell Biology, Maastricht University, Maastricht, Netherlands; Past Chairman of the Dutch Society of Human Genetics; Past Chairman of the Dutch Association of Clinical Genetics Centers; Board Member of the International Chromosome and Genome Society; Past Chairman of the European Society of Human Reproduction and Embryology (ESHRE); Member of the Dutch Health Council.

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Immune Pathways and Immunome as Targets for Breast Cancer Treatment

Dr Giuseppe Curigliano

The Role of Prospective Randomised Trials in Breast Cancer Treatment

Dr Clifford Hudis

New Drugs in Neoadjuvant Breast Cancer Therapy

Prof Sibylle Loibl

Ki67 as a Marker for Making Management Decisions in Breast Cancer

Dr John Bartlett

Targeting BCL-2 for Treating Oestrogen-Positive Breast Cancer

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ESHRE ANNUAL CONGRESS 2015

FEIRA INTERNACIONAL DE LISBOA, LISBON, PORTUGAL

14TH-17TH JUNE 2015

Welcome to the *European Medical Journal* review of the 31st Annual Meeting of the European Society of Human Reproduction and Embryology 2015

The 31st Annual Meeting of ESHRE was held for the first time in Lisbon, Portugal, which proved to be a glorious setting for this momentous event. Lisbon is Europe's second-oldest capital and is steeped in history, having been home to explorers such as Vasco da Gama, Magellan, and Prince Henry the Navigator. Lisbon was the first true world city, the capital of an empire spreading across all continents, from South America to Asia. It is also one of Europe's most soulful, captivating, and picturesque places, built on a series of hills with scenic vistas from every angle.

The significant growth in ESHRE and its congress was emphasised by ESHRE Chairperson Prof Juha Tapanainen in his speech during the opening ceremony, whereby he emphasised the society's global influence. "As usual, participation is made up of many countries in the world and it is quite clear that we are now running a global event," said Prof Tapanainen. "The membership of ESHRE is growing and today we have almost 6,700 members from 115 countries, and one-third of the members come from outside Europe." This has been a record-breaking year for ESHRE, with more participants (approximately 10,000) and more abstracts (1,800) submitted than ever before.

Numerous awards were presented in the closing ceremony on Wednesday 17th June, acknowledging particularly impressive contributions. The Basic

ESHRE ANNUAL CONGRESS 2015

Science Award for oral presentation went to Z. Holubcova (United Kingdom) for the presentation "*High resolution imaging of meiosis in live human oocytes*", while "*The Neurokinin B Receptor Antagonist AZD4901 decreases LH and testosterone secretion in women with PCOS: a randomised, double-blind, placebo-controlled clinical trial*" by J. George (United Kingdom) was awarded the Clinical Science Award for oral presentation.

In addition, L. Matsumoto (Japan) scooped the Basic Science Award for the poster presentation entitled "*Crucial role of hypoxia inducible factor 2alpha in the pregnant uterus*", and the Clinical Science Award for poster presentation was given to A. Ludwin (Poland) for the poster "*Reliability of the ESHRE/ESGE and ASRM classification systems of uterine congenital malformations*".

The congress proved to be highly entertaining and enlightening, with progress from a range of reproductive health topics outlined, disseminated, and discussed through a variety of mediums including symposia, lectures, and poster sessions. Sunday 14th June saw a range of pre-congress courses being held, such as an overview of the latest strategies for improving patient outcomes with individual treatment approaches in assisted reproductive technology. Notable presentations included research displaying the role of dietary habits and nutrition in fertility, evidence showing that women over the age of 44 undergoing *in vitro* fertilisation should use donated eggs, and a study suggesting that endometriosis is associated with an increased risk of serious complications.

During the event there was also time for delegates to participate in lighter activities such as the community evening on Tuesday 16th June, which featured the annual charity run raising funds for patient groups across Europe. ESHRE 2015 was a huge success, leaving attending medical professionals with little doubt about the current state of the field of reproductive health, as well as allowing for significant interaction and discussion. Helsinki will host the next congress in 2016, and we anticipate that the terrific work observed in Lisbon will have been built on and prepared into even more outstanding presentations by this time.

"As usual, participation is made up of many countries in the world and it is quite clear that we are now running a global event."

HIGHLIGHTS

The Burden of Endometriosis for Expectant Mothers

ENDOMETRIOSIS is associated with an increased risk of serious complications, both in the early and later stages of pregnancy, according to a new report presented at ESHRE 2015.

According to the report, which involved a nationwide study of patients in Scotland, women with endometriosis - a relatively common condition whereby cells from the lining of the uterus, or endometrium, are found elsewhere in the pelvic area - carry a greater risk of miscarriage and ectopic pregnancy. Additionally, women with a history of endometriosis whose pregnancies have progressed beyond 24 weeks were found to be at a higher than average risk of complications, including post and antepartum haemorrhage and preterm birth.

"These results indicate that endometriosis predisposes women to an increased risk of early pregnancy loss and later pregnancy complications," said Dr Lucky Saraswat, Consultant Gynaecologist, Aberdeen Royal Infirmary, Aberdeen, UK, as reported in an ESHRE press release on the 15th June. The study utilised discharge data from all of the state hospitals in Scotland, evaluating pregnancy outcomes by cross referencing the records of women with and without a confirmed diagnosis of endometriosis with their maternity records. A total of 14,655 women were included in the analysis, with their medical records followed up for a maximum of 30 years between 1981 and 2010.

"These results indicate that endometriosis predisposes women to an increased risk of early pregnancy loss and later pregnancy complications."

Comparing the reproductive and pregnancy outcomes in 5,375 endometriosis patients with those of 8,280 unaffected women who were pregnant at the same time, the study showed that women with endometriosis had a significantly higher risk of early pregnancy complications than controls. The risk of miscarriage was 76% higher (odds

ratio [OR]: 1.76) and close to three-times higher for ectopic pregnancy (OR: 2.7) in patients with endometriosis, as well as the previously mentioned risk of complications for those with a previous diagnosis of endometriosis.

These new findings should be taken into account by practitioners who are guiding patients with endometriosis through pregnancy, and they may impact on many of the choices made during family planning and pregnancy care. Indeed, in such patients, an increased level of antenatal monitoring is certainly advisable.

Fertility Levels Associated with Nutrition and Diet

FERTILITY and infertility could be affected by dietary habits, a study presented at ESHRE 2015 has confirmed.

The study involved 1,134 men and women attending hospital for 1 year. Fertile couples were defined as those who had produced a child in the past 12 months (cases) and infertile couples as those who had not produced a child (controls). The subjects were asked about their nutritional habits, the results of which were then compared and analysed. A higher percentage of fertile subjects were found to be non-smokers, consumed less alcohol per week, and reported no previous use of drugs, whereas infertile subjects had a higher percentage of these. Furthermore, in the results for diet, fertile subjects reported a more frequent consumption of vegetables, legumes, fruits, and eggs than infertile people, with more fertile men and women eating up to five portions of vegetables and fruit and 2-4 eggs per week, suggesting that eating such foods could lead to improved fertility. The study did, however, note that there were no differences between the two groups regarding red meat, poultry, and fish.

Study investigator Prof Salonia, Director of the Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy, explained why these dietary changes could have such an effect in an ESHRE press release dated 15th June. "Artificial fats such as trans fats can disrupt fertility by increasing insulin insensitivity and inflammation, and thereby disrupting ovulation, conception, and early embryonic development. Studies show that replacing trans fats with monounsaturated and polyunsaturated fats has the opposite effect – by maintaining healthy cholesterol levels, easing inflammation, improving insulin insensitivity, and promoting fertility. Polyunsaturated fats, specifically omega-3 fatty acids found in salmon on walnuts, are also beneficial for fertility. Beans, nuts, legumes, and other plant-based proteins provide a rich source of iron and folate, which are important for follicle development and ovulation."

Exhibitors

1,846

Given the results of this study, Prof Salonia concluded that "a balanced and healthy diet would not only provide a benefit in terms of general health but also in male and female reproductive health."

Same-Sperm Donor Preferred For All Subsequent Conceptions

SAME-SPERM donors in subsequent conceptions to achieve a genetic bond between siblings is of paramount importance to couples who require sperm donation to have children, according to findings from a study presented at ESHRE 2015.

"It is relatively uncharted territory, but it is clear that a genetic link among donor-conceived children is important for aspiring parents."

The study included 34 lesbian and heterosexual couples using sperm donation to start or extend their families, and who were interviewed about their treatment between 2012 and 2013. Nineteen of the couples already had a child through this means, and 15 were in treatment at the time of the study. Investigators who conducted the research, led by midwife Ms Sara Somers, Department of Reproductive Medicine, Ghent University Hospital and Ghent University, Ghent, Belgium, acknowledged the paradox that while sperm donation necessarily implies the genetic detachment of the child from one of its parents, couples are still determined to try and ensure genetic bonds between their children.

In the study, parents described full siblings as having "real" and "unambiguous" kin connections. Some of the couples were particularly unhappy about having to use a new sperm donor if the one they had used for the first child was unavailable, with one couple describing this situation as "a problem". For another, when this became a reality, they were "disappointed" and blamed the hospital for "making the mistake of not informing them about the limited stock of sperm". While other couples were more accommodating about being unable to use the same sperm donor for subsequent children, it remained their preference to use the same donor sperm used for their previous child.

"Donor offspring are increasingly seeking their genetic half siblings through online registries," said Ms Somers in an ESHRE press release dated 16th June. "It is relatively uncharted territory, but it is clear that a genetic link among donor-conceived children is important for aspiring parents." Ms Somers concluded that these findings have implications for sperm banks and clinics in terms of counselling their patients about genetic links as well as in the

identification, storage, and availability of their sperm stocks.

Uterine Transplantation: Women Adjusting Well to Their New Lives

PSYCHOLOGICAL evaluation of nine previously infertile women who had each successfully received a transplanted uterus has revealed that they have all adjusted well since the life-changing procedure. The women were from the Gothenburg programme, led by Prof Mats Brännström, Professor of Obstetrics and Gynecology, University of Gothenburg, Gothenburg and Stockholm IVF, Stockholm, Sweden, which carried out the procedures in October 2014 with the aim of providing a treatment for uterine factor infertility.

Little was known about how the women had adjusted to their new lives, but a new study by psychologist Dr Stina Järvholm, Department of Obstetrics and Gynecology, Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg and colleagues has shown encouraging results during the first year after transplantation, detailed in an ESHRE press release dated 15th June.

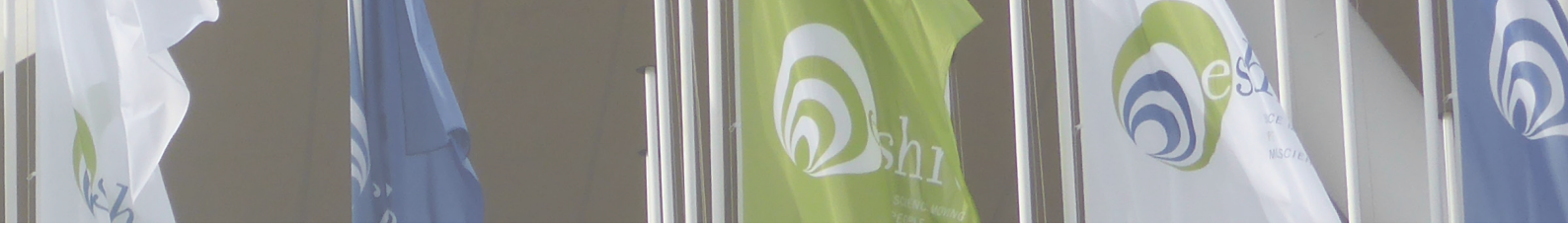
"It will be of the upmost importance to have knowledge of psychological strengths and strains."

Of the 9 patients, who had a median age of 33 years, ranging from 27 to 38, there had been 2 transplantation failures, and 3 of the remaining 7 have since delivered healthy babies. All participants, including the two women whose transplantations had failed, were found to be content with their decision to join the programme, and had incorporated their new circumstances into their daily lives.

A particular feature of the findings was that the women felt as though they now had the same opportunities and hopes that others had in life, with one stating that the main "obstacle to fertility no longer exists", and the participants felt as though their place in everyday life had been restored.

Main programme registrations

8,301



“Women of 44 or older should be fully informed about their real chances of a live birth and counselled in favour of oocyte donation.”

Although each participant said the procedure had mainly had a positive effect on their daily life and provided hope, the 1-year wait between transplant surgery and attempting pregnancy had created stress, as did the new focus on pregnancy and children, which they had previously tried to avoid.

While Prof Brännström did emphasise at the time of the initial reports that uterine transplantation was not yet a technique for mainstream treatment, and was no more than a proof of concept, the treatment will eventually enable women without a uterus (either from birth or following hysterectomy) to be fertile, meaning that this study will provide essential information for the future. As Dr Järvholm explained: “it will be of the upmost importance to have knowledge of psychological strengths and strains.”

Older IVF Patients Should Use Donated Eggs

DOCTORS should advise women over the age of 44 years who are undergoing IVF to use donated eggs, according to Dr Marta Devesa, Department of Obstetrics, Gynecology and Reproductive Medicine, University Hospital Quiron-Dexeus, Barcelona, Spain, who presented the results of a new study at ESHRE 2015.

Similar studies have found that IVF success rates remain at a consistent level when older patients receive donated eggs, leading many to conclude that the age and quality of the egg may be more influential on success than the patient’s age.

To discover how important age is for IVF success, the research team conducted a 12-year study of 4,195 women aged ≥38 years, who received 5,842 cycles of IVF between them, to provide a conclusive indication of predicted live birth rates. Patients were divided into four groups: G1 were aged 38-39 years, G2 40-41 years, G3 42-43 years, and G4 ≥44 years. To accurately evaluate IVF success, the research team calculated the cumulative live birth rate (i.e. after one fresh and any subsequent frozen embryo transfers), according to patient age and the number of eggs initially retrieved.

The results revealed that the probability of freezing spare embryos and the number of embryos frozen decreased with age, and the cumulative live birth rates using fresh and frozen embryos declined dramatically from 23.6% in G1 through to 1.3% in G4. Rates were higher in the cycles where there were excess embryos for freezing, and the main

contribution of embryo freezing was in increasing the live birth rate in the fresh cycle, and not in the frozen embryo transfers. It was concluded that the higher the number of eggs retrieved, the higher the cumulative live birth rate would be.

Dr Devesa attributed the decline in live birth and oocyte retrieval rates to high embryo aneuploidy rates in women aged >42 years, and advised older patients to be mindful that in later frozen cycles there is limited benefit of cryopreservation and frozen embryo transfer. "Indeed, women of 44 or older should be fully informed about their real chances of a live birth and counselled in favour of oocyte donation," said Dr Devesa in an ESHRE press release from 16th June.

Substantial Fall in Rate of Ectopic Pregnancy in ART Patients

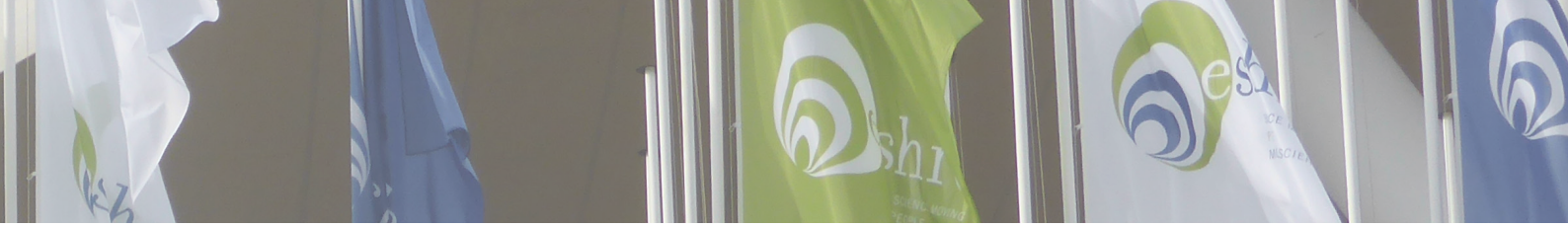
INCIDENCE rates of ectopic pregnancy following assisted reproduction have fallen substantially, according to data presented at ESHRE 2015.

The report, which utilised a nationwide population-based analysis of all pregnancies in the UK that were aided by assisted reproductive technology (ART) from 2000-2012, has found that the rate of ectopic pregnancy following *in vitro* fertilisation (IVF) and intra-cytoplasmic sperm injection (ICSI) progressively decreased throughout the 12 years, almost halving from an overall rate of 20 to 12 cases per 1,000.

When assessed on a yearly basis, the incidence rates of ectopic pregnancy were found to have decreased progressively, year after year.

Ectopic pregnancy occurs when an embryo implants itself outside of the uterus, usually in the fallopian tubes. Studies have found that its risk is elevated in ART conceptions, although the exact reasons for this remain unclear. The aim of this study was not only to determine whether the advances in ART over the past few years have led to a reduction in the incidence of ectopic pregnancy, but also to better understand the risk factors.

1.4% of all ART pregnancies from the study period were found to be ectopic. However, when assessed on a yearly basis, the incidence rates of ectopic pregnancy were found to have decreased progressively, year after year. This decrease in risk was only statistically significant in IVF and ICSI treatments and not in intrauterine insemination, suggesting that advances in IVF and ICSI techniques had positively influenced incidence rates.



Examining the results further, the researchers discovered that the most prominent indicator for ectopic pregnancy was the incidence of tubal factor infertility, which more than doubled the risk (adjusted odds ratio: 2.23). The second most significant risk factor was the transference of multiple embryos in treatment. The use of ovarian stimulation or frozen embryos, however, was found to pose no added risk.

Prof Nikolaos Polyzos, Centre for Reproductive Medicine, Vrije Universiteit, Brussels, Belgium, who presented the findings at ESHRE, said in an ESHRE press release dated 16th June that the apparent decrease in the risk of ectopic pregnancy in ART "appears strictly associated with the reduction in the incidence of tubal factor infertility and the transfer of fewer embryos."

Evidence Suggests No Benefits of the 'Big Freeze' of Embryos

IN VITRO fertilisation (IVF) techniques in which all embryos from an initial treatment cycle are frozen and later transferred as freeze-thawed embryos do not improve outcome, according to results of a study by Dr Ernesto Bosch, Medical Director, Instituto Valenciano de Infertilidad (IVI) clinic, Valencia, Spain, highlighted in an ESHRE press release on 17th June 2015. The findings also suggest that there is no evidence that consistently freezing all embryos and postponing transfer to a subsequent cycle benefits IVF outcome in ongoing pregnancy or live birth rates.

Dr Bosch concedes that previous studies found improved rates of pregnancy in IVF after embryos had been frozen and later transferred. It has been claimed that the approach removes the risk of negative effects that ovarian stimulation has on the uterine environment and embryo implantation. However, Dr Bosch argues that if endometrial receptivity is diminished during ovarian stimulation, the delayed cycle for transferring the embryo, in which the uterus has not been exposed to supra-physiological doses of reproductive hormones, could be a beneficial alternative solution.

The study included 882 patients undergoing their first or second cycle of IVF treatment at the IVI clinic. Two groups were created: 364 patients had an embryo transfer in the initial fresh cycle and 518 patients had all embryos frozen for later transfer. The final comparison showed no differences between ongoing pregnancy rate and live birth rate, and even after adjustments for age and other variables were made there remained no evidence for any impact of freezing.

> "These findings do not support a change in IVF practice moving to a freeze-all strategy in normo-responders in IVF."

"These findings do not support a change in IVF practice moving to a freeze-all strategy in normo-responders in IVF," said Dr Bosch. He further stated that fresh embryo transfer treatment is also more cost-effective and has a lower time to pregnancy rate. Despite this, there is still evidence suggesting that the freeze-all approach lowers the risk of

ovarian hyper-stimulation syndrome. Dr Bosch concluded that although some clinics have introduced freeze-all policies as standard routine in their IVF treatments, this study suggests that there is no clear benefit of doing so.

Academic Performance of ART Children Similar to Spontaneously Conceived Children

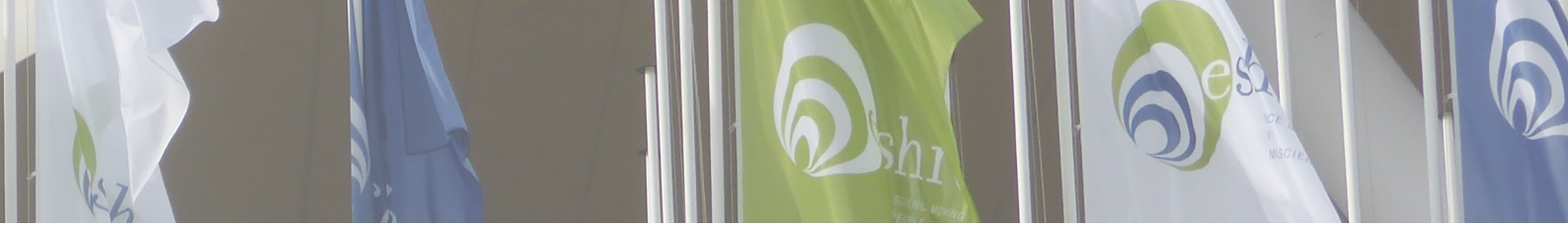
ACADEMIC performance of children conceived using assisted reproductive technology (ART) is no different to that of spontaneously conceived children, providing reassurance for infertile couples. In addition, the study presented at ESHRE 2015 found that ART singletons and ART twins also had comparable performance, meaning that the higher obstetric risk identified in ART pregnancies, particularly in twins, does not affect academic ability.

"Our results now confirm smaller studies which have shown no difference in IQ between ART and non-ART children."

As there were few previous studies that had explored IQ in ART children, the researchers, led by Ms Anne Lærke Spangmose Pedersen, a medical student at the fertility clinic of Copenhagen University Hospital, Hvidovre, Denmark, undertook a national study involving every child conceived by ART and born in Denmark between 1995 and 2000, which represented a total of 8,251 children (4,991 singletons and 3,260 twins). These children were then compared with two control populations of children, one comprising all twins born in Denmark during the same period (n=10,833) and another comprising a randomly selected group of spontaneously conceived singletons (n=10,052), with regard to the results achieved in a general test of academic performance completed by all Danish ninth-grade students (aged 15-16 years).

While initial results demonstrated some discrepancies between the groups, these differences disappeared after statistical adjustments for maternal age, birth weight, gestational age, and social status. "We are pleased to see the results," said Ms Pedersen in an ESHRE press release from 15th June. "The higher rate of twins and preterm birth in ART singletons might have given rise to lower academic test scores. But our results now confirm smaller studies which have shown no difference in IQ between ART and non-ART children. All our four study groups had test scores very close to the average, which is reassuring – and shows the high validity of the study."

These findings are likely to be of great comfort to infertile patients and IVF clinics, and should help to persuade society that ART is a safe procedure from a long-term, child perspective. However, the authors acknowledge that ART is still associated with a



slightly increased risk of congenital malformations and prematurity.

D&C Procedure Causes Increase in Preterm Delivery

DILATATION and curettage (D&C), a common minor surgical procedure used mainly for miscarriage or terminations, has been found to significantly increase the risk of preterm delivery of subsequent pregnancies, which could lead to a further reduction in its use in favour of less invasive treatments.

D&C has generally been considered safe and easy to perform, albeit associated with some rare but serious side-effects. However, an analysis of 21 cohort studies that included almost 2 million women has demonstrated that D&C performed in cases of miscarriage or induced abortion increases the possibility of preterm birth (under 37 weeks) in a subsequent pregnancy by 29%, and of very preterm birth (under 32 weeks) by 69%. The background population risk of preterm delivery is about 6% in women without a D&C, and the results suggest that an earlier D&C would increase this to 7.6%.

These significant increases in incidence were measured against control groups of similar women without a D&C prior to pregnancy, but the risk remained similarly increased (+28%) for D&C even when the control groups were limited to those with a medically managed miscarriage or termination. In women with a history of multiple D&Cs, the risks were even higher.

A proposed explanation from Dr Pim Ankum, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands, who presented the new findings at ESHRE, is that dilatation of the cervix may cause permanent damage that affects cervical tightness, with premature opening of the cervix and subsequent premature birth a consequence. In an ESHRE press release from 16th June, Dr Ankum said that the results "warrant caution in the use of D&C after miscarriage and induced abortion", adding further weight to the case for less invasive treatments such as misoprostol. Gynaecologists in the Netherlands currently still treat 50% of all miscarriages with D&C (around 10,000 procedures each year), and Dr Ankum suggests that other countries probably have similar usage of the procedure.

With Dr Ankum describing preterm birth as "the biggest challenge in western obstetrics", the greater adoption of alternatives to D&C for termination and miscarriage is required, which should reduce the risk to the basic background level.

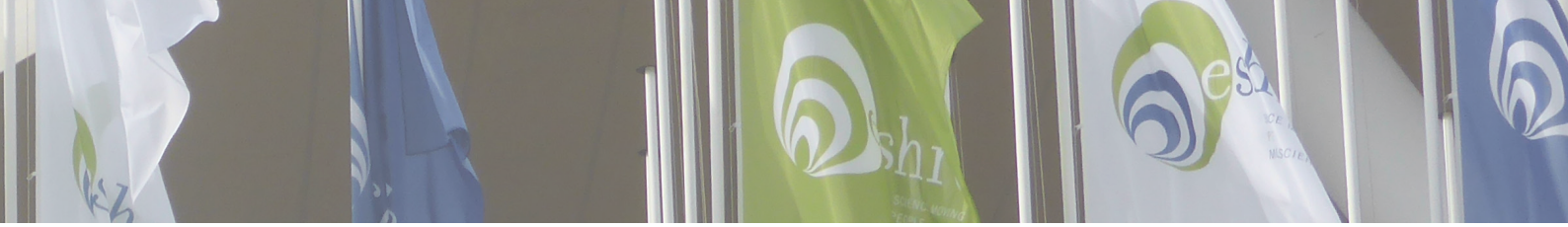
Delegates

10,088

AWARD WINNERS AT ESHRE 2015

Six of the abstracts selected for oral or poster presentation at ESHRE 2015 were awarded prizes by dedicated committees composed of senior scientists and clinicians. There were prizes for best poster and best oral presentation for both basic and clinical science, as well as a prize for best oral presentation delivered by a nurse and a prize for the best poster or oral presentation made by a laboratory technician. There was also a seventh prize, the Fertility Society of Australia Exchange Award, which was an educational travel grant given to one of the participants of ESHRE 2014 so that they may present their data at this year's annual meeting of the Fertility Society of Australia. The clinical and basic science oral presentation awards were both awarded to delegates from the UK, the poster awards were collected by delegates from Japan and Poland, and the remaining three prizes were all won by contributors from Belgium.

The 'Clinical Science Award for oral presentation' was won by Jyothis George who reported the results of a randomised, double-blind, placebo-controlled clinical trial investigating the ability of the neurokinin B (NKB) receptor antagonist AZD4901 to influence luteinising hormone (LH) and testosterone secretion in women with polycystic ovary syndrome (PCOS). The study included a total of 65 women (age: 27±5 years, body mass index: 31.5±6.0 kg/m^2 [both mean ± standard deviation]) who were randomised to one of four treatment arms (placebo or AZD4901 at 20, 40, or 80 mg for 28 days). The results demonstrated that, compared with placebo treatment, the area under the receiver operating characteristic curve for LH exposure was reduced by 52% (95% confidence interval [CI]: 30-67%; p=0.0003), the LH pulse frequency was significantly reduced (3.7±2.1 versus 6.8±2.6 pulses/8 hr; p<0.0001), and serum testosterone levels decreased by 29% (95% CI: 14-41%; p=0.0006) in the 80 mg arm at Day 7. In the 80 mg treatment arm, the reduction in serum testosterone was maintained over the remaining study period (17% decrease from baseline versus placebo at Day 28). These results demonstrate



that manipulation of the kisspeptin–NKB–dynorphin system may be capable of modulating the central pathophysiology of PCOS, with NKB antagonism potentially serving as a therapeutic approach to address LH and testosterone hypersecretion in PCOS and other reproductive disorders in which decreased gonadotropin secretion is desirable.

These results demonstrate that manipulation of the kisspeptin–NKB–dynorphin system may be capable of modulating the central pathophysiology of PCOS...

The 'Clinical Science Award for poster presentation' went to Artur Ludwin for his study investigating the reliability of the ESHRE/ESGE and ASRM classification systems in the diagnosis of uterine congenital malformations. The study analysed prospectively acquired 3D ultrasound scans of the uterus from 112 women of reproductive age (50 consecutive patients with uterine malformations, and 62 randomly selected women without uterine malformations), with the scans being independently assessed by two experienced, blinded raters who used both classification systems and the same ultrasound scanner, methodology of evaluation, and measurements. One of the raters subsequently evaluated the same scans after 2 weeks had passed, and both the inter and intra-rater repeatability of measurements representing the main benchmarks for diagnosis of malformation were estimated, and the reliability/agreement of both systems were calculated and compared. The reliability/agreement of the ESHRE ESGE classification system in diagnoses of uterine malformations (inter-rater po=0.92, κ=0.86, 95% CI: 0.71-1.00; intra-rater po=0.88, κ=0.80, 95% CI: 0.65-0.95) was lower than that of the ASRM system (inter-rater po=0.99, κ=0.98, 95% CI: 0.87-1.00; intra-rater po=0.98, κ=0.96, 95% CI: 0.85-1.00), with the greatest difference between the two systems being the inter-rater reliability/agreement in diagnosis of septate uterus. The consequences of a lower reliability of diagnosis by the ESHRE-ESGE system may be a lack of agreement in the management of uterine malformation and biased research interpretations. The study authors suggest that improvement of the ESHRE-ESGE system is needed, and that the ASRM system (supplemented with absolute morphometric criteria) may be a better temporary solution.

The consequences of a lower reliability of diagnosis by the ESHRE-ESGE system may be a lack of agreement in the management of uterine malformation and biased research interpretations.

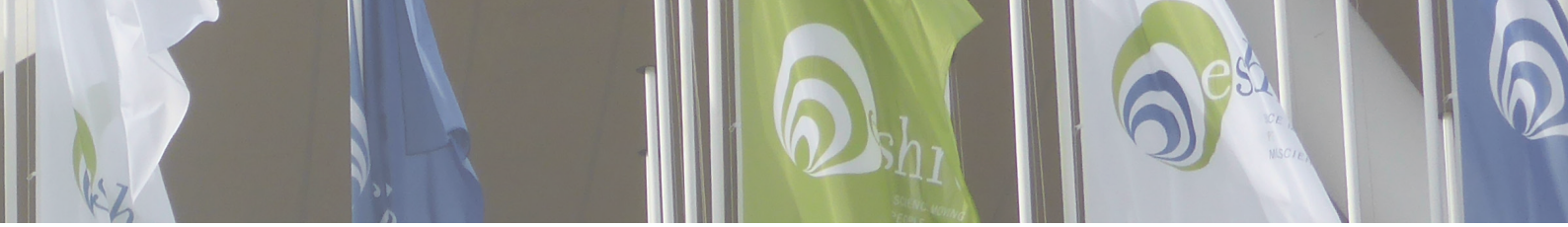
Uterine physiology was also the topic of Leona Matsumoto's abstract that won the 'Basic Science Award for poster presentation' for its description of an essential role for hypoxia-inducible factor 2alpha (HIF-2α) in the pregnant uterus. The role of HIF-2α in this setting is still unknown despite the protein being one of major transcription factors induced by low oxygen tension and it being shown to be strongly induced in the mouse decidua during early pregnancy. The research team created a uterine-specific HIF-2α conditional knockout

mouse model to investigate the effect of the absence of HIF-2α on embryo implantation, decidualisation, placental and fetal growth, and litter size after mating with fertile, wild-type male mice. Whereas control mice exhibited normal litter sizes, HIF-2α null females were infertile and displayed early pregnancy loss with compromised decidualisation. The administration of progesterone improved decidualisation in the HIF-2α-deleted uterus, but it was unable to recover early pregnancy loss in HIF-2α null females. Investigations using human cells will be needed to confirm whether HIF-2α plays a similarly essential role in the human endometrium.

Quantitative image analysis of the time-lapse videos revealed the discrete stages of meiosis and provided insights into the causes of egg aneuploidy.

The question of how human oocytes develop into fertilisable eggs was the subject of Zuzana Holubcova's abstract, which won the 'Basic Science Award for oral presentation' and which involved the high-resolution imaging of chromosomes and the microtubule spindle throughout meiosis in live human oocytes. Errors of chromosome segregation during meiosis are the leading cause of human aneuploidy and pregnancy loss, but most of our knowledge about mammalian oocyte development is derived from studies of mice. Therefore, the research team analysed a total of 374 oocytes from 140 women undergoing ovarian stimulation for intracytoplasmic sperm injection (mean age: 33.5±4.5 years, range: 23-43 years) to generate the first comprehensive data set describing the mechanism of spindle assembly and chromosome segregation in live human eggs. The researchers used immunofluorescence labelling combined with automated confocal microscopy to visualise the changes in chromosomes and microtubules during the maturation of human oocytes into fertilisable eggs. Quantitative image analysis of the time-lapse videos revealed the discrete stages of meiosis and provided insights into the causes of egg aneuploidy. The new findings relating to this fundamental process will hopefully provide a means of further improving fertility treatments.

Analysis of spindle formation in oocytes was also the topic addressed by Sylvie Lierman who won the ART Laboratory Award for a study investigating the effects of long-term androgen treatment in female-to-male transsexual persons (FTMs). The study investigated whether long-term testosterone treatment effected the spindle structure and chromosome alignment of *in-vitro* matured,



cryopreserved oocytes (before or after vitrification) derived from cumulus-enclosed oocytes (CEOs) that had been isolated at the time of ovarian tissue (OT) processing and cryopreservation. It has been shown previously that CEOs can be isolated and *in-vitro* matured to metaphase II following OT processing and cortex freezing in oncology patients, but this has never been described in fertility preservation programmes for FTMs. A total of 680 CEOs were collected from 16 FTMs with a mean age of 24.1±6.2 years and who had received long-term testosterone treatment (53.6±21 weeks). Overall, the results from immunostaining and confocal imaging demonstrated that *in-vitro* matured CEOs do not seem to be morphologically affected by long-term androgen treatment, with spindle structure and chromosomal alignment being normal both before and after vitrification. These results suggest that CEOs can be collected from FTMs during OT cryopreservation, which may help to maximise fertility preservation options for these individuals, although it must be considered that this was solely a morphological study and the parameters describing the biological competence of these oocytes, such as fertilisation capacity and implantation potential, were not analysed.

Public awareness of how fertility can change over time was reported in Ilse Delbaere's abstract that won The Nurses Award for the best oral presentation by a nurse. The cross-sectional study asked whether adolescents, students, and people of reproductive age in Flanders, Belgium, have sufficient fertility-related knowledge to make informed decisions regarding family planning. The study surveyed 989 adolescents (mean age: 15 years), 348 students (mean age: 23 years), and 374 persons of reproductive age (mean age: 35 years, range: 25-45 years) using the Swedish Fertility Awareness Questionnaire (which was translated and adapted according to the study group) and found that all groups overestimated both their fertility and the efficacy of assisted reproductive technology (ART). For example, more than 50% of Flemish adolescents believed that there is a marked decrease in female fertility after 50 years of age; 35% of Flemish students believed there is a marked decrease in female fertility at 40-44 years of age, 12% thought there is a marked decrease at age 45-49 years, and 20% thought there is only a marked decrease after the age of 50 years; similar results were found in people of reproductive age. Furthermore, the majority of the Flemish population surveyed believed that there is a 40-100% chance of becoming pregnant during a single ART cycle. These results indicate that greater education is needed with regard to fertility-related factors, with the apparent lack of knowledge potentially contributing to the tendency to postpone parenthood in Western countries. The limitations of the study were that the participating schools were located in only two of the five provinces of Flanders, and the authors speculate that the difficulty level of the questionnaire may have been too high for the adolescents to fully understand.

Greater education is needed with regard to fertility-related factors, with the apparent lack of knowledge potentially contributing to the tendency to postpone parenthood in Western countries.

Taken together, these findings describe a new CCNE1-dependent, intermediate embryonic cell state balancing between totipotency and differentiation.

The Fertility Society of Australia Exchange Award was won by Maria Krivega who examined the role of cell-cycle-related protein cyclin E1 (CCNE1) in totipotency and early differentiation of human embryonic stem cells (hESCs). The activity of CCNE1 is dependent on the developmental capacity of the embryonic cells and it is abundantly expressed during human preimplantation development. The periodicity of CCNE1 expression is absent in pluripotent cells when compared with somatic cells, and, therefore, constitutive expression of CCNE1 has been suggested to be associated with the undifferentiated state in human embryonic cells. The study found that CCNE1 was ubiquitously expressed in human embryos from embryonic genome activation onwards. Expression of CCNE1 was downregulated in some of the inner cell mass (ICM) cells and all the trophectoderm (TE) cells during blastocyst expansion, and, on Day 6, CCNE1-positive cells were only found in a cluster of ICM cells that were negative for pluripotency and differentiation markers. In embryos (including the early cleavage stages) and in spontaneously differentiating hESCs, CCNE1 was shown to co-localise with the visceral endoderm (VE) marker transthyretin. Overexpression of CCNE1 induced a VE-like phenotype in hESCs. TE cells from outgrowths of plated, expanded blastocysts regained CCNE1 expression and were eventually converted into a pluripotent state. Following addition of CCNE1 siRNA, blastocyst outgrowths degenerated thus proving that CCNE1 is critical for the growth of undifferentiated cells. Taken together, these findings describe a new CCNE1 dependent, intermediate embryonic cell state balancing between to tipotency and differentiation. Cells in this high developmental-potential state are capable of developing into TE and three distinct ICM populations: pluripotent epiblasts, hypoblasts, and a population with a VE-like phenotype. These findings shed new light on the mechanisms responsible for the high developmental-potential of early human embryonic cells, which is a unique characteristic that allows embryos to recover after fragmentation, cryodamage, or (single-cell) biopsy on Day 3 for preimplantation genetic diagnosis. Knowledge of the expression and function of genes involved in these mechanisms should provide a greater understanding of the undifferentiated state of stem cells and allow further improvement of ARTs.

Mátyás Benyó

Assistant Professor, Center of Andrology, Department of Urology, Clinical Centre, University of Debrecen, Debrecen, Hungary.

Q: What first made you decide to pursue a career in reproductive health?

A: When I was a resident in urology I attended a lecture during the annual congress of the European Association of Urology (EAU) about how to choose a subspecialty. An andrologist from England said that the field of andrology attracts fewer young urologists than oncological urology due to the radical surgical procedures involved. This is the same as in Hungary; everyone wants to do laparoscopy and perform radical prostatectomy (RP) and nephrectomy, and thus this field is a little bit overwhelmed. The fields of andrology and reproductive medicine are unfairly undervalued, since the growing incidence of infertility in developed countries provides excellent career opportunities. So I decided to choose this path.

Q: How would you describe the rate of progress since you first began working in this area of medicine?

A: The answer to this question has been partially answered previously. The growing incidence of infertility encourages experts in this field to design new studies and report the most recent results. Better understanding of the connection between infertility and different forms of sexual dysfunction (not just erectile dysfunction [ED] but premature ejaculation as well) increases the success rate of any treatment that assists patients to achieve pregnancy. Furthermore, the complexity of their problem indicates cooperation between specialists. So the multidisciplinary team should include the work of andrologists, gynaecologists, biologists, and also psychologists, since the psychological burden is huge especially on young patients. I welcome the fact that more and more institutes and departments are starting to arrange such teams to the benefit of patients.

Q: How far has our knowledge and understanding of the causes of male sexual dysfunction and infertility developed since you first began research into this area?

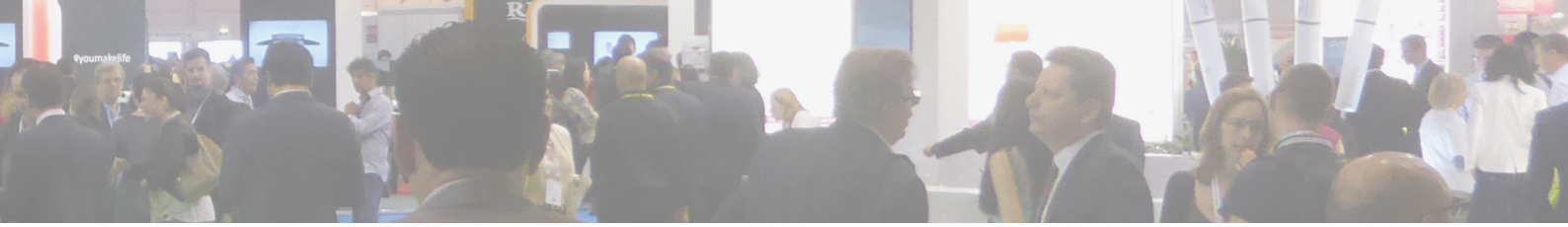
A: Previously, ED was believed to be a mainly urological problem. At the dawn of phosphodiesterase type 5 (PDE5) inhibitors, every patient was administered sildenafil. Nowadays we understand the complex regulation of erection, and the role of cardiovascular (CV) health is receiving proper attention. Unfortunately there are still many patients who turn to their physicians (general practitioner [GP], urologist) for help with ED, and they do not undergo the appropriate evaluation including detailed urological and CV screening, screening hormone, fasting glucose and lipid levels, profiling psychosocial health, and neurological assessment. As leading andrologists in Hungary we take every opportunity available to attend local interdisciplinary congresses and to educate GPs for the sake of the patients.

Q: Do you believe that more needs to be done to encourage men to seek help for ED?

A: Yes, patients need to be informed that ED can be the first sign of potentially malignant CV events, so consultation regarding their symptoms not only improves their sexual health, but can also save their lives. We are about to publish our most recent data of incidence of ED among patients of the GP, outpatients of the urologist, and the ratio of how many of them seek treatment due to erectile problems. According to our data only a minority of males dare to consult their physicians regarding their sexual dysfunction.

Q: Have you noticed any changes in the prevalence of male sexual dysfunction and infertility in recent years? What advice would you give to men to try and limit their risk?

A: It is clear that there is a growing incidence of fertility problems, especially in the developed countries. The exact aetiology is not known, although the role of lifestyle habits (smoking,



drug abuse, obesity) and environmental factors (xenoestrogens, phytoestrogens) have a clear role in the early and late development of male genitals and spermatogenesis. The following can be given as general advice: consume less alcohol, stop smoking, engage in regular physical exercise, have a steady relationship, protect the testicles by avoiding mechanical trauma (too tight pants and underwear), avoid thermal trauma (having a computer in your lap), and avoid unprotected intercourse.

Q: How much improvement has been seen in the treatment of urinary incontinence after prostate surgery since your research first began in this area of medicine?

A: There is one problem with oncologic surgeons (with honourable exceptions); they prefer to focus on the operation itself rather than on the postoperative care. That is why I have started to pay attention to less focussed areas of postoperative care, like thrombosis prophylaxis, ED, and urinary incontinence after RP. Due to my efforts, new methods of thrombosis prophylaxis were applied at our department, which performs the highest number of RPs in Hungary. The rate of urinary continence has also increased, since we arranged tutored pelvic floor muscle training (PFMT) sessions to facilitate continence recovery. The unique aspect of our PFMT: Kriston Intimate Training is that besides tutoring PFMT, it provides the patients with psychological support to accept and deal with the changes caused by the RP. Furthermore, this training is slightly beneficial for the recovery of their erectile function.

Q: Do men require more education in how to detect benign and malignant diseases of the male genital organs?

A: Yes, they need more education, but I think this should be started at elementary school as part of general health education. We should not just focus on one single organ, or screening of different diseases alone, rather we should teach young children how they can prevent diseases and preserve their health. Better understanding of how the reproductive system functions may prevent early smoking and excessive consumption of alcohol. Emphasising the possible negative effects of engaging in sexual activity at a young age and having multiple sexual partners could prevent sexual dysfunction and sexually transmitted diseases.

Q: How would you rate the current state of healthcare in Hungary? Are there any reforms that could be made to improve patient care in the area of reproductive health?

A: Hungary is not a big country, so there is sense in centralising medical care, especially regarding specific fields such as reproductive health. Three of the four medical universities already have centres of andrology performing microsurgical procedures. The improvement of cooperation between assisted reproduction centres and departments of gynaecology and andrology is in progress. Furthermore, the scientific life is also fruitful; we are active at international congresses, members of international associations, and participate in guideline committees. Unfortunately, there is a lack of specialists and medical staff in many departments. The economical support of the treatment of ED should be improved, since the PDE5 inhibitors and penile prostheses are not supported by the health insurance service.

Q: Could you briefly explain the poster session that members of your team are going to present at this year's ESHRE congress?

A: The poster to be presented by our study group presents the correlation between sperm chromatin decondensation, concentration, hyaluronic acid binding capacity, and aneuploidy of semen samples of fertile normozoospermic and infertile oligozoospermic men. Although chromatin condensation occurs in late phases of spermatogenesis, it shows correlation with cytogenetic abnormalities and membrane remodelling, irrespective of the fertility status. It can be explained with a common regulation mechanism, thus malfunction can result in various sperm defects on different levels. However, examined biomarkers cannot replace each other since these reflect different changes in sperm maturation.

Q: What advice would you give to a young medical student beginning a career in reproductive health?

A: My general advice to any student planning to have a medical career is to begin practising in the field during their education at medical university by visiting departments, assisting surgical procedures (microsurgery can be quite spectacular), and attending outpatient consultations. Later – after graduation – the best way to gain additional experience is by applying to scholarship programmes, which are an excellent opportunity to build international relationships for future research exchange programmes. In my personal opinion, reproductive health is one of the most beautiful fields of medicine, since assisting couples to achieve pregnancy is always heart-warming.

Elisabetta Baldi

Associate Professor of Medical Pathology, Department of Experimental and Clinical Biomedical Science, University of Florence, Florence, Italy.

Q: What drew you to the study of reproductive health? Could you give a brief overview of your career path and how it has led you to where you are now?

A: After my PhD in pharmacology at the University of Milan and a post-doctoral fellowship for 2½ years at CWRU in Cleveland, Ohio, USA, where I learnt the techniques for studying signal transduction, I started to work in the Department of Endocrinology at the University of Florence, where I began working on signalling in spermatozoa and its relationship with fertility. At that time (1990), assisted reproduction techniques (ARTs) were in their infancy and a public healthcare service had just opened in Florence in 1989. The project was to understand why some of the sperm samples were unable to fertilise oocytes, and we thought that studying sperm signalling could provide an answer. We were among the first researchers to publish the effects of progesterone on spermatozoa and the first to understand that disruption of sperm signalling in response to the steroid was involved in poor fertilising ability. We then continued to study signalling in other sperm functions (motility, acrosome reaction, and capacitation), as well as mechanisms of sperm DNA damage and its effects on reproduction.

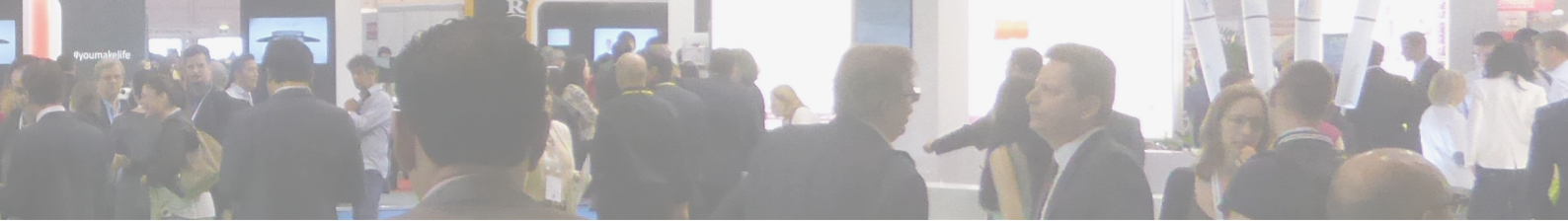
Q: Tell us a little about your current research. What do you personally hope to achieve in the next year?

A: We are currently studying the mechanisms and clinical correlates of sperm DNA damage (DNA fragmentation and base oxidation), as well as the involvement of CatSper (a sperm-specific calcium channel) expression in the fertilising ability of spermatozoa. We hope to discover the mechanisms that lead to development of DNA damage during *in vitro* manipulation of spermatozoa, as well as the effect of such damage on assisted reproduction outcomes.

Q: What changes have you witnessed in the field of reproductive health since your career began? How has it evolved over time and where is it headed in the future?

A: Research in the field of reproduction has changed a lot since the introduction of ARTs. Before ARTs, male infertility was considered a disease to be cured, but now all efforts are devoted to obtaining even a few sperm to be used in intracytoplasmic sperm injection. For this reason, research into sperm biology has evolved. We are still far from achieving optimal results in ARTs (in which success does not exceed approximately 30%) and so there is still a lot to do, especially in gamete and embryo selection and embryo culture before implantation.

Q: How do the treatment and prevalence of reproductive health issues differ between Italy and the rest of Europe? What can Europe learn from the Italian experience?



A: Until a few years ago, Italy had very restrictive laws on the application of ARTs. We only began using donor gametes in the cycles of ARTs this year. Over the years, this legal restriction has led to improvements in gamete selection because the law restricted the number of oocytes that could be inseminated for each cycle to three, and so it was mandatory to select the best three; I believe that we are now ranked among the world leaders, especially in oocyte selection.

Q: Which cultural and lifestyle factors have the most impact on fertility? What can be done to address these issues?

A: There is an increase in the average age of parents, which is certainly a problem, but the increasing amount of pollution is also a problem. In recent years, lifestyle habits have become important as they have led to increases in metabolic syndrome, diabetes, etc. – most of which are due to hectic lifestyles.

Q: What is the biggest challenge facing reproductive health practitioners today, and what must be done to overcome this?

A: Improving success rates is certainly the biggest challenge and something that will have a big economic impact, especially for private ART centres. How do we achieve this? There is a lot of work to be done, but I believe that identifying good markers for the selection of gametes will be successful.

Q: What can governing bodies and other influencers do to promote better sexual health worldwide?

A: There is a lot to do. Firstly, infertility should be recognised as a social problem and as a disease. Currently it is not recognised as such: a large number of people maintain that if God does not want a couple to have children, then they should not try. At present, there is little funding to help research in this field. This is especially true for EU funding: even in Horizon 2020 there is nothing addressing the problem of infertility, despite the fact that the number of infertile couples is increasing and expected to increase further in the future. Funding agencies should help researchers in the field of gamete selection, women's treatment (there is a lot to do because current treatments aimed at increasing the number of follicles are not adequate for all women), and to find pharmacological targets that would increase the fertility status of men. I personally disagree with the idea that women should freeze their oocytes at a young age in order to preserve their fertility for the future and to facilitate their professional career (some American multinational corporations decided to offer payment for oocyte preservation procedures to their female employees in order to allow their career advancement). Something should be done to allow parents to become parents when they want, and not when their career will allow it. To achieve this, governments should help to increase the number of kindergartens and other resources to help family planning.

Q: What do medical congresses such as ESHRE have to offer the field, and what do you feel their focus should be?

A: ESHRE offers the important service of bringing together researchers in the reproduction field every year, organising seminars/courses on technical advancements in reproduction techniques, and financing some research projects. In my opinion, they could do more with regard to the latter point and also try to fund more basic research, i.e. research that does not accomplish immediate achievements but which can be important for future achievements.

Q: What has been your proudest achievement in medicine to date?

A: This is difficult to say. However, my old papers on the effects of progesterone on human sperm and their role in male infertility are highly cited by the international research community, because they represent the first reports showing that anomalies in sperm signalling are important!

"ESHRE offers the important service of bringing together researchers in the reproduction field every year..."

George Anifandis

Lecturer in Human Embryology, Clinical Embryologist, IVF Unit, Department of Obstetrics and Gynaecology, School of Health Sciences, Faculty of Medicine, University of Thessaly, Larissa, Greece.

Q: Why did you originally decide to focus your career on reproductive health?

A: I started studying biology, and following this biology education I took a Masters course with the title 'Applications of Biology in Medicine', and that was the time that I considered that a Masters thesis with the title 'sperm biology' would fit me. Over the following years I graduated from the course and began a PhD thesis relative to reproduction. I think that assisted reproduction will be the tip of the iceberg in reproductive health over the next few years.

Q: How far has our understanding of this field of medicine grown since your career began, and to what extent has treatment improved as a result?

A: Almost 15 years have passed since I began my PhD thesis, so I have 15 years of experience in the field of reproductive health and especially in assisted reproduction. I have to admit that since then the field of assisted reproduction has grown very much, especially concerning the ovarian stimulation protocols and the quality control and assurance of the *in vitro* fertilisation (IVF) lab. Due to this growth the IVF success rates and take-home baby rates have reached a relative maximum level (30%).

Q: As someone whose main area of research is IVF, how much has this procedure developed in terms of safety and efficacy since you first began your research?

A: As I said before, research concerning IVF has gained much attention over the last two decades. Previously the main focus of research was to understand the physiology of reproduction and how our knowledge can be implemented in assisted reproduction. Last year's research was focussed mainly on the techniques and methodologies that will improve the success rates, the survival rates of vitrified oocytes and embryos, and embryo quality in order to transfer the best and euploid embryos. Moreover, another innovation regarding research on assisted reproduction is the pre-implantation genetic diagnosis process, which has also gained attention from many scientists.

Q: Do you see the success rate, accessibility, and cost of this procedure improving in the coming years?

A: I think that success rates are getting better due to the technology in the IVF lab, and the stimulation protocols will enhance the IVF success rates in the forthcoming years. Accessibility may improve in the coming years, but as far as the cost of these procedures is concerned I believe that this depends on the financial policy of every country. In Greece, due to the financial crisis, the cost in most towns has fallen in comparison with previous years.

Q: What more do you think could be done to ensure that IVF treatment is more accessible for couples with fertility problems?

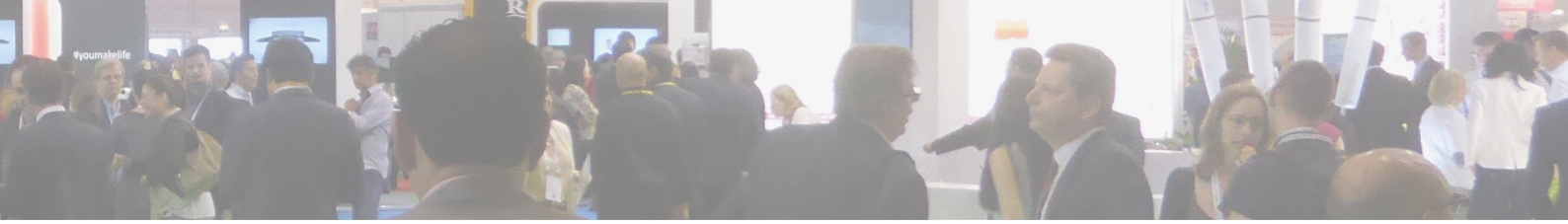
A: I think that we cannot do anything and we should leave things to come to us. What I mean is that at this moment, research is in full power and if something comes up from the research it can be easily implemented in the assisted reproduction field in order to improve either treatment or outcome.

Q: In your opinion, what is the biggest challenge facing those who work in reproductive health today?

A: As far as assisted reproduction is concerned, the biggest challenge is to help subfertile or even infertile couples achieve pregnancy and subsequently be able to take a healthy baby home.

Q: As a clinical embryologist, have you noticed any changes in recent years in the prevalence and types of conditions that you treat?

A: Yes, there is a tremendous improvement in the procedure of embryo selection through the embryoscope, which monitors embryo



development and allows embryologists to select the best embryo without disturbing the developmental conditions. In this way the IVF success rates have been improved by almost 6%.

Q: Do you think that more could be done to educate people on the pressing issues relating to reproductive health?

A: It is bound to be the case that as far as education is concerned many things can and should be done, because education makes us better and helps us to understand any problem from many perspectives. I think that establishing more congresses, educational programmes fostering everyday communication, or even leaflets informing and updating people would be good approaches and potential solutions for successfully educating people on the pressing issues relating to reproductive health.

Q: How would you rate the current standard of healthcare in Greece? Are there any lessons that other countries could learn from the Greek healthcare system?

A: It is difficult to answer this question, but I think that the current standard of healthcare in Greece is at a good level but not excellent due to the economic crisis that has hit our country. Nevertheless, the standard of healthcare and especially of assisted reproduction care is at a very good level.

Q: How important is the annual ESHRE meeting for professionals in reproductive health to keep up-to-date with the latest research?

A: It is the most well-known and exceptional European congress, and stands alongside the American Society for Reproductive Medicine (ASRM) Annual Meeting. Research and novel knowledge are presented at this congress, so participation is highly recommended.

Q: What advice would you give to couples suffering from fertility problems? What are the best options for them to consider?

A: Today almost 15% of couples are suffering from fertility problems. Couples that are seeking conception should seek help from specialist doctors, such as obstetric gynaecologists who are specialised in IVF or assisted reproduction. Almost all couples that have fertility problems will overcome their problem and only 5% will continue to suffer from infertility.

"The biggest challenge is to help subfertile or even infertile couples achieve pregnancy and subsequently be able to take a healthy baby home."

Kristo Ausmees

Medical Director, CEO of Medita Clinic, Tartu, Estonia.

Q: What do you think is the most pressing issue for men's reproductive health at the moment in Estonia, and also in Europe as a whole?

A: There are still two issues that are most important: in society, how to produce better campaigns for education in men's health, healthy lifestyles etc.; and individually, how to divide one's time, not only for work but also for partnership, family, and friends.

Q: You have written multiple articles as a doctor on popular websites; which subjects do you most enjoy writing about and what issues would you most like to draw people's attention to?

"The male reproductive tract works as a whole, i.e. damage in one part can cause disturbances in other parts."

A: In previous decades, my interest was mostly related to middle-aged male reproductive tract pathologies. Before that time I was more interested in semen quality in younger males and related factors. Commonly, my topics are very wide but mostly related to male reproductive pathologies and related co-factors.

Q: What was the reason for your initial interest in andrology and urology, and how did it lead to you specialising in these subjects?

A: That is a good question. At university, I worked as a nurse in the urology department; that was the first connection. Andrology as a (sub)speciality was only conceived 15 years ago, and in that time only a few doctors in Estonia have worked in that field. At some point I made the decision to start my residency, and that was how it began.

Q: Do you believe that the deterioration or negligence of men's health problems are due to a lack of public awareness, as well as information regarding the problems?

A: Absolutely not. In the last decade, public and media interest focussing on men's health has increased the common level of knowledge almost to the maximum level, in my opinion.

Q: What changes would you like to see in your field during the next few years?

A: More treatment possibilities, and increased awareness of the genetic causes and risk factors associated with male reproductive pathologies.

Q: What in your opinion has been the most significant change or discovery in your field over the last 5 years?

A: The knowledge that the male reproductive tract works as a whole, i.e. damage in one part can cause disturbances in other parts.

Q: Some of your work focusses on ageing men and the health problems that follow. Do you think that the age at which men begin to suffer from health problems such as urinary tract disorders, infertility, and erectile dysfunction is decreasing or increasing? If there is a pattern, what would you consider the cause of this to be?

A: There is no clear answer. From one perspective, younger males are having more problems with their sexuality and infertility. On the other hand, most people's lifestyles have changed and therefore chronic age-related diseases, for example prostate hyperplasia, may occur later.

Q: What changes do you think have most significantly advanced the fields of andrology and urology since you began working as a doctor?

A: There have been many changes that have significantly advanced these areas, for example in the knowledge of the diagnostics and treatment for malignant diseases, in the steps in assisted reproductive techniques, and in the growth of public awareness.

Q: What has been the highlight of your career thus far?

A: My career has not ended yet and therefore I would not like to separate any small piece in that way.

Q: What is the most rewarding part of your job?

A: To start and finish every day with smiling, positive colleagues and patients.

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CERVICAL CARCINOMA: CURRENT ROLE OF LAPAROSCOPY

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ABSTRACT

This review aims to analyse and describe the current role of laparoscopy in the treatment of cervical cancer. Laparoscopy has become an important tool in gynaecological oncology. Its general advantages in comparison with open surgery apply to oncological patients as much as they do to benign conditions. Data from retrospective and case-control studies have proven that treatment of early cervical carcinoma is successfully feasible by means of minimally invasive surgery with no compromise of oncological principles nor radicality. Thus, laparoscopy has entered guideline recommendations as an alternative to open procedures when operative therapy is indicated. Nevertheless, laparoscopic radical hysterectomy, as well as lymphadenectomy, remain demanding and require surgeons experienced in both operative oncology and endoscopy.

Keywords: Laparoscopy, gynaecological oncology, early cervical carcinoma, radical hysterectomy, laparoscopic lymphadenectomy.

INTRODUCTION

The incidence of invasive cervical carcinoma has decreased in Europe over the last three decades due to screening programmes, and will probably decrease further with human papilloma virus vaccination. It is currently the fourth most common malignancy in women, behind breast, endometrial, and ovarian cancer, and accounts for only approximately 2% of all malignancies in women. Most invasive cervical carcinomas are at a low clinical stage when diagnosed, i.e. 62% at Stage I and 25% at Stage II.[1] According to current guidelines, primary therapy of the so-called early cervical carcinoma (Stages IA to IIA1) consists of a surgical approach.[2,3] Stage-dependent radical hysterectomy is essentially performed as described in 1974 by Piver et al.[4] and following the historic principles of Schauta and Wertheim and their modifications by Meigs, Latzko, and Okabayashi.[5-7] A widely accepted classification of radical hysterectomy for cervical cancer (CVC) was introduced in 2008 by Querleu and Morrow,[8] which defined four different types of radicality (A-D) based on lateral extent of resection and with subtypes considering nerve preservation and paracervical lymphadenectomy. Lymph node dissection is described separately in this classification as one of four levels (1-4) according to arterial anatomy.

The introduction of laparoscopy into treatment concepts for early CVC aims to reduce the considerable invasiveness and morbidity of these extensive surgical procedures. Pilot reports date back to 1990 when Querleu et al.[9] described pelvic lymphadenectomy in cervical carcinoma, followed in 1992 by the publication of para-aortic lymphadenectomy by Herd et al.[10] and radical hysterectomy by Nezhat et al.[11] Since this pioneering work, the role of minimally invasive surgery in gynaecological oncology has evolved and been the subject of numerous clinical reports and studies.

The aim of this review is to give an overview on data defining the impact of laparoscopic procedures in the framework of surgical therapy concepts for early CVC.

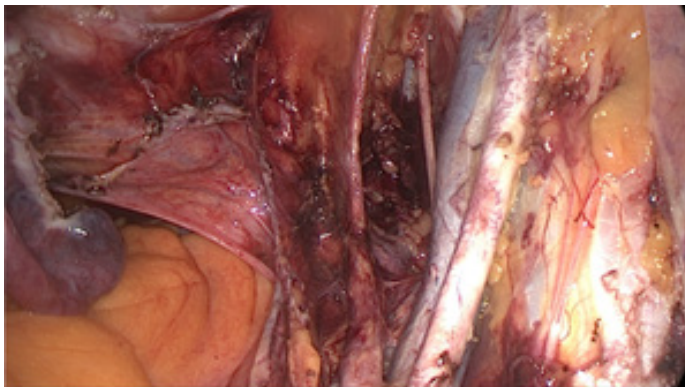

Figure 1: View of right pelvic fossa after laparoscopic lymphadenectomy.

ONCOLOGICAL CONCEPT OF THE SURGICAL APPROACH

Current guidelines recommend operative therapy according to the clinical stage of the disease.[2,3]

Simple hysterectomy is generally recommended in CVC of Stage IA1 without or with up to only one risk factor (G3, L1, V1). If fertility preservation is desired, (*in sano*) conisation with cervical curettage can be performed.[3,12] When risk factors are absent, the risk of lymph node involvement is very low and therefore lymphadenectomy is not indicated.

In Stage IA1 with at least two risk factors (G3, L1, V1), and in Stage IA2 with no more than one risk factor, pelvic lymphadenectomy is performed (Figure 1) because of an elevated risk of approximately 8% for lymph node metastasis.[13] The possibility of sentinel node biopsy may further reduce invasiveness in selected patients at this early stage of the disease, but does not represent a standard procedure. When lymph nodes are negative, extrafascial hysterectomy (Type A, Piver I) or, in the case of fertility preservation, conisation with cervical curettage follows without parametrial resection. In nodal-negative Stage IA2 with more than one risk factor, IB1 and IIA1 require radical hysterectomy with parametrial resection extending medially to the ureter by dissecting the uterine vessels at the ureteral crossing (Type B, Piver II). In the IIA1 stage, the vaginal resection margin must be free of tumour.

Radical hysterectomy with parametrial resection according to Querleu Type C or Piver III (Figure 2) is recommended in Stage IB2 and IIA2 after negative lymph node staging. The lymphadenectomy should be started at the inferior mesenteric artery. If these inframesenteric lymph nodes are positive, the infrarenal para-aortic lymph nodes should be removed and the operation should be stopped. If lymphadenectomy is negative, the radical hysterectomy procedure starts by dissecting the uterine vessels at their origin from the internal iliac vessels, ureteral preparation is performed down to the bladder, sacrouterine and cardinal ligaments are dissected at the sacrum and pelvic wall, and a vaginal cuff is resected.

An indicated lymphadenectomy contributes considerably to the morbidity of the surgery. Therefore, efforts have been made to replace systematic lymphadenectomy with a sentinel approach. Results of several studies indicate that sentinel lymphadenectomy offers a feasible and reliable alternative in patients with a tumour size <2 cm.[14,15] If technetium and blue staining techniques are combined then the sensitivity reaches 93.5%, with a negative predictive value of 99.1%. A Cochrane analysis of 20 studies revealed a sensitivity of 92% and detection rate of 97%. False-negative rates can be further minimised using bilateral sentinel resection and immunohistological ultrastaging.[16] However, because the data derive from retrospective analyses and oncological parameters, the sentinel concept cannot yet be regarded as a clinical standard.[3]

In cases with positive pelvic lymph nodes, and irrespective of the clinical stage, the operative concept is abandoned in favour of radiochemotherapy. Fertility may be preserved by trachelectomy in selected Stage IA/B patients without lymph node involvement,[2,3] and eventually be followed by secondary hysterectomy after accomplishment of pregancy. In Stage IA without lymphangiosis, an *in sano* conisation will probably lead to comparable results with lower morbidity.[12,17,18] Ovaries may be preserved in most premenopausal patients by ovariopexy, although ovariectomy may be necessary in some patients with adenocarcinoma.[3] Adnexectomy should be the procedure in postmenopausal patients with macroinvasive CVC.

THE ROLE OF LAPAROSCOPY

Laparoscopic treatment of early CVC follows the stage-dependent recommendations described above and therefore represents a variation of access rather than a different oncological concept.

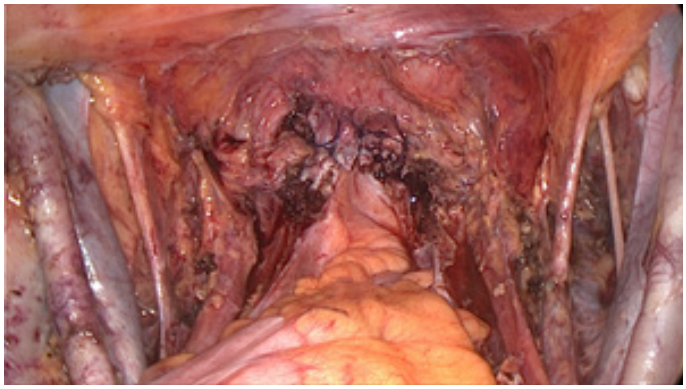

Figure 2: Operative result after total laparoscopic radical hysterectomy with parametrial resection according to Piver III (cervical carcinoma IB2).

The minimally invasive approach, however, promises significant reduction of surgery-induced morbidity when compared with the classical open abdominal procedure.

Safety and Feasibility

Numerous reports have proven both the feasibility and favourable outcome of laparoscopic radical hysterectomy (LRH) and lymphadenectomy in early CVC (Table 1). Most studies refer to Stages IA2 and IB1, but laparoscopy can also be successfully used in higher stages if operative therapy is indicated.[19-34] Conversions to laparotomy are rarely necessary. The minimally invasive procedures tend to last longer than open surgery, with median time differences ranging from 5-76 minutes in the different series, although median overall operating times show a broad range between 92 and 371 minutes. Median blood loss is reported to be between 55-450 ml, which is significantly less than that of open surgery in almost all reported series. Only robotic radical hysterectomy resulted in even lower amounts of bleeding, and this was only in one study.[28] Hospital stay was significantly shorter in all series and decreased to only 2 days in one report.[23] Intra and postoperative complications occurred in approximately 6-10% but did not differ significantly from the open abdominal approach. These complications consisted of problems relating directly to the procedure, such as bleeding or cystotomy, and problems relating to more general events, such as embolism or infections. Long-term complications such as bladder/rectal dysfunction, ureteral stenosis, or fistula occurred in approximately 10% of both laparoscopic and open abdominal cases. Thus, available feasibility data on the laparoscopic approach to early CVC surgery reveal longer durations of the procedures but better short-time outcome with less blood loss, fewer transfusions, shorter hospitalisation, and no increase in complication rates in comparison with open abdominal access.

Oncological Radicality

Oncological radicality has been evaluated considering the number of resected lymph nodes during indicated pelvic lymphadenectomies, as well as the status of vaginal and parametrial resection margins.[19-24,26-30,33,35] Median numbers of resected lymph nodes ranged between 11 and 31, which did not differ from open pelvic lymphadenectomy results. In his large series of 248 patients, Puntambekar et al.[21] reported a median lymph node number of 18. The lowest individual numbers were 9-12, and the highest were 39-61. Remarkable ranges such as 10-61[22] and 12-34[28] may indicate that individual factors beyond surgical radicality or type of surgical access influence the result of lymph node counts. Tumour-free parametrial resection was also generally reported, although most studies dealt with early stages in which parametrial involvement was not to be expected. There are limitations to the existing literature, particularly regarding the results of treating IB2 tumours, and many of the published case series lack data on pathology characteristics and immediate and late outcomes.

While most series were either retrospective or included comparisons with historical controls, the studies by Naik et al.[36] and Simsek et al.[37] concern randomised controlled designs. However, Naik et al.[36] compared laparoscopically assisted radical vaginal but not total laparoscopic hysterectomy with abdominal radical hysterectomy. In this setting there was a clinical short-term advantage confirmed in the laparoscopically assisted treatment group, but surgical radicality was found to be inferior, with smaller vaginal resection margins (1.26 cm versus 2.16 cm) and shorter parametria (1.3 cm versus 2.79 cm). No data on the clinical significance of these findings were reported. Simsek et al.[37] randomised 88 patients to either total laparoscopic (n=35) or open surgery (n=53) and did not find differences concerning the number of resected lymph nodes or tumour-free resection margins. Complete tumour-free resection was also achieved in all cases with parametrial infiltration, which was found in 11.4% of

the laparoscopically treated patients and in 16.9% of the open surgery group.

Oncological Results

Recurrence and survival data are available from few studies (Table 2). Most series focus more on technical feasibility than on oncological outcome, and follow-up times are short. A low recurrence rate of 2.8% after total LRH in Stages IA2-IB1 was reported by Puntambekar et al.[21] in a large series of 248 patients after a median follow-up period of 36 months. Toptas and Simsek[35] found 13.6%, although the number of patients treated was small (22 individuals of whom 3 relapsed after a median follow-up of 42.5 months). Park et al.[29] reported a 5-year recurrence rate of 22% in larger tumours of Stages IB2-IIA2, and Chen et al.[24] found 16.3% after a median follow-up of 36.45 months (range: 8-76) for Stages IA-IIIB. A matched-pairs analysis of 263 patients undergoing LRH versus the same number of patients undergoing an open technique by Nam et al.[27] revealed no higher risk of recurrence (hazard ratio [HR]: 1.28, 95% confidence interval [CI]: 0.62-2.64) or death (HR: 1.46, 95% CI: 0.62-3.43). Even in patients with tumours >2 cm, the HRs were 0.82 (95% CI: 0.31-2.16) and 1.01 (95% CI: 0.35-2.95), respectively, and 5-year recurrence-free survival rates did not significantly differ (92.8% versus 94.4%).

A recently published study by Ditto et al.[34] compared 60 prospective patients undergoing LRH with 60 matched patients undergoing open procedures. As part of favourable feasibility data, the study showed that the execution of LRH or radical abdominal hysterectomy did not influence the site of recurrence (p>0.2) or survival outcomes in terms of the rates of 5-year disease-free survival (p=0.29, log-rank test) and overall survival (p=0.50, log-rank test).

Table 1: Feasibility data on total laparoscopic radical hysterectomy for early cervical cancer.

Study	N	Clinical stage	Conversion to laparotomy, n	Median operating time, min (range)	Median blood loss, ml (range)	Blood transfusion, n	Short-term complications, n	Long-term complications, n
Abu-Rustum et al.[19]	19	IA2-IB1	2	371 (230-600)	301 (75-1,500)	n.r.	2 (bleeding, cystotomy)	n.r.
Ramirez et al.[20]	20	IA2-IB1	0	n.r.	200 (25-700)	1	3 (cystotomy, pulmonary embolus, pneumomediastinum)	2
Puntambekar et al.[21]	248	IA2-IB1	0	92 (65-120)	165	n.r.	15	17
Zakashansky et al.[22]	30	n.r.	0	318.5 (200-464)	200 (100-600)	0	n.r.	n.r.
Frumovitz et al.[23]	35	IA2-IB1	n.r.	344	319	4	6 (postoperative infection)	n.r.
Chen et al.[24]	295	IA-IIIB	5	162 (110-350)	230 (50-1,200)	n.r.	12	31
Malzoni et al.[25]	65	IA1-IB1	n.r.	196 (182-240)	55 (30-80)	n.r.	n.r.	n.r.
Taylor et al.[26]	9	IA2-IB1	n.r.	231.7	161.1	0	0	n.r.
Nam et al.[27]	263	IA2-IIA	n.r.	n.r.	379.6	n.r.	18	24
Chong et al.[28]	50	n.r.	0	211.2 (164-258)	201.9 (53-350)	4	4	n.r.
Park et al.[29]	115	IB2-IIA2	2	n.r.	n.r.	n.r.	n.r.	n.r.
Kong et al.[30]	40	IB-IIA	n.r.	254.5	449.1	n.r.	n.r.	n.r.
Bogani et al.[31]	65	n.r.	2	245	200	4	4	n.r.

n.r.: not reported.

Table 2: Comparative study results of total laparoscopic radical hysterectomy (TLRH) versus open abdominal radical hysterectomy (ARH) and robotic radical hysterectomy (RRH) for early cervical cancer.

Study	Design	TLRH, n	Comparator procedure, n	Median operating time: TLRH vs comparator, min	Median blood loss: TLRH vs comparator, ml	Mean length of hospital stay: TLRH vs comparator, days	Mean number of pelvic lymph nodes removed: TLRH vs comparator, n	Disease-free survival: TLRH vs comparator, %
Abu-Rustum et al.[19]	Retrospective, cohort study	19	195 (ARH)	371 vs 295 (p<0.01)	301 vs 693 (p<0.01)	4.5 vs 9.7 (p<0.01)	25.5 vs n.r.	100 vs n.r.
Zakashansky et al.[22]	Prospective, case-controlled	30	30 (ARH)	318.5 vs 242.5 (p<0.01)	200 vs 520 (p<0.01)	3.8 vs 5.6 (p<0.01)	31 vs 21.8 (p<0.01)	100 vs n.r.
Frumovitz et al.[23]	Retrospective	35	54 (ARH)	344 vs 307 (p=0.03)	319 vs 548 (p=0.009)	2 vs 5 (p<0.001)	14 vs 19 (p=0.001)	n.r.
Nezhat et al.[38]	Prospective, non-randomised	30	13 (RRH)	323 vs 318 (n.s.)	157 vs 200 (n.s.)	2.7 vs 3.8 (n.s.)	25 vs 31 (n.s.)	100 vs 100 (n.s.)
Malzoni et al.[25]	Retrospective	65	62 (ARH)	196 vs 152 (p<0.01)	55 vs 145 (p<0.01)	4 vs 7 (p<0.01)	n.r.	n.s.
Taylor et al.[26]	Retrospective, matched controls 2:1	9	18 (ARH)	231.7 vs 207.2 (n.s.)	161.1 vs 394.4 (p=0.059)	2.9 vs 5.5 (p=0.012)	n.r.	100 vs 100 (n.s.)
Nam et al.[27]	Matched pairs	263	263 (ARH)	n.r.	379.6 vs 541.1 (p<0.001)	12.5 vs 20.3 (p<0.001)	33.6 vs 29.1 (p<0.001)	92.8 vs 94.4 (n.s.)
Chong et al.[28]	Prospective, non-randomised	50	50 (RRH)	211.2 vs 230.1 (p=0.025)	201.9 vs 54.9 (p<0.001)	n.r.	23.1 vs 25 (n.s.)	n.r.
Park et al.[29]	Retrospective	115	188 (ARH)	n.r.	Significantly less in TLRH group (p=0.003)	Significantly shorter in TLRH group (p<0.001)	n.r.	78 vs 77 (n.s.)
Kong et al.[30]	Retrospective	40	48 (ARH)	254.5 vs 246 (n.s.)	449.1 vs 588 (p<0.001)	14.8 vs 18 (p=0.044)	n.r.	97.5 vs 97.9 (n.s.)
Bogani et al.[31]	Prospective, case-controlled	65	65 (ARH)	245 vs 259.5 (n.s.)	200 vs 500 (p<0.001)	4 vs 8 (p<0.001)	n.r.	n.s.
Toptas et al.[35]	Retrospective	22	46 (ARH)	n.r.	n.r.	n/a	28 vs 32 (medians, n.s.)	86.1 vs 90.6 (n.s.)
Ditto et al.[34]	Prospective, propensity-matched comparison	60	60 (ARH)	215.9 vs 175.2 (p<0.001)	50 vs 200	4 vs 6 (p<0.001)	25.4 vs 34.6 (p<0.001)	n.s.

n.s.: not significant; n.r.: not reported; vs: versus

In a published series, disease-free survival rates range between 78% and 100% depending on clinical stage and follow-up, but no study revealed significant differences between laparoscopic and open (or robotically assisted) approaches.[19,22,23,25-31,35,37,38] These results were confirmed by a Health Technology Assessment report from 2010[39] and a systematic review including data from 1,339 patients in 21 studies on laparoscopic treatment.[40]

Nerve-Sparing Concept

Despite the proven advantages of minimally invasive access for CVC surgery, postoperative and long-term morbidity are considerable with regard to bladder, bowel, and sexual dysfunction due to damage of the pelvic autonomic nerves during radical hysterectomy. Nerve-sparing techniques have been introduced to preserve these structures.[41] Identification and conservation of the inferior hypogastric plexus results in significantly less bladder dysfunction and improved sexual results,[42-44] and should therefore be a mandatory approach in order to reduce surgical morbidity. Magnification during laparoscopy or robotic surgery may facilitate identification of the neural structures with measurable impact on bladder function.[45,46]

Laparoscopy in Locally Advanced Disease

Locally advanced cervical carcinoma should be treated by radiochemotherapy.[2,3] Laparoscopy can serve as a means of staging in order to define and document the spread of the disease.

Neoadjuvant chemotherapy followed by surgery is discussed as an alternative to radiochemotherapy. This may result in improved operability but positive data on progression-free and overall survival from a Cochrane analysis in 2012[47] were not reproduced by a more recent meta-analysis.[48] Therefore, recommendations restrict its use to study conditions.[3,47,48] The role of LRH was investigated in this setting compared with abdominal radical hysterectomy and found to be favorable in terms of surgical outcome, and with comparable oncological results.[49,50] Favero et al.[51] found residual disease in 9 of 33 Stage 1B2-IIB patients (27%), mostly cases of adenocarcinoma, during laparoscopic completion surgery after primary radiochemotherapy, and therefore advocated laparoscopic surgery to improve local tumour control. Further studies will define both the role of neoadjuvant regimens as well as the role of the laparoscopic approach in this framework.

Guideline Recommendations

Despite the fact that randomised data on recurrence and survival rates are lacking,[52] the available retrospective and case-controlled results on oncological outcome, together with the feasibility data decribed above, indicate the equivalence of laparoscopic and open approaches. Therefore, current guidelines such as the Scottish Intercollegiate Guidelines Network in 2008,[53] the British National Institute for Health and Clinical Excellence in 2010,[54] and the German S3-Leitlinie zur Therapie des Zervixkarzinoms in 2014,[3] as well as the National Comprehensive Cancer Network (NCCN) Guideline Cervical Cancer[2] recommend LRH in early CVC as an alternative to open radical hysterectomy.

CONCLUSION

The laparoscopic approach to early CVC treatment can be regarded as an alternative to open surgical procedures, with good clinical results as far as feasibility and safety are concerned. The minimally invasive approach may further develop using robotic-assisted surgery, which has been introduced with at least comparable results in recent pilot studies.[55] Well-known advantages of minimally invasive techniques are as relevant for oncological patients as they are for patients with benign conditions. The endoscopic procedure does not represent a new concept, but a variation in access following the same oncological principles of stage-dependent therapy as open surgery. Available data, which are mostly retrospective, confirm a reduction in short-term morbidity without loss of surgical radicality. Long-term and prospective data on recurrence rates and survival are needed. The experience and 'know-how' of the operating surgeon remain of utmost importance for surgical and oncological success.

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INSIGHT INTO BONE METABOLISM AND SKELETAL MASS IN POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex, multifaceted disorder that manifests with obesity, hyperandrogenaemia, hyperinsulinaemia, and possibly hyperoestrogenaemia. These clinical features can cause PCOS to positively influence bone mass, and new relationships between obesity, bone remodelling, and energy metabolism have emerged. Bone mass can also be influenced by interrelated metabolic events that are not necessarily mediated by androgens. This article summarises the current literature with respect to the associations between the diverse clinical components of PCOS and bone.

Keywords: Bone, sex steroid, insulin, growth differentiation factor.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a frequently encountered endocrinopathy that occurs in 15–20% of reproductive-age women when the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine criteria are applied.[1,2] The Androgen Excess Society published a broad consensus statement in 2006 that suggested a practical definition, which integrated the National Institutes of Health and Rotterdam criteria.[2-4] This new definition excluded the phenotype subset of polycystic ovaries (PCO) and ovarian dysfunction without hyperandrogenism. Androgen excess was suggested to be the key component of PCOS related to clinical symptoms and long-term morbidity.

PCOS is a complex disorder with multisystem involvement. In addition to ovulatory dysfunction and dysregulated androgen biosynthesis, this syndrome is characterised by obesity, increased central adiposity, insulin resistance (IR), and glucose intolerance.[1] Various features of this syndrome may influence bone metabolism and skeletal mass. Oestrogen is well known to be a protective factor for maintaining bone mineral density (BMD). The effect of endogenous androgens is less certain. Androgen treatment may not only inhibit osteoclast formation directly but also modulate osteoblast—osteoclast interactions via osteoprotegerin.[5,6] Interrelated metabolic events not necessarily mediated by sex hormones may also influence bone mass. Obesity may exert a positive influence on BMD in women with PCOS due to the beneficial impact of mechanical loading on bone formation. Central adiposity has been reported to be positively associated with maintenance of BMD.[7] Furthermore, hyperinsulinaemia and IR may protect against the development of osteoporosis independent of body mass index (BMI).[8] The purpose of this review is to discuss recently published data regarding the relationship between PCOS and bone metabolism. We have focussed on adult PCOS patients compared with healthy women.

BONE MINERAL DENSITY AND FRACTURE RISK IN PCOS

Several previous studies have compared BMD in premenopausal PCOS patients with healthy controls (Table 1).[9-16] These studies report conflicting results: some authors observed higher BMD in amenorrhoeic PCOS cases, while others observed lower BMD in these patients compared with healthy controls.[11,17] A possible explanation for the lack of agreement between data from these studies is the

differing inclusion of PCO and ovarian dysfunction without hyperandrogenism as criteria for diagnosing PCOS. Furthermore, no attempt was made to control the analysis for age, duration of amenorrhoea, or lifestyle factors. It is also possible that differences in body composition or weight distribution between eumenorrhoeic and oligomenorrhoeic PCOS patients and 'normal' women contribute to the observed differences in BMD. Furthermore, the relative contributions of androgens and oestrogens to BMD in this population were not estimated. It is also of relevance that the majority of these studies included relatively young populations from various ethnic groups. The BMD results could therefore be related to attainment of peak bone mass (PBM), which usually occurs between 25-30 years of age.[18] Genetic and familial factors, race and ethnicity, mechanical loading, and hormonal and nutritional factors also contribute to the variation in PBM in a population.

In general, BMD in women with PCOS appears to be maintained at levels comparable to those observed in healthy controls.[9,10,12-14,16] Additionally, previous studies have reported limited differences in bone metabolic parameters in patients with hirsutism and PCOS versus controls.[9,14,16] Adami et al.[9] evaluated bone turnover markers and BMD in young women (17–33 years) with PCOS, idiopathic hirsutism (IH), or hypothalamic amenorrhoea, as well as in healthy controls. They demonstrated that spine and femoral neck BMDs in PCOS patients were similar to those in healthy controls. In the subgroup of PCOS patients with associated amenorrhoea, spine and femoral neck BMDs were comparable to control values but significantly lower than those in patients with IH and non-amenorrhoeic PCOS, despite comparable oestradiol levels. These data suggest that the negative effects on BMD that likely result from the deleterious effects of anovulation are balanced by higher androgen production in PCOS women, but the percentage of these young patients with suboptimal attainment of PBM (a confounding factor) is not clear. Furthermore, no differences in bone turnover were observed between subjects with either PCOS (amenorrhoeic or eumenorrhoeic) or IH compared with control women. Androgen excess did not seem to be associated with any notable increase in bone formation or reduction in bone resorption when compared with the findings in normal women.

Some studies have included women with PCOS and around 30 years of age, at which point PBM had already been reached.[13,16] Berberoglu et al.[16] examined obese female patients with PCOS or IH and aged 25–35 years, as well as healthy controls matched for age and BMI. Bone turnover markers were similar in all three groups and there were no significant differences in lumbar spine, femoral neck, trochanter, or total hip BMDs between the groups. In contrast to the results of Adami et al.,[9] Berberoglu et al.[16] reported that spine and femoral neck BMDs in the subgroup of PCOS patients with associated amenorrhoea were comparable to those in patients with IH, non-amenorrhoeic PCOS, and healthy controls. McCleary[13] measured lumbar BMD by quantitative computed tomography (QCT) at the central skeleton and did not observe any significant difference between PCOS cases aged 35 years or older (mean age: 47.6 years) and control patients in any univariate comparisons, nor were any significant differences found in any multivariate-adjusted comparisons.

Many previous studies have included PCOS patients with excessive body weight (Table 1). Good et al.[10] focussed on women with PCOS and a BMI ≤26 kg/m^2 and examined BMD and fat distribution in 12 lean women with PCOS compared with 10 healthy controls. There was no statistically significant difference in total body BMD between PCOS patients and controls. Kassanos et al.[15] used peripheral QCT to show significant improvement in the volumetric cortical BMD of the tibia without alterations in metabolic bone status, geometry, or strength in PCOS patients (especially the lean ones) compared with normal controls. This result suggests a higher bone material quality and stiffness in PCOS patients.[15] Possible associations between bone structure and BMI, sex hormones, and insulin were not investigated in the study.

To our knowledge, BMD levels and fracture risk in postmenopausal women with previous PCOS have only been evaluated in a single prospective study.[19] The study group was relatively lean (median BMI: 25 kg/m^2) and patients had been diagnosed with PCOS prior to menopause. This long-term follow-up study demonstrated that older women with PCOS (age range: 61–78 years), who have most likely been exposed to high androgen levels for several decades and who have reached the postmenopausal period, display comparable BMD and incidence of fractures (56% of women with PCOS versus 41% of controls) as age and BMI-matched controls. The prospective measurement of

BMD is complicated by the possible use of different dual X-ray absorptiometry scanners over time.

FACTORS INFLUENCING BONE MINERAL DENSITY IN PCOS

Many previous studies have suggested that a relatively high oestrogen concentration, IR, hyperandrogenaemia, and obesity are crucial, bone-growth stimulating factors in PCOS.[9,10,20,21]

Body Composition and Insulin Resistance

Obesity is prevalent among women with PCOS. Based on available literature, obesity appears to affect bone metabolism through several mechanisms. First, adipocytes and stromal cells in fat tissue express P450 aromatase and are capable of converting adrenal and ovarian testosterone and androstenedione into 17β-oestradiol and oestrone, respectively. Oestrogen levels are strongly related to BMD. Secondly, mechanical loading stimulates bone formation by decreasing apoptosis and increasing proliferation and differentiation of osteoblasts and osteocytes through the Wnt/β-catenin signalling pathway.[22] Mechanical loading also inhibits adipogenesis by downregulating peroxisome proliferator-activated receptor gamma.[23]

Table 1: Characteristics of published studies examining the relationship between polycystic ovary syndrome (PCOS) and bone mineral density (BMD) in premenopausal women.

Study (year)	Design	Study Population Mean age (years), Mean BMI (kg/m^2)	BMD	Bone Turnover
Adami et al.[9]	Hypothalamic amenorrhoea (n=26) versus idiopathic hirsutism (n=24) versus PCOS (n=51) versus controls (n=35) **PCOS definition** 1990 NIH criteria	Women aged 17–33 **PCOS** Age = 24.2±4.9 BMI = 23.5±4.8 **Idiopathic hirsutism** Age = 22.7±4.4 BMI = 23.9±4.8 **Hypothalamic amenorrhoea** Age = 23.6±4.5 BMI = 23.0±3.0 **Controls** Age = 29.0±6.5 BMI = 21.7±3.2	BMD (by DXA at spine, femoral neck, Ward's triangle) comparable in PCOS versus controls. Amenorrhoea associated with lower BMD. BMD associated with testosterone.	No differences in PCOS versus controls. Increased bone metabolism in hypothalamic amenorrhoea.
Good et al.[10]	PCOS (n=12) versus controls (n=10) **PCOS definition** 1990 NIH criteria	Non-Hispanic women **PCOS** Age = 28.5±7.0 BMI = 22.4±2.3 **Controls** Age = 28.9±8.3 BMI = 22.0±2.2	Total body BMD comparable in patients versus controls. Hip and rib BMD increased in PCOS. BMD associated with testosterone.	
Noyan et al.[12]	PCOS (n=29) versus controls (n=17) **PCOS definition** 2003 Rotterdam criteria	Women in good health **PCOS** Age = 28.2±2.8 BMI = 27.7±6.7 **Controls** Age = 29.5±2.0 BMI = 27.0±5.0	BMD (by DXA at femoral neck, lumbar spine, trochanter, Ward's triangle, and total BMD) comparable in PCOS versus controls. BMD associated with insulin, oestrogen, and testosterone.	

Table 1 continued.

Study (year)	Design	Study Population Mean age (years), Mean BMI (kg/m^2)	BMD	Bone Turnover
McCleary[13]	PCOS (n=104) versus controls (n=97) **PCOS definition** Chronic anovulation and clinical evidence of elevated androgen (identified by the presence of hirsutism) or biochemical evidence of elevated androgens (testosterone >2 nmol/l or LH:FSH ratio >2.0)	Women aged ≥35 **PCOS cases** Age = 47.1±6.1 BMI = 32.0±8.6 **Controls** Age = 48.2±5.7 BMI = 28.3±6.4	BMD (by QCT of the lumbar spine) comparable in PCOS versus controls.	
Kassanos et al.[15]	Lean PCOS (n=15) versus obese PCOS (n=15) versus controls (n=15) **PCOS definition** 2003 Rotterdam criteria	Women aged 17–35 **PCOS** *Lean cases* Age = 26.5±3.6 BMI = 22.3±2.6 *Obese cases* Age = 28.5±4.1 BMI = 32.3±2.87 **Controls** Age = 26.7±4.4 BMI = 23.6±3.2	PCOS women (especially lean ones) had higher cortical BMD of distal tibia (by pQCT) in comparison with controls.	
Berberoglu et al.[16]	PCOS (n=42) versus idiopathic hirsutism (n=23) versus controls (n=20) **PCOS definition** 2003 Rotterdam criteria	**PCOS** Age = 29.6±3.7 BMI = 36.5±3.7 **Idiopathic hirsutism** Age = 30.4±3.7 BMI = 36.4±4.2 **Controls** Age = 30.9±4.1 BMI = 37.6±4.4	BMD (by DXA at lumbar spine, femoral neck, trochanter, total hip) comparable in PCOS versus idiopathic hirsutism versus controls.	No differences PCOS versus idiopathic hirsutism versus controls. GDF-15 negatively correlated with OCL and positively correlated with urine DPD.

BMI: body mass index; DPD: deoxypyridinoline; DXA: dual X-ray absorptiometry; GDF: growth and differentiation factor; OCL: osteocalcin; pQCT: peripheral quantitative computed tomography; QCT: quantitative computed tomography; NIH: National Institutes of Health.

Hyperinsulinaemia, independent of BMI, may protect against the development of osteoporosis.[8,11] It may also stimulate osteoblast activity directly and indirectly via suppression of the production of sex hormone-binding globulin (SHBG) and insulin-like growth factor-binding protein (IGFBP).[24] Lower SHBG and IGFBP concentrations may be responsible for the increased bioavailability of sex hormones and insulin-like growth factor (IGF).[24] Insulin further stimulates hepatic IGF-1 production.[8,24] Therefore, obesity and IR may act synergistically on bone metabolism and stimulate bone formation.

In contrast, obesity may increase bone resorption through upregulation of proinflammatory cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor-α, which are capable of stimulating osteoclast activity through regulation of the receptor activator of nuclear factor kappa-B ligand (RANKL)/RANK/osteoprotegerin signalling pathway.[25] Obesity is also associated with a significant increase in serum leptin and decrease

in adiponectin.[26,27] Serum leptin concentrations in women with PCOS have been reported to be higher than or similar to those in weight-matched controls.[28,29] The action of leptin on bone appears to be complex, with both positive and negative effects reported. Leptin acts on osteoblasts via two different neural pathways.[30] In the first, leptin activates the sympathetic nervous system by decreasing serotonin synthesis. Consequently, osteoblast proliferation is inhibited and both RANKL expression and bone resorption is increased. The second pathway functions via the activation of cocaine and amphetamine-regulated transcription and inhibits bone resorption. In addition, leptin increases expression of the osteoblast-specific *Esp* gene, and thus reduces osteocalcin bioactivity because the intracellular protein tyrosine phosphatase encoded by *Esp* favours osteocalcin carboxylation. It appears that the action may depend on current leptin status and the mode of the action (central or peripheral effects). In animal models, adiponectin has been reported to inhibit osteoclastogenesis, reduce bone resorption, and increase bone mass.[31] Not all women with PCOS are obese, although many patients (either overweight or not) have a high waist-to-hip ratio that is associated with abdominal fat mass, high body weight, IR, and other components of metabolic syndrome. These factors could, at least in part, explain the positive correlation observed between waist-to-hip ratio and BMD.[32] Furthermore, hyperandrogenaemia increases muscle mass and has therefore been postulated to influence BMD indirectly through skeletal loading.[33]

Sex Hormones

The protective effects of oestrogen on BMD have been well studied, but the effects of androgens are less certain, particularly in women. Androgens can influence bone directly by activation of androgen receptors (ARs), or indirectly after aromatisation of androgens into oestrogens within the extraglandular tissues, with subsequent activation of oestrogen receptors (ERs). Osteoblasts, osteoclasts, and mesenchymal stromal cells that differentiate toward the osteoblast lineage all express both ARs and ERs, with a predominance of ARs on osteoblasts.[34] As described above, fat tissues can convert testosterone and androstenedione into 17β-oestradiol and oestrone, respectively. Similarly, osteoblast-like cells in bone are also capable of transforming androgens into oestrogens.[34] In addition, these cells can convert testosterone into dihydrotestosterone due to 5α-reductase activity. It has been suggested that androgens may reduce IL-6 production, inhibit the production of prostaglandins, and suppress the effect of parathyroid hormone on osteoblasts.[34]

The influences of oestrogen and androgen on the remodelling of the skeleton are distinct. Oestrogens are thought to maintain adult bone mass predominantly through inhibition of bone resorption by osteoclasts. Interestingly, recent data have demonstrated that oestrogen treatment has no direct effect on osteoclasts whereas an indirect stimulation of osteoprotegerin was observed in osteoblasts.[35] Conversely, non-aromatisable androgens, such as 5α-dihydrotestosterone, increase bone mass by stimulating differentiation and maturation of osteoblasts, and thus also stimulate bone formation. However, recent data have demonstrated both the direct inhibition of osteoclast formation and bone resorption by androgen treatment and an indirect effect through increased expression of osteoprotegerin by osteoblasts.[35]

Androgens increase bone mass in specific skeletal compartments. At the periosteum, oestrogen suppresses new bone formation whereas androgens stimulate this process. Conversely, at the endosteum, oestrogen stimulates bone formation but androgens strongly suppress it.[36] Thus, androgens act to expand cortical bone and maintain trabecular bone. Additional support for this notion is reported in some studies.[10,15] Good et al.[10] demonstrated significant regional differences in BMD, with female PCOS patients having higher BMD in the left arm, right arm, and left ribs. This difference may be related to higher concentrations of androgens in women with PCOS, which is supported by a significant positive correlation between total BMD and androgen levels. Kassanos et al.[15] showed significant improvement in the volumetric cortical BMD of women with PCOS.

Recently, however, Vanderschueren et al.[37] demonstrated that the traditional endocrine model, with stimulatory effects of androgens in men and inhibitory effects of oestrogens in women, should be reconsidered in the context of recent findings. In both sexes, androgens may stimulate periosteal bone formation and low levels of oestrogen may affect the mechanical sensitivity of the periosteum, either directly or, more likely, indirectly via upregulation of IGF-1. There may be an interaction between oestrogens and androgens that affects BMD determination in women with

PCOS. Higher concentrations of endogenous oestrogens may inhibit periosteal bone apposition through interaction with mechanical loading (with ER-β effect) or IGF-1 secretion.[38]

Most studies have reported a lack of association between testosterone levels and BMD.[9,10,16] The use of imprecise testosterone assays and local androgen activation within bone tissue may explain this outcome.[39,40] However, no differences in bone turnover were observed in patients with PCOS (amenorrhoeic or eumenorrhoeic) or the control groups.[9,16] Therefore, any long-term and persistent effects of higher androgen levels on bone formation may be limited. Possible escape mechanisms should be evaluated in future studies.

Oestrogens play a key role in the development and maintenance of an appropriate bone mass in women, and their levels are strongly related to BMD.[41] Girls with premature adrenarche have a higher bone mineral content (BMC) compared with those not yet at this stage.[42] BMC and increased bone remodelling, which have been explained by high oestrogen, growth hormone, and IGF-1 levels, persist throughout the growth spurt in girls, even when growth velocity declines. Oestrogen secretion in women with PCOS is characterised by a chronic, high level of secretion without the cyclical pattern. Among women with PCOS and amenorrhoea or oligomenorrhoea, oestrogen concentrations are lower than in healthy females.[11,15] However, it seems that women with PCOS and menstrual irregularities have higher levels of oestrogen than women without PCOS but with menstrual irregularities, and they may not suffer from the same degree of hypoestrogenism. There is also enhanced peripheral aromatisation of androgens into oestrogens. An increased production of oestradiol from oestrone in extraovarian tissues, combined with decreased SHBG levels, results in increased quantities of biologically available oestradiol. These factors contribute to a state of functional hyperoestrogenism in PCOS patients.[43] Therefore, it is not intuitive that, in women with PCOS, there would be any difference in BMD compared with women with regular menstrual cycles.

GROWTH AND DIFFERENTIATION FACTORS AND OSTEOPROTEGERIN IN PCOS

Growth and differentiation factors (GDFs) belong to the transforming growth factor-β superfamily. GDF-9, known to be secreted by oocytes in human primary follicles, is essential for normal folliculogenesis. GDF-9 enhances pre-antral follicle growth by upregulating production of androgens by theca cells, and promotes follicular survival during this early stage by suppressing apoptosis of granulosa cells and follicular atresia.[44] There is controversy regarding levels of GDF-9 expression in patients with PCOS. Some authors report no significant difference in either plasma GDF-9 levels during the early follicular phase or GDF-9 expression in oocytes between patients with PCOS and controls. In contrast, other studies show delayed and decreased expression of GDF-9 during the early follicular stage in ovarian tissues of patients with PCO or PCOS.[16,45-47] The expression of GDF-9 has been demonstrated to decrease greatly in animal models of hyperinsulinism, which is also a crucial factor in the development of PCOS.[48] A mutational analysis of the coding region of GDF-9 has revealed variants in GDF-9 to be in association with PCOS.[49] These genetic differences may contribute to the aberrant follicular development in PCOS. It is not known whether plasma GDF-9 concentrations correlate with GDF-9 production in the ovaries because non-ovarian expression of GDF-9 mRNA has been reported in various tissues.[50] A lack of correlation between GDF-9 and bone markers in PCOS patients has been demonstrated by Berberoglu et al.[16]

GDF-15 is a stress-induced cytokine that provides prognostic information about cardiovascular events (CVEs) and mortality.[51] In addition, a study found that GDF-15 significantly promoted osteoclastic differentiation in a concentration-dependent manner following its secretion from adjacent osteocytes during disuse and/or ischaemia in bone.[52] In accordance, GDF-15 concentrations in women with PCOS were reported to be negatively correlated with osteocalcin and positively correlated with deoxypyridinoline levels in a recent study.[16] Plasma GDF-15 levels, however, were similar in PCOS, IH, and control groups, and were not correlated with testosterone and dehydroepiandrosterone sulphate concentrations. The authors have suggested that osteoblast-derived osteocalcin and GDF-15 have endocrine functions that affect glucose homeostasis.[16] Furthermore, they postulate that the reciprocal regulatory effects of GDF-15 and osteocalcin on either bone or energy metabolism makes its effect on BMD difficult to investigate.

Osteoprotegerin functions as a soluble decoy receptor and inhibitor for RANKL. It competes with RANK for RANKL binding and consequently inhibits osteoclastogenesis. Recent studies report decreased or unchanged osteoprotegerin levels in PCOS patients versus controls.[53,54] However, it must be noted that neither serum RANKL levels nor the serum RANKL/osteoprotegerin molar ratio were different in PCOS patients compared with the non-hyperandrogenic controls.[53] Osteoprotegerin levels have also been positively associated with vitamin D, BMD, and testosterone,[54] and are an independent predictor of CVEs, which makes their impact on BMD difficult to study.[55]

VITAMIN D AND PCOS

Vitamin D deficiency that adversely affects bone mineralisation, bone remodelling, and BMD is common in women with PCOS.[56] Supporting an association between vitamin D deficiency and PCOS, parathyroid hormone levels were increased in some studies, although this could not be reproduced in others.[9,57,58] It seems, however, that the prevalence of vitamin D deficiency is similar in women with and without PCOS.[56] It is possible that the high prevalence of vitamin D deficiency in PCOS is related to obesity. Previous reports established an inverse relationship between BMI, insulin, IR, and vitamin D status.[57,58] However, BMD is preserved despite decreased 25-hydroxyvitamin D levels. This may be related, at least in part, to serum 1,25-dihydroxyvitamin D concentrations in women with PCOS, which appear to be maintained at levels comparable to those observed in controls. This active vitamin D stimulates activity of aromatase in osteoblasts.[59]

CONCLUSION

Strong data on BMD and fracture risk in PCOS are lacking. BMD and fracture risk were, however, not increased in the studies. The relationship between BMD and the androgen:oestrogen equilibrium in patients with PCOS with menstrual cyclicity offers important insights into the potential synergies between oestrogens and androgens in protecting against osteoporosis. In contrast, the absence of any notable differences in BMD and bone turnover markers between patients with PCOS and healthy controls suggests that any direct effects of androgens on bone formation may be limited. The diverse components of the syndrome may influence bone mass through interrelated metabolic events not necessarily mediated by androgens. Understanding this crossregulation between bone and energy metabolism may offer a novel endocrine perspective on bone metabolism in PCOS. Additional long-term, prospective studies of BMD in PCOS patients and data on fracture risk in postmenopausal women with PCOS are required.

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VARICOCELE: A REVIEW

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ABSTRACT

The link between varicocele and infertility was first reported by Celsius in the first century AD, but it was not widely acknowledged until Tulloch and colleagues reported the improvement of sperm parameters in 26 of 30 patients undergoing varicocelectomy. The World Health Organization also reported that varicocele was present in 25% of patients with abnormal sperm parameters and 12% of patients with normal sperm parameters. Varicocele is the most frequently encountered surgical disease causing male infertility.

Keywords: Male infertility, varicocele, varicocelectomy.

INTRODUCTION

The link between varicocele and infertility was first reported by Celsius in the first century AD.[1] A varicocele was described as a 'bag of worms' by Dubin and Amelar in 1970,[2] although a more scientific definition would be: an abnormal venous dilatation of the pampiniform plexus caused by various aetiologies. The first usage of the word 'varicocele' was by Curling in 1843.[3] Varicocele remained out of the spotlight until Tulloch and colleagues[4] reported the improvement of sperm parameters in 26 of 30 patients undergoing varicocelectomy. The World Health Organization reported that varicocele is present in 25% of patients with abnormal sperm parameters and 12% of patients with normal sperm parameters.[5] The condition is commonly encountered in the left testicular side: 90% of cases are isolated in the left side and only 2% are isolated in the right side; this phenomenon can be explained by the venous anatomy.[6]

AETIOLOGY

There are three commonly accepted theories:

- Differences between left and right testicular venous drainage anatomy: The left internal spermatic vein has about 8-10 mm H_2O higher blood pressure and relatively slower blood flow compared with the right side. This is mainly caused by the drainage of the left testicular vein to the left renal vein with a perpendicular angle, whereas in the right testicular vein the drainage is to the vena cava at a steeper angle. This causes less cranial venous drainage.
- Venous reflux: Reflux to the pampiniform plexus is caused by the lack of valves in the internal spermatic vein and/or the reflux caused by venous collateral flow. The lack of valves in the junction of the left renal and internal spermatic veins was shown by Shiraishi and Naito.[7] Braedel and colleagues[6] demonstrated a lack of venous valves in 73% of 659 male patients with varicocele.
- Partial obstruction of testicular veins: This phenomenon, called 'the nutcracker phenomenon,' occurs when a specific vein is compressed by arteries. In proximal type, the left renal vein is compressed by the aorta and the superior mesenteric artery. In distal type, the left common iliac artery compresses the left common iliac vein.

PATHOPHYSIOLOGY

The underlying pathophysiology can be summarised in the following hypotheses. It is

proposed that varicocele is associated with increased testicular blood flow; Grasso Laenza and colleagues[8] reported increased blood flow in patients with varicocele compared with healthy controls in colour Doppler studies. An increase in interstitial fluid was described in animal models of varicocele formation,[9] with the increased number of leukocytes potentially attributable to this increase. In addition, a higher testicular temperature may inhibit spermatogenesis. In both animal models and humans, an increased intra and intertesticular temperature was shown in the presence of varicocele.[10,11] Increased venous pressure caused by reflux may contribute to decreased testicular flow,[12] while Tek et al.[13] demonstrated a higher apoptotic index in patients with varicocele (0.693 versus 0.206; $p<0.05$). Varicocele also causes impairment of the blood—testis barrier,[14] which leads to the formation of anti-sperm antibodies.[15]

DIAGNOSIS

Diagnosis is made using physical examination. Physical examination should be completed in both sitting and standing positions inside a warm and comfortable room. The scrotum should be inspected first and then palpated to evaluate spermatic cord and testes. The diagnosis is finally made by observing and/or palpating a dilated or tortuous segment.[16] Varicocele can be classified as one of three grades:

Grade 1: Dilated veins are palpable only with Valsalva manoeuver.

Grade 2: Dilated veins are seen whilst the patient is standing but not without Valsalva manoeuver, the physician can palpate dilated veins.

Grade 3: Tortuous and dilated veins can be seen in scrotum skin.

Radiology can assist in diagnosis: scrotal ultrasonography (USG) is not indicated for diagnosis of varicocele, but can be helpful if the diagnosis is inconclusive, such as in patients with morbid obesity, previous scrotal surgery, or accompanying ipsilateral hydrocele. The spermatic cord and epididymis can be evaluated with conventional USG. The assessment of testicular volume can also be helpful for indicating a need for intervention. Nowadays, because of the wide usage of Doppler USG and the subjectivity of physical examination, Doppler USG has become a standard diagnostic tool. It is recommended that patients should have at least one diagnostic imaging modality combined with physical examination. Through the use of Doppler USG, blood flow to the testes and reflux can be evaluated. Hirsh et al.[17] graded varicocele according to the reflux observed in Doppler USG:

Grade 1: No spontaneous venous reflux.

Grade 2: Intermittent spontaneous venous reflux.

Grade 3: Continuous spontaneous venous reflux.

However, an objective grading system is lacking and a validated Doppler grading system is needed. The most sensitive test for diagnosing varicocele is venography, but, due to its invasiveness and relatively high cost, it is no longer recommended unless a therapeutic occlusion is planned. The success rate of the occlusion procedure is as high as 85% for recurrent varicoceles.[18] Scrotal thermography is another tool for diagnosis although it is not a widely used method, mainly due to the widespread use of Doppler USG. This method can be used to document elevated temperature of the scrotum due to varicocele.

TREATMENT INDICATIONS

Guidelines from the European Association of Urology[19] recommend treatment in the case of clinical varicocele, oligozoospermia, infertility lasting longer than 2 years, and unexplained infertility. The American Urological Association recommends treatment when the following criteria are met: palpable varicocele on physical examination (may be validated via ultrasound), known infertility, normal female fertility potential, and abnormal semen parameters.[20]

TREATMENT OPTIONS

The main aim of treatment is to disrupt internal spermatic venous drainage whilst preserving arterial and lymphatic blood flow. There are several defined methods, the most widely used of which are the inguinal,[21] subinguinal, and microscopic subinguinal approaches. Although not very commonly used, retroperitoneal,[22] scrotal,[23] and laparoscopic approaches[24] have been described. Furthermore, a minimally invasive method, angiography, and embolisation for internal spermatic veins, have also been defined.[25]

TREATMENT OUTCOMES

In 2009, a meta-analysis suggested that varicocele repair did not improve the likelihood of conception.[26] After the meta-analysis was published, it was greatly criticised for heterogeneity among enrollment and having normal semen parameters of patients suffering from infertility.[27-29] Furthermore, the study included one of the most debatable topics in urology, the subclinical varicocele, which will be discussed in more detail below. In contrast to this meta-analysis, several papers published in subsequent years advocated the use of varicocelectomy to improve sperm parameters and reported improvement of sperm parameters and paternity rates.[30]

Subinguinal varicocelectomy can yield high improvement rates: 75.2% improvement in semen parameters and 45.5% in pregnancy rates.[31] Abdel-Meguid et al.[30] treated patients with varicocelectomy and followed their progress. They revealed a significant improvement in the treatment arm of the study, and a 3.04-times higher rate of spontaneous pregnancy.[30] There is a debate as to whether varicocele repair improves pregnancy rates. Nilsson et al.[32] reported the first comparative study of varicocele repair and did not reveal a significant difference in pregnancy rates among patients treated with varicocele repair. Krause and colleagues[33] conducted a study of 65 men treated with sclerotherapy and revealed no significant improvement compared with those who received no treatment. However, both studies have been criticised for low cohort size and poor follow-up.

On the other hand, Okuyama et al.[34] compared patients treated using Palomo repair with those who received no treatment and revealed significantly higher pregnancy rates in the treatment arm (30% versus 18%; $p<0.05$). Schlegel[35] wrote a review on varicocele treatment outcomes and revealed a 33% pregnancy rate in patients with varicocele repair compared with 16% in the no-treatment group. Varicocele can cause scrotal pain and some surgeons prefer surgery to treat this painful disease. In several studies pain was shown to be resolved with conservative treatment (e.g. oral anti-inflammatory medicine and limiting physical activity) in 5-15% of patients.[36,37] Several studies reported success rates between 75-91%.[38-44]

PROGNOSTIC FACTORS

Not all patients improve after a successful varicocelectomy. Patients with a disease history <2 years and who have high-grade varicoceles are more likely to improve following varicocelectomy. Patients with a total motile sperm count <5 million, who have genetic disorders, altered follicle-stimulating hormone (FSH)/testosterone levels, small testis volume, or concomitant metabolic syndrome have worse treatment outcomes.[44-46]

COMPLICATIONS

Recurrence rates can be as high as 29% with high ligations and about 2% with microscopic techniques. When optical enhancement is not an option, hydrocele may be observed in 7-10% of cases following varicocelectomy. Following the improvement of the technique with microscopy, one of the most feared complications, testicular atrophy, is encountered at a rate of only 1%. Ilioinguinal nerve damage may be seen with the loss of sensation in the scrotum and inner thigh. Haematoma and infections may be encountered in about 2-5% of cases.[46,47]

Patients with Non-Obstructive Azoospermia

The need for varicocelectomy in patients with non-obstructive azoospermia is debatable. Palpable varicocele can be found in approximately 4-14% of cases.[48] Even after varicocele repair, most of these men will be subfertile.[48] One of the most comprehensive meta-analyses was conducted by Weedin and colleagues,[49] and stated that 39% of a total of 233 patients had motile sperm in semen analysis performed after successful varicocelectomy. They concluded that histopathological examination was a key factor in treatment success, with those with maturation arrest being the most likely to benefit from surgery, whereas Sertoli-cell-only syndrome had the worst outcome ($p<0.001$). Daitch et al.[50] proposed a higher rate of pregnancy following surgical repair. Esteves et al.[51] reported better outcomes following intracytoplasmic sperm injection therapy.

Patients with Subclinical Varicocele

Subclinical varicocele is defined as varicocele that is only diagnosed radiologically. Several investigators have conducted trials concerning treatment of subclinical varicocele and do not recommend treatment.[28,29,52] Both the European

and American Urological Associations do not recommend treatment of subclinical varicocele.[20]

Paediatric and Adolescent Varicocele

Indications for treatment are: varicocele associated with small testis, additional testicular condition affecting fertility, bilateral palpable varicocele, pathological sperm quality, and symptomatic varicocele.[53] Çayan and colleagues[54] compared studies of paediatric varicocelectomy and proposed that a 10% volume difference in the affected testis should be the indication for surgical repair; they reported that 53% of patients gained normal sperm volume following the operation before 14 years of age. With the subinguinal microscopic approach, recurrence rates are usually <10%.[55-57]

KEY MESSAGES

- The WHO reported that varicocele was present in 25% of patients with abnormal sperm parameters and 12% of patients with normal sperm parameters.
- Diagnosis is made using physical examination. Nowadays, because of the wide usage of Doppler USG and subjectivity of physical examination, Doppler USG has become a standard diagnostic tool. It is recommended that patients should have at least one diagnostic imaging modality combined with physical examination.
- The main aim of treatment is to disrupt internal spermatic venous drainage while preserving arterial and lymphatic blood flow. There are several methods defined, with the most widely used being the inguinal, subinguinal, and microscopic subinguinal approaches.
- Patients with a disease history <2 years and with high-grade varicoceles are more likely to improve following varicocelectomy.
- Not all patients improve after a successful varicocelectomy. Patients with a total motile sperm count <5 million, who have genetic disorders, altered FSH/testosterone levels, small testis volume, or concomitant metabolic syndrome have worse treatment outcomes.
- Both European and American Urological Associations do not recommend the treatment of subclinical varicocele.
- Following the improvement of the technique with microscopy, one of the most feared complications, testicular atrophy, is encountered at a rate of only 1%.
- Several papers advocate for varicocelectomy in improving sperm parameters, and report improvement of sperm parameters and paternity rates.

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VARICOCELE IN MALE INFERTILITY: CURRENT STATUS OF SURGERY TECHNIQUES

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ABSTRACT

Varicocele is the most common cause of male infertility and is generally correctable, or at least improvable, through various surgical techniques. Although several different techniques for varicocele repair have been described in the literature, microsurgical subinguinal varicocelectomy is recognised as the gold-standard approach for varicocelectomy due to high success rates with minimal complications. This article reviews the current status of the effects of varicocelectomy techniques on male infertility and the recurrence and complication rates associated with these techniques.

Keywords: Varicocele, varicocelectomy, microsurgical varicocelectomy, laparoscopic varicocelectomy, open varicocelectomy.

INTRODUCTION

Varicocele is the abnormal dilatation of the pampiniform plexus due to the inversion of venous blood flow within spermatic veins.[1] Although the cause of varicocele is multifactorial, the most popular mechanism today is increased abdominal pressure during childhood and early adolescence.[2] Pathological dilatation of the veins that drain the testicles leads to increased temperature in the seminiferous tubules and decreased sperm quality.[3] The most common clinical symptom of varicocele is male infertility, and less commonly testicular pain or palpable mass.[4]

Varicocele is the most frequent cause of male infertility. This condition can be detected in 19-41% of patients with primary infertility and 45-81% of those with secondary infertility.[5] Some researchers have hypothesised that impaired venous drainage causes an increase in venous stasis and a decrease in arterial blood flow, thus inducing hypoxia and deficiency in testicular microcirculation. Also, it is thought that this hypoxia could be responsible for defective energy metabolism at the mitochondrial level, which causes dysfunction of both Leydig and germinal cells.[6,7] Other researchers have suggested that varicocele is associated with increased sperm DNA damage, and that this sperm pathology may be secondary to varicocele-mediated oxidative stress. Varicocelectomy can reverse this sperm DNA damage. However, the exact pathophysiology of varicocele remains unknown.[8,9]

Accepted indications for the treatment of varicocele are men with infertility and scrotal pain or men with discomfort.[10] Scrotal pain is generally treated with conservative methods such as scrotal support, limited physical activity, and anti-inflammatory drugs, but this has been met with poor resolution rates and a surgical approach is rarely performed. Varicocelectomy is frequently performed for infertility due to varicocele.[11] Guidelines relating to varicoceles and infertility have been put forth by the American Urological Association,[12] and more recently by the American Society for Reproductive Medicine.[13] Both reports recommend varicocele repair in cases of clinically palpable varicocele with documented infertility, one or more abnormal semen parameter, and in the setting of normal or potentially correctable female fertility. The duration of infertility also seems to be important. In a recent study it was shown that couples with infertility of >2 years duration had a significantly higher pregnancy rate after varicocelectomy compared with couples with an

untreated varicocele. In couples with a shorter duration of infertility, such a difference was not observed.[14] In men with subclinical varicocele or normal semen analysis, the treatment of varicocele has not been recommended because its effectiveness has not been shown.[12] The aim of this review is to present the current status of the effects of varicocelectomy techniques on male infertility, as well as their recurrence and complication rates.

TREATMENT OPTIONS FOR VARICOCELE

The basis of varicocele treatment is the blockade of the internal spermatic venous drainage of the testicle while preserving the internal spermatic artery, the vasal and deferential vessels, and the spermatic cord lymphatics.[10] Various techniques have been introduced and practised for varicocele repair. These techniques can largely be classified into two categories: surgical and radiological approaches. Radiological treatment has been used as an alternative to surgical repair with the aim of less invasiveness and better opportunity to control the small collaterals that may not be detected during surgery. The modalities of the radiological approach are retrograde embolisation or sclerotherapy and antegrade sclerotherapy.[11]

VARICOCELECTOMY TECHNIQUES

A number of different techniques have been described to treat varicocele. The type of intervention chosen depends mainly on the experience of the surgeon.[15] Complications of varicocele repair include hydrocele formation, recurrence of the varicocele, and, rarely, testicular atrophy. The rates of recurrence, complications, and pregnancy that are associated with open, microsurgical, and laparoscopic varicocelectomy techniques are summarised in Table 1[16-22] and Table 2.[23]

Open Retroperitoneal, Inguinal, or Scrotal Varicocelectomy

The aim of open surgical techniques is the ligation of the internal spermatic vein superior to the internal ring. A number of different localisations have been described for open varicocelectomy. These include:[1] retroperitoneal (high) ligation of the testicular artery and vein above the internal inguinal ring (Palomo technique);[2,24] high ligation of the vein while sparing the artery (Bernardi technique);[3,25] and ligation of the cremasteric and internal spermatic veins as they travel within the inguinal canal as structures of the spermaticcord (Ivanissevich technique).[26]

Table 1: The rates of recurrence of varicocele, formation of hydrocele, and pregnancy in comparative studies of varicocelectomy (open, laparoscopic, and microsurgical techniques).

Study	Surgical technique (n)	Recurrence, %	Hydrocele, %	Pregnancy, %
Cayan et al.[16]	Palomo (232) Microsurgical high inguinal (236)	15.51 2.11	9.09 0.69	33.7 42.8
Bebars et al.[17]	Palomo (65) Laparoscopic (128)	10.8 3.9	4.6 2.3	43 51
Ghanem et al.[18]	Palomo (109) Microsurgical subinguinal (304)	7 0	6.4 1.6	N/A N/A
Watanabe et al.[19]	Palomo (50) Microsurgical subinguinal (61) Laparoscopic (33)	12 0 6.1	10 0 3.03	35.8 50.9 40.4
Al-Kandari et al.[20]	Open inguinal (40) Microsurgical subinguinal (40) Laparoscopic (40)	17.5 2.5 22.5	17.5 0 25	28 40 30
Al-Said et al.[21]	Open inguinal (92) Microsurgical subinguinal (112) Laparoscopic (94)	17.4 3.6 26.6	4.3 0 8.5	31 38 33
Abdel-Maguid, Othman[22]	Open subinguinal (80) Microsurgical subinguinal (82)	11.3 0	8.7 1.2	21.2 37.8

N/A: not available.

Table 2: Rates of pregnancy, recurrence of varicocele, and formation of hydrocele associated with different surgical techniques, arranged according to descending order of pregnancy rate.[23]

Surgical technique	No. of studies analysed	Pregnancy rate (range), %	Recurrence of varicocele (range), %	Formation of hydrocele (range), %
Microsurgical subinguinal	13	44.75 (33.8-51.5)	2.07 (1.4-14.8)	0.72 (0.3-1.6)
Microsurgical inguinal	6	41.78 (40.8-42.8)	9.47 (0.7-15.2)	0.29 (0.0-0.7)
Palomo	4	34.21 (33.5-36.0)	12.5 (7.3-15.5)	7.58 (4.6-9.0)
Inguinal	6	30.06 (20.0-31.5)	15.65 (3.57-17.5)	7.47 (4.3-17.5)
Laparoscopic	9	27.53 (13.1-40.0)	11.11 (4.0-26.5)	7.57 (1.7-12.7)

Table 3: The rates of recurrence of varicocele, formation of hydrocele, and pregnancy in studies comparing microsurgical inguinal and subinguinal varicocelectomy.

Study	Microsurgical technique (n)	Recurrence, %	Hydrocele, %	Pregnancy, %
Gontero et al.[31]	Inguinal (50) Subinguinal (45)	8.0 4.9	0 0	48.9 50.0
Orhan et al.[32]	Inguinal (147) Subinguinal (65)	0.68 3	0 0	41 33

Several studies have shown that, although the recurrence and complication rates of open varicocelectomy techniques were higher than those of the microsurgical techniques, the pregnancy rates of the open techniques were lower (Table 1). Cayan et al.[16] compared the Palomo technique (n=232) with microsurgical high inguinal varicocelectomy (n=236) in patients with primary infertility and abnormal semen analysis. They noticed that the recurrence rates of the Palomo and microsurgical techniques were 15.5% and 2.1%, respectively. Hydrocele formation rates of the Palomo and microsurgical techniques were found to be 9.0% and 0.6%, respectively. Although the complication and recurrence rates of the Palomo technique were higher than those of microsurgical techniques, the pregnancy rate of the Palomo technique was lower than that of the microsurgical (33.7% and 42.8%, respectively). Abdel-Maguid and Othman[22] compared open subinguinal (n=80) and microsurgical subinguinal (n=82) techniques. They reported that the rates of varicocele recurrence and formation of hydrocele with open varicocelectomy were found to be higher than with the microsurgical technique (11.3%, 8.7% and 0%, 1.2%, respectively). None of the patients who underwent microsurgical subinguinal varicocelectomy had a recurrence of varicocele. The rates of pregnancy in the open and microsurgical group were found to be 21.2% and 37.8%, respectively. They also noticed that postoperative mean sperm count and motility improved significantly in both groups: 42.7% and 67.1% of the microsurgical subinguinal varicocelectomy group and 23.7% and 33.8% of the open subinguinal varicocelectomy group showed a ≥50% improvement in sperm count and motility, respectively, after 1 year. The results of these two studies were similar to other studies that compared open and microsurgical varicocelectomy.[18-22]

Microsurgical Inguinal or Subinguinal Varicocelectomy

The microsurgical approach to varicocelectomy has become a popular treatment because it identifies small spermatic veins, the testicular artery, and lymphatics more effectively, thus substantially decreasing recurrence and complication rates.[6] In three randomised controlled studies comparing open, laparoscopic, and microsurgical techniques, the lowest rates of recurrence and hydrocele formation were found in the patients who underwent a microsurgical technique. Although two[19,21] of these studies included only patients with infertility and abnormal semen analysis, the other study[20] also included patients with pain and normal semen analysis. Watanabe et al.[19] noted that the recurrence rates associated with the Palomo (n=50), laparoscopic (n=33), and microsurgical (n=61) approaches were 12%, 6.1%,

and 0%, respectively; the rates of hydrocele formation were: 10%, 3%, and 0%, respectively. The authors found that the pregnancy rates associated with the Palomo, laparoscopic, and microsurgical techniques were 35.8%, 40.4%, and 50.9%, respectively. Al-Kandari et al.[20] found that the recurrence rates in the open (n=40), laparoscopic (n=40), and microsurgical (n=40) varicocelectomy groups were 17.5%, 22.5%, and 2.5%, respectively; the rates of hydrocele formation were: 17.5%, 25%, and 0%, respectively. The authors also reported that the pregnancy rates of open, laparoscopic, and microsurgical techniques were 28%, 30%, and 40%, respectively. Al-Said et al.[21] reported that the recurrence rates of open (n=92), laparoscopic (n=94), and microsurgical (n=112) approaches were 17.4%, 26.6%, and 3.6%, and that the rates of hydrocele formation were 4.3%, 8.5%, and 0%, respectively. The pregnancy rates after open, laparoscopic, and microsurgical techniques were found to be 31%, 33%, and 38%, respectively.

The most current microsurgical approaches are subinguinal[27] and inguinal varicocelectomy.[28] Microsurgical subinguinal varicocelectomy was introduced by Marmar et al.[29] in 1985, and was then modified by Goldstein et al.[30] in 1992. Since then it has become the gold-standard technique in adults.[2] In studies comparing subinguinal and inguinal techniques, although the rates of pregnancy were similar, the rates of recurrence were discordant (Table 3).[31,32] Gontero et al.[31] noted that the rates of recurrence after inguinal (n=50) and subinguinal (n=45) microsurgical varicocelectomy were 8.0% and 4.9%, respectively. The pregnancy rates of the inguinal and subinguinal group were found to be 48.9% and 50.0%, respectively. Conversely, Orhan et al.[32] found that the rate of recurrence in the inguinal (n=147) microsurgical group was lower than in the subinguinal (n=65) group (0.6% and 3.0%, respectively). The pregnancy rates of the inguinal and subinguinal group in this study were 41% and 33%, respectively.

Laparoscopic Varicocelectomy

The laparoscopic transperitoneal Palomo varicocelectomy was introduced in the early 1990s.[33] Since then it has gained wide acceptance as a safe, simple, and minimally invasive procedure in both adults and children.[34] Earlier studies reported that, although the rates of hydrocele formation and recurrence of varicocele in the laparoscopic approach were lower than in open varicocelectomy, the rate of pregnancy in laparoscopic varicocelectomy was higher than in open varicocelectomy.[17,19] However, the results of ensuing studies were incompatible with the results of these two studies. Al-Kandari et al.[20] noticed that the rates of recurrence and hydrocele in laparoscopic varicocelectomy were 22.5% and 25.0%, respectively. These rates in open varicocelectomy were only 17.5% and 17.5%, respectively. Al-Said et al.[21] reported that the rates of recurrence and hydrocele in the laparoscopic approach were higher than in the open approach. In these two studies, the rates of pregnancy in laparoscopic and open varicocelectomy were found to be similar.

VARICOCELECTOMY IN ADOLESCENTS

Varicocele is not common in children: in adolescents the prevalence ranges from 13.7-16.2%.[35] However, it is believed that the population of boys with varicoceles represents the same population of adults with varicoceles. Varicocele progressively affects the testis, resulting in atrophy and abnormal semen.[36] The recommended indications for varicocele repair in children and adolescents are:[1,37] varicocele associated with a significantly small ipsilateral testis;[2] additional testicular conditions affecting fertility;[3] bilateral palpable varicoceles;[4] pathological sperm quality (in older adolescents);[5] varicocele associated with a supranormal hormone response to the gonadotropin-releasing hormone stimulation test;[6] symptomatic varicocele (i.e. causing physical discomfort).

Microsurgical subinguinal varicocelectomy was undertaken in adolescents by Lemack et al.[2] in 1998, and is now increasingly applied in children and adolescents. Many studies have described significant improvement in semen quality after varicocelectomy in both adults and adolescents.[36,38,39] However, it must be considered that 50-80% of male patients with a varicocele never have problems with fertility.[40] Therefore, further studies comparing observation with surgical intervention are needed to refine the current indications for varicocele repair in the adolescent male.

CONCLUSION

Varicocele is the most common identifiable and treatable cause of male infertility. Varicocelectomy should be considered in the case of a clinical

varicocele, oligospermia, infertility duration of >2 years, and otherwise unexplained infertility in the couple. Several varicocelectomy approaches that differ according to surgical technique (such as open, laparoscopic, and microsurgical) and localisation (such as high inguinal, inguinal, and subinguinal) are available for treatment of varicocele. Current evidence indicates that microsurgical varicocelectomy is the most effective and least morbid method among the varicocelectomy techniques. Many studies have shown that the optimal surgery technique for the treatment of varicocele is the microsurgical approach. The complication and recurrence rates of microsurgical varicocelectomy are lower than those of both laparoscopic and open varicocelectomy and the pregnancy rate is higher than that following the other techniques. Although laparoscopic varicocelectomy is feasible, it must be justified in terms of cost-effectiveness.

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SPERM ANEUPLOIDY AND DNA INTEGRITY: A REVIEW

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ABSTRACT

Male factors leading to infertility account for at least half of all cases of infertility worldwide. The purpose of this review is to highlight the importance of sperm DNA integrity. A systematic literature search was performed up to January 2015 in order to determine the impact of sperm DNA integrity and of the techniques used to determine it. Only articles presenting sperm aneuploidy together with DNA fragmentation studies are discussed. We also discuss several causes and risk factors that have been identified as having detrimental effects on sperm genetic integrity. Aneuploidy and sperm DNA fragmentation (sDNAfrag) analyses show promising results in determining the sperm genetic status. However, more studies must be performed to develop a technique that can simultaneously verify the sperm DNA integrity and haploidy before introduction into routine clinical practice. Once sperm is subjected to the current technologies it cannot be immediately used in assisted reproduction treatments. However, recent studies have shown that an improved protocol of sperm selection can result in sperm with very low levels of sDNAfrag, rendering the risk of selection low.

Keywords: Sperm aneuploidy, sperm DNA integrity.

INTRODUCTION

The infertility onus has been increasing over recent decades and it is estimated to affect 15% of couples worldwide.[1] There is conspicuous evidence that male partners account for the aetiology of half the cases. The evaluation of male infertility is based on routine semen analysis, which measures both semen production and sperm quality. However, normal values of these parameters do not accurately mirror the fertilisation capability of the sperm. Moreover, there are numerous known causes of male infertility that this analysis provides no information about. One certain factor influencing male fertility is the integrity of sperm DNA.[2] Increasing concern regarding the transmission of genetic diseases through intracytoplasmic sperm injection (ICSI)[3] has spurred investigations into the genomic integrity of the male gamete. Possible sperm nuclear alterations include: A) abnormal chromatin structure; B) chromosomes with microdeletions; C) aneuploidies; and D) DNA strand breaks.[4]

Sperm DNA integrity is not evaluated routinely in an assisted reproduction technology (ART) laboratory, although it has been recognised as an important clinical parameter in infertile patients.[5] A variety of assays have been developed to measure sperm DNA fragmentation (sDNAfrag). While some approaches detect breaks in DNA strands, others assess the vulnerability of the DNA to denaturation.[6] Previous reports have indicated that natural pregnancy is impaired when increased levels of sDNAfrag are detected.[7,8] The threshold value is dependent on the technique used to determine sDNAfrag.[9,10] Nevertheless, it seems to have a prognostic value with regard to ART outcomes.[11,12] On the other hand, sperm aneuploidy has been evaluated by fluoresence *in situ* hybridisation (FISH) and is considered a major cause of pregnancy loss, aneuploid births, and developmental defects.[13] Recent reports

demonstrate a significant increase of the sperm aneuploidy rate in infertile men when compared with fertile counterparts, although this did not exceed 2% with regard to chromosomes X, Y, 18, and 21.[14,15] Due to the importance of these two parameters, sDNAfrag and aneuploidy, this review will focus on those studies that have evaluated both parameters within the same biological samples.

Controversial data have been obtained but, overall, studies provide clear evidence for a significant increase in both the rate of chromosome aneuploidy and the percentage of sDNAfrag in infertile patients when compared with fertile donors, as well as a positive correlation between the two parameters. Increases in both parameters have been reported in infertile patients with abnormal semen analysis results. Patients with low sperm counts (oligozoospermic) display a higher frequency of sperm aneuploidies and higher percentage of sDNAfrag,[16] although these are even higher in patients presenting with severe testicular damage[17] and in oligoasthenozoospermic men.[18]

Concerning morphology, teratozoospermic patients show an increase in sperm chromosome aneuploidy and sDNAfrag.[19-21] Rare sperm morphological alterations that affect <1% of infertile male patients have also been studied. Patients with the severe aberration of macrocephalic—multi-flagellated sperm syndrome exhibit high rates of aneuploidy.[22-25] Another rare condition termed globozoospermia, which is characterised by a lack of the acrosomal vesicle and associated structures, has returned conflicting results: some researchers did not find a correlation between globozoospermia and aneuploidy although they observed elevated percentages of sDNAfrag,[26,27] whereas others have demonstrated that globozoospermia is associated with increased sDNAfrag levels and increased frequencies of sex chromosome aneuploidy.[22,28-31] Increases in sperm aneuploidy and sDNAfrag were also reported for sperm with large-head vacuoles,[32] although others observed no clear link between these sperm features and this particular sperm abnormality.[33] The same observations were found in patients with severe-to-total asthenozoospermia presenting with dysplasia of the fibrous sheath and head abnormalities; these sperm showed high levels of aneuploidy and sDNAfrag.[34] It therefore seems that sperm aneuploidy and sDNAfrag rates are increased in infertile men with multiple morphological anomalies, regardless of the type of teratozoospermia.[19,21-23,25] In addition, increased sperm aneuploidy rates were observed in sperm with higher sDNAfrag in patients with each of the three major types of altered semen analysis values (count, morphology, and motility).[35]

Males of couples presenting with recurrent spontaneous abortion have also been evaluated for sperm aneuploidy and sDNAfrag, with studies showing increases in both parameters and sDNAfrag being significantly correlated with the percentage of sperm aneuploidy.[36] However, even though significant differences have been shown, there is no consensus regarding the correlation between these parameters and miscarriage[37] or their correlation with embryo chromosomal anomalies in these couples.[38] Unfortunately, patients showing significantly increased sperm aneuploidy rates were excluded from the embryo aneuploidy study.[38] Transmission of a damaged sperm cell to the oocyte has its risks and thus more studies in patients presenting with recurrent miscarriage or implantation failure should be performed and enlarged.

ICSI has become a powerful technique in overcoming male infertility. However, selection of the spermatozoon is necessary prior to the technique being attempted. Driven by the objective of improving embryological and clinical outcomes, non-invasive methods were developed to select for clinical use those sperm that are free of DNA damage. As the selection of sperm continues to be performed based on morphology and motility, sperm assortment under high magnification can improve this selection[39] and has also been shown to decrease sperm aneuploidy and sDNAfrag levels,[17] with this decrease being accentuated with hyaluronic acid treatment.[40]

In addition to the classic sperm preparation techniques, approaches such as magnetic-activated cell sorting (MACS) have also been applied.[41] Using semen from normozoospermic men, it has been demonstrated that density gradient centrifugation (DGC) and MACS can effectively decrease sperm aneuploidy rates and sDNAfrag levels. This study also showed that the decrease of both parameters was correlated, and that the decrease after MACS was more apparent.[42] Using MACS followed by DGC and swim-up can substantially reduce sDNAfrag levels, which was also true in cases with abnormal morphology, motility, vitality, and membrane integrity.[41] These results reinforce the need for more studies to be conducted in patients with altered semen parameters.

INFLUENCE OF CHROMOSOMAL ABNORMALITIES

Spermatogenesis is a complex biological process that can be influenced by chromosomal abnormalities. Although some of these anomalies impair spermatogenesis apparently due to germ cell degeneration,[43] others may license the achievement of spermatogenesis with sperm production. However, the presence of structural or numerical chromosomal abnormalities can interfere with normal spermatogenesis and cause male infertility due to abnormal conventional sperm parameters. Moreover, these patients may be at a higher risk of transmitting a chromosomal anomaly to their progeny. Due to this augmented jeopardy, and since conventional semen parameters do not provide information about the nuclear status of sperm, several studies in carriers of chromosomal abnormalities were performed in order to assess the male gamete risk of aneuploidy and DNA fragmentation. We have summarised the different chromosomal anomalies and their consequences on sperm aneuploidy and DNA fragmentation in Table 1.

Table 1: Effects of different chromosomal anomalies on sperm aneuploidy and DNA fragmentation.

Chromosomal anomaly	Carrier	Sample type	Effects on sperm aneuploidy and DNA fragmentation	Reference
47,XYY	Extreme OAT	Testicular biopsy	Two-thirds of the cells with an XYY constitution Aneuploidy rate (FISH): • 3.93% in round and elongated spermatids • 0.91% in late spermatids and spermatozoa High rate of germ cell degeneration High rates of DNA fragmentation (TUNEL) in spermatids and spermatozoa	44
9qh+++ polymorphism	Severe OAT	Ejaculate	Increased rates of disomy for Chr X, Chr Y, and Chr 18 (FISH) Increased DNA fragmentation (77.81%) (TUNEL)	45
t(7;8)(p12;p22) t(13;15)(q31;q26.2) t(6;8)(q27;q24.1) rob(13;14)(q10;q10)	Three reciprocal and one Robertsonian translocation	Ejaculate	Increased aneuploidy (FISH) Increased DNA fragmentation (TUNEL) 2-5-times higher proportion of spermatozoa with unbalanced Chr content and fragmented DNA than among those with normal balanced content	46
46,XY,t(3;6) (p24;p21.2),inv(8) (p11;2q21.2)	Normal spermiogram	Ejaculate	No difference regarding Chr aneuploidy rates (FISH) Increased DNA fragmentation (TUNEL)	47
46,XY	45 infertile men	Ejaculate	Chromosomally abnormal sperm cells more likely to display DNA fragmentation (SCDt) Lower sperm count and motility increased the percentage of chromosomally abnormal sperm (FISH)	48
46,XY,t(6;10;11) (q25.1;q24.3;q23.1)	Asthenozoospermic patient	Ejaculate	Five-fold increased level of aneuploidy of Chr 13, 15, 18, 21, 22, X, and Y (5.3-fold for disomy and 1.7-fold for diploidy) (FISH) No difference regarding DNA fragmentation (TUNEL)	49
Mosaicism 45,X and 47,XYY Mosaicism 45,X; 46,XY and 47,XYY	Normal spermiogram Teratozoospermia	Ejaculate	Significant increase in frequency of XY disomic and diploid spermatozoa (FISH) Significant increase in diploidy and autosomal aneuploidy (FISH)	50

Chr: chromosome; OAT: oligoasthenozoospermia; FISH: fluorescence *in situ* hybridisation; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling assay; SCDt: sperm chromatin dispersion test.

Previous studies evaluated sDNAfrag and sperm aneuploidy in spermatozoa of infertile patients with both numerical and structural chromosomal abnormalities. The majority of the studies reported an increased incidence of aneuploidy in sperm with fragmented DNA compared with those with intact DNA. In this regard, although infertile males with a chromosomal abnormality showed a significant association between sperm chromosome abnormalities and sDNAfrag, they also presented with a wide spectrum of detrimental effects on the male fertility status. The conflicting results obtained by different authors (Table 1) may be due to the fact that different and variable numbers of chromosomes and different sDNAfrag detection techniques were investigated in these studies. Moreover, the number of sperm cells evaluated may also explain the different results. Due to these conflicting results, there is a need to further investigate the relationship between meiotic segregation, DNA fragmentation, and conventional sperm parameters using a full panel of chromosomes and the same sDNAfrag detection technique. Moreover, there is still the need to confirm these results in a larger number of carrier patients. Because these patients have a low probability of being able to conceive naturally, they should be enrolled in genetic counselling programmes in order to reduce the risk of genetically abnormal offspring and be advised about prenatal diagnosis and preimplantation genetic diagnosis.

INFLUENCE OF ANEJACULATION

Male infertility may also be due to the inability to ejaculate semen; despite producing sperm, some men are not empowered to expel it. This sexual disorder is commonly designated as anejaculation and can be caused by psychological or physical factors, with the latter occurring due to neurogenic or obstructive reasons. With the main goal of retrieving sperm for artificial insemination, several treatment options for men with anejaculation are available.[51,52] Studies have shown that therapies for the treatment of anejaculation due to spinal cord injury, such as penile vibratory stimulation, produce a decreased sperm concentration,[53] an increase in sperm aneuploidy rates of approximately 1.5-2.4-times for chromosomes 13, 18, and 21, and about 2.2-2.4-times for chromosomes X and Y, as well as an increase in the rate of sDNAfrag.[53,54] Although the use of a minimally invasive percutaneous vasal sperm aspiration procedure in these patients has been shown to increase sperm motility, these patients still exhibited poorer semen quality and higher rates of sDNAfrag and sperm chromosomal aneuploidies when compared with healthy donors.[53,54] However, the effect on sDNAfrag may be overcome by the use of testicular sperm aspiration;[55] although the same is not true for aneuploidy.[52]

INFLUENCE OF AGE

Another factor shown to impact semen quality and sperm genetic integrity is the age of the male. Unlike women, male fertility varies from man to man and age is not a good predictor, as men may experience spermatogenesis for up to 95 years.[56] The introduction of ART and innovative medicines for erectile dysfunction allow paternity for elderly men. However, with increasing paternal age, the amount and motility of sperm cells decreases, testicular histological architecture deteriorates, and, as well as decreased fecundity, there may also be an increased risk of transmitting a heritable disease to progeny. A recent review reports evidence that male ageing after 40 years is associated with decreased sperm concentration, motility, and fecundity, and with an increase in sperm aneuploidy, sDNAfrag, sperm DNA mutations and epigenetic changes, altered pregnancy, and offspring prone to autosomal diseases and several neurocognitive disorders.[57] To investigate the impact of paternal ageing on sDNAfrag and sperm chromosomal abnormalities, several experiments have been conducted. Comparing testicular tissue and semen from 35 men aged 65-102 years, investigators observed a 1.29% increase in the aneuploidy rate of postmeiotic cells only when spermiogenesis was arrested, but no influence when complete. Furthermore, sDNAfrag did not seem to increase with age. Therefore, it was concluded that advanced male age does not represent any specific risk.[56] Another study in 97 non-smokers aged 22-80 years found no association between age and frequency of aneuploidy. Nevertheless, these authors disclosed a 5-fold increase in sDNAfrag, with a negative correlation to sperm motility and a positive correlation to sexual abstinence.[58] In a more recent report comparing 140 infertile patients aged 24-76 years with 50 men with proven fertility and aged 25-65 years, authors observed that male age did not affect sperm morphology, motility, sDNAfrag, or disomy. However, increasing male age was associated with a decrease in semen volume and sperm vitality,

and with an increase in sperm concentration and sperm diploidy.[21]

INFLUENCE OF EXTERNAL FACTORS

Male infertility may be innate for several reasons, but may also be acquired. The major risk factors associated with acquired male infertility include medical treatments and medicines, lifestyle habits, and environmental and occupational influences.[59] Several therapies also appear to play an important role in acquired male infertility due to their gonadal toxicity.[60] The threat of infertility due to these causes is related to the risk factor, amount, and time of exposure (Table 2). Dividing cells are the preferential target of these risk factors, which makes them very injurious to male spermatogenesis. Because mitosis of spermatogonia and meiosis of spermatocytes ensue during the course of adult life, these processes are susceptible to the permanent or temporary effects of these risk factors. Even though there is much evidence in the literature for the overall effect of these risk factors on the male reproductive function, only a few studies have looked into their possible simultaneous consequences on sperm aneuploidy and sDNAfrag; a summary of these studies can be found in Table 2.

Table 2: Adverse effects of various aneugenic agents with respect to sperm aneuploidy and DNA fragmentation.

Type of exposure	Aneugenic agent	Effects on sperm aneuploidy and DNA fragmentation	Study type	Reference
Occupational	Carbaryl	No relation to semen parameters although some not significant morphological defects Increased Chr X and Chr Y disomy, increased Chr 18 disomy, increased frequency of Chr X, Chr Y, and Chr 18 nullisomy (FISH) Increased sperm DNA fragmentation (TUNEL)	*In vivo*	61
	Ionising radiation	Decreased motility and viability, and increased morphological abnormalities No significant incidence of sperm aneuploidy (FISH) Increased sperm DNA fragmentation (TUNEL and SCSA) Increased hypermethylated sperm (immunodetection of 5-methylcytosine)	*In vivo*	62
Environmental	Seasonal air pollution with reactive polyaromatic hydrocarbons	Reduced sperm motility and normal morphology Increased risk of sperm aneuploidy (FISH) Increased DNA denaturation (SCSA)	*In vivo*	63
	Episodic air pollution	No relation to semen parameters No association with increased sperm aneuploidy (FISH) Increased sperm DNA fragmentation (SCSA)	*In vivo*	64
	Perfluorinated compounds	Alteration of sperm parameters Increased disomy and diploidy (FISH) Increased sperm DNA fragmentation (TUNEL)	*In vivo*	65
Medical treatment	Cancer and antineoplastic therapy (chemo and/or radiation)	Increased rate of structural and numerical Chr abnormalities Long-lasting-to-permanent sperm DNA fragmentation	Review	66
	Hormonal (FSH)	Improves sperm parameters of oligozoospermic patients Reduces aneuploidy Reduces sperm DNA fragmentation	Review *In vivo*	67

FISH: fluorescence in situ hybridisation; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling assay; SCSA: sperm chromatin structure assay; Chr: chromosome; FSH: follicle-stimulating hormone.

With regard to lifestyle habits such as alcohol consumption and smoking, there is a lack of reports on these two parameters. Cigarette smoke and alcohol are considered aneugenic agents.[68] However, there are conflicting results concerning DNA integrity, with some authors showing an increase in sDNAfrag[69] whereas others observed no effect.[70] It is therefore necessary to further study sperm aneuploidy and sDNAfrag in the same pool of patients with excessive alcohol consumption and smoking. Little is acknowledged about the origins of human sperm aneuploidy and sDNAfrag, particularly with regard to the impact of environmental and occupational exposures. Although all of the studies included in this review (Table 2) seem to be consistent with respect to occupational and environmental factors increasing sDNAfrag, the same cannot be said about its association with sperm aneuploidy because inconsistent results have been obtained.

Cancer treatment has been improving over recent decades, with many patients now experiencing long-term survival.[71] With this rise in life expectancy, concern over the quality of life for the surviving patients is a fundamental matter. Efforts have been made to preserve male reproductive function.[72] Meanwhile, sperm aneuploidy rates and sDNAfrag indices may afford means of evaluating genomic damage that might prove useful in genetic counselling efforts.[73] The authors of a review on this topic state that both chemotherapy and radiotherapy augment the rate of structural and numerical chromosome abnormalities, and patients who could preserve or restore their fertility status present with long-lasting-to-permanent sDNAfrag.[66] Nonetheless, broader investigations are necessary for each anti-cancer agent and on the variety of compounds used in combination with those agents that theoretically protect the male reproductive function. Gonadotrophins have been used empirically for the treatment of idiopathic male infertility, and have been shown to improve ART outcomes.[74] The impact of this treatment on sperm genomic integrity was considered and researchers found that hormonal treatment with follicle-stimulating hormone improved sperm parameters and reduced sperm aneuploidy and sDNAfrag in oligozoospermic patients.[67] Albeit preliminary, this study opens doors for future investigations.

Exposure to potential reproductive risk factors can affect sperm cells by either inducing breaks in the DNA or affecting sperm chromosomes, altering both their number and structure. However, more epidemiological studies should be conducted, especially taking into account the evaluation of semen quality both prior to and following exposure. In addition to the external risk factors presented here, numerous others may affect sperm DNA integrity and may need to be disclosed and studied. Moreover, it is necessary to take into account the transience of lifestyles and other types of exposure, which may mean that sperm damage can vary over an individual's lifetime.

INFLUENCE OF CRYOPRESERVATION

Along with the improvement of ART, preservation of sperm has been a widely used procedure (mainly for sperm donation)[75] for men undergoing vasectomy or at risk of azoospermia.[76,77] However, cryopreserved sperm from infertile men displayed greater DNA fragmentation and decreased motility and fertility compared with that of fertile donors, when used *ad infinitum* after cryopreservation, with the longest use reported after 28 years of storage.[78] Damaged sperm was also revealed to be likely to be less cryoresistant.[79] Cryopreservation in liquid nitrogen has been commonly used in ART. However, the risks of its contamination are now being appreciated and solved with the use of closed cryopreservation straws. In a study carried out in a cohort of 30 healthy donors, sperm was cryopreserved using the conventional protocol with liquid nitrogen and lyophilisation. This research revealed that both methods decreased sperm viability, motility, and morphology, and did not induce any change in aneuploidy and diploidy rates. Moreover, no statistically significant difference in sDNAfrag was observed before or after lyophilisation. Despite this observation, a statistically significant decrease in sDNAfrag after cryopreservation in liquid nitrogen was detected. The authors suggested combined studies with different physical (warming regimens) and chemical (antioxidants and zinc in the cryopreservation media) exposure environments.[80]

Many factors may have varied side-effects on male fertility, and so fertility preservation is the only option for fatherhood in several types of patients. Therefore, it is of utmost importance to continue and extend studies on the integrity of cryopreserved sperm, but also to not forget prepubescent patients whose only option is to preserve the germinal tissue. Studies on the ability of the oocyte repair system to restore sperm-

contributed imperfections should also be pursued, even after cryopreservation. The clinical impact of increased levels of sperm aneuploidy and sDNAfrag should also be taken into account in further studies, as correlations may be an adjunct in predicting ART outcome.

CONCLUSION

ARTs have improved in the preceding decades, which has pushed newborn rates to their highest percentage ever. For a couple to be infertile, it is usually due to a combination of both partners' fertility, which brings the issue of gamete quality to the fore. Apart from the female's contribution, studies of males and male gametes must be properly addressed before the treatment is initiated. In this report we have discussed the possible relationship between sDNAfrag and sperm aneuploidy in several causes of infertility. Although there seems to be a trend towards a positive correlation, the studies that addressed both parameters are debatable. One of the reasons for the conflicting results may be the use of different methods to evaluate sDNAfrag and sperm aneuploidy, which may produce different results. Regarding sperm aneuploidy, FISH appears to be a reliable method to measure sperm aneuploidy rates.[81] However, the limitation lies in the fact that only 2-4 of the 23 human chromosomes are analysed. These studies would probably benefit from the use of probes for multiple chromosomes. Some incongruity in results may also be the consequence of not evaluating both parameters at the same time in the same sperm cell or sample, which is necessary to definitely endorse a clear link between the two parameters. There seems to be no doubt that the most severe underlying reason for male infertility is the risk of sperm containing damaged DNA. This cell may not eventually be repaired by the oocyte, which will decrease ART outcomes or transmit an anomaly to the embryo.

Despite not having been the purpose of this review, we cannot fail to notice throughout all of the literature reviewed that, while numerical and structural alterations to chromosome segregation are likely to arise during meiosis, sDNAfrag may occur at any point during spermatogenesis. Unlike spermatogonia, spermatocytes (due to DNA breaks during chromosome recombination) and early spermatids (due to chromatin remodelling) display a high DNA repair ability, whereas mature spermatids and testicular sperm do not exhibit such a need.[82] Moreover, sDNAfrag seems to be related to different nuclear regions, which are either related to the type of chromosomes with gene-rich areas or to the amount of histones and protamines.[41]

More studies should be conducted, both in testicular tissue and ejaculate, in order to reveal if increased frequencies of sDNAfrag are due to abortive spermatogenesis. The presence of sperm with damaged DNA may be a failed attempt by the germ cells to complete apoptosis.[83] Besides sperm nuclear domains and abortive spermatogenesis, another point of view is that a low rate of aneuploid sperm coincides with a high rate of spermatocyte-I degeneration.[44] The emerging data therefore seem to support the idea that both sDNAfrag and aneuploidy are correlated not only with the severity of the male infertility but also with poorer ART outcomes.

As a final remark, the genetic integrity of sperm assessed through sDNAfrag and sperm aneuploidy analysis should remain the standard semen analysis. Meanwhile, although selecting sperm for ART based on MACS-DGC-swim-up[41] will reduce the risk of selecting damaged sperm based on their morphology and motility, patients will remain predisposed and susceptible to changes in the genomic integrity of the sperm DNA, and therefore should be offered genetic counselling or, alternatively, should seek prenatal diagnosis and/or preimplantation genetic diagnosis due to the risk of conceiving offspring with genetic anomalies.

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SPERM MOTILITY AND VIABILITY: OVERVIEW OF THE CELLULAR AND PHYSIOLOGICAL ASPECTS THAT SUPPORT THESE FUNCTIONS

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ABSTRACT

This review briefly summarises the cellular and physiological aspects of sperm motility (SM) and viability from the point of view of male fertility/infertility. We discuss the SM patterns and maturation processes during the epididymal transit, including the effects of seminal plasma proteins, and while moving through the female reproductive tract. In connection with SM and viability, the oxidative stress, the mitochondrial markers of SM and related predictive value of the proportion of motile sperm, and the effect of male age on sperm function are reviewed within the current literature. Furthermore, some of the potential techniques to determine molecules involved in sperm motion are presented. Other key points are sperm maturation and the markers of sperm maturity, including sperm-hyaluronic acid binding and DNA integrity, as well as the proportion of hyaluronic acid-bound sperm with respect to sperm morphology and tyrosine phosphorylation. Finally, proteins regulating SM and assessment approaches of sperm viability are pointed out in this review.

Keywords: Human sperm, motility, viability, fertilisation potential.

INTRODUCTION

Infertility is a troubling medical condition with social and economical influences for couples who desire pregnancy. In fact, the data collected from all Society for Assisted Reproductive Technologies member clinics indicated that 17% of assisted reproductive technology patients were diagnosed with only male factor infertility in 2012.[1] However, the aetiology for male factor infertility is multifactorial. Most studies focus on sperm motility (SM), viability (SV), and DNA integrity since these parameters are important characteristics of sperm function. Therefore, the purpose of this article is to provide an overview of human SM and SV in the concept of sperm function, including fertilising potential.

SPERM MOTILITY AND MATURATION

Normal development of sperm plays an essential role in enabling reproductive capacity. Spermatozoa acquire motility and fertility in the epididymal lumen which is a complex microenvironment. Phosphorylation, glycosylation, and further processing are several of the posttranslational modifications that sperm proteins undergo during epididymal transit, resulting in changes in protein function, ultimately leading to mature spermatozoa.[2]

The comparison of seminal plasma proteome in fertile and infertile men showed that ten seminal proteins are significantly up-regulated in the infertile group, in line with statistically significant differences in motility and sperm count between fertile and infertile men.[3] Thus, it has been proposed that a variety of peptides in seminal

fluid appear to have a role in SM.[4] In fact, there is increasing recognition of the role that peptides present in seminal plasma, such as seminal plasma-neutral endopeptidase and aminopeptidase N, have in determining SM (for a review[4]). Nonetheless, there is not much known about their specific roles in male fertility/infertility.

The activation of sperm is not entirely completed upon release from the male genital tract and is further modified while moving through the female reproductive tract. Once deposited inside the female reproductive tract, spermatozoa seek to reach the oocyte first and acquire hyperactivated progressive motility, defined as moving actively, either linearly or in a large circle, regardless of linear speed.[5] Spermatozoa gain hyperactive motility upon arrival to the oviduct.

Sperm movement is generated by the flagellum which constitutes more than 90% of the length of the mammalian spermatozoon.[6] Paoli et al.[7] described SM as the result of the propagation of transverse waves along the flagellum in a proximal-to-distal direction producing a hydrodynamic impulse that pushes the spermatozoon through the female genital tract to penetrate the cumulus and zona pellucida of the oocyte. Most mammalian sperm display two types of physiological motility: activated motility, as is seen in freshly ejaculated sperm, and hyperactivated motility, as is seen in most sperm recovered from the site of fertilisation.[8] However, these motility patterns are temporary and may vary in a time-related fashion. Turner et al.[9] defined that the flagellum of an activated sperm generates a symmetrical, lower amplitude waveform that drives the sperm in a relatively straight line. Once sperm from most species becomes hyperactivated, the flagellar beat becomes asymmetrical and of higher amplitude, which results in circular or figure-eight trajectories.[10] The propagation of a calcium-induced wave produced from the opening of calcium channels along the flagella is a necessary milestone in sperm maturation and makes hyperactivated motility possible.[11]

Hyperactivated motility and the associated protein tyrosine phosphorylation in the flagellum are initiated during *in vitro* capacitation, and their maintenance in zona pellucida-bound spermatozoa is of importance. Previously, we showed that the levels of capacitation-related tyrosine phosphorylation in the sperm neck and principal piece, a pattern that is a marker of sperm activation, increases in a time-related manner.[12] Progesterone hormone was previously described to initiate an immediate increase in intracellular calcium within human sperm cells through potentiation of CatSper channel[13] and also rapidly triggers hyperactivated motility and initiation of the acrosome reaction. Sperm intracellular pH (pHi) is a key regulator for the initiation of motility and hyperactivation. Even the basal SM is pH-sensitive since dynein's ability to hydrolyse adenosine triphosphate (ATP) and provide axonemal bending greatly increase with the rise of pHi.[11]

There have been numerous attempts to identify pharmacological agents such as theophylline that might improve SM, particularly in testicular sperm extraction cases where the isolating of a clean preparation of usable sperm is difficult due to the declined tendency of the sperm to detach from the testicular tissue owing to its limited motility.[14] The study indicated that 64 out of 65 patients (98.5%) showed a significant improvement in thawed testicular SM when theophylline is used, but this result has to be confirmed by independent laboratories. In addition, hyaluronic acid in the medium increased the velocity and retention of motility and viability in freshly ejaculated as well as in cryopreserved–thawed human spermatozoa.[15,16] The effects of hyaluronic acid upon sperm are likely to be receptor mediated due to the presence of the hyaluronic acid receptor in human sperm. We also found that the tyrosine phosphorylation patterns of sperm bound either to zona pellucida or to hyaluronic acid were similar.[12] Therefore, we think that there is a common regulatory pathway of tyrosine phosphorylation related to sperm ability. It is possible that such a regulatory pathway originated in the synchronous formation of the zona pellucida and hyaluronic acid receptors in the sperm plasma membrane, following the remodelling process related to the progress of spermiogenesis.

SPERM MOTILITY AS AN IMPORTANT FACTOR IN MALE FACTOR INFERTILITY

Studies demonstrated that SM correlates well with fertilisation and pregnancy rates after intrauterine insemination or *in vitro* fertilisation.[17,18] Based on 358 semen samples from a group of men reflecting the general male population, Larsen et al.[19] reported that the concentration of motile spermatozoa, defined as spermatozoa with curvilinear velocity

>25 µm/s, was the most significant and independent computer-assisted semen analysis parameter in predicting the chance of natural conception.

SM is also an important parameter when investigating men for potential environmental exposures or male infertility. Exposure to environmental and occupational toxicants may adversely affect SM and motion characteristics, and thus male reproductive potential is affected by environmental factors during sperm development or epididymal storage. Thus, there have been previous attempts to develop methods for preservation of SM by various semen extenders for semen analysis or assisted reproduction.[20] We have previously demonstrated that the use of phenylmethylsulfonyl fluoride and 4°C conditions during shipping for next-day semen analysis methods can preserve various sperm attributes, including sperm concentration, heat shock chaperone protein levels, chromatin maturity, DNA integrity, and sperm shape.[21-23] In a recent publication we reported that loading sperm with the MitoTracker® reagent allows the delayed assessment of SM based on the analysis of mitochondrial activity.[24]

OXIDATIVE STRESS AND SPERM MOTILITY

Oxidative stress can be initiated by a variety of factors in the male germ line including infection, age, obesity, and exposure to a variety of adverse environmental influences. It is caused by an excess of reactive oxygen species that damages proteins, lipids, and DNA in human spermatozoa and is considered a major cause of impaired sperm function.[25,26] One of the first functions affected by oxidative stress and lipid peroxidation is SM. It is known that sperm is responsible for reactive oxygen species production.[27] It was reported that regulation of reactive oxygen species levels is involved in sperm capacitation, motility acquisition, and acrosome reaction.[28,29] Agarwal et al.[30] showed that infertile men have reduced semen parameters and elevated reactive oxygen species levels compared to proven fertile men who have established a pregnancy recently or in the past, suggesting that measurement of reactive oxygen species levels in the seminal ejaculates might provide clinically-relevant information to clinicians.

High levels of nitric oxide are associated with alterations in sperm function, particularly with decreased motility.[5,19,31,32] Studies indicated that hydrogen peroxide was the most cytotoxic oxygen metabolite; that superoxide and hydroxide probably also played a role in the immobilisation of spermatozoa by reactive oxygen species in preventing SM loss under such circumstances.[33] Even though the axonemes are reported to be affected, mostly as a result of ATP depletion,[28,29] the molecular mechanism underlying this loss of motility in spermatozoa under oxidative stress is not clearly known.

Uribe et al.[34] have shown that *in vitro* peroxynitrite causes decreased motility and mitochondrial membrane potential in human spermatozoa, compromising vital functions of the male gamete without affecting viability.[34] Oxidative stress results in redox-dependent protein modifications, such as tyrosine nitration and S-glutathionylation. Thus, in a very recent paper, Morielli et al.[35] showed that oxidative stress promotes a dose dependent increase in tyrosine nitration and S-glutathionylation, and alters motility and the ability of spermatozoa to undergo capacitation in normozoospermic sperm samples from healthy individuals. Furthermore, Talevi et al.[36] demonstrated that *in vitro* treatment of human spermatozoa with zinc, D-aspartate, and coenzyme Q10 exerts a direct protective effect on SM, kinetics, lipid peroxidation, and DNA fragmentation during handling, extended culture, and cryopreservation, particularly during assisted reproductive procedures.

Ebner et al.[37] presented a sperm-selection chamber called the Zech-selector that exclusively enables the accumulation of spermatozoa with fast progressive motility without exposure to centrifugation stress. Since the spatial separation of spermatozoa of highest motility happened in a time-dependent manner, the idea is that spermatozoa of fast progressive motility are those most likely to be DNA intact.[38] Spermatozoa showing numeric (17.4% of patients without aneuploidy) or structural chromosomal abnormalities (90% of patients without strand-breaks) are reported to be separated most effectively with this sperm processing technique.[38] However, the efficiency of this technique in oligoasthenoteratozoospermic patients is not yet known.

MITOCHONDRIAL DEFECTS AND SPERM MOTILITY

During spermatogenesis there is a significant reduction of mitochondria number per cell due to

mitochondrial DNA (mtDNA) replication arrest. Mitochondria may supply sperm with energy for several purposes, including motility.[32] Experimental evidence suggesting an association between mitochondrial functionality and sperm quality was presented in previous papers.[6,39] Indeed, the structural and functional defects in sperm mitochondria, and the presence of mutant mtDNAs are associated with decreased SM in men.[7,40]

Mutations in the mitochondria have been implicated in infertility[41] via new candidate genes such as human GALNTL5 and are suggested to result in male infertility with the reduction of SM.[42,43] Tian et al.[44] suggest that mtDNA copy number and epigenetic factors including LINE-1 element may be linked to semen quality via SM. This aspect also brings the idea of the assessment of the genetic and epigenetic modifications as diagnostic information in addition to the information gained by the routine semen analysis in the evaluation of male infertility, especially in patients with potential risks.[44]

MALE AGE

There is an increasing trend for older men to have children. The advanced age-associated increases in sperm defects are a big concern, in particular for older men who are attending assisted reproductive clinics in order to father children. Using data from 90 studies (93,839 subjects), a systematic review and meta-analysis to quantify the effect of male age on motility revealed that male age is associated with a decrease in the percentage of motility and the percentage of progressive motility.[45] Thus, it has been suggested that DNA fragmentation and progressive motility would be better diagnostic parameters during fertility treatments of ageing couples.

Schmid et al.[46] identified that older men (≥65 years) had significantly higher levels of zinc, copper, and calcium in their sperm and higher levels of sulphur in their seminal fluid than younger men (≤28 years). Both higher sperm calcium and copper were reported to be associated with lower SM and increased frequency of DNA fragmentation. In addition, seminal plasma sulphur was found to be negatively associated with SM and structural aberrations, and positively associated with DNA fragmentation. These findings demonstrated that there are differences even in the elemental composition of whole sperm and seminal plasma between younger and older men and that these elements are quantitatively associated with increased risks for poorer semen quality and genomic defects in the sperm of older men.[46]

Furthermore, the lifestyle and environmental factors may adversely affect human health and reproductive performance. Kumar et al.[47] reported that there is a non-significant lowering of sperm count and total progressive motility between tobacco smokers and non-smokers among the oligozoospermic patients. Besides deterioration in sperm count, total progressive motility and normal sperm morphology is observed among alcohol consumers who had oligozoospermia. However, no statistical significance is observed, possibly due to low frequency of alcohol consumers in the study population. In another study, cigarette smoking and alcohol consumption separately and combined were found to have a deleterious effect on sperm parameters and sperm DNA fragmentation.[48]

This also brings up the possibility that the increased use of certain new technologies such as portable computers may decrease male fertility. Avendaño et al.[49] examined the effect of portable computers on human spermatozoa *in vitro.* They have demonstrated that laptop computers connected wirelessly to the internet decrease the sperm progressive motility and increase the proportion of sperm with DNA fragmentation. However, the mechanisms involved in mediating the decrease in SM and DNA integrity require further investigation.

DETERMINATION OF PROTEINS INVOLVED IN HUMAN SPERM MOTILITY

SM can be affected by several molecules. Lin et al.[50] showed that nerve growth factor could promote human SM *in vitro* by increasing the movement distance and the number of A-Grade spermatozoa in a dose-dependent manner. Although the number of such related publications has risen dramatically in the past few years, unfortunately there is as yet no comprehensive model of the myriad molecular mechanisms controlling SM. Two clear signalling pathways are known to be involved in SM regulation: cyclic monophosphate/protein kinase A pathway and calcium signalling. There is an increasing number of papers reporting several proteins to be necessary for sperm flagellum movements. This also brings on different approaches to study SM and determine the proteins that support human SM. Currently, the

use of patch clamp technique and mathematical analysis of calcium dynamics,[51,52] the use of imaging and fluid mechanics simulation of sperm swimming to reveal the influence of media viscosity in SM,[53] and the analysis of protein phosphatase 1 complexes[54] can be counted as some of these potential techniques. More recently, Amaral et al.[55] have suggested a high-throughput differential proteomic strategy to identify proteins involved in SM (de)regulation.

SPERM VIABILITY ASSESSMENT

SV can be described with multiple criteria that collectively contribute to sperm-oocyte activation and fertilisation, and also further developmental steps during embryogenesis. There are several proteins to be potentially used as markers for SV. Most of them rely on the sperm membrane proteins since the fusion of the sperm and oocyte membranes is one of the critical steps for *in vivo* fertilisation. While this mechanism provides many proteins that may be studied as markers of SV, it is important to note that this fusion step is bypassed in intracytoplasmic sperm injection (ICSI) by the embryologist. Even if the ICSI method is used, the mature and viable sperm has to be selected accurately and predictably for injection into the ooplasm. Thus, it is necessary to identify markers that are vital to sperm function downstream of sperm-oocyte fusion. In addition, proteins involved in the sperm DNA fragmentation have been considered as potential markers for poor viability.[56] There is still an increasing effort to identify markers that possess a functional role during sperm maturation and also following delivery to the oocyte, including sperm-derived mRNA and micro-RNA transcripts.[57]

We have previously shown that sperm that are able to bind to hyaluronic acid are mature and have completed the spermiogenetic processes of sperm plasma membrane remodelling, cytoplasmic extrusion, and nuclear histone-protamine replacement, as well as demonstrating high DNA integrity. In addition, we determined an increase of the Tygerberg normal spermatozoa in the hyaluronic acid bound sperm fractions.[58] The increase in hyaluronic acid-selected spermatozoa with normal morphology attributes was also associated with concomitant improvements in the sperm maturity biochemical markers. Clearly, only viable sperm exhibited hyaluronic acid binding.[59,60] Thus, hyaluronic acid binding by human sperm indicates cellular maturity and also viability. This sperm-hyaluronic acid binding assay conforms to the basic idea that sperm DNA integrity should always be studied in motile sperm.

CONCLUSION

In conclusion, the physiology of SM and SV are very complex functions, and there are several molecules reported to regulate these sperm functions. In fact, special care has to be taken in the advent of assisted reproductive technologies such as ICSI that require the bypass of SM. However, this might also increase the genetic defects passed to subsequent generations. Thus, further studies are required to highlight the molecular mechanisms underlying these processes, to decrease possible negative outcomes and concerns in assisted reproductive technologies.

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FERTILITY PRESERVATION IN PATIENTS WITH ENDOMETRIAL CANCER

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ABSTRACT

Endometrial carcinoma (EC) is the most common gynaecological cancer, with 2-14% of cases occurring in women <40 years of age. When considering the increase in the delay of pregnancy in developing countries, the incidence of EC in nulliparous women is likely to increase. Younger women with EC have a chance to preserve their fertility due to the probability of being diagnosed with early-stage and low-grade endometrioid carcinomas. However, it should be noted that the most important step of fertility preservation in patients with EC is patient selection. The appropriate clinical criteria should include: a) well-differentiated histology; b) absence of myometrial invasion; c) absence of extrauterine or pelvic and pre-aortic lymphatic spread; and d) absence of synchronous ovarian tumour. There is no consensus regarding endometrial suppression therapy or the follow-up period for fertility preservation in women with EC. Therefore, this review aims to evaluate the current literature.

Keywords: Endometrial cancer, fertility preservation, fertility sparing, progestin, conservative treatment.

INTRODUCTION

Endometrial carcinoma (EC) is the most common gynaecological malignancy amongst women, with a lifetime risk of 2.6% in developed countries.[1] It is predominantly seen in the postmenopausal period; however, 15-25% of cases are premenopausal[2] and 2-14% of cases affect women <40 years of age.[3] The endometrial tumours of those of a younger age are low-grade ECs that are usually diagnosed at earlier stages and therefore have an excellent prognosis, with a 98% 10-year disease-free survival rate.[3] The incidence of nulliparity in premenopausal women with EC is 55%.[4] The standard treatment of EC is comprehensive surgical staging, which includes total hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymphadenectomy.[5] For younger patients who have not completed pregnancy, this kind of definitive surgical management is not an acceptable option.

For gynaecological and non-gynaecological cancers of premenopausal women, a new concept termed 'oncofertility', which describes a multidisciplinary approach involving oncology and reproductive endocrinology, has come to prominence, especially during the last decade.[6] Young patients should be counselled by oncologists in the decision-making process with updated information for both surgical treatment and chemotherapeutic drugs with gonadal toxicity. The age of the patient, stage of EC, life expectancy, previous history of infertility, risk of progression and recurrence, duration of treatment, and when to consider hysterectomy should be discussed with patients before suggesting a fertility-preserving management option.

EC of young patients is usually related to unopposed excessive oestrogen exposure with risk factors such as infertility, obesity, polycystic ovary syndrome (PCOS), and chronic anovulation; which is described as 'Type 1' endometrial cancer. As this type of EC is reported to be highly hormone-dependent due to it being well differentiated, conservative fertility-sparing medical treatment strategies are also discussed for special patient populations.[7,8] Considering the aetiology of EC, evaluation of the risk of underlying infertility before planning pregnancy is recommended, and assisted reproductive technology (ART) is suggested in selected groups to shorten the treatment period.[8] In clinical studies,

medroxyprogesterone acetate (MPA), megestrol acetate (MA), hydroxyprogesterone acetate, 17α-hydroxyprogesterone caproate, norethindrone, gonadotropin-releasing hormone (GnRH) agonists, aromatase inhibitors, levonorgestrel-releasing intrauterine devices (LNG-IUDs), hysteroscopic excision procedures, and photodynamic treatment have been tried in selected patient groups.[6,7,9,10]

PATIENT SELECTION

Patient selection criteria for fertility-sparing therapy in complex atypical hyperplasia (CAH) and EC are identified in several clinical studies. Young patients still of child-bearing age, who accept the risks of unresponsiveness, progression, and recurrence, and who are convinced to attend follow-ups regularly are ideal candidates for conservative management. The patient should also be convinced that this is not the standard treatment, and comprehensive surgery will be planned after achievement of pregnancy or in case of failure in conservative management. The appropriate clinical criteria are: a) well-differentiated histology (Grade 1 Stage 1A), b) absence of myometrial invasion, c) absence of extrauterine or pelvic and pre-aortic lymphatic spread, and d) absence of synchronous ovarian tumour.[11] The age of the patient is unrelated to the possibility of remission, recurrence, progression, or pregnancy, and therefore there is no recommended age limit for fertility-sparing treatment.[7] However, storage of oocytes and likelihood of pregnancy decreases in patients >40 years of age.[12,13] Kudesia et al.[12] reported a live birth rate of >30% in a small-population *in vitro* fertilisation group who underwent fertility-sparing management, with a median age of 38.5±4 years. Besides the patient's age, ovarian reserve tests, such as follicle-stimulating hormone and anti-müllerian hormone levels, and antral follicle count should be considered in order to predict the likelihood of pregnancy or response to ART.[13]

Patient selection is an important step for fertility preservation in EC because hormonal therapy of EC with Lynch syndrome is ineffective and, therefore, fertility-sparing management is not an appropriate option for these patients.[14-16] Clinicians should consider Lynch syndrome in younger patients with a low body mass index (BMI) or undifferentiated and dedifferentiated carcinomas.[17] Transvaginal ultrasound (US) and computed tomography (CT) for evaluation of extrauterine spread and magnetic resonance imaging (MRI) for evaluation of myometrial invasion are recommended.[18] MRI is described as the most useful imaging technique to determine myometrial invasion, with a sensitivity of 57% and specificity of 96% when diagnosing whether a tumour is restricted to the endometrium.[19]

Co-existing synchronous ovarian tumours are not rare in EC cases. In a recent report by Song et al.,[20] the incidence of synchronous ovarian cancer (OVC) in women with EC and <40 years of age was reported as 4.5%. However, Walsh et al.[21] reported an incidence of 25% among women <45 years and these cases were mostly endometrioid adenocarcinomas. The presence of synchronous ovarian tumours with EC is more common in patients with a high BMI, and they generally have low-grade endometrioid tumours and favourable prognosis.[22-24] Laparoscopic evaluation with adnexal exploration and peritoneal cytology is recommended before starting conservative treatment.[25] Although preoperative imaging and diagnostic laparoscopy may help to detect adnexal involvement, 15% of synchronous tumours display normal preoperative imaging and 15% of patients display benign-appearing ovaries at the time of surgery. Therefore, patients should be informed about the risk of disease progression at the end of medical treatment due to the risk of occult OVC.

HORMONE TREATMENT

Type 1 ECs that commonly affect younger age groups are defined as well-differentiated carcinomas and therefore hormone receptor expression is expected to be positive in most cases. The most commonly used progestin agents are MPA and MA.[2,7] In a recent meta-analysis, Gunderson et al.[26] reported that in EC patients: 49% received MPA, 25% received MA, and 19% received an LNG-IUD. There is no consensus on the ideal agent, dosage, or duration of treatment in the literature. MPA is used with doses of 200-600 mg daily; while some studies report that a 200 mg daily dose of MPA is an appropriate regimen,[27] others advocate that a better response is observed with the high-dose regimen.[11,28,29] MA treatments have been given at a daily dose of 160 mg or 320 mg in clinical studies and they were both considered effective, with response rates >80%.[18,30,31] GnRH agonists are used mostly as an adjuvant drug to progestin therapy or can be used alone.[18,32,33] LNG-IUDs have also been found to be effective in clinical studies but this may not be a good option for fertility-seeking

patients. MA was found to be associated with a higher probability of remission and lower progression rates in a recent meta-analysis by Koskas et al.,[7] and was recommended as a first-line agent for medical treatment. However, Park et al.[34] suggested that, despite similar response rates to MA, the long-term outcomes of MPA treatment were more successful and with lower recurrence rates.

The minimum duration of treatment for regression is 3 months, with a median of 4-6 months.[2] At least 12 months of progesterone treatment is recommended for these patients.[35] Remission probability within 12 and 24 months of treatment is reported as 78% and 81.4%, respectively. Therefore, if no remission is attained by this time, radical surgery should be considered because the likelihood of remission after 12 months of treatment is not significant.[7] Response to progestin treatment strongly depends on the hormonal receptor status, and better response is seen in low-grade tumours.[36] Compared with CAH, the response rate is lower while the disease persistence rate is higher in EC.[26] Increased BMI is associated with lower response rates.[34] Gunderson et al.[26] reported a 77.7% response rate to hormonal therapy, with a durable response rate of 53.2% after 39 months of follow-up. Similarly, Park et al.[34] also reported a response rate of 77.7%, with a 68% rate of 5-year recurrence-free survival.

HYSTEROSCOPIC TECHNIQUES

Hysteroscopic resection of tumoural tissue followed by progesterone treatment has also become an option in conservative management. It is advocated that the addition of surgical resection to medical therapy shortens the time to remission and reduces recurrence rates, therefore giving the possibility of a faster return to fertility.[37] Gradual resection of the lesion also helps to provide pathological assessment of the depth of invasion. Mazzon et al.[10] report a pregnancy rate of 67% using a three-step technique: resection of the lesion, resection of the adjacent endometrial tissue, and resection of the myometrium, followed by MA administration with a dose of 160 mg/dl for 6 months. Laurelli et al.[38] treated patients with hysteroscopic resection of the lesion and the myometrium below, followed by oral MA for 6 months or LNG-IUD for 12 months. Arendas et al.[37] reported two cases of hysteroscopic resection of endometrial and endomyometrial tissue followed by use of oral MPA and a LNG-IUD, with no recurrence reported within 4 years of follow-up.

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is an alternative option in gynaecological malignancies and involves selective destruction of tumour tissue by using a selective tumour photosensitiser, haematoporphyrin derivative, and laser light.[39] Choi et al.[9] evaluated 16 patients undergoing PDT for EC during a mean follow-up period of 78 months; the final response rate was reported as 68% and the pregnancy rate was reported at 57% among 7 patients who attempted to become pregnant.

FOLLOW-UP PROTOCOL

Follow-up of patients is essential in order to intervene in cases of persistence, progression, recurrence, and also in cases of co-existing synchronous OVC. First biopsy is recommended after 12 weeks of treatment because 12 weeks is considered the minimum time for response.[11] Follow-up protocols should be repeated every 3-6 months and the patient should be evaluated with pelvic examination, endometrial biopsy, hysteroscopy, testing for cancer antigen 125 (CA-125), and imaging techniques such as transvaginal US, MRI, or CT.[2,8] Endometrial sampling with dilatation and curettage (D&C) after removal of an LNG-IUD was reported to be more accurate than endometrial aspiration biopsy with an LNG-IUD in place, with a diagnostic concordance of 32.1% between examinations, while endometrial aspiration biopsy detected only three of the nine cases of endometrial adenocarcinoma detected by D&C.[40] D&C also reduces the tumour burden when compared with endometrial biopsy.[41] A flow chart describing the management of women with EC or CAH and eligible for fertility-sparing treatment is shown in Figure 1.

There is research into new biomarkers for predicting the response or recurrence rates for EC. Human epididymis protein 4, with a cut-off value of 70 pmol/l, was found to be superior to CA-125 with a cut-off of 35 U/ml as a predictor of recurrent disease.[42] Annexin-A2 expression was found to be effective in the prediction of recurrent disease in EC.[43] The younger patients, those with previous pregnancy, infertility, or treatment with MA were found more likely to achieve remission.[7] The risk of progression was investigated as

well, and it was found that the risk increased 3.95-fold when other medical therapies (MPA, hydroxyprogesterone caproate, norethisterone acetate, GnRH agonists, LNG-IUD, combination of GnRH agonists and LNG-IUD, bromocriptine, natural progesterone, ovulation induction, and oral contraceptives) were used in comparison with the use of MA.[7] However, clinicians should note that the study reported not only the progression outcomes of EC but also the progression outcomes of atypical endometrial hyperplasia. A recent systematic review of literature that compared outcomes of progestin therapy with CAH and Grade 1 endometrial adenocarcinoma revealed that the complete response rate was significantly higher in the CAH group (48%) than in the endometrial cancer group (66%); recurrence rate and persistent/progressive disease rate were higher in patients with carcinoma, 35% versus 23%, and 25% versus 14%, respectively. However, there was no difference between the reproductive outcomes.[26]

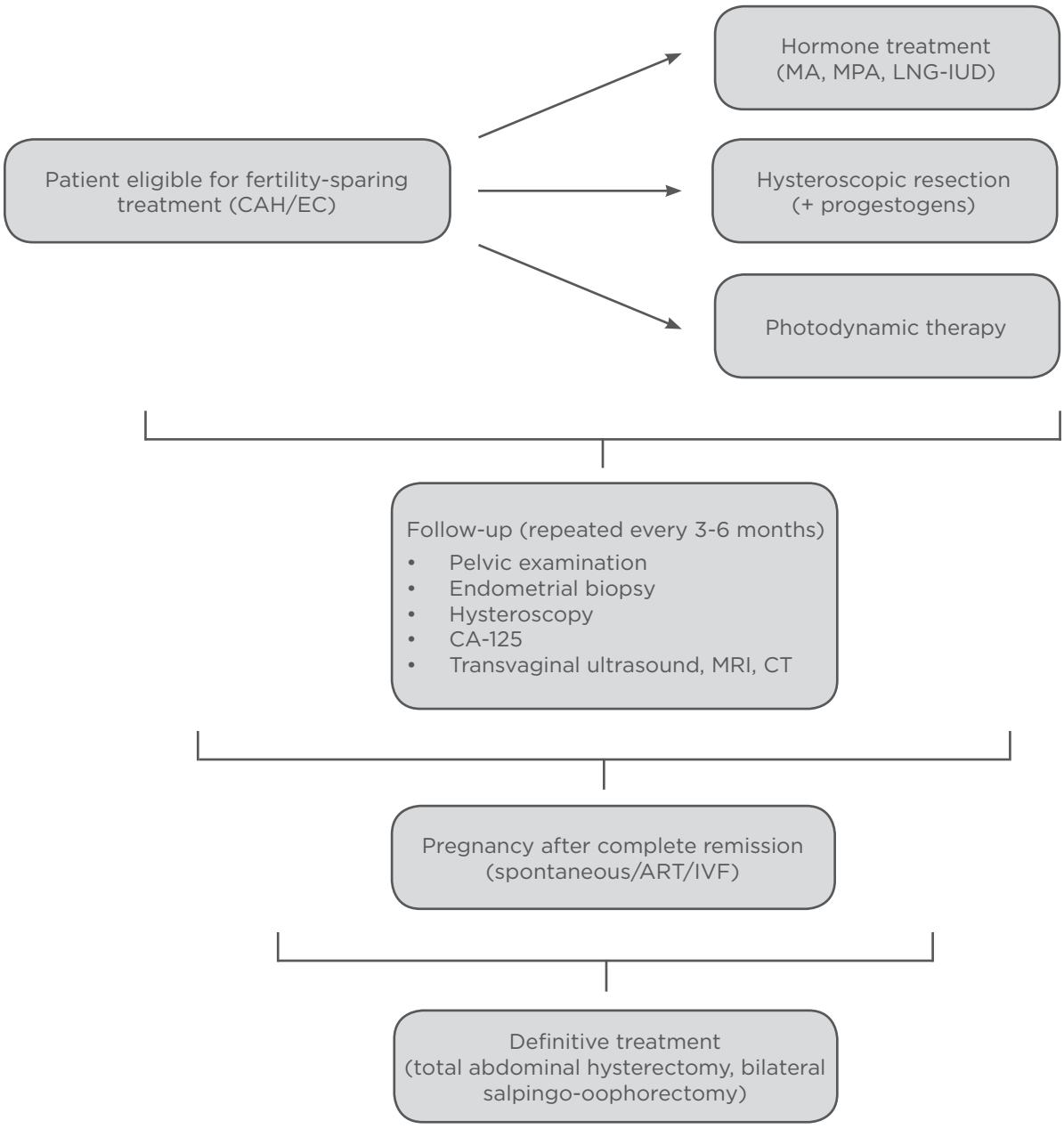

Figure 1: Flow chart describing the management of women with endometrial cancer (EC) or complex atypical endometrial hyperplasia (CAH) and eligible for fertility-sparing treatment.
MA: megestrol acetate; MPA: medroxyprogesterone acetate; LNG-IUD: levonorgestrel-releasing intrauterine device; CA-125: cancer antigen 125; MRI: magnetic resonance imaging; CT: computed tomography; ART: assisted reproductive technology; IVF: *in vitro* fertilisation.

PREGNANCY RATES

According to a meta-analysis by Koskas et al.,[7] 31.6% of the patients became pregnant. Among these patients, 54.1% achieved pregnancy by ART, 26.1% had spontaneous pregnancy, and 19.8% were not stated. Gallos et al.[44] found that the live birth rate was higher after ART (39.4%) than after spontaneous pregnancy (14.9%). ART is suggested especially in patients with PCOS, a higher BMI, and previous history of infertility, and it does not have a negative impact on the prognosis.[35] In a prospective study including CAH and low-grade early-stage EC, although complete remission of all cases had not been achieved, attempting pregnancy was allowed because it was thought that pregnancy could create an environment similar to a high-dose progesterone therapy. Live births occurred in this study and, after birth, persistent disease or EC relapse requiring definitive treatment was detected. With a median follow-up of 98 months, all patients were alive and free from disease.[45]

There are diverse and experimental fertility preservation options, such as gestational surrogacy,[12] embryo cryopreservation, oocyte cryopreservation, and ovarian tissue banking.[6] For women without partners, ovarian stimulation and oocyte cryopreservation may constitute an alternative because they do not require the use of a surgical procedure such as ovarian tissue cryopreservation.[46] However, ovarian tissue cryopreservation should be considered for oncological cases in which ovarian stimulation will cause a delay in treatment.[6] In a series of 11 patients diagnosed with different types of cancer, four patients achieved live births after treatment by oocyte vitrification.[47] The literature states that, for patients with EC who received fertility-preserving treatment, there is no increased risk of recurrence with the drugs used for ovulation induction.[35,48] Tamoxifen cannot be used because of its stimulatory effect on the endometrium.[13] Radiotherapy and chemotherapy are not a part of fertility-preserving treatment in endometrial cancer, therefore endometrial damage caused by these approaches is not a subject of fertility-preserving treatment in endometrial cancer. However, recurrent curettages and progestin use may cause intrauterine synechiae and disturbances in the endometrial lining, which may disrupt fertility.[49]

CONCLUSION

ECs in patients ≤40 years of age are not common. However, delayed pregnancy is likely to increase in the future, especially in developing countries. Fertility sparing in patients with EC should be an option in young prospective mothers. However, it should be considered that there has not been a consensus in the management of these patients. Appropriate patient selection, management, and follow-up protocols should be standardised after future prospective trials.

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NEW CONCEPTS IN THE THERAPEUTIC MANAGEMENT OF MYOMA

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ABSTRACT

The therapeutic management of symptomatic uterine fibroids is based on three pillars: surgery, pharmacotherapy, and interventional radiotherapy. Modern myoma treatment is personalised and should involve an interdisciplinary approach according to the patient's wishes and pathology.

Keywords: Myoma, uterine fibroids, ulipristal acetate (UPA), myoma enucleation, hysterectomy.

INTRODUCTION

Uterine leiomyomas or 'fibroids' (also known as myomas) are common, benign tumours of the myometrium.[1] Their growth is dependent on the female sex hormone.[2,3] Data from the USA show that up to 40% of all 35-year-old Caucasian women have myoma, whereas in African-American women it occurs even more frequently (estimated at 60% in this age class), and at the age of 50 years the prevalence of myoma reaches up to 70% in Caucasian women and >80% in African-American women.[4] More than 50% of all myomas are asymptomatic and do not require any therapy. The size of the myoma does not necessarily correlate with the severity of symptoms: even large myomas may not cause any symptoms. However, the localisation of the myoma is crucial in determining the extent and nature of symptoms. Myomas remain the predominant cause of hysterectomy for benign indications (about 50%).[4] Symptoms that require therapeutic intervention are: anaemia due to uterine bleeding, dysmenorrhoea, bulky symptoms (dyspareunia, pelvic pain, constipation, urge incontinence, pollakiuria, nocturia, symptoms of overactive bladder, compression of the ureter and subsequent hydronephrosis, and pyelonephritis leading to renal dysfunction), and acute pelvic pain in necrotic myoma. Another group of patients who should undergo appropriate therapy are patients with subjective wellbeing but with subfertility due to myoma.[5] Undiagnosed myoma can be a cause of recurrent miscarriage and sterility. Intrauterine myoma or polyps are diagnosed in 28.7% of women with recurrent miscarriage.[6]

In myoma patients suffering from bleeding-related anaemia, a submucosal localisation of the fibroid is most likely. Bleeding symptoms are due to dysregulated angiogenesis.[7] Bulky symptoms are found in patients with subserous and large intramural myoma. Bulky symptoms depend on the size and especially on the localisation of the myoma (e.g. due to the application of pressure on the intestine or bladder). In contrast to dysmenorrhoea, pelvic pain due to bulky symptoms occurs irrespective of the stage of the menstrual cycle.[8,9]

Modern myoma treatment consists of three therapeutic pillars: surgery, pharmacotherapy, and radiological interventions (high-intensity focussed ultrasound [HIFU] and myoma embolisation). The predominant therapeutic aim is to improve quality of life (QoL). Three aspects have to be taken into account in order to establish adequate therapeutic management:

- Does the patient wish to conceive?
- What is the size, number, and localisation of the myoma?
- What are the predominant symptoms?

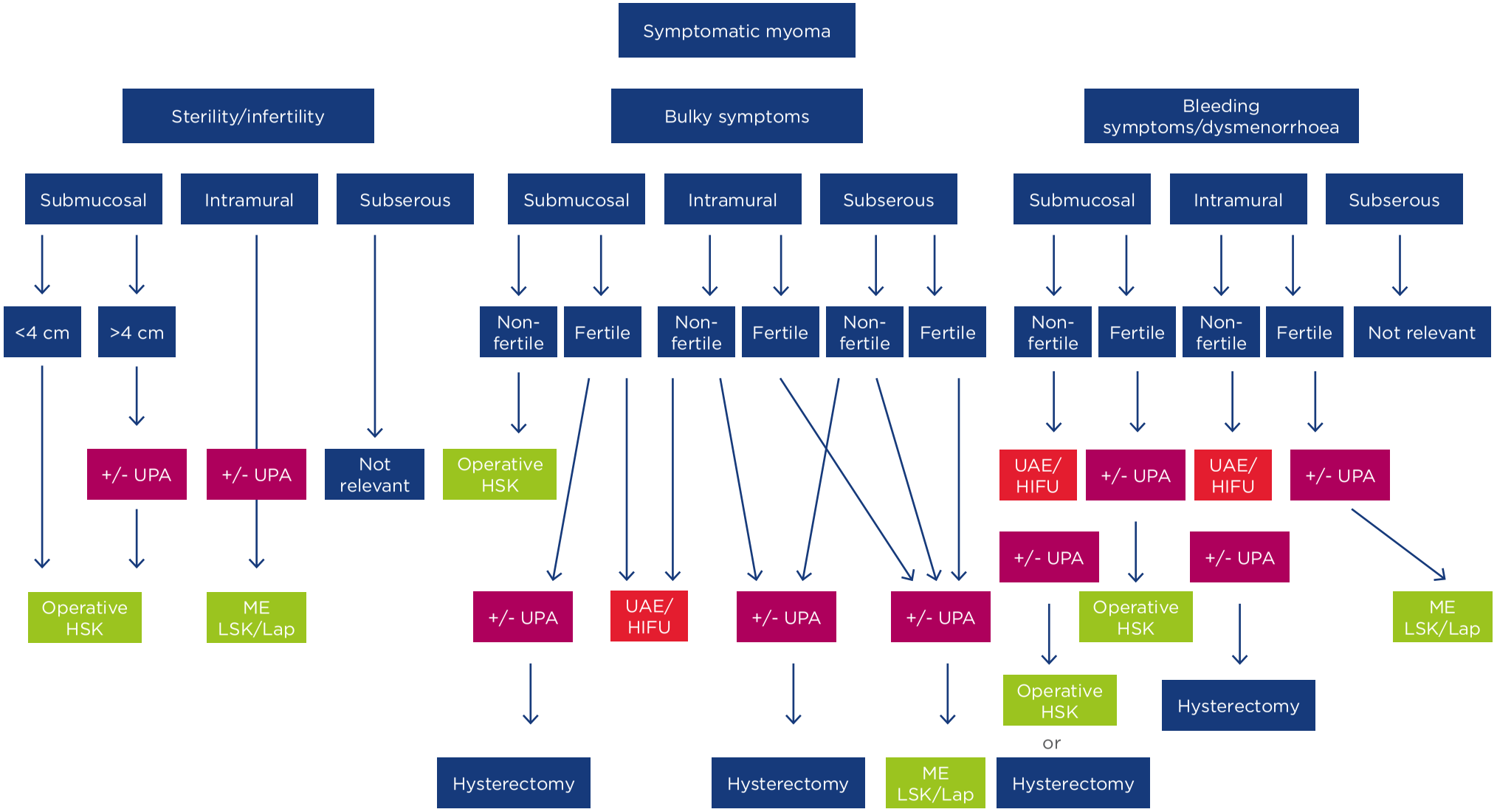

Figure 1: Algorithm for the personalised therapy of symptomatic myoma according to the patient's symptoms and respecting their wish to conceive.
ME: myoma enucleation; UPA: ulipristal acetate; UAE: uterine artery embolisation; HIFU: high-intensity focussed ultrasound; HSK: hysteroscopy; Fertile: wish to conceive; Non-fertile: no wish to conceive; LSK: laparoscopy; Lap: laparotomy.
+/- UPA refers to preoperative UPA treatment for either one or two cycles in order to achieve shrinkage and amenorrhoea.

Menopause and the expected interval until menopause have to be considered, as well as contraindications for surgery (comorbidity or if the patient refuses surgery). Modern therapeutic management of symptomatic myoma consists of personalised concepts tailored to the individual situation of the patient (Figure 1).

Surgery

An elaborate preoperative work-up is essential prior to surgical therapy for myoma. Six aspects must be clarified:

i. The symptoms must be attributed to the myoma.
ii. In uterus-preserving procedures, three groups of patients must be differentiated: asymptomatic patients who present for subfertility, symptomatic patients who wish to conceive in the future, and symptomatic patients who wish to maintain their uterus irrespective of reproduction and menopausal stage.
iii. The aim of the procedure must be clearly defined (symptom control versus fertility improvement).
iv. The ideal operative access must be defined.
v. The reasonableness of preserving the uterus in patients with completed family planning and perimenopausal patients must be estimated.
vi. The patient must be informed about alternatives to the procedure.

In any case and even in postmenopausal women, the patient's wish to preserve their uterus must be respected.

FERTILITY-PRESERVING OPERATIVE METHODS

Hysteroscopic Enucleation of Myoma

The operative method depends on the localisation of the myoma. Submucosal myomas are usually resected via hysteroscopy. Most patients present with bleeding disorders (the diagnosis of submucosal myoma is established in 59.8% of myoma patients with hypermenorrhoea),[10] or with sterility and recurrent spontaneous abortions.[6,11] Another common symptom in submucosal uterine fibroids is dysmenorrhoea. A possible pathophysiological mechanism is dysregulated uterine contractility.[12] Intrauterine fibroids or polyps are diagnosed in more than 20% of all patients presenting with infertility.[6,13] After hysteroscopic resection of intrauterine myoma, pregnancy rates reach about 50%[14] and bleeding symptoms are resolved in 70-99% of cases.[11]

The European Society for Gynaecological Endoscopy (ESGE) defines three subtypes of intrauterine myoma:

- Type 0: the myoma is located completely in the uterine cavity or pedunculated.
- Type 1: <50% of the myoma is located in the myometrium.
- Type 2: >50% of the myoma is located in the myometrium.

This classification is crucial in order to estimate the chance for complete resection of the myoma prior to the procedure. According to European Society of Hysteroscopy (ESH) in 2012, a complete hysteroscopic resection of intrauterine myoma is achieved in the following percentages:

- Type 0: 96-97%
- Type 1: 86-90%
- Type 2: 61-83%

The hysteroscopic resection should not be postponed in infertility patients with diagnosed submucosal myoma. Even small intrauterine myomas (<1.5 cm) without any symptoms should be removed, as the operability and rate of complete resection correlates inversely with the size of the myoma.[15] Hysteroscopic resection in large myomas (>4 cm) and Type 2 submucosal myomas requires special expertise. Long operating time and intraoperative injury of vessels are risk factors for symptoms of fluid overload, especially in large myomas. In some cases of very large fibroids, hysteroscopic resection might have to be performed in two procedures rather than one.[16]

Laparoscopic Enucleation of Myoma

The impact of myomectomy of non-submucosal fibroids on fertility and sterility remains controversial. In the case of intramural fibroids <4 cm that do not affect the endometrium, a possible influence on infertility has been reported but data are inconsistent. In a recent retrospective study, Yan et al.[17] reported that non-cavity-distorting intramural fibroids >2.85 cm in their largest dimension significantly impair the delivery rate of patients undergoing assisted reproduction (*in vitro* fertilisation/intracytoplasmic sperm injection). One possible pathomechanism may be abnormal uterine peristalsis in myoma patients, which can be improved by myomectomy.[18] In a prospective study of a cohort of assisted-

reproduction patients, laparoscopic enucleation of intramural fibroids correlated with improved rates of pregnancy compared with those receiving no procedure (56.5% versus 41%, respectively); however, this analysis did not reach statistical significance.[19] Strictly subserous fibroids <5 cm do not seem to have any significant impact on fertility and outcome of the pregnancy.[20,21] Nevertheless, patients wishing to conceive should be informed about the possibility of increased myoma growth during pregnancy, which may enhance myoma-related complications (necrosis, preterm birth, obstetric obstacle, etc.).

Unfavourable factors for laparoscopic enucleation of myoma are: size >5 cm in diameter, localisation in the anterior wall, deep infiltration of the uterine muscle, uterus multimyomatosus with >4 fibroids, and gonadotropin-releasing hormone (GnRH) agonist therapy prior to surgery.[22] Uterine functional integrity is mandatory, especially in patients who wish to conceive. Another important factor is the risk of uterine rupture during pregnancy and delivery following enucleation of myoma. Incidence of uterine rupture in pregnancy after enucleation of myoma is estimated to be 1%. Risk factors for uterine rupture are excessive electrocoagulation, haematoma, and fistula in the myoma scar and suture cavities.[23] For this reason primary laparotomy is advised in large myomas >8 cm in diameter when the uterus should be preserved.[24]

Hysterectomy

Hysterectomy remains an effective treatment option in peri and postmenopausal patients, as well as patients opting for a definite therapy for myoma-related symptoms and who do not wish to become pregnant. Patients who suffer from bulky symptoms (pollakiuria, constipation, meteorism, tenesmus, pain, dyspareunia, hydronephrosis, etc.) rather than bleeding symptoms, which can often be managed with drugs or radiological intervention, benefit from hysterectomy as the appropriate therapeutic regimen.

If cervical dysplasia can be ruled out, and if there is no history of former cervical dysplasia, hysterectomy can be performed supracervically (laparoscopic supracervical hysterectomy [LASH]). Regarding peri and postoperative complications, operating time, blood loss, and recovery, LASH shows significant advantages compared with abdominal hysterectomy and total laparoscopic hysterectomy (TLH).[25] Women undergoing LASH report significantly lower pain levels and faster resumption of normal daily activities and sexual activity in comparison with those undergoing TLH.[25,26] The patient must be informed of a 10% risk of menstrual spotting after LASH due to remaining cervical endometrioid cells.[27]

Currently, power morcellation performed during LASH is controversial due to suspected tumour cell spreading in cases of undiagnosed uterine sarcoma. The risk of tumour cell spreading of undiagnosed uterine malignancies is estimated to be <0.2%.[28,29] The prevalence of 'smooth-muscle tumours of unknown malignant potential' is even more infrequent. According to recent data, tumour cell spreading by power morcellation of undiagnosed sarcoma is associated with upstaging of the sarcoma and is suspected of worsening the prognosis.[30] However, data describing accidentally morcellated uterine sarcoma are scarce. Reporting, follow-up, and subsequent meta-analysis of as many cases as possible should be conducted.[29,31]

Preoperative risk reduction requires a careful selection of patients appropriate for LASH. Postmenopausal patients with fast-growing myoma >6 cm in diameter showing sonographic hypervascularisation and heterogeneous structure are at risk and should not undergo morcellation.[32] However, what is the alternative? As a result of the current discussion, all patients with sonographically diagnosed myoma would be considered candidates for abdominal hysterectomy, even those with the desire to conceive, in order to avoid the potential risk of morcellation of undiagnosed malignant tumours because even abdominal enucleation of myoma may cause tumour cell spreading. As a consequence, all therapeutic improvements regarding conservative or radiological interventional myoma therapy have to be questioned. While the risks of morcellation of undiagnosed uterine sarcoma may be serious, the positive aspects of LASH have been demonstrated. The advantages regarding sexual function derive from the preservation of cervical glands that play an important role in lubrication. There is a significant reduction of genital organ prolapse and dehiscence as a result of LASH,[33] with some authors reporting significant advantages regarding bladder function after LASH when compared with total hysterectomy after a 1-year follow-up.[34] However, the data concerning bladder function after LASH in comparison with total hysterectomy remain limited and controversial, and must be

interpreted with care. As a result, the patient has to be informed meticulously about the risk of power morcellation and the alternative of abdominal total hysterectomy must be discussed. Laparoscopy reaches its technical limits in very large uteri and in patients with excessive adhesions. Some patients are not appropriate for laparoscopy if pneumoperitoneum during laparoscopy is a risk due to adipositas per magna or cardiovascular problems.[35]

In perimenopausal patients suffering from bleeding rather than bulky symptoms, the indication for hysterectomy must be established with care. On the one hand, other pathologies (e.g. endometrial hyperplasia or malignancy and perimenopausal bleeding disorders) and preoperative histological diagnosis should be considered. On the other hand, myoma-related symptoms may resolve when attaining postmenopause. Consequently, in specific cases, medical therapy (e.g. ulipristal acetate [UPA], see below) may relieve symptoms until postmenopause and hysterectomy can be avoided.

In patients who do not wish to conceive but opt for myomectomy, their wish to preserve the uterus must be respected and informed consent is mandatory. In cases of large or multiple uterine fibroids, myomectomy may bear a higher risk of intra and postoperative complications and requirement of surgical re-intervention compared with hysterectomy. Furthermore, the risk of accidental tumour cell spreading must be mentioned when opting for myomectomy. In cases with adenomyosis, myomectomy alone is not sufficient to alleviate the symptoms.[36]

Uterine Artery Embolisation and High-Intensity Focussed Ultrasound

Uterine artery embolisation (UAE) was introduced in 1995. The procedure offers an established fertility-preserving alternative to surgery and aims to reduce myoma-related symptoms, such as bleeding symptoms and bulky symptoms. UAE results in myoma necrosis and shrinkage but not in complete eradication of the myoma. Embolisation of fibroid perfusion is performed via a transfemoral catheter and application of small (500 μm) polyvinyl particles, for example under radiological control. UAE is a valuable alternative in cases where there are contraindications for surgery, or if the patient refuses a procedure. In specific cases of very large myoma, preoperative UAE can be helpful in order to reduce intraoperative blood loss and to reduce the risk for hysterectomy in patients who want to undergo myomectomy.[37] Adverse events (AEs) are severe ischaemic pain that can persist for some days, post-embolisation syndrome, fever, endomyometritis, sepsis, and hysterectomy. In a recent meta-analysis including seven randomised controlled trials, UAE is associated with a higher rate of minor complications and an increased likelihood of requiring surgical intervention 2-5 years after UAE when compared with primary surgery (myomectomy and hysterectomy). According to the data, 15-32% of UAE patients require further surgery within 2 years.[38]

Hormone-based fibroid medication (e.g. GnRH agonists) should not be administered for at least 12 weeks prior to UAE due to the risk of uterine artery spasms that might disturb the procedure.[39] UAE should not be performed in women who wish to conceive and especially not in patients who present for infertility or sterility due to uterine fibroids. A significant risk of miscarriage following UAE was demonstrated (up to 64% in some studies), as well as risk of preterm delivery, abnormal placentation, and postpartum haemorrhage. In contrast with evidence-based enucleation of myoma, a significant reduction in ovarian function and an increased risk for amenorrhoea has been demonstrated.[40,41]

HIFU is a non-invasive thermoablation of symptomatic uterine fibroids that can be performed in an outpatient setting. Volumetric sonification induces homogenous tissue heating with subsequent necrosis of the fibroid. The sonification system is regulated via magnetic resonance imaging (MRI) feedback mechanisms, thus AEs are rare. HIFU is considered efficient and well tolerated in a selected patient cohort, and AEs like persistent pain or injury of neighbouring organs are reduced to a minimum. Myoma-related symptoms and the size of the myoma are significantly reduced. MRI offers exact therapy planning. MRI real-time observation of energy transmission ('sonification') generates a 'mapping' of the applied temperatures in fibroid tissues. Thus, applied temperatures are measured in order to regulate and optimise the therapy. In contrast to UAE, there is no radiation exposure in HIFU. Myoma volume can be reduced by 13.5-46% after 6 months. Efficiency regarding reduction of myoma-related symptoms and significant improvement in QoL can be assessed.[42]

Data regarding fertility and pregnancy after HIFU are limited.[43] Prospective data, and particularly randomised studies, comparing fertility and pregnancy outcomes with conventional, well-established myomectomy are missing. Apart from long-term effects regarding pregnancy and obstetric outcome, AEs on endometrium, tubal factor, and ovarian function can be hypothesised.[43] In UAE and HIFU, the patient's informed consent is mandatory, particularly in infertility and sterility patients. The patient should be informed about the lack of histopathological examination of the fibroid in conservative treatment options. Although the histological diagnosis of malignant tumours instead of suspected myoma is rare (0.1-0.2%), any alternative or conservative treatment involves the risk of undetected malignancy.[28]

Therapeutic Drugs

A higher concentration of the progesterone receptor has been found in fibroid tissue compared with normal myometrium.[44] Classical therapeutic drugs administered to myoma patients are progestins and GnRH agonists. Progesterone receptor modulators inhibit proliferation and induce apoptosis of myoma cells.[45] The cyclic use of progestins[46] can be administered to patients with bleeding symptoms. Oral contraceptive intake reduces the risk of developing myoma.[47] However, progestins do not reduce myoma growth and may even promote myoma cell growth.[7] Therefore, a clear differentiation in the treatment of myoma-induced bleedings must be made between mere symptom control, inducing amenorrhoea, and a causal therapy, reducing myoma volume.[1] In rural settings in developing countries, symptom relief achieved by the administration of progestins may, in contrast to GnRH analogues, offer a cost-effective therapeutic option. Until 2012, GnRH agonists were the only therapeutic drugs approved for the treatment of myoma. The use of GnRH analogues reduces fibroid size and bleeding symptoms.[48] GnRH agonists act by reducing serum oestrogen concentrations to a postmenopausal level.[49] However, these drugs show severe climacteric side-effect profiles, e.g. hot flushes, mood swings, loss of libido, vaginosis, depression, and bone loss. GnRH agonists should be administered 3-4 months prior to surgery and they are not recommended for long-term use.[50] Main indications are hysteroscopic resections of large submucosal and intramural fibroids. However, some authors claim that there are difficulties in fibroid preparations in myoma laparoscopy after GnRH agonist treatment.[27]

Therapeutic effects cease after stopping the medication and a high recurrence rate of fibroid growth has been observed.[51] The selective progesterone receptor modulator (SPRM) UPA was introduced in 2012.

Two large, randomised, double-blind, Phase III trials (PEARL I and PEARL II) demonstrated the effect of UPA administration for 13 weeks. A cessation of bleeding symptoms after 7 days and a 51% reduction in fibroid size were achieved.[52,53] After cessation of UPA treatment, the reduced fibroid size persists for up to 6 months. Preoperative UPA administration offers a positive effect via 'auto-transfusion' and may enable laparoscopic surgery of large myomas by preoperative shrinkage. SPRMs induce amenorrhoea while maintaining endogeneous oestrogen secretion.[7] With a daily administration of SPRMs, serum levels of oestrogen range in the low follicular phase and climacteric side-effects, such as thromboembolic effects and bone loss, do not occur.[53] The most frequent side-effects reported in women receiving multiple treatment courses are headaches, nasopharyngitis, abdominal pain, and hot flushes.[45,54]

Long-term treatment with UPA may cause sonographic endometrial thickening and progesterone receptor modulator-associated endometrial changes (PAEC). PAEC occurs in approximately 50% of all patients and is reversible 1-2 months[55,56] after cessation of UPA treatment. In cases of persistent endometrial changes 3 months after ending the UPA treatment, targeted hysteroscopic biopsy of the endometrium must be performed. These sonographic signs are generally not associated with endometrial hyperplasia but rather represent cystic glandular dilatation. Although conception during UPA intake is improbable due to suppression of ovulation, alternative contraception (especially barrier methods) must be additionally advised. The menstrual cycle also recovers quickly after ending UPA administration.[45,52] The benefits of UPA, particularly in myoma patients suffering from bleeding symptoms, are an integral part of routine myoma therapy. Future concepts of UPA administration include long-term use in order to bridge the time gap until menopause and to avoid hysterectomy. Currently, two cycles of UPA for 13 weeks with a therapy-free interval are approved.

The PEARL III study investigated the administration of up to four 3-month courses of UPA. The off-treatment period between each UPA course included one menstrual bleed and the beginning of a second bleed. In order to explore any effect on the reversibility of PAEC or timing and magnitude of the next menstruation off-treatment, 10 days of treatment with the progestin norethisterone acetate or placebo were administered after each cycle of UPA. There were improvements in amenorrhoea rates and fibroid volume reduction with successive treatment courses, while the incidence of side-effects showed a tendency to decrease after several courses. No cases of endometrial hyperplasia or adenocarcinoma were reported, although there was an increase in non-physiological pathological features in endometrial biopsies.[54]

CONCLUSION

Modern myoma therapy aims at individual patient-tailored concepts. New drugs and radiological therapies offer interesting alternatives to surgery. Therapeutic management of symptomatic myoma is based on interdisciplinary concepts involving gynaecologists, gynaecological surgeons, fertility specialists, and interventional radiologists. Patients should therefore be referred to specialised centres. Future therapeutic concepts include long-term medication without side-effects, resolving bleeding and bulky symptoms, and innovation in minimally invasive surgery and radiological interventional therapies.

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ROLE OF NUCLEAR RECEPTORS IN SPONTANEOUS AND RECURRENT MISCARRIAGE

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ABSTRACT

Although early pregnancy loss is a common complication of human reproduction, a significant proportion of miscarriages still happen for unknown reasons. Nuclear receptors are key players in trophoblast invasion and metabolism; therefore, their agonists and ligands are a promising target for the prevention of miscarriage. This review gives an overview of the existing data and literature concerning the involvement of nuclear receptors in maintaining a viable pregnancy.

Keywords: Miscarriage, recurrent miscarriage, nuclear receptor, peroxisome proliferator-activated receptor (PPAR), retinoid X receptor (RXR), liver X receptor (LXR), thyroid hormone receptor (THR).

INTRODUCTION

Spontaneous miscarriage occurs in 12-15% of known pregnancies, whereas 30% of all conceptions are lost between implantation and the sixth week. The risk of subsequent miscarriage increases with maternal age and with the number of previous miscarriages.[1] Recurrent miscarriage (RM) is defined as three or more consecutive miscarriages. The risk of recurrent spontaneous miscarriage is much higher in patients with previous losses: the risk of miscarriage after two consecutive losses is 17-25% and the risk of miscarrying a fourth pregnancy after three consecutive losses is 25-46%. Yet there remains an unsolved problem: up to 50% of cases of recurrent losses do not have a clearly defined aetiology.[2]

ROLE OF ESTABLISHED UNDERLYING CAUSES

Chromosomal abnormalities linked to maternal age are common risk factors for miscarriage.[3] Approximately 50-60% of early spontaneous miscarriages are associated with a chromosomal anomaly of the conceptus. The most common abnormality is aneuploidy, with autosomal trisomy accounting for >50% of chromosomally abnormal abortuses.[4] In the case of RM, multiple underlying causes have been identified besides karyotype changes: uterine pathologies such as uterus arcuatus, which is a uterus with a fundal impression and which accounts for 15% of all women with RM;[5] endocrine dysfunctions, e.g. thyroid disorders; and autoimmune diseases, e.g. acquired or inherited thrombophilic disorders.[6] Investigations have shown that some RM patients remain in a permanent prothrombotic state outside pregnancy.[7]

Apart from the reasons mentioned above, the cause of RM remains unknown in up to 50% of cases.[8] Therefore, identification of possible risk factors is a focus of current research. This article summarises evidence for the known implications of nuclear receptors in spontaneous miscarriage and in RM. For readers with further interest in the physiological roles of nuclear receptors in pregnancy, we recommend the following reviews: McCarthy et al., 2013 (role of peroxisome proliferator-activated receptor [PPAR]);[9] Beltowski and Semczuk, 2010 (role of liver X receptor [LXR]);[10] and Mark et al., 2009 (role of retinoid X receptor [RXR]).[11]

ROLE OF NUCLEAR RECEPTORS IN RECURRENT MISCARRIAGE

The large ligand-activated nuclear receptor superfamily includes PPAR, the retinoic acid receptors (RARs), RXR, the thyroid hormone receptors (THRs), LXR, the vitamin D3 receptors, and the steroid receptors. These receptors all function as transcription factors and, after ligand activation, they bind DNA as homo or heterodimers and regulate gene expression.[12] A recent investigation showed that some of these receptors are crucially involved in the process of spontaneous and recurrent miscarriage.

ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS IN MISCARRIAGE

This group of nuclear receptors is named for their ability to induce hepatic peroxisome proliferation in mice. Three PPAR isoforms (α, β/δ, and γ) have been identified to date and are encoded by different genes. PPARs are a key regulator of cell differentiation, the cell cycle, and the induction of apoptosis.[13,14] PPARγ is the isoform with the greatest influence on metabolism and is a strong regulator of the immune system and a key player in carcinogenesis. PPARγ binds to a specific DNA response element as a heterodimer with the RXR.[14]

PPARγ is of great interest in the field of miscarriage research for many reasons, such as its role in the regulation of fatty acid storage, glucose metabolism, and insulin sensitivity. In addition, PPARγ is involved in trophoblast differentiation and invasion,[15] as well as being a key player in anti-inflammatory processes,[16] and so this receptor can make an impact on the process of miscarriage in multiple ways. PPARγ disposes of a wide range of natural and synthetic ligands, such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J2, fatty acids including oxidised lipids, monounsaturated fatty acids such as oleic acid, and polyunsaturated fatty acids like linoleic acid and arachidonic acid.[17-19] In addition to their importance in miscarriage research, the clinical relevance of these receptors can be seen by the fact that synthetic PPARγ agonists, such as thiazolidinediones (TZDs), are already in widespread use in diabetes therapy in order to improve insulin sensitivity.[12]

PPAR is not only a key player in metabolism, however, as studies on PPAR-null mutant mice revealed the central role of PPAR in fetal development and placentation. PPAR enhances the invasion of the placental trophoblast and therefore plays a major role in maintaining a viable pregnancy.[15-20] In a human *in vitro* model, cytotrophoblast invasion was abrogated in a dose-dependent manner by PPARγ stimulation, with its blockade leading to increased extravillous trophoblast (EVT) invasion.[21,22] Furthermore, PPARγ stimulation altered the differentiation of syncytiotrophoblast (ST),[20] and PPARγ ligands induced human chorionic gonadotropin (hCG) production in human trophoblasts.[23]

Further studies in miscarriage research have revealed that PPAR activation is connected with leptin: PPAR and leptin are two important adipose tissue factors involved in the regulation of energy metabolism.[24] Leptin is a regulator of satiety and energy homeostasis. It is synthesised in adipose tissue and also in the placenta, especially in the ST and EVT.[25] Proinflammatory cytokines, such as tumour necrosis factor alpha (TNFα) and interleukin 1 (IL-1), may also directly induce leptin gene expression. Toth et al.[26] demonstrated that in normal and disturbed pregnancy there seems to be regulation of leptin triggered by nuclear hormone receptors such as PPARs and their coactivators. Enhanced expression of PPAR/RXR was identified in EVTs and STs of miscarriage. Leptin expression in the ST was lowest in miscarriages and highest in mole pregnancies.

Leptin induces hCG production in trophoblast cells.[27] Leptin production is upregulated during normal pregnancy, and leptin gene expression is regulated by a variety of hormones including oestrogen, which is responsible for the upregulation during pregnancy. Decreased leptin levels are associated with miscarriage.[28] The leptin-mediated secretion of proinflammatory cytokines such as IL-1, IL-6, TNFα, and prostaglandin E2 (PGE2) is inhibited through PPAR activation.[29,30] With regard to the dynamic interaction between PPARγ and leptin, there may be a potential strategy for intervening in the process of miscarriage: activation of PPARγ by natural ligands or TZDs inhibits leptin gene expression and leptin release both *in vivo* and *in vitro*.[31,32] PPARγ agonists positively regulate hCG, leptin, and human placental lactogen.[20] If these mechanisms are of any benefit in the prevention of miscarriage then they should be evaluated in future studies.

ROLE OF RETINOID X RECEPTOR IN MISCARRIAGE

The RXR consists of three isotypes that are referred to as RXRα, RXRβδ, and RXRγ.[33] All three RXR isotypes are potentially important in maintaining a viable pregnancy, as they are all involved in cell proliferation, cell differentiation, embryonic patterning, and organogenesis,[34] but it is especially RXRα that is a key regulator during embryogenesis and morphogenesis.[35] The heterodimer of PPARγ and RXRα regulates the uptake of fatty acids in trophoblasts, which is essential for embryonic development and production of placental steroid hormones.[16,36] Furthermore, both partners promote trophoblast differentiation, possibly because they induce the secretion of important hormones such as hCG, leptin, and lactogen.[20] Homozygous RXRα-null mice die between embryonic days (E)13.5 and E16.5,[37,38] which highlights the role of RXRα during embryonic development.

Potentially, RXRα represents a potent target in the treatment of RM: invasion of cytotrophoblasts is indirectly correlated with the concentration of RXRα and PPARγ.[16] Enhanced expression of RXRα in EVTs and villous trophoblasts of miscarried placentas was recently identified, and an increased number of apoptotic EVT is present in miscarried placentas.[35] RXR and its heterodimeric partner RAR can be activated by vitamin A derivatives termed 'retinoids'.[39] A likely conclusion is that RXRα plays an important role in the induction of apoptosis. Downregulation of RXRα, as observed in choriocarcinoma cells and trophoblasts, may serve as a protection against apoptosis and miscarriage.[35] In addition, increased retinoic acid, which is the main agonist of RXR, has an inhibitory effect on genes essential for implantation in the glandular epithelium (GE).[40] These results are in line with our findings of RXR upregulation in GE of miscarriage: the nuclear receptors PPARγ and RXRα are negatively correlated in the decidual tissue cells of physiological pregnancy, whereas this correlation is lost in miscarriage. Because expression of PPARγ is unchanged in abortive tissue compared with normal controls, we assume that upregulation of RXRα in abortive tissue is responsible for the loss of negatively correlated PPARγ/RXRα expression.

Combination of PPARγ with RXRα is essential for trophoblast differentiation, with the receptor complex inducing the secretion of gestational hormones such as hCG, leptin, and lactogen.[20,41] The heterodimer further regulates the uptake of fatty acids in trophoblasts, which is crucial for the production of placental steroid hormones and fetal growth.[16,36] Because invasion of cytotrophoblasts is indirectly correlated with the concentration of RXRα and PPARγ,[16] and the latter plays a specific role in trophoblast differentiation, function,[15,42] and fetal development,[18] the replacement by RXRα is likely to disturb physiological development during pregnancy. Furthermore, the isotype of RXRα plays an essential role during embryogenesis and morphogenesis,[43] and protects against apoptosis in trophoblasts, and so the enhanced expression of RXRα in miscarriage is twice as disruptive in early pregnancy. Expression of RXRα is increased in GE and trophoblasts during miscarriage and correlation analysis shows that increased LXR and RXR expression takes place during miscarriage, whereas LXR and PPARγ are upregulated simultaneously in regular GE. The loss of physiological correlation in nuclear receptors is supposedly responsible for the deficit in the regular function of trophoblasts and embryonic tissue.

ROLE OF LIVER X RECEPTOR IN MISCARRIAGE

LXR is a physiological regulator of lipid and cholesterol metabolism that also acts in an anti-inflammatory capacity. Because LXRs control diverse pathways in development, reproduction, metabolism, and inflammation, they have potential as therapeutic targets.[44] LXRs are expressed in human and mouse trophoblasts and the placenta from early gestation,[45] and are regulators of trophoblast invasion[46] and maternal–fetal cholesterol transport,[10,47] which makes them key players for successful placentation and embryonic development.[48]

LXR expression is downregulated in the ST of the placenta of a spontaneous abortion. However, the difference is greatest in the decidua of miscarriage; in the decidua of RMs there is no expression of LXR at all. Therefore, the downregulation of LXR could be a signal of excessive oxidative stress in the ST of spontaneous abortions. In RM, however, there is a strong immune modulation component and additional mechanisms, which, together with oxidative stress, can cause abortion.[49] Strong downregulation of LXR in the EVT and no significantly altered

expression in the ST occurs in RM. Therefore, pregnancy loss occurs in RM before oxidative damage reaches the ST layer of the placenta.

In addition, double-immunofluorescence staining showed that LXR, as well as RXRα and PPARγ, is expressed by the EVT, and RXRα and LXR showed co-expression in the same EVT cells. In the ST, a positive correlation for the combination of LXR/PPARγ occurs in abortions and there is a negative correlation for LXR/RXRα.[48] LXR activation with synthetic or natural ligands inhibits trophoblast invasion *in vitro*,[42] therefore correlation of LXR and RXRα might be a sign of increased maternal–fetal cholesterol transport. Plösch et al.[47] showed that LXR upregulation leads to increased expression of the LXR target genes *ABCG1* and *ABCA1*. This mechanism is believed to increase the cholesterol flux from mother to fetus.[47] This may be indicative of pronounced demand during embryogenesis, as cholesterol is crucially involved in neural pattern formation via hedgehog proteins and in brain development.[50-52]

The GE of the uterus and the EVT form the decidua. The GE is known to be crucial for blastocyst implantation and decidualisation in pregnancy,[53] and it further provides a nutrient-rich environment to support embryonic development until the placenta is functional.[54] Expression of PPARγ and LXR is unchanged in the GE of miscarriage: expression changes in these receptors are restricted to trophoblasts. In the GE of physiological pregnancy, a positive correlation between LXR and PPARγ was demonstrated (Knabl et al., unpublished data): here we can speculate that LXR and PPARγ are upregulated simultaneously in regular GE. As this correlation was not found in abortive tissue, increased LXR and RXR expression can be seen in miscarriage. Proper function of the GE plays a key role in implantation of the conceptus and decidualisation of the uterine stroma.[53,55] As increased LXR signalling reduces synthesis and secretion of hCG from trophoblast cells,[56] and decreases trophoblast invasiveness by matrix metalloproteinase 9,[57] these effects may be a consequence of a disturbed function in GE.

ROLE OF THYROID HORMONE RECEPTORS IN MISCARRIAGE

Thyroid hormones are essential for the maintenance of pregnancy, and a deficiency of maternal thyroid hormones has been associated with early pregnancy loss.[58] The ligands of THRs play a major role in trophoblast differentiation and fetal neurodevelopment.[59] Thyroid hormones bind to specific nuclear receptors. Two genes, *THRA* (*NR1A1*) and *THRB* (*NR1A2*), encode the isoforms THRα and THRβ which code for the four ligand-binding thyroid receptors THRα1, THRβ1, THRβ2, and THRβ3, and the four non-ligand binding receptors.[60,61] While the isoforms THRα1, THRα2, and THRβ3 are widely expressed, the expression of THRβ2 is restricted to the hypothalamus and pituitary gland.[62] The hormone T_3 is the high-affinity ligand of THR and thereby regulates gene transcription.[63] After this hormone has bound to the ligand-binding site, the THR switches to its active form and recruits specific co-activators such as SRC1-3 and PGC-1.[64] A two-fold increase in miscarriage and stillbirth rates can result from untreated hypothyroidism.[65] Hyperthyroidism and autoimmunity can also have severe effects on pregnancy outcome. Therefore, the maintenance of a euthyroid state is crucial during pregnancy and necessary for the prevention of disturbed placentation syndromes such as pre-eclampsia and intrauterine growth restriction.[65] Results obtained by our group show that expression of the THRs: THRα1, THRβ2, THRβ1, and THRβ2 is downregulated in abortive placentas, which also leads to miscarriage. The THRs are predominantly expressed in decidual stromal cells.[66] Only THRβ2 is also expressed in EVT cells. PPARγ expression was also investigated by our group and we identified an upregulation of PPARγ in miscarriage.[16,35,66,67] Interestingly, a recent study showed that activation of PPARγ signalling via rosiglitazone induced a strong downregulation of both THRα and THRβ in both brown adipose tissue and in rats *in vivo*.[68] Based on these results, we may speculate that the downregulation of THRs is also mediated by activated PPARγ, and probably the RXR system, in abortion.

SUMMARY

Nuclear receptors are key players in maintaining a viable pregnancy and play an important role in spontaneous miscarriage and RMs:

- The expression of the nuclear receptors PPARγ, RXRα, LXR, and THRs is altered in miscarriage: this group of nuclear receptors is important for embryogenesis and trophoblast invasion.
- Enhanced expression of PPAR/RXR was identified in the EVTs and STs of miscarriage. Expression of PPARγ and LXR was unchanged

in the GE of miscarriage: expression changes of these receptors are restricted to trophoblasts.

- RXRα expression is increased in miscarriage in the GE and trophoblasts, and correlation analysis showed that increased LXR and RXR expression takes place in miscarriage, whereas LXR and PPARγ are upregulated simultaneously in regular GE. The loss of physiological correlation in nuclear receptors is supposedly responsible for the deficit in regular function in trophoblast and embryonic tissue.
- LXR expression is downregulated in ST and EVT in spontaneous miscarriage. A strong downregulation of LXR in the EVT and no significantly altered expression in the ST occurs in RM. Therefore, pregnancy loss occurs in RM before oxidative damage reaches the ST layer of the placenta.
- Expression of the THRs: THRα1, THRα2, THRβ1, and THRβ2 is downregulated in abortive placentas, which also leads to miscarriage. The THRs are predominantly expressed in decidual stromal cells.

Future research should focus on the investigation of existing agonists and antagonists in the prevention of miscarriage in order to bring experimental data towards achieving clinical improvement.

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THE EPIDEMIC OF TWINS: THE CHALLENGE IN OBSTETRICS AND GYNAECOLOGY

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ABSTRACT

In the past 50 years, the incidence of multiple pregnancy has increased dramatically due almost exclusively to two factors: delayed childbearing and assisted reproduction techniques (ARTs). Although the clinical guidelines and protocols currently endorsed by scientific societies have systematised the obstetric care required in multiple pregnancy, the effects of obstetric care beyond the perinatal period have seldom been evaluated. Twin deliveries also involve additional difficulties derived from the need for 'simultaneous' care of two fetuses during the expulsive period, during which the second twin is particularly vulnerable due to potential complications. Chorionicity, gestational age at birth, ART, birth order, and some parental variables, such as maternal age, have been studied as possible sources of neurodevelopmental delay in twins.

Keywords: Multiple pregnancy, assisted reproduction, perinatal outcomes, preterm birth.

MULTIPLE PREGNANCY

Simultaneous development of two or more fetuses in the uterus is a normal phenomenon in lower mammals but is rare in humans. Evolutionary adaptation of the maternal organism is aimed at developing a single fetus. Therefore, multiple pregnancy, though not strictly pathological, represents a deviation from the norm and, to some extent, a phylogenetic regression. If we consider that the first hominid species with human reproduction patterns appeared 2.5 million years ago, then we can assume that the increased number of multifetal pregnancies observed in the last 50 years will carry a biological price. In this sense, Keith[1] wondered 30 years ago whether the maternal and neonatal risks posed by iatrogenic multiple pregnancy were justified.

Multiple pregnancy increases first trimester symptoms such as nausea, headache, dizziness, and cramps, while hyperemesis gravidarum, threatened abortion, gestational hypertension, gestational diabetes, anaemia, venous insufficiency, and sciatic pains are more frequent. Hypervolume in the final months produces palpitations, persistent constipation and frequent urination, oedema and vascular compression in the legs and vulva, and dyspnoea. Maternal weight gain in multiple pregnancy is higher than in singleton pregnancy, approximately 20 kg.[2]

The risk of maternal complications such as preeclampsia, diabetes, premature rupture of membranes, and anaemia increases with the number of fetuses.[3,4] In addition, the risk of maternal death is 3.6-times higher in multiple gestations (plurality-specific pregnancy mortality ratio: 20.8 deaths per 100,000 multifetal pregnancies versus 5.8 deaths per 100,000 singleton pregnancies) independent of race and socio-cultural status.[5] From a psychological point of view, multiple births are also a source of destabilisation and social disruption in relationships. Such situations cause stress, depression, and frequent mood swings. The studies published to date suggest that these problems are present in one of every three or four families. Postpartum depression is 40% more likely in cases of multiple pregnancy.[6]

The most common complication of multifetal pregnancy is preterm birth.[7] Birth occurs before 37 weeks in 50% of twin pregnancies and in 99% of triple pregnancies, which results in perinatal morbidity and mortality. The risk of cerebral palsy is four-times higher in twins than in single births.[8] The probability of dying in the first year of life due to prematurity is seven-times higher in the case of twin pregnancies, and the proportion with low birth weight is up to seven-times higher.

CAUSES

During the past 50 years, the incidence of multiple pregnancy has increased due almost exclusively to two factors: delayed childbearing and assisted reproduction techniques (ARTs).[9,10] Age is considered an independent risk factor for natural multiple pregnancy.[11] The probability of conceiving more than one fetus increases between the age of 35-39 years and declines thereafter, except among the black population in whom an increasing trend is observed after this age.[12] In these populations, higher levels of endogenous follicle-stimulating hormone (FSH) cause multiple ovulations and, consequently, multiple pregnancies.

On the other hand, ARTs are the only possibility of conceiving for some women, and so treatments in assisted reproduction centres include high doses of FSH to allow the development of more than one egg per cycle, or intrauterine transfer of more than one embryo simultaneously, in order to increase the chances of pregnancy during a cycle of ovarian stimulation. Results of ART are very often evaluated only in terms of implantation rates and pregnancy rates per cycle, and ignore everything related to pregnancy and multiple birth complications, prematurity of newborns, or the economic or psychological overload that appears when more than one child is delivered.

The role of ART as the primary aetiological factor for multiple pregnancy is clear, as is the interest of fertility centres in preventing its occurrence. Commercial interest in private centres and the economic interest of the pharmaceutical industry make it very difficult to consider the biological, economic, social, and psychological costs relating to multiple pregnancies, transferring multiple embryos, and hyperstimulating ovarian cycles.[13] Given the extraordinary consequences of these procedures and the lack of communication between ART specialists and obstetricians, medical societies should promote new regulations. When a multiple pregnancy is detected, the obstetrician is usually the one who has to warn the future parents about the physical, social, psychological, and economic consequences. However, accurate information should be provided to women prior to ART. Actions such as the official promotion of single-embryo transfer or the compulsory and systematic registration of final perinatal outcomes after ART performed in fertility centres should be taken into account.

OBSTETRIC VARIABLES AND LONG-TERM OUTCOMES IN TWINS

Psychoevolutionary development, skill acquisition, behaviour, and school achievement are subject to the influence of a number of variables. Some of these variables, which affect the perinatal period, have often been associated with a poor outcome. On the other hand, the increasing number of multiple pregnancies in recent years has raised a particular concern about the problems associated with such pregnancies in terms of prematurity and adverse perinatal outcomes.

It should be noted that although the scientific literature is riddled with studies conducted in twins, most of them stem from the paradigm of medicine and 'differential psychology' introduced by Galton in the 19th century.[14] This scientist delved into the debate between the innate and the acquired, and focussed on ascertaining the impact of heredity and environment on certain health traits. A smaller number of studies in twins report that these children are a unique population group with particular needs and an intrinsic complexity amenable to specific clinical, social, and educational research. Although the clinical guidelines and protocols currently endorsed by scientific societies have systematised the obstetric care required in this type of pregnancy, the effects of obstetric care beyond the perinatal period have seldom been evaluated. Most of these guidelines are based on expert recommendations rather than randomised studies.[5]

It is worth mentioning that pregnancy risks, clinical management, and subsequent outcomes are very different for monochorionic and dichorionic twin pregnancies. Therefore, determination of chorionicity is required in order to correctly stratify perinatal risk according to the type of twin pregnancy. Even the duration of the multiple pregnancy depends on chorionicity. On the one hand, women with monochorionic,

uncomplicated twin pregnancies should know that elective birth from 36 weeks and 0 days does not appear to be associated with an increased risk of serious adverse outcomes, and that continuing uncomplicated twin pregnancies beyond 38 weeks and 0 days increases the risk of fetal death. On the other hand, women with dichorionic, uncomplicated twin pregnancies should be aware that elective birth from 37 weeks and 0 days does not appear to be associated with an increased risk of serious adverse outcomes, and that continuing uncomplicated twin pregnancies beyond 38 weeks and 0 days increases the risk of fetal death.

Delivery of twins involves additional difficulties derived from the need for 'simultaneous' care of two fetuses during the expulsive period, during which the second twin is particularly vulnerable due to potential complications. In this regard, we believe that it has not been adequately evaluated whether twin deliveries, as currently performed, provide the same chances to both fetuses and whether this has any impact on subsequent stages of development.[15] It is very difficult to define the period in which obstetric variables started to be considered important for normal neuropsychological development in children. As early as 1955, Pasamanick and Lilienfeld[16] demonstrated an association between some maternal and fetal factors and delayed child development, and postulated the hypothesis of the 'continuum of reproductive casualty'. The observed relationship between certain neuropsychological disorders and some obstetric complications, such as placenta previa or obstructed, multiple, or premature delivery, led these authors to hypothesise the existence of a continuum of casualty in which an extreme of severe, lethal fetal involvement (cases of abortion and fetal or perinatal death) and a number of other conditions that involve sublethal damage, possibly including cases of cerebral palsy, epilepsy, mental retardation, learning and developmental difficulties, and behavioural disorders, could be recognised. Many studies have subsequently been able to document the existence of neurological and cognitive development problems in children exposed to obstetric and perinatal risks such as prematurity, low birth weight, and perinatal hypoxia;[17] one study has demonstrated that, even when these children have apparently normal psychometric results, there are often learning difficulties and special educational needs.[18] It should be taken into account that any association between prematurity and psychological development may be due to the causes of prematurity rather than to prematurity itself.[19]

Because a significant growth in brain mass occurs during the final weeks of gestation, during which corticomedullary differentiation processes are also completed, the risk of impaired neurological and cognitive development is greater in late preterm infants compared with term infants.[20] Behavioural problems,[21] neurodevelopmental delay, and difficulty acquiring reading skills[22,23] have been reported. In agreement with these studies conducted in single pregnancies, a statistically significant correlation between twins' gestational age at birth and intelligence quotient scores at 6 years of life has been reported. In addition, scores were systematically higher in children born beyond 37 weeks of gestational age in all school achievement areas evaluated.

In some studies, the use of ART has been associated with the occurrence of neurodevelopmental delay in children.[24] Although results are conflicting, assisted reproduction protocols do not appear to be associated with severe cognitive impairment or significant neurodevelopmental delay, but their potential relationship to mild delay or impairment should be investigated.[25] Because one of the most common complications of ART is the high multiple pregnancy rate, assessment of the impact of these techniques on academic achievement, intelligence, and neuropsychological maturity areas in twins is of special interest.

Various studies have associated advanced maternal age with better academic achievement[26] and better scores in the neurocognitive evaluation of children.[27] Some authors[28] suggest that this association is mediated by the existence of favourable social and familial determinant factors associated with advanced maternal age, which allow for child development in a more educational and stable environment. The differences between children born first and second according to the type of delivery have also been investigated. Some authors have reported a greater risk of perinatal morbidity in vaginal delivery of the second twin.[29-32] Other studies, however, could not show any benefit of elective caesarean section over vaginal delivery of the second twin.[33-35] It appears difficult to reach conclusions in this regard. While delivery by caesarean section prevents a poorer score in development areas for the second-born twin, the

impact of the type of delivery can vary with regard to academic achievement, neuropsychological development, and intelligence. It was revealed that vaginal delivery was associated with better scores in the areas of reading accuracy, total reading, phonetic orthography, visual orthography, calculation, writing, articulatory language, expressive language, spatial structuring, visual perception, non-verbal development, and matrices. Based on these results, vaginal delivery appears to be the most advantageous option.

Twin delivery, however, involves a certain hierarchy in birth conditions so that, although vaginal delivery is globally associated with better results in the reported areas, when the hierarchy involved in birth order is introduced then circumstances where the second twin benefits from delivery by caesarean section are detected. Published results[32] suggest a disadvantage to the second twin, with the differences in the perinatal period noted by other authors[36-38] possibly being reflected at school age. At this point in time, management of the risks and interests of each infant and the mother should be thoroughly agreed upon.

Multiple pregnancy is a source of medical complications for mothers and newborns. Long-term effects on child development are not negligible, are a source of psychological maladjustment and destabilisation of the family, and generate a significant economic burden. The economic cost of a multiple pregnancy may be 10-30-times the cost of a single *in vitro* fertilisation cycle, which is the main aetiological factor. The increase in multiple pregnancies can be described in terms of an epidemiological health alert. It is necessary to develop clinical protocols for single-embryo transfer and legislative tools in order to reduce maternal morbidity, perinatal mortality, and disability resulting from the prematurity caused by multiple births.

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