Issn 1110 -6352

THE EGYPTIAN JOURNAL OF FERTILITY AND STERILITY

Volume 18 Number 1 January 2014

EFSS

EDITOR : MOHAMED YEHIA

The Egyptian Journal Of Fertility And Sterility

The Official Journal of the Egyption Fertility and Sterility Society (**EFSS)**

Instrucions To Authors

SUBMISSION OF PAPERS

Manuscripts should be written in English, typed with double spacing, submitted and, where possible, on a disk. Figures and diagrams should, if possible be used instead of tables. The work shall not be published elsewhere in any language without the written consent of the editor in chief. The articles published in this journal are protected by copyright. Contributors should submit their papers and disk to:

Editor in chief
Prof dr. Mohamed Yahia
Prof. ob & gynecology, Ain Shams University
Email: mysoliman@gmail.com

Asst. Editor:
Prof. Ahmed Badawy,
Prof. ob & gynecology, Mansoura University.
Email: ambadawy@yahoo.com

Preparation of manuscripts

- Papers should be typed double- spaced, on white paper, size A4 (210 x 297 mm). upper, lower, right and left margins should have a minimum of 25 mm.
- The pages should be numbered consecutively, beginning with the title page, each section of the manuscript should commence on a new page, in the following sequence: title page; abstract, synopsis, and key words, main text (ending with acknowledgments); references; tables; and legends for illustrations.

Title page

The title page should contain:

1. The title itself, and subtitle if any.
2. The number(s) of the author(s), first name(s) mentioned and highest academic degree).
3. The number(s) of the department(s) and/ or institution(s) from which the study originated.
4. The name and full address (including telephone and tele-fax numbers) of the “corresponding” author.
5. A “running title” of maximum 40 characters, including word spaces.

Abstract, Synopsis and Key words

- Page 2 of the manuscript. shou’d carry an Abstract not exceeding 250 words. A structured abstract is required for original research articles; excluded are case reports and brief communications. The structured abstract should contain the following headings (each of them beginning a new paragraph): Background and aim: (main question or hypothesis), Methods (Study design, number and type of subjects, treatment, and type of statistical analysis), Results (outcome of study and statistical significance, if appropriate). Conclusions (those directly supported by data, along with any clinical implications).
- The abstract should be followed by 3 - 7 key words or short phrases for Indexing purposes. Key words should be separated by semicolons.
- Synopsis: A ~ummary of the abstract in maximum of 30 words to be printed in the table of contents mainly describing the conclusions.

Main Text

- The text is conventionally divided into sections headed; Introduction, Material and Methods, Results, and Discussion. Lengthy papers may require sub-headings for clarification, particularly in the Results and Discussion sections.
- When reporting research on human beings, the authors must include an assurance that the work was approved by a medical ethics committee and that the subjects gave their informed consent to participate. do not repeat in the text all the data displayed in the tables or illustrations, do not repeat detailed data (numbers) of results in the discussion section. Avoid unqualified statements and conclusions that are not supported by the data.

Acknowledgments

Acknowledgments should only be made to funding institutions and organizations and, if to persons, only to those who have made substantial contributions to the study.

References

- References should be numbered consecutively (Arabic n merals) in the order in which they appear in the text. In the text section, the reference numbers should be given in parentheses. References within tables or legends should be numbered in accordance with the order in which they appear in the text.
- Avoid abstracts as references. Unpublished observations and personal communications -may not be used as refer - ences, but may be cited within parentheses in the text. Only papers published or in press should be numbered and .included in the reference list. Use the form of references adopted in index Medicus i.e., the Vancouver Style

Examples of correct form of references

1- Standard journal article

List all authors when six or less. When seven or more, list only first six and addetal. Toppozada MK, Gaafar AA, Shaala SA. In - vivo inhibition of the human non pregnant uterus by prostaglan din E2. Prostaglandins, 1974; 8: 401 - 406.

2- Books:

(a) Personal author: Speroff L, Glass RH, Kase NO. clinical gynecologic endocrinology and infertility. 4th edition, Baltimore, Williams & Wilkins; 1988: 105

(b) Chapter in book; Wilhelmsson L, Norstrom A, Tjugum 1, Hamberger L. Interaction between prostaglan dins and catecholamines on cervical collagen. In: Toppozada M., Bygdeman ‘. M., Hafez ESE, Eds. Prostaglandins and fertility regulation. Advances in reproductive health care. Lancaster, England, MTP Press Ltd., 1985 : 75 - 80.

3- Agency publication

National Center for Health Statistics. Acute conditions: incidences and associated disability, United States July 1908 - June 1909. Rockville. MD.: National Center for Health Statistics, 1972.

Tables

Tables should be typed on separate sheets. They should be numbered consecutively (in Roman numerals) and should be provided with a brief title. Vertical and horizontal lines should not be used within the body of the table.

Illustrations

All figures must be clear and submitted either as glossy black and white photographs or as graphic reproductions (Two complete sets); freehand or typewritten lettering is unacceptable. Roentgenograms and similar material should be submitted as photographic prints. Letters, numbers and symbols must be clear and large enough to remain visible after size-reduction for printing.

Each figure should have on its reverse side, lightly written by pencil, the numerical order (Fig. #), the name(s) of the author(s), and the correct orientation, e.g., an arrow pointing to the top. Do not mount it on cardboard, or use clips or tapes.

Photomicrographs must have an internal scale marker (or the magnification factor must be given in the legend). Any symbols, arrows or letters used should be in strong contrast with the background. Previously published illustrations must be acknowledged, giving the original source; with a written permission from the copyright-holder to reproduce the material. No permission is required for documents in the public domain.

For illustrations in colour, colour negatives or positive tran parencies must be supplied . .Legends for illustrations should be typed on a separate page, using Arabic numerals corresponding to the illustrations.

Proofs

Proofs will be sent for the correction of typographic errors only. No change in make-up can be accepted. Proofs not returned within 10 days will be considered approved by the author.

The Egyptian Journal of Fertility and Sterility has no page charges and offers no free reprints. The cost of printing illustrations in colour will be charged to the author(s). Significant changes in the printed proofs will also be charged to authors.

Contents :

Letter from the Editor:

Dear colleagues,

As this issue comes up to your hands, you will notice we tried to incorporate your previous suggestions. We welcome your comments as well as the scientific activity to be incorporated in the upcoming issues.

The society is planning a wide variety of activities during this year. Our activities will include meetings in Ismailia, Mahala and Damietta governorates, in addition to our Annual International conference in Cairo. We certainly welcome any invitation from local activity and we will try to participate in them.

As always we pray to god to keep Egypt safe and to grant its prosperity.

Editor in Chief,
Prof. Mohamed Yehia

INFERTILITY AND ENDOMETRIOSIS: A GLOBAL PERSPECTIVE

Dominique de Ziegler, MD[1], [3]VanessaGayet, MD, [1]Hanadi Mohmmde1 Alain Daher, [1]PietroSantulli, MD[1], 2Charles Chapron, MD[1], [2]
[1]Université Paris Descartes – Assistance PubliqueHôpitaux de Paris, CHU Cochin, Dept Of Obstetrics & Gynecology and Reproductive Medicine II, Paris, France.
[2]Cochin Institute, CNRS UMR 8104, INSERM U1016, Paris, France.

Abstract

Endometriosis adversely affects fecundity by sets of different mechanisms acting at the level of the pelvic cavity, ovaries and the uterus itself. Surgery for endometriosis facilitates the chances of natural conception for the ensuing 6-18 months. It is therefore necessary to ensure that natural conception is possible (sperm, tubes) and that the age of the patient and her ovarian reserve status permit to dedicate 6-18 months for attempting a natural conception. Surgery offers no benefit on ART outcome and may actually often harm by further compromising ovarian function and responses to COS. Temporary ovarian suppression using either, GnRH-a for 3-6 months or OC for 6-9 months corrects the alterations seen in the endometrium in case of endometriosis and optimizes ART outcome.

Key words: Endometriosis, endometrioma, infertility, fertilization, implantation, ovarian reserve, AMH

Introduction

Endometriosis is a disease of unknown origin causing pelvic pain and infertility 1. While the links between endometriosis – location and extension – and pain are relatively well established, the exact cause of infertility still eludes our understanding in most cases. The number of mechanisms by which endometriosis can interfere with fecundity are too numerous to list here. For clarity sake, we elected to regroup them by the actual territory in which they are expressed (Fig. 1). This classification is functionally important as it actually outlines the respective efficacy of the various therapeutic measures envisioned depending on the individual circumstances encountered.

Pelvic cavity inflammation and in vivo conception

In the pelvic cavity, the effects of endometriosis mainly amount to an activation of sets of inflammatory processes that collectively interfere with the sperm-oocyte interactions. The net result of such effects is therefore a decrease in the capacity of conceiving naturally, or 'in vivo'.
Fig. 1From: de Ziegler et al. Lancet 2010;376:730-8.

Ovarian endometriosis and responses to COS
Fig. 2 From: Vercellini et al. Human Reprod 2009;24:254-69

Fig. 3. ART outcome of women
with and without endometriosis

Uterine alterations and endometrial receptivity

Throngs of recent data have concurred to show that the endometrium – the 'eutopic endometrium' – is abnormal in case of endometriosis5. The functional alterations encountered in case of endometriosis are seen as likely to impede the quality of endometrial receptivity and thus, account for the reduced ART outcome typically described in these cases67. Of paramount importance is the characteristic form of resistance to progesterone, which isprone to beleaguer the sequence of secretory changes normally seenduring the luteal phase8. Remarkably, the endometrial anomalies described in endometriosis were all shown to regress following the suppression of ovarian function, using either GnRH-a 9, or the OC pill10.
Clinical trials have indicated that 3-6 months of pre-ART ovarian suppression with GnRH-a significantly improved ART outcome11. Remarkably, GnRH-a treatment did not decrease the magnitude of ovarian response to COS in comparison to controls who received no pre-ART treatment11. The latter finding likely resulted from the careful observance by these authors 11 of a proper recovering phase – a 2-week delay since the last administration of long-acting GnRH-a – before starting COS.

We recently demonstrated that a similar normalization of endometrial receptivity could be achieved in endometriosis by suppressing ovarian function with OC, as illustrated in Fig 312.Women who received pre-ART administration of OC (red columns) maintained the pregnancy rates encountered in controls even when endometriomas were present at the time of oocyte retrieval. On the contrary, women who did not receive pre-ART treatment with OC has significantly lower outcome when endometriomas were present. When using pre-ART treatment with OC, it is necessary to observe a 6-day recovery interval – OC-COS – for assuring that the ovarian response to COS is not impeded. Moreover, the OC pill causing a profound suppression of endogenous LH 13 mandates using some form of LH effect during the late stage of COS14. In our hands, we reported that the ovarian suppression using OC offered optimal pregnancy rates, while causing far less side effects than ovarian suppression using GnRH-a 12.

Conclusion

Infertility in case of endometriosis mandates the deployment of a global approach in order to tailor individual therapeutic options offered according to each patient's best interests. Early on in the infertility workup it is worth assessing whether that patient's infertility is best approached by (i) surgery, or (ii) ART. The algorithm used at Cochin is outlined in Fig. 4. Infertility-wise, surgery enhances the chance of conceiving naturally during the ensuing 6-18 months. Hence, it is first necessary to determine whether natural conception is possible before scheduling the surgical procedure. Therefore, it is necessary to assess the sperm quality (>1Mi motile sperm/specimen) and tubal status. It is also indispensable to determine Fig. 4. Infertility and endometriosis: therapeutic algorithm.

Conversely, when the patient's age and/or ovarian reserve status precludes the possibility of waiting for 6-18 months after surgery or when the sperm and/or tubal status are incompatible with natural pregnancy, ART should be undertaken immediately. In these latter cases, pre-ART suppression of ovarian function is recommended using either GnRH-a for 3-6 months or, OC for 6-9 weeks. In case of pre-ART use of OC, it is necessary to allow an OC-COS interval of 6 days for permitting the proper recovery of ovarian function. It is also necessary to assure an LH effect using either minihCG or hMG in the late stage of COS to counteract the profound gonadotropin suppression – including LH – induced by OC. In principle, surgery is not warranted prior to ART, as it has been shown to either not help or, most often bear detrimental consequences for ART outcome.

The new rule proclaiming **'no-surgery-before IVF'** knows certain exceptions however. Indeed, surgery is to be considered before ART in case of: (i) pelvic pain ;(ii) hydrosalpynges;(iii) large endometriomas (5-7cm) and; (iv) when doubts exist as to exact nature of the ovarian pathology seen on pelvic imaging. Strict abidance to this algorithm, will be ultimately avoid that the patient's final treatment of infertility and endometriosis solely depends on the primary activity of doctor who was first encountered, as it is still too often the case.

Dominique de Ziegler, MD
Professor and Head
Reproductive Endocrinology and Infertility
Hôpital Cochin – Port Royal
53 Ave de l'Observatoire
75014 Paris, France
E-mail: ddeziegler@orange.fr

References

1. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet 2010;376:730-8.
2. Vercellini P, Somigliana E, Vigano P, Abbiati A, Barbara G, Crosignani PG. Surgery for endometriosis-associated infertility: a pragmatic approach. Hum Reprod 2009;24:254-69.
3. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod 2011;26:1616-24.
4. Streuli I, de Ziegler D, Gayet V, et al. In women with endometriosis anti-Mullerian hormone levels are decreased only in those with previous endometrioma surgery. Hum Reprod 2012;27:3294-303.
5. Wang G, Tokushige N, Fraser IS. Nerve fibers and menstrual cycle in peritoneal endometriosis. Fertility and sterility 2011;95:2772-4.
6. Giudice LC, Telles TL, Lobo S, Kao L. The molecular basis for implantation failure in endometriosis: on the road to discovery. Annals of the New York Academy of Sciences 2002;955:252-64; discussion 93-5, 396-406.
7. Pabona JM, Simmen FA, Nikiforov MA, et al. Kruppel-like factor 9 and progesterone receptor coregulation of decidualizing endometrial stromal cells: implications for the pathogenesis of endometriosis. The Journal of clinical endocrinology and metabolism 2012;97:E376-92.
8. Aghajanova L, Velarde MC, Giudice LC. Altered gene expression profiling in endometrium: evidence for progesterone resistance. Seminars in reproductive medicine 2010;28:51-8.
9. Kamada Y, Nakatsuka M, Asagiri K, et al. GnRH agonist-suppressed expression of nitric oxide synthases and generation of peroxynitrite in adenomyosis. Hum Reprod 2000;15:2512-9.
10. Choi YS, Cho S, Lim KJ, et al. Effects of LNG-IUS on nerve growth factor and its receptors expression in patients with adenomyosis. Growth Factors 2010;28:452-60.
11. Surrey ES, Silverberg KM, Surrey MW, Schoolcraft WB. Effect of prolonged gonadotropin-releasing hormone agonist therapy on the outcome of in vitro fertilization-embryo transfer in patients with endometriosis. Fertility and sterility 2002;78:699-704.
12. de Ziegler D, Gayet V, Aubriot FX, et al. Use of oral contraceptives in women with endometriosis before assisted reproduction treatment improves outcomes. Fertility and sterility 2010;94:2796-9.
13. Damario MA, Barmat L, Liu HC, Davis OK, Rosenwaks Z. Dual suppression with oral contraceptives and gonadotrophin releasing-hormone agonists improves in-vitro fertilization outcome in high responder patients. Hum Reprod 1997;12:2359-65.
14. Meldrum DR, Scott RT, Jr., Levy MJ, Alper MM, Noyes N. Oral contraceptive pretreatment in women undergoing controlled ovarian stimulation in ganirelix acetate cycles may, for a subset of patients, be associated with low serum luteinizing hormone levels, reduced ovarian response to gonadotropins, and early pregnancy loss. Fertility and sterility 2009;91:1963-5.

UTERUS TRANSPLANTATION: NON-HUMAN PRIMATE RESEARCH AND HUMAN CASES

Liza Johannesson MD, PhD[1], Anders Enskog MD, PhD[2], Niclas Kvarnström MD[3], Mats Brännström MD, PhD[1,4]

[1]Department of Obstetrics and Gynecology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[2]Department of Anesthesiology and Intensive Care, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[3]Sahlgrenska Transplant Institute, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Abstract

One of the still untreatable subgroups of female infertility is the group of women suffering from absolute uterine factor infertility (AUFI), which is due to either absence of the uterus or that a present uterus is non-functional in terms of ability to provide the necessary local environment that is required for initiation/continuation of pregnancy. Uterus transplantation (UTx) may become a future treatment for women with AUFI. This review summarizes the AUFI patient populations that may become candidates for UTx and the non-human primate research experiments, which have paved the way for the experimental human UTx cases that are currently underway. Additionally, the few human cases that have been performed and the ethics around human UTx will be commented. It is further pointed out that experimental human UTx should only be done under a strict scientific protocol by a group with extensive research experience in the field and in a facility with expertise in advanced extraperitoneal gynecologic surgery, transplantation surgery, reproductive medicine and high-risk obstetrics.

Key words: human; infertility; non-human primate; transplantation; uterus

Introduction

Parenthood is unquestionably one of the most important expectations in life. Consequently, infertility is a worldwide public health issue and the prevalence in women aged 20-44 years is approximately 9 %, with a range of 3.5–16.7 % depending on specific populations . While many infertile couples overcome their situation and become parents, some after infertility treatment and others through adoption or surrogacy, many couples remain involuntary childless. One such group is the women with absolute uterine factor infertility (AUFI), because of lack of the uterus or presence of a non-functional uterus. The causes of AUFI will be further discussed below. Transplantation of organs/tissues is not only restricted to those with life-threatening illnesses. In recent years, also quality-of-life enhancing transplantation types, such as transplantation of the face, hand and larynx, have entered the clinical arena. It is quite possible that uterus transplantation (UTx), as a treatment of AUFI, will soon be added to this list of quality-of-life enhancing types of transplantation. In addition, UTx will be a life-propagating type of transplantation, similar to ovarian transplantation . Research in the field of UTx has been very active during the last decade, covering experiments in many animal models including rodents , rabbits and large domestic species . Important issues that have been resolved in these studies are uterine tolerability to cold and warm ischemia, pregnancy in non-rejection setting , rejection mechanisms , suitable immunosuppression and pregnancy after allogeneic UTx . The research on UTx has lately come to include also a substantial number of studies performed in non-human primate species and these studies are described and discussed below.

Absolute uterine factor infertility (AUFI)

Absolute uterine factor infertility (AUFI) is either congenital or acquired and can also be grouped into absolute or relative. The group of women with AUFI constitutes approximately 3-5 % of the infertile female popu-

Mats Brännström
Department of Obstetrics and Gynecology
Sahlgrenska University Hospital
SE-41345 Gothenburg, Sweden
E-mail: mats.brannstrom@obgyn.gu.se

lation.The size of this group has been estimated to be around 13 000 only in the UK . This would correspond to around 17 000 in Egypt and 80 000 women with AUFI in the Middle East region.

One large subgroup of women with congenital AUFI is the women with Müllerian duct anomalies (Fig. 1). The Müllerian ducts are of mesodermal origin and are the primary roots of the internal female reproductive organs. During fetal life, the ducts differentiate to form the Fallopian tubes, uterus, uterine cervix and the upper 2/3 of the vagina. The incidence of uterine malformations in the general population is estimated to be around 4.3% . Naturally, the incidence of Müllerian duct anomalies is higher among infertile women and in those having recurrent spontaneous abortion, rates up to 15% have been reported . It should be clearly stated that a majority of these congenital uterine malformations are not associated with infertility, as further discussed below.

The most prevalent type of congenital uterine anomaly among infertile women is the septate uterus , which is the result of incomplete resorption of the central parts of the Müllerian ducts after fusion (Fig. 1). The septate uterus constitutes around 1/3 of all uterine malformations . Spontaneous abortion is seen in about 80% of pregnancies in untreated septate uteri . Hysteroscopic resection is an effective treatment and markedly decreases the rate of spontaneous abortion, but a small proportion of these women remain infertile .
The second most common type of Müllerian duct anomaly is the bicornuate uterus (Fig. 1), where disturbed fusion of the Müllerian ducts, gives rise to bilaterally fully developed uterine horns with a single cervix and vagina. This malformation represents around 1/4 of all uterine malformations . The rate of spontaneous abortion among women with bicornuate uteri is around 35% . Surgery may normalize the increased rate of spontaneous abortion , but a large number of women with bicornuate uteri will not be able to carry a pregnancy to the third trimester.

The less common uterine malformation type of unicornuate uterus (Fig. 1) comprises around 10% of the uterine malformations . Disturbed development of one of the Müllerian ducts results in the unicornuate uterus, with or without (Fig. 1) a contralateral rudimentary uterine horn. Such a rudimentary horn could be either communicating or non-communicating. It should be noted that there often exists renal agenesis on the contralateral side of unicornuate uterus . The unicornuate uterus is associated with infertility in some cases and any pregnancy in a unicornuate uteri should be considered a high-risk pregnancy since this malformation is associated with increased risk of preterm labour (43%), spontaneous abortion (34%) and ectopic pregnancy (4%) . A rudimentary uterine horn that contains functional endometrium should be surgically removed, in order to prevent menstrual cycle-related pain but surgery does not improve the pregnancy potential .

A total failure of fusion of the Müllerian ducts results in a didelphic uterus (Fig. 1), i.e. two separate uterine horns without a common cavity. This malformation represents around 10% of the total group of uterine malformations . In didelphic uterus, the duplication of the vagina and the cervix may be partial or complete. The usual form is that of two separated uteri but with the endocervical canals fused at their distal ends. The potential to establish a pregnancy in a didelphic uterus is decreased and in case of pregnancy, around 30% will end in miscarriage and the total live birth rate is only around 50% . Surgery does not improve the pregnancy potential of the didelphic uterus . There exist reports of simultaneous pregnancies in each didelphic horn, with long intervals between deliveries , that would indicate origin in different ovulatory cycles.

The most extensive type of Müllerian duct anomaly is uterine agenesis, which represents around 3% of all these congenital uterine malformations and it is seen in around one in every 5000 newborn girls . Typically, these women have a rudimentary solid bipartite uterus in combination with absence of the vagina above the hyminal ring. The syndrome is generally named the Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome or simply the Rokitansky syndrome. Three subtypes of the MRKH syndrome exist. The typical subtype, which goes without extrauterine malformations, is present in around 50%. The atypical MRKH subtype, with associated malformations in the renal and auditory system, is present in around 20%. In the remaining 30% of MRKH patients a severe form exists with typical malformations also in the skeleton of the upper back and neck . The outcome of gestational surrogate pregnancies with MRKH patients as the genetic mothers does not indicate any increased malformation risk in offspring.

Leiomyoma is probably the most common cause of AUFI (Fig. 1). There exist reports of prevalence figures, among reproductive-aged women of selected populations, as high as 20-40% . However, a more true prevalence may be that of around 5.5%, which was reported in a random sample of Swedish women between 25 and 40 years . In that study, a higher prevalence (8%) was seen in the subgroup of older women between 33 and 40 years of age. A comparable prevalence was seen in Caucasian women in the United States, but with a 2-fold higher prevalence in Afro-American woman . In total, approximately 1% of all women between 30 and 34 years and around 2.5 % of those between 35 and 39 years have been hysterectomized due to leiomyoma in the United States . Hysterectomy, secondary to leiomyoma, is naturally the most obvious group of leiomyoma-related AUFI. However, more often leiomyoma will lead to infertility in a woman that still has her uterus , with both structural and biochemical factors contributing to this leiomyoma-related infertility . In a thorough review of a large IVF population, it was shown that larger (> 4 cm) and subendometrial leiomyoma are those that cause infertility . A large extent of the leiomyoma-related infertile population can be treated by myomectomy , but some remain infertile.

One cause of hysterectomy during fertile age is cervical cancer, which worldwide is one of the most common gynaecological malignancies in females , but with considerably lower incidence in countries with cervical cytology screening programs. Around 30-40% of cervical cancer patients in developed countries are of fertile age at diagnosis . Tumors of low stage can be treated by uterine-sparing surgery (conization, trachelectomy) but radical hysterectomy is the recommended treatment for larger stage Ib-IIa tumors. The ovaries can be spared in squamous cell carcinoma of the cervix because the risk of ovarian metastasis is very low.

Emergency peripartum hysterectomy is performed to save the life of the mother in situations of severe bleeding due to uterine rupture/atony, invasive malplacentation or uncontrolled bleeding at caesarean section. The incidence of hysterectomy at delivery is today around 50 in 100 000 deliveries , but an increasing rate is seen due to the more frequent use of caesarean section as mode of delivery.

Intrauterine adhesions generally occur secondary to intrauterine infections (primarily genital tuberculosis) or surgical abortions . If the intrauterine adhesions are left untreated an infertility rate of >50 % can be expected . Hysteroscopic lysis of adhesions can restore the uterus cavity but the majority of women with severe intrauterine adhesions remain infertile in spite of surgery .

Non-human primates and UTx

No group of animals has a greater resemblance, concerning anatomy and physiology of the reproductive organs, to the human female than female non-human primates. In higher primates, the uterus is simplex, with a single cavity. The arterial inflow of the non-primate uterus is alike the human uterus, mainly through the uterine arteries, but with extensive blood flow also through the vaginal and ovarian arteries. The venous outflow is through several uterine and vaginal veins and especially through the ovarian veins, which converge into two major veins before their inlet into the caval vein and the left renal vein. The ageing female non-human primate exhibits a natural menopause, not characteristic of most other higher animal species . Two species of non-human primates have been subjected to research involving UTx, the macaque and baboon. In the sections below, the existing non-human primate UTx research experience is summarized

Avascular UTx

Pioneering work in non-human UTx research was performed more than 40 years ago, by James Scott and co-workers. The experiments on avascular UTx included both autologous and allogeneic trials in rhesus macaques . A midline low abdominal incision was made to perform subtotal hysterectomy and bilateral salpingectomy en bloc. The ovaries and cervical stump was left in situ. No information is given concerning flushing of the graft or ischemic times. At transplantation, the cervical stump was sutured (interrupted 4-0 catgut sutures) to the lower uterine segment over a splint and the fimbriaes of the oviducts were reattached to their normal positions on the ovaries, by single 4-0 sutures. To accomplish vascularization of the graft the omentum was brought into the pelvis and wrapped around and sutured to the uterus and oviducts, so that the omentum completely enveloped these organs. This procedure would enable revascularization of the uterus plus oviducts through ingrowth of vessels from the omentum. In the four rhesus macaques with autologous avascular UTx, 3 out of 4 resumed menstruation but no pregnancy was seen in spite of 10 months of breeding attempts. At autopsy, the uterus had normal size and gross morphology. Histology showed normal endometrium in all cases. However, all oviducts were nonpatent with fibrosis of the tissue. The allogeneic avascular UTx attempts were without immunosuppression. Consequently, the four grafts showed progressive rejection-associated changes, with abundant uterine necrosis during the second postoperative week.

Autologous UTx

Autologous transplantation is usually conducted early in organ-specific experimental transplantation research, in order to only test the effects of the surgical approach and of ischemia reperfusion on the survival of the graft. In the more complex allogeneic transplantation, effects of rejection and immunosuppression to avoid rejection are factors that are added to this complex experimental situation. Thus, autologous or syngeneic (between genetically identical individuals) UTx

have also been used in initial research on UTx. Due to absence of inbred primate strains, all these initial non-human primate UTx experiments have involved autologous UTx.

In preparation for the first human UTx case, performed in Saudi Arabia in year 2000, some experiments involved UTx-like experiments in the baboon . The experiments involved 16 female nulliparous baboons of 2-4 years age and with an average weight of around 16 kg. Through a midline incision, hysterectomy with partly preserved vasculature was performed and the uteri were flushed with Euro-Collins solution through the veins. The first 8 animals underwent end-to-end uterine vessel anastomosis but occlusions at this site was seen in majority of the cases . It is not specifically stated at what level the end-to-end anastomosis was done or what size of sutures were used. Our own experience of UTx in the baboon , is that the uterine artery has an inner diameter of less than one mm at the level above the branching of the umbilical artery and that the uterine veins also are fairly tiny. In the second part of the study of Fageeh and co-workers , also involving 8 animals, the surgical technique was altered to end-to-side anastomosis between the uterine vessels and the internal iliac vessels. The technique was not described in any detail, but it was stated that vascular patency was demonstrated in 90 % of the anastomotic sites and in general that survival of the grafts were seen with good long-term vessel patency. This description of graft survival was based gross morphological observations, such as evaluation of color and texture, carried out in conjunction with euthanasia 6-12 weeks after UTx. Importantly, morphological assessment by light and electron microscopy, was not performed and facts concerning the important functional parameter of restored menstruation was not reported.

In our initial study on autologous UTx in the baboon, also the ovaries, the Fallopian tubes and vessel pedicles of the ovarian veins and the uterine arteries/anterior branches of the internal iliacs, were included in the uterine graft . The reason for including also the ovaries in the transplantation procedure was that external perineal inspection of the animals could easily detect resumed ovarian cyclicity and thereby ovarian graft function. Menstruation, as an added parameter, would provide data on uterine functionality. In these experiments, we simplified the transplantation procedure by performing extensive preparatory work of the vasculature of the internal genital tract on the back-table. The ends of the vascular pedicles of the uterine arteries and the ovarian veins were first anastomosed side-to-side on the back-table to create one arterial end and one uterine end (Fig. 2a) using 8-0 and 9-0 sutures, respectively. The uterus was brought back into the abdomen of the animals and the two vascular ends of the graft were then unilaterally anastomosed end-to-side to the external iliac vessels (Fig. 2a) using 8-0 sutures.

In these initial experiments , the total duration of surgery (organ recovery, organ flushing with back-table preparation, transplantation) of the 10 female baboons was somewhat longer than 6h, whereof the retrieval took almost 3h. The duration of anastomosis surgery, when the graft gradually is warmed, was just over 1 hour and the total ischemic period was almost 3h. In spite of the long surgical time, the long-term animal survival was 90 %, with one baboon dying within 12h after surgery. That animal experienced multiple arrythmic episodes during surgery and post mortem examination showed cardiomyopathy. Ovarian cyclicity was resumed in 5 out of the 10 animals, but menstruation was only seen in 2 animals. These two animals were followed for more than one year and menstruation continued normally. Histological examination of the tissue after euthanization, around 18 months post UTx, confirmed normal uterine morphology, but with presence of extensive adhesions around the entire internal genital tract. It should be pointed out that the low success rate of 20%, which may have been caused by relative stenosis at the vascular anastomosis lines, was in spite of that the surgical team had extensive training of UTx procedures in both the swine and sheep models.

In our follow-up study on autologous UTx in the baboon , we initially repeated the surgical procedure of our first non-human primate UTx paper . Among these initial 6 animals, two animals died shortly after surgery because of bleeding/peritonitis. The surviving animals resumed cyclicity within 4 months but no menstruation occurred. At euthanization, viable ovaries were seen but the uteri had shrunken into small nodules of fibrotic tissue, with no vascular connection to the ovaries. Since both the ovaries and the uterus were supplied by the same blood flow of the uterine arteries in that transplantation model, the results indicate that postsurgical blood flow was insufficient for continued uterine viability but that the ovaries survived the ischemia and established new blood supply by neoangiogenesis. This is compatible with that avascular transplanted ovarian tissue, showed survival both in the baboon model and in the human .

Since the results were poor in terms of graft survival in the initial series of this second paper on baboon UTx everal modifications were tested to possibly improve the results. An experienced transplant surgeon joined the team, and the technique was modified so that the organ was preflushed with heparinized saline which was

followed by flushing at back-table with proper organ preservation solution (HTK solution) and parts of the main trunks of the internal iliac arteries were included in the graft to achieve a larger arterial anastomosis end, with one of the arterial ends of the graft first coupled end-to-end to a posterior internal iliac artery (Fig. 2b) and this arterial construct was then anastomosed end-to-end to the proximal end of the internal iliac artery (Fig. 2b). An extended dissection of the venous pedicle, up to and including patches of the vena cava/left kidney vein, was performed to enable establishment of the venous anastomosis to the external iliac vein utilizing a graft vein with a wall of reasonable thickness. The animal survival with this modified technique was 100 % and 80 % of these animals resumed cyclic hormonal patterns. Importantly, the success rate of the grafts increased 3-fold from the original study since 60 % of the animals presented with resumed regular menstruation . However, despite repeated attempts with timed mating of these animals, no pregnancy occurred. Tubal blockage was seen in all animals at post-mortem analysis and it is likely that this had occurred shortly after transplantation as a consequence of surgical or ischemic trauma to the oviducts of the graft. These results of bilateral tubal blockage are in line with those seen after avascular UTx in the rhesus macaque . The surgical time for graft recovery was around 2.5h and anastomosis surgery at transplantation took around 1h. The total surgical time was still somewhat longer than 6h, mostly due to an extended cold ischemic period on the back-table, during flushing and blood vessel reconstructions.

The cynomolgus macaque has also been used for autologous UTx experiments . This non-human primate has a considerably smaller body size than the baboon, and in the two animals used in the initial UTx study of this species , the bodyweights were 3.6 and 4.2 kg. The small size of the animal is probably one factor underlying the long surgery time with uterine recovery extending over 6-8h. The recovery surgery included isolation of the uterus with bilateral dissection of the uterine arteries and the largest uterine veins, which in one case was the deep uterine veins and in the other case the superficial uterine veins. Back-table preparation included flushing with heparinized saline and trimming of the vessels. Vascular anastomosis was accomplished bilaterally with the uterine vessels anastomosed mainly end-to-side to the external iliac vessels, but with occasional end-to-end anastomosis to the internal iliac artery. The anastomosis surgery was complicated since most of the vessels had a diameter of less than 1 mm, and this may be one explanation to the long total durations of surgery of over 10 h with warm ischemia for more than 5 h in both cases. Even if one animal died shortly postoperatively, the demonstration of restored menstruation after 4-5 months in the other animal showed the feasibility of this UTx technique also in this smaller non-human primate species.

In a follow-up study, four cynomolgus macaques underwent autologous UTx . The total surgery duration was between 12.5 and 17.5 h, with warm ischemia varying from 4 to 6 h. Depending on specific anatomy and vessel sizes of each animal, either bilateral or unilateral uterine vessel anastomosis end-to-side to the external iliacs were accomplished. By the use of fluorescent indocyanine technique it was demonstrated that also unilateral uterine vessels anastomosis was sufficient to perfuse the entire uterus. However, the long-term survival of the animals was only 25% and it is not stated what specific anastomosis technique was used in the surviving case that also demonstrated menstruations within 6 months after surgery. Another study from the same research group investigated the contribution of uterine blood flow feeded via the uterine arteries, as compared to the ovarian arteries, after transection of the vagina . In that study, indocyanine green fluorescent imaging was used with clamping of either vessel and analysis of the uterine hemodynamics showed that only clamping of the uterine vessels, but not the ovarian vessels, decreased uterine perfusion.

Recently, the first pregnancy ever after any type of UTx procedure in a non-human primate species was reported in a study of autologous UTx, involving two cynomolgus macaques with differences in the specific vascular anastomosis techniques used . The animal with unilateral anastomosis of the uterine artery and ovarian vein to the external iliacs did not resume spontaneous menstruation and no pregnancy occurred. In the other case, with resumption of menstruation, bilateral uterine artery anastomosis was accomplished and venous outflow was through one deep uterine vein and the contralateral ovarian vein. Natural mating resulted in a pregnancy that developed normally until placental abruption occurred near term. A live offspring was delivered but it showed signs of fetal distress. This is the first case of offspring after UTx in any primate species.

Allogeneic UTx

The first published report of vascular allogeneic UTx in rhesus macaques, used an initial set of animals to study the pelvic anatomy and to perform sham retrieval and transplantation of the uterus . Subsequently five allogeneic transplantations of the uterus, including vascular pedicles of aorta and vena cava, were performed. This technique is similar to graft recovery from a deceased donor since extensive parts of the central vascular bed

can be included in the graft. The anastomosis site of the recipient was chosen depending on the specific anatomy of each individual animal, following a preferred hierarchy of internal iliac vessels, external iliac vessels, common iliac vessels and aorta together with vena cava. As immunosuppressant, cyclosporine was used but neither doses nor blood levels were described in the paper. It should be pointed out that since no results in terms of animal or graft survival were reported, it is not known whether this single immunosuppressant therapy effectively prevented rejection, without any serious systemic side-effects.

We have also performed allogeneic UTx in a non-human setting, using the baboon model with transplantation of a uterine graft from a living donor . The study included 18 animals undergoing uterus donation surgery. Uterus recovery surgery included excision of the oviducts and ovaries from the uterine graft, dissection of the ureters to separate the uterine arteries with inclusion of the major parts of the internal iliac arteries and dissection of the ovarian veins with inclusion of caval/left renal vein patches. The recovery surgery extended over around 3h and importantly the surgical and immediate postoperative survival was 100%. Various immunosuppression protocols were tested and it was found that induction therapy with anti-thymocyte globulin (ATG) followed by triple immunosuppression with tacrolimus, mycophenolate and corticosteroids was compatible with long-term graft survival. The short-term survival rate (i.e. the surgical survival) was 100% also in the recipients, which also experienced surgical times of around 3h. Following transplantation, all recipients showed episodes of rejection, which were resolved when treated properly. Although hormonal cyclicity reappeared in some animals (40%) as a sign of wellbeing, none showed resumed menstruation during the study period.

In another study of allogeneic UTx in the baboon, using a graft from a deceased donor, anastomoses were performed with the aorta and vena cava of the graft to the recipient´s aorta and vena cava (our unpublished results). The immunosuppression protocol was an identical induction plus triple immunosuppression protocol as described above. Although episodes of graft rejection have occurred, these have been successfully treated and graft survival over 12 months is now established.

Human UTx

The experience in human UTx research is because of obvious reasons limited. Some years ago, we conducted a study on human uterine tissue to assess the tolerability to cold ischemic preservation . Small tissue pieces of hysterectomy specimens, with the tissue samples containing myometrium and endometrium, were subjected to cold ischemia for times up to 24 h. Uterine tissue that had been preserved in proper preservation solutions showed well-preserved ultramorphology, and spontaneous as well as prostaglandin-induced contractions. These findings would indicate that a human uterus is relatively tolerable to cold ischemia.

In the context of human UTx, it is appropriate to discuss alternative sources of organs. The general field of transplantation surgery has developed immensely during the last decade and shortage of organs is a universal problem, with many patients dying while awaiting transplantation of suitable organs. The source of organs/tissues for transplantation is mainly brain-dead, heart-beating donors. The organ shortage has led to more usage of non heart-beating donors and living donors. Both the number of deceased and living donors varies between different regions due to organisation, cultural, religious and legal issues. Today, live organ donation is practiced in kidney, partial liver and single lung transplantation.

In a human UTx situation, both living and deceased donation can be considered, with advantages with either approach. The uterus retrieval and transplantation procedures, especially with an organ recovered from a live donor, are complicated. The difficulties lie in that long vascular pedicles have to be obtained bilaterally and to establish arterial and venous anastomoses have to be achieved bilaterally. Although dissection of the uterine arteries with mobilization from the ureters is a standard procedure in abdominal radical hysterectomy, the dissections of the thin-walled uterine veins that run over or under the ureters are complicated and time-consuming. The potential benefits of live donation are the possibility to plan the surgical procedure in advance, with both parties of the transplantation in the best possible physical condition, and that the organ, being exposed to minimum ischemic time, will be in a favorable state as shown in large series of kidney transplants . Naturally, the source of potential uterus donation candidates in the family could be large, since several female family members will have completed their childbearing or be of the immediate postmenopausal age. Other possible advantages of live donor UTx would be that the graft can undergo extensive preoperative evaluation providing an opportunity to choose an organ with no underlying disease/malfunction, such as myoma, polyp, human papilloma virus (HPV) infection or intrauterine adhesions. A requirement of a donor uterus from a living donor should be previous pregnancy and confirmed live birth, to demonstrate a favorable potential for normal pregnancy.

The most obvious advantage of organ retrieval from a deceased donor is naturally that the surgical risk imposed on a healthy donor is avoided. There is also the obvious advantage that the extent of the vasculature at organ retrieval can be increased and this may make it possible to create more favorable sites for vessel anastomosis at transplantation into the recipient, with also the possibility to perform only one arterial (aorta) and one venous (vena cava) anastomosis. However, in uterus retrieval from female multiorgan donors, the uterus will most likely be retrieved after procurement of all other traditional/vital types of transplantation organs, such as the kidneys, liver and the heart. This may negatively affect the extent of vasculature that can be retrieved.

Lately, the field of tissue engineering technologies has been expanded and successful reconstruction of several different tissues, such as the trachea and the oesophagus, have been presented . This technique of engineering new organs/tissues from synthetic materials or decellularized natural tissues may also be a solution for organ demand at UTx, and a great advantage would be that the recipient´s own stem cells would be used to colonize the matrix and the recipient would not need any immunosuppressant therapy.

There exist two human studies that have looked at techniques for human uterus recovery. The first study, carried out in New York City, attempted to recover the uterus with inclusion of complete internal iliac vessels bilaterally as part of a multi-organ donation . During a period of six months, there were almost 150 multi-organ procurements in females that were identified as potential donors of a uterus. Donor inclusion criteria included age (16-45 years), recent normal tests for cervical dysplasia and HPV infection, regular menses, no previously known infertility and normal uterine anatomy according to results of recent magnetic imaging. Exclusion could be on basis of infectious disease (human immunodeficiency virus, hepatitis), leiomyoma larger than 10 mm, gynecologic cancer, endometriosis or malformations of the uterus. The intention was to secure the complete internal iliac vessels up to the bifurcation of the common iliacs, for possible anastomosis to the internal iliac vessels of a recipient. It should be noted that only seven cases were included in the study since some were excluded based on the criteria mentioned above and since donation of the uterus for research purposes was only accepted by the immediate family of the multiorgan donor in nine of the 150 cases. In the seven organ retrievals that were carried out, the intended vessel pedicles were procured in two women and in the remaining five cases only the anterior portion of the iliacs were included in the graft. In two of the cases a unilateral loss of the uterine vessels led to use of the ovarian vein. It is worth noticing, as also stated above, that only 6% of potential donor families consented to donation of the uterus specifically . The low consent rate can naturally be explained by the fact that this was a research study rather than actual donation and that the concept of uterine and UTx is new and largely unheard of. However, it may also be related to that the uterus carries a symbolic feature of woman-/mother-hood and people may regard uterus donation more questionable than donation of other types of organs. It may well be that unwillingness to include a uterus on the list of consented organs from deceased young females may in the future lead to shortage of uterus grafts from deceased donors.

The second study on the issue of uterus recovery, evaluated the feasibility of uterus retrieval from live donor by to some extent simulating a retrieval operation and to estimate the uterine vessels recovered during a radical hysterectomy . This study, performed by our group, involved extra dissection of the uterine veins at radical hysterectomy. The results were that blood vessel dissection of the vessels added around 30 min to a radical hysterectomy procedure. No differences were detected regarding peri- or postoperative morbidity. The free ends of the procured uterine arteries were around 65-70 mm in length and the corresponding free ends of the uterine veins had lengths of 50-55 mm. Since the inter-external iliac artery distance was around 90 mm, the vascular lengths that were obtained would provide enough extents for bilateral end-to-side anastomosis to the external iliac vessels.

Up until today, 6 human UTx attempts have been performed, with the last 4 performed by us in Sweden in September-October 2012. Our cases (our unpublished results) involved live uterus donation from close relatives (mothers, aunt) to young females with AUFI. We have ethical permission to perform a case series of 10 patients and the surgeries will be completed during the spring of 2013. All our patients have undergone IVF treatment prior to UTx and have embryos cryopreserved, in preparation for embryo transfer that will be performed around 12 months after UTx.

The first human UTx trial was performed in year 2000 in Saudi Arabia . A 26-year-old woman, who had previously undergone an emergency peripartum hysterectomy due to life-threatening bleeding, underwent UTx with a graft including the uterus and oviducts. The graft was from an unrelated 46-year-old live donor, who was undergoing elective surgery because of bilateral benign ovarian cysts. The vascular pedicles of the uterine vessels were short and at back-table both uterine arter-

ies and veins (3 at each side) were elongated by segments of the saphenous veins. The anastomoses were performed with the extended pedicles of the uterine arteries and veins bilaterally end-to-side to the external iliac vessels of the recipient. Initially, the surgery and the postoperative period of the donor and the recipient were without major complications. Immunosuppressive treatment followed a standard triple therapy regimen of cyclosporine, azathioprine and prednisolone. One episode of acute rejection was successfully treated with ATG. The endometrium responded to sequential estrogen and progesterone treatments with proliferation and withdrawal bleeding. However, uterine prolapse was evident after 3 months and hysterectomy had to be performed since the uterus showed signs of general necrosis. The graft vessels were thrombosed and the authors speculated whether poor uterine fixation caused uterine prolapse. This would lead to compressed and bended uterine blood vessels, with predisposition of thrombosis formation. Our opinion is that this first human UTx case was performed prematurely, considering the scarcity of published research data on UTx at that time and also considering the team's minimal preparatory UTx research, which only included a small number of autologous UTx procedures in the baboon.

In august 2011, the world's second human UTx attempt was performed in Turkey, when a 21-year old MRKH-patient received a uterus from a 22-year-old deceased donor . The recovery from a multiorgan donor lasted around 2 h and the vascular pedicles included bilateral uterine arterial and venous supply, up to and including the common iliac vessels. The transplantation procedure in the recipient took around 5.5 h and included bilateral end-to-side anastomosis of the common iliac vessels of the graft to the external iliac vessels. Immunosuppression was by only ATG for 6 days and introduction of triple immunosuppression (tacrolimus, mycophenolate, prednisolone) from day 7. The patient had her first menstruation already 20 days after transplantation and the graft has so far survived with regular menstrual pattern for 12 months.

Conclusion

Even if UTx is a great scientific task and a complicated medical procedure, it may well be that the ethics surrounding UTx represents the greatest challenge towards general acceptance of UTx both among health professionals, politicians and the public. The ethics regarding UTx involves essential issues concerning reproduction, parenthood, health costs and medical advancements. In the light of that moral, legal and religious restrictions differ between cultures and countries, UTx may be accepted under some conditions whilst others might find it unacceptable.

As UTx constitutes both a new surgical procedure, proposed as a treatment for AUFI, but at the same time being a non-life-saving organ transplantation procedure, the ethical analysis of the procedure should be assessed by stringent and thorough criteria. A risk-benefit analysis of UTx should include no less than four parties; the donor or her immediate family in case of a uterus from a deceased donor, the recipient, the partner of the recipient, and the possible future child.

The alternatives to UTx to gain motherhood for patients with AUFI are adoption or use of gestational surrogacy, of which the latter also is complex in ethical terms. While these two alternatives provide an excellent option for a large number of infertile couples, they may in some cultural, societal, legal or religious settings be severely restricted or considered inappropriate. Given that UTx is a quality-of-life enhancing transplantation procedure, the safety precautions in clinical introduction must be rigorous. Because of this we have considered the extension of the research on UTx to include a non-human primate model vital and subsequently as of today we have many years of experience with non-human primate UTx. Since no other animal model provides such a resemblance to the human regarding reproductive anatomy and physiology the non-human primate models are considered to be the final step prior to clinical introduction aiming to make the initial human UTx attempts as safe as possible. The inclusion of non-human primate was also one of the issues stated in the FIGO ethical guidelines, as being desirable before human implementation . It should also be pointed out that any team that plan to perform human UTx in the future should undergo extensive training and methodological development with the use of large animal models, and these experiments should involve, not only the surgical training and procedure, but all possible aspects of the UTx procedure and the subsequent pregnancy including psychological evaluation and follow-up of all parties involved.

Because of the delicate ethical issues surrounding UTx, the treatment should only be considered for those women with uterine factor infertility, for whom no other alternative treatment is available. Another important point considering UTx is that in spite of the six human attempts performed so far, with reported success after 12 months in one case , the definition of a truly successful human UTx procedure is the birth of a healthy baby from an allogenically transplanted uterus. It is quite likely that this may be reported within the next few years considering the initial human cases and that

preparations of human UTx trials are underway in at least 5 more centers worldwide.

It should be pointed out, that experimental human UTx should only be done under a strict scientific research protocol after approval by the local or national ethics board. The team performing experimental human UTx should have extensive research experience in the UTx field and it should only be performed in an institution with a large transplantation program. Several experts have to be involved, with transplant surgeons and experienced gynecologic oncology surgeons performing the surgery, but also with essential roles of subspecialists in reproductive medicine to perform in vitro fertilization before UTx and subspecialists in feto-maternal medicine to take care of any pregnancy.

Figure legends

Figure 1: Schematic drawing of the different uterine malformations and other diseases that underly uterine infertility. The red cross (upper left corner) indicates the nonexistence of the uterus, which is seen in patients with the MRKH syndrome and in patients that have been hysterectomized.

Figure 2: Schematic drawing of vessel anastomoses used in baboon UTx experiments. In panel a), the side-to-side unified ovarian veins and uterine arteries are anastomosed end-to-side to the external iliac vessels. In panel b), the side-to-side unified ovarian veins and the end-to-end unified uterine arteries are anastomosed end-to-side to the external iliac vein and end-to-end to the internal iliac artery, respectively.

Fig1

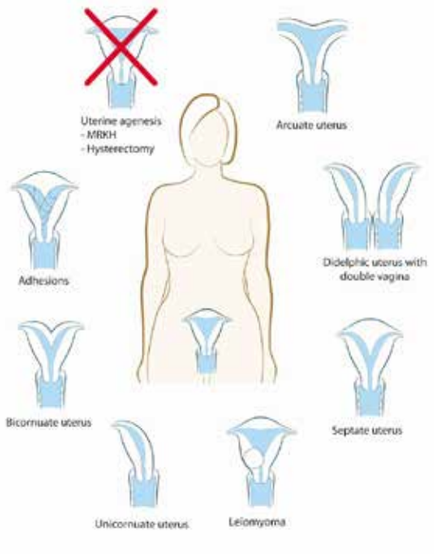


Fig 2a

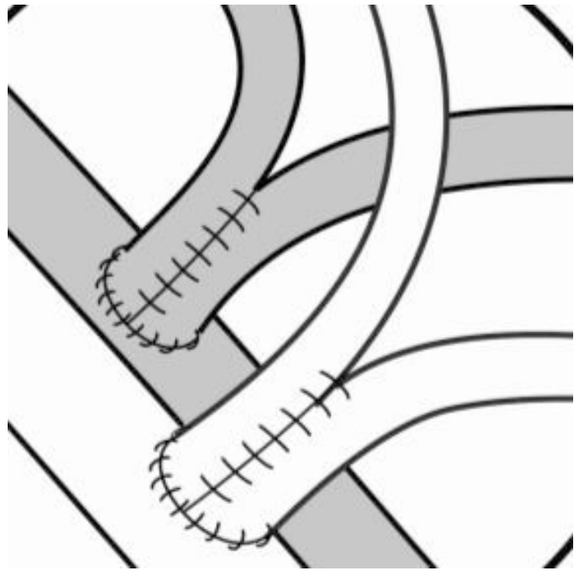
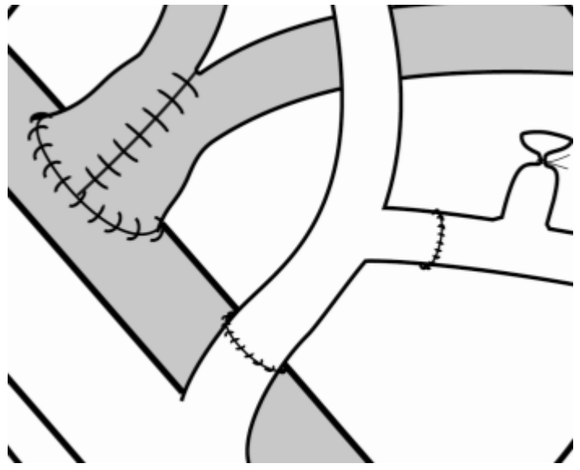


Fig 2b



References

1. Boivin, J., et al., International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod, 2007. 22(6): p. 1506-12.
2. Silber, S.J., G. Grudzinskas, and R.G. Gosden, Successful pregnancy after microsurgical transplantation of an intact ovary. N Engl J Med, 2008. 359(24): p. 2617-8.
3. Silber, S.J. and R.G. Gosden, Ovarian transplantation in a series of monozygotic twins discordant for ovarian failure. N Engl J Med, 2007. 356(13): p. 1382-4.
4. Gosden, R.G., Ovary and uterus transplantation. Reproduction, 2008. 136(6): p. 671-80.
5. Racho El-Akouri, R., G. Kurlberg, and M. Brannstrom, Successful uterine transplantation in the mouse: pregnancy and post-natal development of offspring. Hum Reprod, 2003. 18(10): p. 2018-23.
6. Diaz-Garcia, C., et al., First report on fertility after allogeneic uterus transplantation. Acta Obstet Gynecol Scand, 2010. 89(11): p. 1491-4.
7. Wranning, C.A., et al., Pregnancy after syngeneic uterus transplantation and spontaneous mating in the rat. Hum Reprod, 2011. 26(3): p. 553-8.
8. Sieunarine, K., et al., Uterine allotranspiantation in the rabbit model using a macrovascular patch technique. Int Surg, 2008. 93(5): p. 288-94.
9. Wranning, C.A., et al., Fertility after autologous ovine uterine-tubal-ovarian transplantation by vascular anastomosis to the external iliac vessels. Hum Reprod, 2010. 25(8): p. 1973-9.
10. Avison, D.L., et al., Heterotopic uterus transplantation in a swine model. Transplantation, 2009. 88(4): p. 465-9.
11. Ramirez, E.R., et al., Pregnancy and outcome of uterine allotransplantation and assisted reproduction in sheep. J Minim Invasive Gynecol, 2011. 18(2): p. 238-45.
12. Racho El-Akouri, R., et al., Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. Hum Reprod, 2003. 18(10): p. 2024-30.
13. Diaz-Garcia, C., et al. The effect of warm ischemia at uterus transplantation in a rat model. Acta Obstet Gynecol Scand, 2012. DOI: 10.1111/aogs.12027.
14. Groth, K., et al., Rejection of allogenic uterus transplant in the mouse: time-dependent and site-specific infiltration of leukocyte subtypes. Hum Reprod, 2009. 24(11): p. 2746-54.
15. El-Akouri, R.R., et al., Rejection patterns in allogeneic uterus transplantation in the mouse. Hum Reprod, 2006. 21(2): p. 436-42.
16. Wranning, C.A., et al., Rejection of the transplanted uterus is suppressed by cyclosporine A in a semi-allogeneic mouse model. Hum Reprod, 2007. 22(2): p. 372-9.
17. Groth, K., et al., Cyclosporine A exposure during pregnancy in mice: effects on reproductive performance in mothers and offspring. Hum Reprod, 2010. 25(3): p. 697-704.
18. Grimbizis, G.F., et al., Clinical implications of uterine malformations and hysteroscopic treatment results. Hum Reprod Update, 2001. 7(2): p. 161-74.
19. Sieunarine, K., et al., Possibilities for fertility restoration: a new surgical technique. Int Surg, 2005. 90(5): p. 249-56.
20. Raga, F., et al., Reproductive impact of congenital Mullerian anomalies. Hum Reprod, 1997. 12(10): p. 2277-81.
21. Acien, P., Embryological observations on the female genital tract. Hum Reprod, 1992. 7(4): p. 437-45.
22. Saravelos, S.H., K.A. Cocksedge, and T.C. Li, Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. Hum Reprod Update, 2008. 14(5): p. 415-29.
23. Homer, H.A., T.C. Li, and I.D. Cooke, The septate uterus: a review of management and reproductive outcome. Fertil Steril, 2000. 73(1): p. 1-14.
24. Pabuccu, R. and V. Gomel, Reproductive outcome after hysteroscopic metroplasty in women with septate uterus and otherwise unexplained infertility. Fertil Steril, 2004. 81(6): p. 1675-8.
25. Buttram, V.C., Jr., Mullerian anomalies and their management. Fertil Steril, 1983. 40(2): p. 159-63.
26. Papp, Z., et al., Reproductive performance after transabdominal metroplasty: a review of 157 consecutive cases. J Reprod Med, 2006. 51(7): p. 544-52.
27. Nahum, G.G., Uterine anomalies. How common are they, and what is their distribution among subtypes? J Reprod Med, 1998. 43(10): p. 877-87.
28. Michalas, S.P., Outcome of pregnancy in women with uterine malformation: evaluation of 62 cases. Int J Gynaecol Obstet, 1991. 35(3): p. 215-9.
29. Fedele, L., et al., Reproductive performance of women with unicornuate uterus. Fertil Steril, 1987. 47(3): p. 416-9.
30. Donnez, J. and M. Nisolle, Endoscopic laser treatment of uterine malformations. Hum Reprod, 1997. 12(7): p. 1381-7.
31. Taylor, E. and V. Gomel, The uterus and fertility. Fertil Steril, 2008. 89(1): p. 1-16.
32. Nohara, M., et al., Twin pregnancy in each half of a uterus didelphys with a delivery interval of 66 days. BJOG, 2003. 110(3): p. 331-2.
33. Folch, M., I. Pigem, and J.C. Konje, Mullerian agenesis: etiology, diagnosis, and management. Obstet Gynecol Surv, 2000. 55(10): p. 644-9.
34. Aittomaki, K., H. Eroila, and P. Kajanoja, A population-based study of the incidence of Mullerian aplasia in Finland. Fertil Steril, 2001. 76(3): p. 624-5.
35. Oppelt, P., et al., Clinical aspects of Mayer-Rokitansky-Kuester-Hauser syndrome: recommendations for clinical diagnosis and staging. Hum Reprod, 2006. 21(3): p. 792-7.
36. Petrozza, J.C., et al., Congenital absence of the uterus and vagina is not commonly transmitted as a dominant genetic trait: outcomes of surrogate pregnancies. Fertil Steril, 1997. 67(2): p. 387-9.
37. Stewart, E.A., Uterine fibroids. Lancet, 2001. 357(9252): p. 293-8.
38. Borgfeldt, C. and E. Andolf, Transvaginal ultrasonographic findings in the uterus and the endometrium: low prevalence of leiomyoma in a random sample of women age 25-40 years. Acta Obstet Gynecol Scand, 2000. 79(3): p. 202-7.
39. Marshall, L.M., et al., Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstet Gynecol, 1997. 90(6): p. 967-73.
40. Farquhar, C.M. and C.A. Steiner, Hysterectomy rates in the United States 1990-1997. Obstet Gynecol, 2002. 99(2): p. 229-34.
41. Farhi, J., et al., Effect of uterine leiomyomata on the results of in-vitro fertilization treatment. Hum Reprod, 1995. 10(10): p. 2576-8.
42. Oliveira, F.G., et al., Impact of subserosal and intramural uterine fibroids that do not distort the endometrial cavity on the outcome of in vitro fertilization-intracytoplasmic sperm injection. Fertil Steril, 2004. 81(3): p. 582-7.
43. Benecke, C., et al., Effect of fibroids on fertility in patients undergoing assisted reproduction. A structured literature review. Gynecol Obstet Invest, 2005. 59(4): p. 225-30.
44. Somigliana, E., et al., Fibroids and female reproduction: a critical analysis of the evidence. Hum Reprod Update, 2007. 13(5): p. 465-76.
45. Quinn, M.A., et al., Carcinoma of the cervix uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet, 2006. 95 Suppl 1: p. S43-103.
46. Sonoda, Y., et al., A fertility-sparing alternative to radical hysterectomy: how many patients may be eligible? Gynecol Oncol, 2004. 95(3): p. 534-8.
47. Kwee, A., et al., Emergency peripartum hysterectomy: A prospective study in The Netherlands. Eur J Obstet Gynecol Reprod Biol, 2006. 124(2): p. 187-92.
48. Schenker, J.G. and E.J. Margalioth, Intrauterine adhesions: an updated appraisal. Fertil Steril, 1982. 37(5): p. 593-610.
49. Yu, D., et al., Asherman syndrome--one century later. Fertil Steril, 2008. 89(4): p. 759-79.
50. Fernandez, H., et al., Fertility after treatment of Asherman's syndrome stage 3 and 4. J Minim Invasive Gynecol, 2006. 13(5): p. 398-402.
51. Martin, L.J., K.D. Carey, and A.G. Comuzzie, Variation in menstrual cycle length and cessation of menstruation in captive raised baboons. Mech Ageing Dev, 2003. 124(8-9): p. 865-71.
52. Scott, J.R., R.M. Pitkin, and M.E. Yannone, Transplantation of the primate uterus. Surg Gynecol Obstet, 1971. 133(3): p. 414-8.
53. Fageeh, W., et al., Transplantation of the human uterus. Int J Gynaecol Obstet, 2002. 76(3): p. 245-51.
54. Enskog, A., et al., Uterus transplantation in the baboon: methodology and long-term function after auto-transplantation. Hum Reprod, 2010. 25(8): p. 1980-7.
55. Wranning, C.A., et al., Auto-transplantation of the uterus in the domestic pig (Sus scrofa): Surgical technique and early reperfusion events. J Obstet Gynaecol Res, 2006. 32(4): p. 358-67.
56. Dahm-Kahler, P., et al., Transplantation of the uterus in sheep: methodology and early reperfusion events. J Obstet Gynaecol Res, 2008. 34(5): p. 784-93.
57. Wranning, C.A., et al., Transplantation of the uterus in the sheep: oxidative stress and reperfusion injury after short-time cold storage. Fertil Steril, 2008. 90(3): p. 817-26.
58. Johannesson, L., et al., Uterus transplantation in a non-human primate: long-term follow-up after autologous transplantation. Hum Reprod, 2012. 27(6): p. 1640-8.
59. Diaz-Garcia, C., et al., Ovarian cortex transplantation in the baboon: comparison of four different intra-abdominal transplantation sites. Hum Reprod, 2011. 26(12): p. 3303-11.
60. Donnez, J., et al., Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet, 2004. 364(9443): p. 1405-10.
61. Kisu, I., et al., A new surgical technique of uterine auto-transplantation in cynomolgus monkey: preliminary report about two cases. Arch Gynecol Obstet, 2011.
62. Mihara, M., et al., Uterus autotransplantation in cynomolgus macaques: intraoperative evaluation

Mats Brännström

of uterine blood flow using indocyanine green. Hum Reprod, 2011. 26(11): p. 3019-27.
63. Kisu, I., et al., Indocyanine Green Fluorescence Imaging for Evaluation of Uterine Blood Flow in Cynomolgus Macaque PLoS ONE 2012. 7(4): p. e35124. doi:10.1371/journal.pone.0035124.
64. Mihara, M., et al., Uterine autotransplantation in cynomolgus macaques: the first case of pregnancy and delivery. Hum Reprod, 2012. 27(8): p. 2332-40.
65. Del Priore, G., S. Schlatt, and J. Malanowska-Stega, Uterus transplant techniques in primates: 10 years' experience. Exp Clin Transplant, 2008. 6(1): p. 87-94.
66. Wranning, C.A., et al., Short-term ischaemic storage of human uterine myometrium--basic studies towards uterine transplantation. Hum Reprod, 2005. 20(10): p. 2736-44.
67. Perico, N., et al., Delayed graft function in kidney transplantation. Lancet, 2004. 364(9447): p. 1814-27.
68. Badylak, S.F., et al., Engineered whole organs and complex tissues. Lancet, 2012. 379(9819): p. 943-52.
69. Del Priore, G., et al., Human uterus retrieval from a multi-organ donor. Obstet Gynecol, 2007. 109(1): p. 101-4.
70. Johannesson, L., et al., Vascular pedicle lengths after hysterectomy: toward future human uterus transplantation. Obstet Gynecol, 2012. 119(6): p. 1219-25.
71. Ozkan, O., et al. Preliminary results of the first human uterus transplantation from a multiorgan donor. Fertil Steril, 2012. DOI: S0015-0282(12)02254-6 [pii]
72. 10.1016/j.fertnstert.2012.09.035.
73. Nyachieo, A., et al., The baboon as a research model for the study of endometrial biology, uterine receptivity and embryo implantation. Gynecol Obstet Invest, 2007. 64(3): p. 149-55.
74. Milliez, J., Uterine transplantation FIGO Committee for the Ethical Aspects of Human Reproduction and Women's Health. Int J Gynaecol Obstet, 2009. 106(3): p. 270.

TRANSVAGINAL ULTRASOUND ELASTOGRAPHY IN DIAGNOSIS OF ENDOMETRIAL PATHOLOGY IN POSTMENOPAUSAL BLEEDING

[1]Ehab Sadek, [1]Mohammed Nezar, [1]Emad Fyalla, [2]Talal Amer, [1]Ahmed El-Menayyar
[1]Department of Obstetrics and Gynecology
[2]Department of Radiology, Faculty of Medicine, Mansoura University, Egypt

Abstract

Objective: The aim of this work was to assess the role of transvaginal ultrasound elastography as a new technique in the diagnosis of endometrial pathology in women with postmenopausal bleeding.

Method:A prospective comparative study was conducted at Obstetrics & Gynecology and Radiology Departments in Mansoura University Hospital. In 35 postmenopausal women with vaginal bleeding, transvaginal ultrasound elastography was performed and the results were compared to the pathological results from dilatation and curettage (D&C).

Results: There was a significant difference of elastography image of endometrium described by elastography index (EI) between patients with normal (23%) or atrophic (31%) endometrium confirmed by pathological examination and women with abnormal findings like endometrial hyperplasia (23%), polyp (17%) or cancer (6%). EI in the group with normal or atrophic endometrium was 0 or 1 point and in those with endometrial pathology was from 2 to 4 points.

Conclusion: Elastography could be helpful as a new non- invasive diagnostic technique in differentiating endometrial pathologies in postmenopausal women.

Key Words: elastography / postmenopausal bleeding / dilatation and curettage (D&C)and endometrial cancer.

Introduction

Postmenopausal bleeding is bleeding that occurs 12 or more months after cessation menstruation in menopausal age and accounts for 5% of all gynecologic office visits. While it is not always a symptom of cancer, the exclusion of endometrial hyperplasia and carcinoma is the key issue in the evaluation of patients with postmenopausal bleeding. The primary evaluation of postmenopausal women who present with abnormal uterine bleeding includes a medical history and a pelvic examination. Investigative studies, such as a uterine biopsy, ultrasound, hysteroscopy or dilation and curettage, may be required. Treatment will depend on the cause determined. The most important point is that irregular perimenopausal or postmenopausal bleeding should not be ignored or assumed to be a normal phenomenon [1].

The preliminary investigation for women presenting with postmenopausal bleeding is transvaginal ultrasonography, which is used to identify those with a thickened endometrium [2]. A thin endometrium (less than 5 mm)is widely accepted as a good predictor of a normal endometrium[3] but this has a low specificity for the detection of endometrial carcinoma[4].Other investigations include hysteroscopy, dilatation and curettage (D&C), aspiration sampling and saline infusion sonography.[4]
Elastography is a non-invasive method in which stiffness or strain images of soft tissue are used to detect or classify tumors. A tumor or a suspi-

Ehab Sadek, MD
Department of Obstetrics and Gynecology
Faculty of Medicine,
Mansoura University, Egypt
Tel 0020502255473
Email ehabsadek@yahoo.com

cious cancerous growth is normally 5-28 times stiffer than the background of normal soft tissue. When a mechanical compression or vibration is applied, the tumor deforms less than the surrounding tissue i.e. the strain in the tumor is less than the surrounding tissue. Elastograms (images of tissue strain) have been shown to be affected by the degree of adherence of the tumor to its surroundings, indicating a potential to extend elastography to tumor mobility characterization to improve diagnostic accuracy and surgical guidance [5].

Aim of the study

The aim of our study was to assess the role of transvaginal ultrasound elastography as a new technique in diagnosis of endometrial pathology in women with postmenopausal bleeding.

Patients and method

A prospective comparative study was conducted between May 2013 and December 2013 on thirty five patients with postmenopausal bleeding who were admitted at Obstetrics & Gynecology Department in Mansoura University Hospital and were referred to undergo transvaginal ultrasound elastography with and without an intrauterine catheter to assess whole endometrial length and the elastography images were saved by the use of ElastoScan software in (Hitachi – 7500) ultrasound equipment and compared with histopathological results made after that by dilatation and curettage (D&C).

Endometrium was described by Elastography Index (EI) presented by Swiatkowska-Freund & Preis [6] to be able to describe the elastography images by numbers and to make it easier to evaluate the images and to perform statistical analysis as follows: purple was assigned 0 point, blue was assigned 1 point, green 2 points, yellow 3 points and red 4 point.

In all the patients the standard D&C procedure was performed and the obtained material was sent for pathological examination. Normal or atrophic endometrium was considered as normal result. Polyp, hyperplasia and endometrial cancer were qualified as abnormal findings. The results of elastography presented as Elastography Index of endometrium were compared to the pathological results.

Results

Table 1 shows the relationship between type of lesion and patients characteristics as regard age, nulliparity, Diabetes, hypertension and obesity. Data presented in table 1 shows that increasing age, nulliparity, DM&HTN and obesity are risk factors for development of endometrial hyperplasia and carcinoma in women with postmenopausal bleeding.

Table 2 shows that in 54% of the patients (19 women) no significant pathology was found as normal (8 patients – 23%) or atrophic (11 women– 31%) endometrium was described by pathologist. In eight cases (23%) endometrial hyperplasia was diagnosed, in six cases (17%) endometrial polyp and in two cases (6%) endometrial cancer. The significant difference in EI was found between patients with normal finding results (normal or atrophic endometrium) and women with pathologic findings (endometrial cancer, hyperplasia or polyp). P value is significant > 0.05 with sensitivity about 100%.

Data presented in table 2 suggested, that normal and atrophic endometrium is evaluated as 0 or 1 point of EI, and abnormal tissue is softer, coded as green, yellow or red and assessed as 2, 3or 4 points.

Table (1)

The demographic parameters of the patients before starting of treatment.

Type of lesion	Age						Nulliparity		DM&HTN		Obesity (BMI <30)	
	()45-55		()56-65		>65							
	No	%	No	%	No	%	No	%	No	%	No	%
Atrophy and normal (total no 19)	7	37	7	37	5	26	3	17	4	21	4	21
Hyperplasia (total no 8)	3	38	4	50	1	12	2	25	4	50	5	63
Polyp (total no 6)	4	67	2	33	0	0	0	0	1	17	2	33
Endometrial carcinoma (total no 2)	0	0	1	50	2	50	1	50	2	100	2	100

DM = diabetes mellitus HTN = hypertension BMI = body mass index

Table (2)

presents EI in groups of patients with different pathological findings.

Result of D&C	Number	%	EI	P value
Normal	8	23	0-1	< 0.05
Atrophic	11	31	0-1	
Hyperplasia	8	23	3	
Polyp	6	17	2-3	
Endometrial cancer	2	6	4	
Total	35	100	0-4	

Discussion

Elastography proved to be very helpful in management of the liver, breast or other organs cancer, where neoplasmatic transformation causes changes of the tissue elasticity as tumors are usually harder than the healthy organ [7]. In a case of uterus, as a relatively hard organ, elastography showed the endometrial pathologies as softer than normal or atrophic endometrium [8]

Many women undergo dilatation and curettage (D&C) for clinical symptoms or sonographic findings, the procedure performed under general anesthesia brings significant complications risk for the patient.In many cases the pathological examination reveals no pathology and the patients do not require any treatment [9] .The use of transvaginal ultrasound elastography would be very useful and might help to avoid these unnecessary invasive procedures.

There are some publications presenting results of studies assessing the elastography significance in the imaging of the uterine cervix changes taking place before delivery [10, 11, 12, 13]. It was found useful in detecting the patients with better chances for labor induction success. Similar study is being conducted to check the usefulness of elastography in predicting preterm delivery. Only three publications were found in the literature concerning application of elastography in gynecology [8, 14, 15]. Authors proved, that in relation to the hard uterus and cervix, softer pathologic tissues were easy to present in the elastoscan [14, 15].

There is no available publication in the literature to assess the sensitivity of elastography in detecting endometrial pathologies in upper uterine segment so we performed a new technique by applying displacement to upper uterine segment by inflating a pediatric rubber catheter with fluid inside and obtaining a second image of uterine wall under displacement which revealed that any endometrial pathology in upper uterine segment was detected by the elastography.

In our study group the sensitivity of this method in diagnosis of endometrial pathology in postmenopausal bleeding was 100% as no patient with any endometrial pathology had EI below 2 in agreement with the study done by Krzysztof1 P, et al., 2011 [8]. Elastography has key benefits as it provides a simple, low-cost alternative to magnetic resonance imaging (MRI) for the diagnosis of uterine, cervical and pelvic disorders, unlike conventional ultrasound, holds potential for distinguishing between fibroids and adenomyosis in the uterus and could be used to distinguish endometrial cancer from benign uterine disorders such as hyperplasia [16].

Conclusion

These preliminary results suggest that transvaginal ultrasound elastography may be used for differentiation between endometrial pathologies in postmenopausal bleeding which helps in early diagnosis and management especially in cases of endometrial carcinoma improving their survival rate. More research is needed to confirm these findings and develop more quantitative methods to assess lesions.

References

1. Chin-Yuan Hsu, Chih-Ping Chen and Kung-Liahng Wang. Assessment of Postmenopausal Bleeding. International Journal of Gerontology. 2008; 2:55-59.
2. Scottish Intercollegiate Guideline Network (SIGN). Guideline 6 Investigation of Post-menopausal Bleeding. Scottish Intercollegiate Guideline Network: Edinburgh. 2002; 61:8-12.
3. Van Der Bosch T, Van Schoubroeck D, Domali E, [et al.]. A thin and regular endometrium on ultrasound is very unlikely in patients with endometrial malignancy. Ultrasound Obstet & Gynecol. 2007; 29: 674–679.
4. John Wiley & Sons, Ltd. Ultrasound ObstetGynecol. 2011; 38: 233–236.
5. Mendelson EB, Chen J and Karstaedt P. Assessing tissue stiffness may boost breast imaging specificity. Diagnostic Imaging. 2009; 31:15-17.
6. Preis K, Świątkowska-Freund M, Pankrac Z. Elastography in the examination of the uterine cervix before labor induction. Ginekol Pol. 2010; 81: 757-761.
7. Rago T, Santini F, Scutari M, [et al.]. Elastography: new developments in ultrasound for predicting malignancy in thyroid nodules. J ClinEndocrinolMetab. 2007; 92: 2917-2922.
8. Krzysztof1 P, Katarzyna1 Z, Malgorzata1 S, [et al.]. The role of elastography in the differential diagnosis of endometrial pathologies. Ginekol Pol. 2011; 82: 494-497.
9. Tsuda H, Kawabata M, Yamamoto K, [et al.]. Prospective study to compare endometrial cytology and transvaginal ultrasonography for identification of endometrial malignancies. GynecolOncol.1997; 65: 383-386.
10. Amaguchi S, Kamei Y, Kozuma S, [et al.]. Tissue elastography imaging of uterine cervix during pregnancy. J Med Ultrasonics. 2007; 34: 209-210.
11. Preis K, Świątkowska-Freund M, Pankrac Z. Elastography in the examination of the uterine cervix before labor induction. Ginekol Pol. 2010; 81: 757-761.
12. Świątkowska-Freund M, Preis K. New methods of ultrasonographic assessment of uterine cervix before an induction of labor. Gin Pol Med Project. 2010; 3:9-15.
13. Swiatkowska-Freund M, Preis K, Pankrac Z. Ultrasound elastography in assessment of uterine cervical consistence during pregnancy. Arch Perinat Med. 2010; 16: 175-177.
14. Hobson M, Kiss M, Varghese T, [et al.]. In vitro uterine strain imaging: preliminary results. J Ultrasound Med. 2007; 26: 899-908.
15. Thomas A, Kummel S, Gemeinhardt O, [et al.]. Real-time sonoelastography of the cervix: tissue elasticity of the normal and abnormal cervix. Acad Radiol.2007; 14: 193-200.
16. Varghese T, Kliewer M and Zagzebski J. Elastographic Imaging of the Cervix and Uterine Wall. Wisconsin Alumni research foundation WARF: P03009 US.2007; 7297116:1-12.

EFFECT OF ENDOMETRIAL THICKNESS AND ECHOGENIC PATTERN ON THE SUCCESS OF INTRACYTOPLASMIC SPERM INJECTION (ICSI) CYCLE

Waleed El-Refaie, Ahmed Shabana, Mohamed S. Abdelhafez , Mostafa Alkhiary, Osama M. Warda
Department of Obstetrics and Gynecology,
Mansoura University, Mansoura, Egypt

Abstract

Objective: Evaluation of the endometrial thickness and pattern on the day of human chorionic gonadotropin (HCG) administration as a predicting factor for the clinical pregnancy rate, the implantation rate, and the miscarriage rate in intracytoplasmic sperm injection (ICSI) cycle.

Patients and method: A retrospective study performed in Fertility Care Unit (FCU) at Mansoura University Hospital, Mansoura, Egypt. Couples were recruited for management of infertility for different causes by ICSI through controlled ovarian hyperstimulation (COH) with pituitary down regulation by either gonadotropin releasing hormone (GnRH) agonist or antagonist. The endometrial thickness was defined as the distance from the point of the endometrial-myometrial junction on one side to the same point on the other side and patients were divided into three groups regarding the endometrial thickness into three group ; group 1 were the endometrium thickness was less than 7 mm , group 2 thickness from 7 mm to 14 mmand group 3 were thickness above 14 mm. After fertilization through ICSI, 2-4 good quality embryos were transferred transcervically 2-5 after oocyte retrieval. The main outcomes were clinical pregnancy rate (CPR), implantation rate (IR) and miscarriage rate (MR).

Results: Clinical pregnancy rate increased from 25% among patients with an endometrial thickness less than 7 mm to 69.2% among patients with an endometrial thickness of 7– 14 mm. In the group of patients with an endometrial thickness more than 14 mm, the clinical pregnancy rate declined to 52.2% with a statistically significant difference among the three groups. Also the implantation rate among the three group show stastically significant difference as it increased from 15.28 ± 28.73 in patients whom endometrial thickness less than 8 mm to 35.58 ± 30.57 in those whom endometrial thickness between 8-14 mm then decline to 25.36 ± 30.58 in patients whom endometrial thickness lmore than 14 mm. Howevere there was no stastically significant difference as regard the miscarriage rates among the three groups.

Conclusion:clinical pregnancy rate and implantation rate were significantly lower in endometrial thickness less than 7 mm with either trilaminar pattern and echogenic non trilaminar pattern. Also we conclude that the clinical pregnancy rate and implantation rate were higer in endometrial thickness more than 7 mm being highest between 7-14 mm with trilaminar pattern and slightly declined but still significantly high in thickness more than 14 mm with trilaminar pattern. However the miscarriage rate showed no significance among the three group and in both endometrial echogenic pattern.

Key Words: Endometrial thickness, endometrial echogenic pattern, intracytoplasmic sperm injection, ICSI, ICSI outcome.

Osama M Warda MD
Professor of Obstetrics & Gynecology
Faculty of medicine- Mansoura University
Tel.01066153212
E-mail: osamawarda@gmail.com

Introduction

In vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI) has been frequently performed worldwide and its success is determind by many factors, and one of them is the endometrial receptivity which is an essential component of implantation that is necessary for the success of the treatment. (1)

Endometrial thickness asessed by transvaginal ultrasonography is a popular indicator for endometrial receptivity. However the studies done to prove the relationship between endometrial thickness and IVF-ICSI outcome have shown conflicting results. Some studies proved no association between endometrial thickness and pregnancy (2, 3). Other studies have shown a significant relationship (4-5), while others have reported controversial results (6, 7).

Beside endometrial thickness the endometrial echogenic pattern may be predictor as non-triple-line endometrial pattern seems to be a prognostic sign of a less favorable outcome, while a triple-line pattern appears to be associated with pregnancy (8–9).

In this study, we examined the correlation between endometrial thickness and pattern (individually and together) and IVF/ICSI outcome. Our objective was to investigate whether there is predictive value for combined analysis of endometrial thickness and pattern for the success of IVF/ICSI cycles or not.

Patients and method

The participants of this study were chosen from the couples recruited for management of infertility for different causes by intracytoplasmic sperm injection (ICSI). A written informed consent was taken from each couple selected to participate in the study. The inclusion criteria were:

1. Woman age is < 40 years.
2. Body mass index (BMI) is 19-35 kg/m2.
3. No uterine abnormalities, myoma or previous uterine surgery.
4. The couple should have at least 2 good quality cleavage-satge embryo (good quality cleavage-stage embryos display stage-specific cell division, have blastomeres of fairly equal size with few to no cytoplasmic fragments).

A full, precise history was taken from each participant, and the cause of infertility was reported. Thorough clinical examination (including general, abdominal, and pelvic examination) was performed as well. The semen analysis of the husband was checked according to the WHO (2010) guidelines. The results of the previously done hysterosalpingography, laparoscopy and hysteroscopy were evaluated. Basal (day 3) serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) was assayed for each woman within 3 cycles of the scheduled IVF/ICSI cycle. A TVS scan was performed and any deviation from normal pelvic anatomy was looked for and reported.

According to the indication in each patient, either the long GnRH agonist (GnRHa) protocol or the GnRH antagonist flexible protocol was used for COH as following:

- If the long GnRHa protocol was used, the GnRHa preparation (Triptorelin) was administered subcutaneously in a dose of 0.1 mg/day starting in the mid-luteal phase (day 21) of the preceding cycle then the dose was reduced to half the dose (0.05 mg/day) from the day of ovarian stimulation till the day of HCG administration. Ovarian stimulation using gonadotropin preparation was commenced on day 3 of the next cycle (stimulation cycle) after ensuring adequate pituitary and ovarian suppression (serum E2 < 50 pg/ml), and performing TVS scan to confirm absence of ovarian cysts, and presence of endometrial thickness < 3 mm. The gonadotropin was given daily by deep intramuscular injection and the starting dose and type depended on the age of the woman, baseline FSH levels, BMI and previous trials. TVS scan was performed regularly for monitoring the follicular development (folliculometry); starting from day 8 of the cycle and repeated every 2-3 days. The dose and type of gonadotropin was modulated according to ovarian response.
- If the GnRH antagonist flexible protocol will be used, ovarian stimulation using gonadotropin preparation was commenced on day 3 of the stimulation cycle after performing TVS scan to confirm absence of ovarian cysts. The gonadotropin was given daily by deep intramuscular injection and the starting dose and type depended on the age of the woman, baseline FSH levels, BMI and previous trials. TVS scan was performed regularly for monitoring the follicular development (folliculometry); starting from day 8 of the cycle and repeated every 2-3 days. The dose and type of gonadotropin was modulated according to ovarian response. The GnRH antagonist preparation (Cetrorelix) was administered subcutaneously in a dose of 0.25 mg/day when a leading follicle reaches 14 mm in diameter and continued till the day of HCG administration.

The cycle was cancelled when poor ovarian response (< 4 follicles not reaching 18 mm correlated with serum E2 level < 400 pg/ml) is detected during follow up visits after counseling the couple regarding the success rates. The cycle was also cancelled when there is a high risk for ovarian hyperstimulation syndrome (i.e. more than 30 follicles, steep rise in serum E2, or ovarian size > 8 cm).

When there were at least 3 leading follicles > 18 mm in diameter, final oocyte maturation was induced by intramuscular administration of 10000 IU of HCG. The total number of follicles ≥ 12 mm in diameter by TVS, serum E2 level and endometrial thickness and pattern were evaluated on day of HCG administration.

Endometrial thickness was measured through TVS in the mid-sagittal plane of the uterine body. The endometrial thickness was defined as the distance from the point of the endometrial-myometrial junction on one side to the same point on the other side.

Endometrial pattern was also assessed by describing the type of relative echogenicity of the endometrium compared with the adjacent myometrium. Endometrial pattern noticed was either:

- **Triple line echogenicity pattern:** Which means hypoechoic endometrium with well-defined hyperechoic outer walls and a central echogenic line.
- **Non triple line echogenicity pattern:** Which means either isoechoic endometrium with poorly defined outer walls and central echogenic line, or homogeneous hyperechoic endometrium with absent central echogenic line.

After HCG injection by 34-36 hours, oocyte retrieval will be performed through transvaginal aspiration of follicles under TVS guidance followed by endometrial preparation for embryo transfer (ET) by giving 300 mg/day natural progesterone supplement (100 mg/day intramuscular supplement + 20 mg/day oral supplement) + 4 mg/day estradiol supplement.

After fertilization through IVF/ICSI, 2-3 good quality embryos were transferred transcervically 3-5 days after oocyte retrieval. If there is ≤ 5 good quality cleavage-stage embryos, ET were performed on day 3 after oocyte retrieval while if there is > 5 good quality cleavage-stage embryos, embryos will be left to reach the morula or blastocyst stage and ET will be performed on day 4 or 5 after oocyte retrieval.

The luteal phase support was continued by the same regimen started on the day of oocytes retrieval until 2 weeks after ET. Biochemical pregnancy will be documented by performing quantitative serum β-HCG assay 2 weeks after the ET and a level of ≥ 50 mIU/ml was considered positive indicator of pregnancy. Cases with positive pregnancy test will be examined by TVS 2-4 weeks later (4-6 weeks after ET) to document clinical intrauterine pregnancy which is defined as presence of at least one intrauterine gestational sac with fetal pole and cardiac activity on TVS scan at 4-6 weeks after the ET. Implantation rate (IR) will be calculated by dividing the number of gestational sacs on TVS scan at 4-6 weeks after ET by the number of transferred embryos. The study outcomes will be:

- **Clinical pregnancy rate (CPR):** Number of clinical pregnancies divided by the number of ET procedures.
- **Implantation rate (IR):** Number of gestational sacs on TVS scan at 4-6 weeks after ET divided by the number of transferred embryos.
- **Miscarriage rate (MR):** Number of first trimester miscarriages divided by the number of clinical pregnancies.

Statistical analysis was performed using Statistical Package for Social Sciences, Version 16.0 (SPSS, Inc., Chicago, Ill., USA) for Windows. Continuous variables were analyzed as mean ± standard deviation (SD) or median (interquartile range). Differences among continuous variables with normal distribution were analyzed by t test, one way analysis of variance test (ANOVA) and Chi-Square test ($\chi 2$ test). For continuous variables without normal distribution, non-parametric tests were used and differences were analyzed by the Mann-Whitney U-test and Kruskal Wallis test. Correlations between different parameters were determined by using Pearson's correlation coefficient (Pearson's r) and Spearman's correlation coefficient (Spearmann's rho). P value ≤ 0.05 was considered statistically significant, P value ≤ 0.01 was considered highly significant, and P value ≤ 0.001 was considered very highly significant.

Results

One hundred CC resistant PCOS patient were equally The total participants included in the study were 168 women. About half of the patients (49.1%) were in their first ICSI trial, while 26.4% had previous one trial and 24.5% had previous ≥ 2 trials. Among the 146 patients, most of patients (75.5%) were < 35 years old while 24.5% were ≥ 35 years old. The mean BMI of the study group was 30.06 kg/m2. Only 11.8% of the patients were of average weight while 31.8% were overweight and 56.4% were obese. Most of patients (70%) complained of primary infertility while 15.5% complained of secondary infertility and 14.5% complained of relative infertility (the woman can get pregnant but is unable to carry through to live birth). The mean duration of infertility was 6.97 years. The main

cause of infertility was male factor (50.9%) followed by PCOS (30.9%) then tubal factor (18.2%) then endometriosis (10.9%) then uterine factor (6.4%) and in 15.5% of couples, the infertility was unexplained. The ultrasonographic and biochemical characteristics and the COH and ICSI outcomes of the study group are summarized in table 1.

There was no dropped out cases in any step in the study. Out of the total 168 patients, 14 patients (12.7% of total patients) had cycle cancellation (11 patients due to poor ovarian response, and 3 patients due to risk of OHSS) while the remained 154 patients continued the cycle and underwent oocyte retrieval.

Out of the 154 patient who underwent oocyte retrieval, 146 patients had ET, while the other 8 patients (8.33% of patients who underwent oocyte retrieval) did not have ET (2 patients due to absence of oocytes in follicular fluid, 2 patients due to non occurrence of fertilization after ICSI, one patient due to non occurrence of cleavage after ICSI, one patient due to arrest of cleavage after ICSI, and 2 patients due to moderate OHSS before ET).

Out of the 146 patients who had ET, 70 patients had negative pregnancy test while the other 76 patients had positive pregnancy test (4 of them had biochemical pregnancy only while the others 72 patients had clinical pregnancy).

The endometrial thickness of the study population ranged from 4 mm to 16 mm the patient were classified into 3 groups according to endometrial thickness (less than 7 mm, from 7 mm to 14 mm and more than 14 mm. there was no significant difference in the three group as regard age, BMI, days of stimulation number of oocyte retrieved and oocyte maturation. But there was significant difference regarding dose of FSH, E2 level and numbers of oocyte retrieved.

Clinical pregnancy rate increased from 25% among patients with an endometrial thickness less than 7 mm to 69.2% among patients with an endometrial thickness of 7– 14 mm. In the group of patients with an endometrial thickness more than 14 mm, the clinical pregnancy rate declined to 52.2% with a statistically significant difference among the three groups. Also the implantation rate among the three group show statistically significant difference as it increased from 15.28 ± 28.73 in patients whom endometrial thickness less than 8 mm to 35.58 ± 30.57 in those whom endometrial thickness between 8-14 mm then decline to 25.36 ± 30.58 in patients whom endometrial thickness more than 14 mm. However there was no statistically significant difference as regard the miscarriage rates among the three groups.

We tried to declare the effect of combined endometrial thickness and pattern on clinical pregnancy rate, so we calculated clinical pregnancy rate according to the endometrial pattern in each endometrial thickness group. The clinical pregnancy rates and implantation rate was higher in patients whom endometrial pattern show trilaminar pattern howevere no significant difference was found regarding the miscarriage rate.

Table (1)
Ultrasonographic and biochemical characteristics and COH and ICSI outcomes of the study group (n = 146):

Parameters	Mean ± SD	Median (range)
Bilateral AFC	19.68 ± 8.67	16 (6-35)
Serum TSH (uIU/ml)	2.05 ± 1.17	1.74 (0.38-6)
Serum prolactin (ng/ml)	13.03 ± 6.88	11.9 (0.5-33.6)
Basal serum FSH (mIU/ml)	6.18 ± 2.11	5.81 (1.09-15.02)
Basal serum LH (mIU/ml)	6.92 ± 5.33	5.3 (1.9-41.7)
FSH/LH ratio	1.18 ± 0.68	0.98 (0.08-3.68)
Total FSH dose (IU)	2691.1 ± 673.45	2550 (1575-4875)
Stimulation days (days)	11.53 ± 1.63	11 (8-17)
Number of follicles ≥ 12 mm in diameter by TVS on day of HCG administration	17.88 ± 6.85	18 (3-35)
Peak serum E2 (pg/ml)	3496.58 ± 1718	3000 (976-8919)
Endometrial thickness by TVS on day of HCG administration (mm)	10.63 ± 4.4	10 (4-17)
Number of oocytes retrieved	11.95 ± 5.07	11 (3-25)
Oocyte maturation rate (%)	83.78 ± 15.6	87.5 (31.25-100)
Fertilization rate (%)	78 ± 18.06	80 (25-100)
Good quality embryos (%)	81.29 ± 20.11	84.62 (27.27-100)
Implantation rate (%)	25.68 ± 30.94	0 (0-100)

Table (2)
Comparison between women with endometrial thickness < 8 mm, those with endometrial thickness 8-12 mm and those with endometrial thickness > 12 mm as regard age, BMI and COH and ICSI outcomes:

Parameter	Endometrial thickness on day of HCG administration			P value
	< 7 mm	**7-14 mm**	**> 14 mm**	
Age (years)	29.67 ± 4.81	27.54 ± 4.19	28.78 ± 4.28	0.057
BMI	30.11 ± 4.06	30.25 ± 3.47	29.93 ± 3.13	0.907
Total FSH dose (IU)	2808.33 ± 76 0. 31	773.08 ± 68 0. 14	2476.09 ± 51 3. 51	**0.030**
Stimulation days (days)	11.71 ± 1.66	11.62 ± 1.77	11.26 ± 1.41	0.375
Number of follicles ≥ 12 mm in diameter by TVS on day of HCG administration	12.5 ± 4.85	18.42 ± 5.1	22.87 ± 6.37	**< 0.001**
Peak serum E2 (pg/ml)	2005.55 ± 62 2. 56	3035.69 ± 65 2. 85	5573.43 ± 12 63 .5 8	**< 0.001**
Number of oocytes retrieved	8.33 ± 3.34	11.65 ± 4	16.04 ± 4.67	**< 0.001**
Oocyte maturation rate (%)	84.38 ± 14.92	80.45 ± 15.67	86.93 ± 15.82	0.116
Fertilization rate (%)	81.34 ± 14.45	76.11 ± 21.93	76.65 ± 16.42	0.293
Good quality embryos (%)	81.28 ± 20.39	83.46 ± 19.85	78.83 ± 20.26	0.528
Clinical pregnancy rate (%)	12/48 (25%)	36/52 (69.2%)	24/46 (52.2%)	**< 0.001**
Implantation rate (%)	15.28 ± 28.73	35.58 ± 30.57	25.36 ± 30.58	**0.001**
Miscarriage rate (%)	4/12 (33.3%)	4/36 (11.1%)	6/24 (25%)	0.170

Table (3)
Clinical pregnancy rate with the 2 endometrial patterns in the 3 endometrial thickness groups:

		Endometrial thickness			P value
Endometrial pattern		**< 7 mm**	**7-14 mm**	**> 14 mm**	
	Triple line	4/10 (40%)	32/42 (76.2%)	12/22 (54.5%)	**0.047**
	Non triple line	8/38 (21.1%)	4/10 (40%)	12/24 (50%)	0.056
P value		0.218	**0.026**	0.758	

Table (4)
Implantation rate with the 2 endometrial patterns in the 3 endometrial thickness groups:

		Endometrial thickness			P value
Endometrial pattern		**< 7 mm**	**7-14 mm**	**> 14 mm**	
	Triple line	23.33 ± 30.63%	36.11 ± 26.97%	30.3 ± 36.96%	**< 0.001**
	Non triple line	13.16 ± 28.25%	33.33 ± 44.45%	20.83 ± 23.18%	0.862
P value		**0.607**	**0.019**	**0.034**	

Table (5) :
Miscarriage rate with the 2 endometrial patterns in the 3 endometrial thickness groups:

		Endometrial thickness			P value
Endometrial pattern		**< 7 mm**	**7-14 mm**	**> 14 mm**	
	Triple line	1/4 (25%)	3/32 (9.4%)	2/12 (16.7%)	0.592
	Non triple line	3/8 (37.5%)	1/4 (25%)	4/12 (33.3%)	0.911
P value		0.665	0.349	0.346	

Discussion

There are conflicting results regarding the association between endometrial thickness and IVF-ICSI outcome. In one study there was a significant relationship between endometrial thickness measured on the day of hCG injection and clinical pregnancy rate (10). On the other hand, Bassil showed that there is no significant correlation between endometrial thickness measurement and clinical outcome of IVF claiming that endometrial thickness has no prognostic value in IVF cycles (11).

There is no endometrial thickness cut off above which successful pregnancy occurrs. In one study, no pregnancies occurred when the endometrial thickness was less than 7 mm [12], whereas other studies reported clinical pregnancies at endometrial thickness of 6 mm [13-14], however Oliveira et al. have reported that there was no clinical pregnancy when the endometrial thickness was less than 7 mm (12).

Our study showed a positive correlation between endometrial thickness and clinical pregnancy rate and implantation rate. To our knowledge, this study has agreed with previous studies (15, 16, and 17). We found that the clinical pregnancy rate in group 1 (endometrial thickness ≤7 mm) was significantly lower (25%) than groups 2 (69.2%) and 3(52.2%) , and the implantation rate was significantly lower in the first group (15.28%) than the second (35.58%) and third group (25.36%) .

Although Weissman reported a high miscarriage rate with increased endometrial thickness (>14 mm) [18], in the present study, there was no significant difference regarding the miscarriage rate between the three group. Thus, our findings support those of some previous studies in which increased endometrial thickness (>14 mm) did not have a detrimental effect on clinical outcome.

In this study, clinical pregnancy rate and implantation rate were the highest with the trilaminar endometrium when compared with other pattern, and the difference was statistically significant. Kuc et al., 2011 reviewed 583 cycles of ICSI with different protocols of controlled ovarian hyperstimulation and reported that endometrial echogenicity was a significant predictor of pregnancy in the long GnRH agonist protocol (19). However, several studies have demonstrated no prognostic value of endometrial pattern for pregnancy (20, 21). In the other hand we found that the miscarriage rate showed no statistical significant difference between the three groups.

In this study, we tried to evaluate the role of combined endometrial thickness and pattern on clinical pregnancy rate, implantation rate and miscarriage rate. The endometrial thickness of 7–14 mm with trilaminar appearance produced the highest clinical pregnancy rate (76.2%) compared to pregnancy rates in other endometrial thickness and pattern groups. The implantation rate was higher in the group of endometrium thickness of 7-14 mm with trilaminar appearance (36.11%) and group of endometrium thickness of more than 14 mm with trilaminar pattern (30.3%) compared to the group of endometrial thickness of less than 7 mm (23.33%) .

Conclusion

From the results of our study, we can conclude that clinical pregnancy rate and implantation rate were significantly lower in endometrial thickness less than 7 mm with either trilaminar pattern or echogenic non trilaminar pattern. Also we conclude that the clinical pregnancy rate and implantation rate were higer in endometrial thickness more than 7 mm being highest between 7-14 mm with trilaminar pattern and slightly declined but still significantly high in thickness more than 14 mm with trilaminar pattern. However the miscarriage rate showed no significance among the three group and in both endometrial echogenic pattern.
Larger studies are needed to make a definitive conclusion.

References

5. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. Nat Rev Genet. 2006; 7(3): 185-199.
6. De Geyter C, Schmitter M, De Geyter M, Nieschlag E, Holzgreve W, Schneider HP. Prospective evaluation of the ultrasound appearance of the endometrium in a cohort of 1186 infertile women. Fertil Steril. 2000; 73(1): 106-113.
7. Dietterich C, Check JH, Choe JK, Nazari A, Lurie D. Increased endometrial thickness on the day of human chorionic gonadotropin injection does not adversely affect pregnancy or implantation rates following in vitro fertilization-embryo transfer. Fertil Steril. 2002; 77(4): 781-786.
8. Rinaldi L, Lisi F, Floccari A, Lisi R, Pepe G, Fishel S. Endometrial thickness as a predictor of pregnancy after in-vitro fertilization but not after intracytoplasmic sperm injection. Hum Reprod. 1996; 11(7): 1538-1541.
9. Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, and Awartani K. The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer (IVF-ET) outcome. Reprod Biol Endocrinol. 2008; 6: 37.
10. Weissman A, Gotlieb L, Casper RF. The detrimental effect of increased endometrial thickness on implantation and pregnancy rates and outcome in an in vitro fertilization program. Fertil Steril. 1999; 71(1): 147-149.
11. Bassil S. Changes in endometrial thickness, length and pattern in predicting pregnancy outcome during ovarian stimulation in in vitro fertilization. Ultrasound Obstet Gynecol. 2001; 18(3): 258-263.
12. Gonen Y, Calderon I, Dirnfeld M, Abramovici H. The impact of sonographic assessment of the endometrium and meticulous hormonal monitoring during natural cycles in patients with failed donor artificial insemination. Ultrasound Obstet Gynecol 1991;1:122–6.
13. Ja¨ rvela¨ IY, Sladkevicius P, Kelly S, Ojha K, Campbell S, Nargund G. Evaluation of endometrial receptivity during invitro fertilization using three-dimensional power Doppler ultrasound. Ultrasound Obstet Gynecol 2005;26:765–9.
14. Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, and Awartani K. The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer (IVF-ET) outcome. Reprod Biol Endocrinol. 2008; 6: 37.
15. Bassil S. Changes in endometrial thickness, length and pattern in predicting pregnancy outcome during ovarian stimulation in in vitro fertilization. Ultrasound Obstet Gynecol. 2001; 18(3): 258-263.
16. Oliveira JBA, Baruffi RLR, Mauri AL, Petersen CG, Borges MC, Franco JG Jr:Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme after ovarian stimulation and gonadotrophin releasing hormone and gonadotrophins. Hum Reprod 1997,
17. 12:2515-2518.
18. Gonen Y, Capsper RF: Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). J In Vitro Fert Embryo Transfer 1990,7:146-152.
19. Coulam CB, Bustillo M, Soenksen DM, Britten S: Ultrasonographic predictors of implantation after assisted reproduction. Fertil Steril 1994, 62:1004-1010.
20. Kovacs P, Matyas S, Boda K, Kaali SG. The effect of endometrial thickness on IVF/ICSI outcome. Hum Reprod. 2003; 18(11): 2337-2341.
21. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. Fertil Steril. 2007; 87(1): 53-59.
22. Zhang X, Chen CH, Confino E, Barnes R, Milad M, Kazer RR. Increased endometrial thickness is associated with improved treatment outcome for selected patients undergoing in vitro fertilization-embryo transfer. Fertil Steril. 2005; 83(2): 336-340.
23. Weissman A, Gotlieb L, Casper RF: The detrimental effect of increased endometrial thickness on implantation and pregnancy rates and outcome in an in vitro fertilization program. Fertil Steril 1999, 71:147-149.
24. Kuc P, Kuczynska A, Topczewska M, Tadejko P, Kuczynski W. The dynamics of endometrial growth and the triple layer appearance in three different controlled ovarian hyperstimulation protocols and their influence on IVF outcomes. Gynecol Endocrinol 2011;27(11):867–73..
25. Merce´ LT, Barco MJ, Bau S, Troyano J. Are endometrial parameters by three-dimensional ultrasound and power Doppler angiography related to in-vitro fertilization/embryo transferoutcome? Fertil Steril 2008;89:111–7.
26. Noyes N, Hampton BS, Berkeley A, Licciardi F, Grifo J, Krey L. Factors useful in predicting the success of oocyte donation: a 3-year retrospective analysis. Fertil Steril 2001;76:92–7.

THREE-DIMENSION ULTRASOUND STUDYING THE ROLE OF CESAREAN SECTION IN PROTECTING LEVATOR ANI INTEGRITY

[1]R.M.Rashed, [1]Abd- Elraof Oun, [1]Mazen Abd El Raouf El Zahry, [1]Mofied Fawzy, [2]Abd el Aziz A.oun
[1]Obstetric and Gynecology Department,
[2]Radiology Department,
Faculty of Medicine, Al-Azhar University, Cairo, Egypt

Abstract

Objective: The aim of this study was to study the role of caesarean section in protecting LAM integrity by using perineal three-dimensional (3D) ultrasound.

Patients and method: A prospective cross-sectional observational study using perineal three-dimensional (3D) ultrasound. This study includes 104 primipara women with no past history of previous vaginal surgery in obstetric and gynecology department Of Sayed Galal university hospital, faculty of medicine ,Al-Azhar University In Cairo, between June 2012 and November 2012,Women who delivered vaginally were considered as group A and women who had a caesarean section were classed as group B. Within group A, the women were also subdivided in relation to the mode of delivery as follows: spontaneous delivery, vacuum extraction and forceps extraction, whereas in group B distinction was made between elective and emergency caesarean section Three-dimensional perineal ultrasound was performed between 48 and 72 hours postpartum. The axial plane at the level of minimal hiatal dimension and tomographic ultrasound imaging were used to determine LAM biometry and defect.

Results: In all 104 women participated: 54 (51.9%) women delivered in group A (46) spontaneous deliveries, (6) vacuum extractions, (2) forceps extraction) and 50(48.1%) in group B (36) elective and (14) emergency caesarean sections). All biometrical indices of levator ani hiatus were higher after vaginal delivery compared with post-caesarean section values ($P < 0.001$). Except for LAM thickness. The presence of levator ani defects was significantly higher in group A (22/54, 40.7%) in comparison to group B (2/50, 4%) ($P < 0.001$), with a strong positive association between vaginal delivery and levator trauma (relative risk7.1; 95%CI2.7420.18).

Conclusion: there was strong positive association between vaginal delivery and levator trauma despite this; emergency caesarean section seems to have no complete preventive effect on LAM trauma. While elective cesarean section completely protect LAM integrity.

Key Words: Three-Dimension Ultrasound, Cesarean Section, Levator Ani

Introduction

The strength of the muscles of the pelvic floor and other supporting structures of the pelvic organs are affected by various events that occur during a woman's lifetime. Pregnancy and childbirth have a pronounced influence on maternal anatomy and physiology.1 the physiological processes of pregnancy and parturition must involve dramatic adaptations to the vagina and pelvic floor to allow marked distension at delivery and to return to a near pre-pregnant state after parturition. The recovery process, however, may not be complete. Vaginal parity has been identified as an important risk factor for pelvic organ prolapse.1–3 the levator hiatus, defined by the puborectalis/pubococcygeus muscle and pubic bone, is the largest potential hernia portal in the human body.4 The area of the levator hiatus, which

Abd-Elraof Oun, MD,
Department of Obstetrics & Gynecology,
Faculty of Medicine, Al-Azhar University, Cairo, Egypt

varies widely in size from 6 to 35 cm2 during the Valsalvamanoeuvre,5 needs a distension of between 25% and 245%to allow the passage of the fetal head (for an average cross sectional area of 68 cm2, based on Caucasian biometric data).10.It is conceivable that enlargement of the hiatus, even without gross muscle trauma, may lead to pelvic organ prolapse and may be an alternative mechanism in the pathogenesis of this common gynecologic problem.4 The involvement of the LAM on morbidity of the pelvic floor has been widely investigated. The finding that enlarged hiatus is associated with genital prolapse has suggested that LAM trauma or an enlargement of the urogenital hiatus, influences pelvic support.7,8 It has become clear recently that major trauma to the levator ani muscle is a consequence of vaginal childbirth in a considerable minority of women. The most obvious form of trauma, i.e. avulsion of the anteromedial aspects of the pubovisceral muscle off the arcus tendineus, can be detected on palpation, as described by Howard Gaineyin9 19431, There is no doubt, that caesarean section, whether primary or secondary, can lead to significant and occasionally major morbidity and even mortality. For now we have no means of determining whether this risk outweighs the risk of attempted vaginal childbirth in a given patient, and this must be reflected in the advice that we provide to our patients. Regarding prolapse, pregnancy and childbirth are well documented as major risk factors10-11. Caesarean delivery is associated with less need for surgical correction of incontinence or prolapse10 and seems protective against symptomatic prolapse12. Recent studies using magnetic resonance imaging and ultrasonography have found levator trauma (avulsion injury) in 15–30% of parous women who delivered vaginally.13-17 One reason for our continuing ignorance is that most levator ani trauma is occult at the time of childbirth, and that palpation of levator trauma requires significant training18, 19. Fortunately, diagnosis has now become much easier with the help of modern imaging methods. Recent advances in sonographic imaging technology allow the study of anal sphincter and levator ani muscle morphology20-21. The use of this technology sheds new insights into the sequelae of childbirth to the pelvic floor22. It has been shown recently that translabial three dimensional (3D) ultrasound can be utilized to document major levator ani trauma, both in symptomatic and a symptomatic women23,24 and before and after childbirth25. The prevalence seems to range between15 and 35% of vaginally parous women, with maternal age at first delivery a predictor both in MRI26and ultrasound25studies. Whether and how vaginal delivery is responsible for pelvic floor morbidity is a controversial debate that is still wide open. If it is true that all women undergo pelvic floor stretching during delivery, not all of them suffer injury. The latency of symptoms and the multifactorial aetiology of prolapse and of urinary and faecal incontinence do not facilitate an understanding of the true role of vaginal birth. However, There is no doubt that delivery is the most stressful and dangerous event that the pelvic diaphragm is submitted to during a woman's life. On other hand, Whether or not elective cesarean section prevents these symptoms in the long-term is still unclear. After the first delivery, women who had delivered vaginally have two-fold more incontinence than those who had delivered by cesarean section. However, this protective effect of cesarean deliveries on urinary incontinence decreases with age and is not present in older women27 .Furthermore vaginal delivery may stretch and/or load the pelvic floor tissue beyond the physiological and in this way may lead to irreversible changes in tissue properties which play an important role in the urethral support continence mechanism 28.so, the aim of our research is to study the role of caesarean section in protecting LAM integrity by avoiding fetal passage through the birth canal.

Patients and methods

A prospective cross-sectional observational study was designed to compare the levator ani integrity following vaginal delivery and caesarean section, using perineal three-dimensional (3D) ultrasound. This study includes 104 women with no past history of previous vaginal surgery in obstetric and gynecology department of Sayed Galal university hospital, Faculty Of Medicine, Al-Azhar University In Cairo, between June 2012 and November 2012, A total of 110 primigravida women were recruited from the emergency room, of Maternity Sayed Galal Hospital. The inclusion criteria were: 1) primigravida with singleton pregnancy, 2) between 36 and 40weeks of gestation, 3) at least 18 years of age, 4) no previous pregnancies of more than 20 weeks of gestation.5) able to give informed consent. Exclusion criteria were: 1) complicated pregnancies (e.g. multiple pregnancy, macrosomia, fetal growth restriction, fetal malformations, hydramnion, oligohydramnion), 2) women with perineal or vaginal operation in past medical history,3) age less than 18 years, 4)and inability to communicate with care providers because of mental illness, and difficulties. After vaginal delivery or caesarean section, women were then asked to participate in this study. Each woman was carefully informed about the aim and design of the study. Instructions regarding the perineal 3D ultrasound were given .Oral informed consent was obtained before any investigation took place. All 3D ultrasound were performed by the same experienced investigator, highly trained in pelvic 3D ultrasound. Consenting women underwent a perineal ultrasound scan in bed, within the first 3 day post partum. The examination was performed in dorsal lithotomy position (the woman lying on her back with bent knees positioned above the hips and spread apart, without the use of stirrups) after voidingThe probe was positioned longitudinally, parting the vulvar labia in the area of the fourchette and perineal body, with minimal pressure being applied. Before beginning the examination, women were asked to cough to part the labia, to expel air bubbles and to ensure good contact between the transducer and tissue. No specific precautions concerning filling status of the rectum are made. The transducer axis was oriented in the mid-sagittal plane to visualize, from right to left, the symphysis pubis, the urethra (distinguishable by the hypoechogenic mucosa and submucosa layers), the bladder, the vaginal walls, the distal part of the rectum with anorectal junction, the proximal part of the anal canal and, posterior to the latter structure, a hyperechogenic spot representing the puborectalis sling. The method of obtaining hiatal dimensions used in our Study was performed according to the study published by Dietz et al.29 and found to be reproducible by others.30.31The plane of minimal hiatal dimensions was identified in the mid-sagittal plane, evident as the minimal distance between the hyperechogenic posterior aspect of the pubic symphysis and the hyperechogenic anterior border of the levator ani muscle, just posterior to the anorectum. When a satisfactory two-dimensional ultrasound image was achieved, the 3D volume was obtained automatically by the3D function of the system, The axial plane at the level of minimal hiatal dimensions between the pubic bone and the dorsal aspect of the puborectalis sling, was used to determine mid-sagittal (hAP) and coronal (hLL) diameters of the levator hiatus, hiatal area (hA) and hiatal circumference (hC). The maximal thickness of the pubococcygeus-puborectalis muscle was measured by cranially moving the plane of minimal hiatal dimension until it was possible to visualise the maximal thickness of the muscle and take a measurement on each side, close to the rectum.29Levator avulsion was diagnosed whenever a discontinuity was detected between the inferior pubic ramus and the puborectalis muscle on tomographic ultrasound imaging. The detection of levator defects by three-dimensional pelvic floor ultrasonography has been shown previously to be highly reproducible.24To assess levator ani integrity, we used tomographic multislice ultrasound imaging with 2.5-mm slice intervals, from 5 mm below to 12.5 mm above the plane of minimal hiatal dimensions. This produced eight slices for each woman despite the fact that we obtained the volumes with the woman in a resting position, we decided to consider slices at and above the plane of minimal hiatal dimensions only, as proposed by Dietz andShek32 for the volumes obtained on pelvic floor contraction. We documented each discontinuity (a break in the normal texture of the pubocoggygeal-puborectalis muscle, evident as an ultrasound hypo/anechogenic lesion interrupting the hyperechogenic course of muscle fibres) involving the pubococcygeus-puborectalis muscle, recognisable in the coronal C-plane slice (unilateral if the defect involves one side, bilateral if both sides are damaged). To standardize the diagnosis of a puborectalis sling injury and to differentiate true lesions from artifacts, we decided to diagnose an abnormality (meant as ultrasound discontinuity) evident in at least three consecutive slices above the plane of minimal hiatal dimension, as a LAM defect, All characteristic data on women were collected from a specific database program available in our hospital and analyzed using the Excel program (Microsoft Office Excel 2007).The Student's t test and the Mann–Whitney U test were performed to compare continuous parametric and non parametric variables respectively. The proportion of categorical variables was analyzed for statistical significance by using Fisher's exact test. Statistical significance was considered to have been reached when the P-value was <0.05.

Results

During the study period, a total of 110 primiparae were considered eligible for participation in the study and gave their consent for ultrasound examination.6 women complained of pain and discomfort during the Scan and refused to finish the ultrasound evaluation, as a result 104 women completed the examination and were included for data analysis. Women who delivered vaginally were considered as group A and women who had a caesarean section were classed as group B. Within group A, the women were also subdivided in relation to the mode of delivery as follows: spontaneous delivery, vacuum extraction and forceps extraction, whereas in group B distinction was made between elective and emergency caesarean section.54patients (51.9%) women delivered in group A :(46) spontaneous deliveries, (6) vacuum extractions, (2) forceps extraction) and 50patients(48.1%) in group B (36) elective and(14)emergency caesarean sections).

Figure S1. Intact levator ani after elective cesarean section

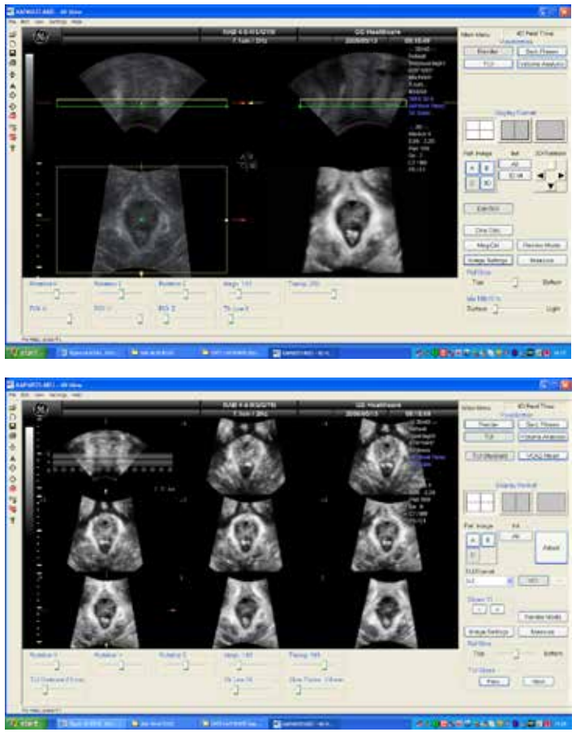


Figure 1. The multiplanar mode (A) and the TUI slices (B) show an intact levator ani after elective cesarean section

There was no statistical difference between the two groups for patient characteristics (age, body mass index [BMI]) and gestational age (Table 1).

Table (1)
Demographic characteristics of group A and group B

Characteristics	Group A (n = 54)	Group B (n = 50)	P-value
Age (years)	24.76 (±5.51)	25.3 (±4.4)	>0.05
BMI(KG\M2)	22(15–34)	22(14–44)	>0.05
GESTATIONAL AGE (WEEK)	39 (34–43)	39 (34–42)	>0.05

Data are expressed as mean ± SD, median (range). Group A, vaginal deliveries; group B, caesarean sections.

All biometrical indices of levator ani hiatus were higher after vaginal delivery compared with post-caesarean section values (P < 0.001). Except for LAM thickness. The thickness of the LAM both on the left and on the right side did not significantly vary between the two groups (Table 2).

Table (2)
Biometric indices of LAM of group A and group B

Biometrical indices	Group A (n = 54)	Group B (n = 50)	P-value
hAP(cm)	5.8 (±0.7)	4.7 (±0.8)	<0.001
hLL(cm)	3.8(3.1–5.4)	3.5 (2.4–5.5)	<0.001
hA(cm2)	13.85 (8.4–22.0)	11.12 (5.5–31.33)	<0.001
hC(cm)	16.12 (11.20–18.85)	12.86 (9.1–20.04)	<0.001
Rt levator thickness(cm)	0.69(0.3–1.24)	0.64 (0.29–1.20)	>0.05
lt levator thickness(cm)	0.72 (0.31–1.80)	0.67 (0.29–1.10)	>0.05

Data are expressed as median (range) or mean ± SD.Group A, vaginal deliveries; group B, caesarean sections; hAP, midsagittal(antero-posterior, AP) diameter of the hiatus (h) delimited bylevator ani muscle; hLL, coronal (latero-lateral, LL) diameter of the hiatus (h) delimited by levator ani muscle; hA, area (A) of the hiatus(h) delimited by levator ani muscle; hC, circumference (C) of the hiatus delimited by levator ani muscle.

Figure 2. Levator ani trauma after spontaneous delivery

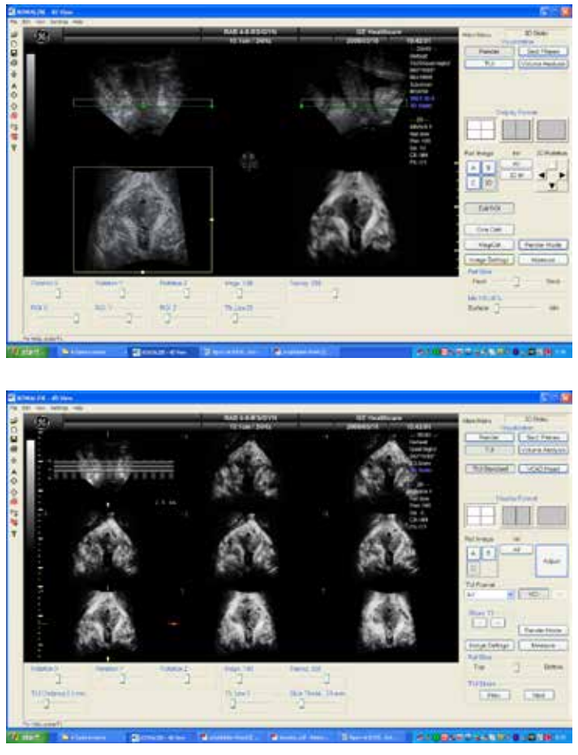


Figure S2. Levator ani defect early postpartum in a patient had spontaneous vaginal delivery

(In the multiplanar mode, the axial plane (lower left) and the rendered image (lower right) show a unilateral levator discontinuity (**) on the right side of pubococcigeal-puborectalis muscle.

- Eight slices obtained with TUI in coronal-C plane in the same patients: the discontinuity (arrow) is demonstrated in at least three consecutive slices at and above the plane of minimal hiatal dimension (frames *,-1,-2,-3).

Similar findings were observed when comparing a subgroup of group A (spontaneous vaginal delivery) with a subgroup of group B (elective caesarean section) (see Table 3),

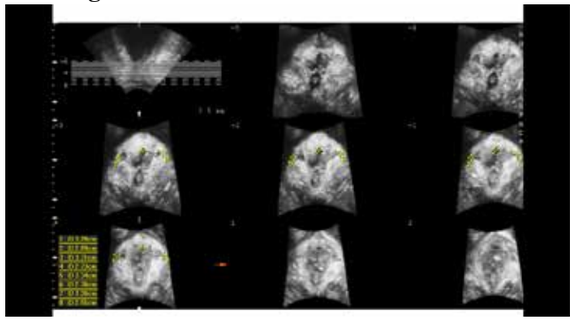
Table (3)
biometrical indices of LAM after spontaneous deliveries vs. elective cesarean section

Biometrical indices	Spontaneous deliveries	Elective cesarean section	P value
hAP(cm)	5.9 (±0.8)	4.9 (±0.8)	<0.001°•
hLL(cm)	4.0 (3.0-5.5)	3.6 (2.6- 5.7)	<0.001*•
hA(cm2)	14.33 (8.6-22.4)	10.49 (6.0-31.3)	<0.001*
hC(cm)	15.61 (11.28-18.93)	13.23 (10.53-20.08)	<0.001*•
Levator thickness right (cm)	0.7 (0.4-1.29)	0.66 (0.33-1.24)	>0.05
Levator thickness left (cm)	0.68 (0.34-1.44)	0.65 (0.4-1.02)	>0.05

Data are expressed as median (range), mean ± standard deviation

hAP: midsagittal (antero-posterior, AP) diameter of the hiatus(h)delimited by levator ani muscle; hLL: coronal(latero-lateral, LL) diameter of the hiatus (h) delimited by levator ani muscle;hA: area (A) of the hiatus (h) delimited by levator ani muscle;hC: circumference (C) of the hiatus delimited by levator ani muscle;

Figure 3. Levator ani defect after emergency cesarean section due to intrauterine asphyxia during late first stage of labour



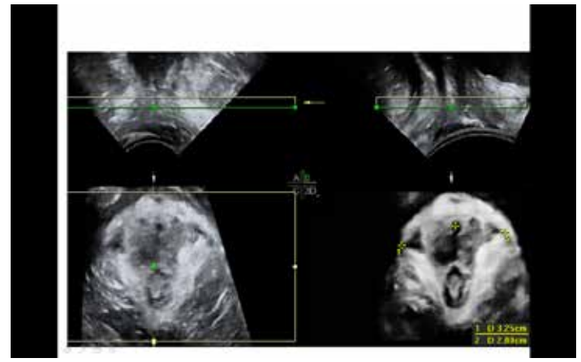


Figure 3. Levator assessment after an emergency cesarean section due to intrauterine asphyxia during late first stage of labour

A: Acquisition screen shows the orthogonal plane (lower left) and the rendered volume (lower right): the measurement of 'levator-urethra gap' (bottom right hand corner) is > 2.5 cm in both sides

B: In the same patient the TUI slices (* slice is the reference plane, the plane of minimal hiatal dimension; slices-1,-2,-3,-4,-5 correspond respectively to slice at 2.5 mm, 5 mm, 7.5 mm, 10 mm, 12.5 mm above the reference plane): the measurements of 'levator-urethra gap' at and above the plane of minimal hiatal dimension are > 2.5 cm in both sides of LAM.

and these two groups were also comparable in age , BMI and gestational age The presence of levator ani defects was significantly higher in group A (22/54, 40.7%) in comparison to group B (2/50, 4%) (P < 0.001), with a strong positive association between vaginal delivery and levator trauma(relativerisk7.1;95%-CI2.7420.18).18 and 2 unilateral defects, while, 6 and 2 bilateral defects were found in group A and group B, respectively.

In group A, unilateral defects were found to be significantly more frequent than bilateral (P = 0.009).Overall, 22 levator defects were found in group A: 18 (39.1%) after spontaneous delivery, see (figure 2), 2 (33%) after vacuum extraction and 2 after the forceps extraction in the study. In group B, 2 levator defects were observed, all of them after emergency caesarean section with complete cervix dilatation. These caesarean sections were performed on one woman because of intrauterine asphyxia before the active pushing phase of the second stage of labour had begun (see Figures 3), and in one woman because of the arrest of fetal head descent during the late second stage of labour (Figure 4).

Figure 4: Levator ani defect after emergency cesarean section due the arrest of fetal head descent during the late second stage of labour

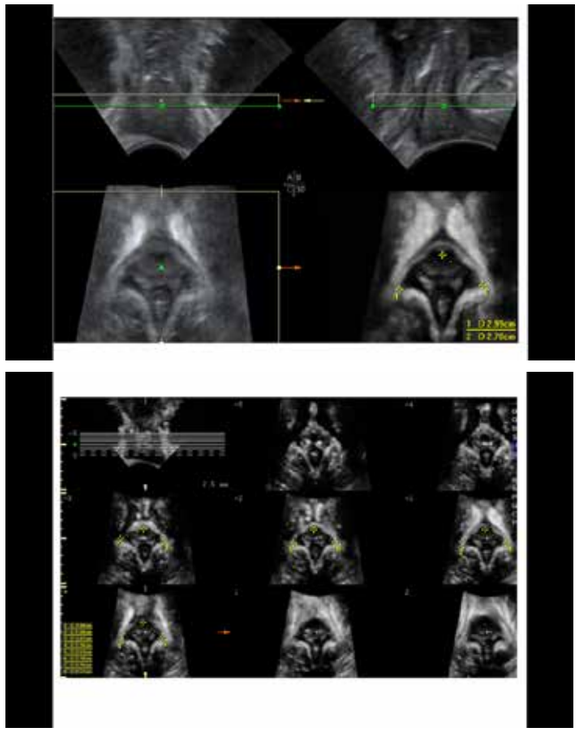


Figure 4. Levator assessment after an emergency cesarean section due the arrest of fetal head descent during the late second stage of labour

A. Acquisition screen shows the orthogonal plane (lower left) and the rendered volume (lower right): the measurements of 'levator-urethra gap' (bottom right hand corner) are >2.5 cm in both sides. B. In the same patient the TUI slices (* slice is the reference plane, the plane of minimal hiatal dimension; slices -1,-2,-3,-4,-5 correspond respectively to slice at 2.5 mm, 5 mm, 7.5 mm, 10 mm, 12.5 mm above the reference plane): the measurement of 'levator-urethra gap' confirms the presence of abnormalities in both sides of LAM

In no woman was it documented that the head had descended below the level of the ischial spines before caesarean delivery. None of these 2 women had previously given birth, and none had attempted vaginal delivery in the index pregnancy before caesarean delivery. Consequently there was no potential for them to have had application of vacuum or forceps before caesarean delivery. Comparing levator ani biometry after elective and emergency caesarean section, no significant difference on any level was found, These groups were comparable in terms of age (P = 0.07) and BMI (P = 0.87), and in terms of gestational age (P < 0.006). Our results show that the presence of levator ani defects was significantly higher after emergency caesarean section (2/14, 14.2%), than after elective caesarean section, (see figure 1) (0/36) (P = 0.004), and that elective caesarean section protects from LAM defect with a relative risk of 0.03 (95% CI 0.001–0.683) in comparison with emergency caesarean section. The j-statistic for agreement between examiners was performed on a subgroup of40 women and gave j = 0.899 (P < 0.005).

Discussion

Tomographic ultrasound imaging in conjunction with3D pelvic floor ultrasound allows definition of morphological abnormalities of the LAM to a previously unattainable degree. The findings of our study suggest that after vaginal delivery a woman is seven times more likely to develop LAM defects than after a caesarean section. Despite this, we also found LAM abnormalities following emergency caesarean section. Our results were in agreement with result of Albrich33 et al, 2011. In our caesarean section group, 2 women were found to have a levator defect, all of them after a caesarean section performed before the active pushing phase of the second stage of labour had begun and before fetal head engagement. The finding of a levator tear after caesarean section was comperable to finding of Albrich et al., 2011. However our finding was absolutely unexpected in terms of the data published in the current literature. Furthermore, it is difficult to understand how tearing of the levator could occur before crowning of the fetal head because the distension of the puborectalis muscle does not appear to be necessary before crowning.34Nevertheless, we considered it to be important to describe and discuss our data, considering the limitations and the possible bias of this cohort trial. First, the prevalence of LAM defects in our study is high: 39.1% while the highest reported in literature to date was by Albrich et.al. 2011: 39.5% in comparison with 18.8% reported by Valsky et al., 35 during the same postpartum period and using the 3D perineal ultrasound assessment. Over-diagnosis could be a possible explanation, when you consider that every test produces false-positive results, especially one that is so operator-dependent. The decision to include all recognizable LAM abnormalities could also explain the difference in our results. Indeed, we have defined 'defect' and not avulsion as the assessed LAM abnormality, assuming that 'avulsion' is considered to be the complete detachment of the muscle from the bone. The ultrasound assessment of complete and incomplete trauma is sometimes difficult, because of the complex and3D nature of the levator hiatus occupying a warped (non-Euclidean) plane.36 Recently, Dietz et al.37 proposed a method to define partial and

complete trauma of the LAM: they considered a complete avulsion to be if all three central slices (slice at plane of minimal hiatal dimension plus the two above) were abnormal, and partial avulsion was diagnosed when any three to eight slices were abnormal. Considering these criteria for diagnosis of avulsion, we have included complete and partial avulsion in our results. The ultrasound differential diagnosis between a muscular tear and the presence of a fluid collection, e.g. a haematoma, is also sometimes unclear. LAM defect occurred more frequently (but not siginificantly so) on the right side, but were often bilateral, the latter diagnosed more commonly than has been observed previously24, 25. This increased prevalence of bilateral defects may have been due to the improved detection of smaller abnormalities with tomographic imaging. Defects seemed best defined on levator ani contraction, and the use of volumes obtained on contraction may also have improved the detection compared with previously used methodology24.From our data it also seems that bilateral avulsion (more than30% of all defects in both groups), is not as rare as previously de scribed.15, 35 This can be considered acceptable in light of the inclusion of complete and partial avulsion. We cannot exclude the possibility that those women who underwent an emergency caesarean section may have begun in adequate voluntary pushing for some time before complete dilatation, considering that it appears to be more plausible for maternal expulsion forces to generate an injury rather than uterine contractions. This could create a bias when it comes to interpreting the data. The discussion about the possible protective effect of an elective c-section on the pelvic floor is still in progress: Altmann and colleagues38 describe that incontinence symptoms are more common following spontaneous vaginal delivery when compared with cesarean section 10 years after first delivery. However, cesarean section is not associated with a major reduction of anal and urinary incontinence38 (altmann et al. 2007).on the other hand, Boyles et al.39 found in a study in5599 primiparous women that indeed vaginal delivery increases the risk of urinary incontinence, but they could not show a statistical difference in the incidence of urinary incontinence among those women who had elective cesarean deliveries (6.1%), women who had cesarean deliveries after laboring (5.7%), and women who had cesarean deliveries after laboring and pushing(6.4%) (Boyles et al. 2009)39.The lack of ante partum scans also includes the possibility of the presence of asymmetric levators as a normal anatomic variant in nulliparous women. The only study demonstrating the presence of levator abnormalities after caesarean section was published by Novellas et al.40 They assessed primiparous women with MRI shortly after a caesarean section had been performed and they reported abnormalities (defined as hyper signal of the muscle, thinning or thickening, or rupture of the muscular insertion) in the pubococcygeus-puborectalis muscle of those women. They demonstrated that women experiencing active labour during a caesarean section had 2.7 times more abnormalities than women undergoinga caesarean without being in labour (including the emergency group of women with an average cervical dilatation of 6.2 cm [range 3–10 cm] and an average duration of labour of 5.65 hours [range 2–10 hours]).A methodological aspect of our study and Albrich33 et al. 2011 were to investigate the structure of the LAM just after delivery. This is in contrast to most of the published studies, whereby its biometry was evaluated at least 6 weeks postpartum.15,41-43 The minimal discomfort of 3D perineal sonography allowed us to evaluate the LAM even in the delicate and sensitive period of the early postpartum days. The fact that only 6 women complained of pain or discomfort during the assessment and that 104 women completed the examination, confirms the broad feasibility and acceptance of this assessment procedure. In addition, to scan the women at this time was favorable in terms of logistics because they were still in hospital. Such early ultrasound assessment allows us to evaluate the soft pelvic tissue immediately after the acute trauma, before tissue transformations and the remodeling process have occurred in the postpartum period. However, this timing of the assessment only permits us to evaluate the LAM morphology at rest: the movement of the pelvic floor (contraction or Valsalva manoeuvre) could not be performed correctly by all women immediately postpartum because of pain related to episiotomy, perineal lacerations, caesarean wound, uterine contractions or simply the discomfort related to recent delivery. Therefore the lack of dynamic volumes during pelvic floor muscle contraction could also be a possible explanation for our unexpected findings, considering that avulsions appear to be more de fined during contraction of the muscle.44Tomographic ultrasound imaging and quantification of defects will hopefully allow us to investigate the etiology and pathogenesis of levator ani trauma in greater detail, in order to define personal risk factors and the impact of obstetric variables. It is expected that more detailed assessment of trauma will increase the power of a longitudinal study aims to assess the prevalence and extent of trauma and its association with obstetric parameters in women after their first vaginal delivery.

Conclusion

From our results we can conclude that there was strong positive association between vaginal delivery and levator trauma, and associated with a higher risk of enlarging the pelvic diaphragm hiatus, in comparison with caesarean section. From our findings it seems that the beginning of labour itself may play a role in levator morphological changes. Also, we can conclude that, emergency caesarean section seems to have no complete preventive effect on LAM trauma. While elective cesarean section completely protect LAM integrity.

References

1. Mant J, Painter R, Vessey M. Epidemiology of genital prolapse: Observations from the Oxford Family Planning Association Study. Br J Obstet Gynaecol 1997; 104:579–85.
2. Hendrix SL, Clark A, Nygaard I, Aragaki A, Barnabei V,
3. McTiernan A. Pelvic organ prolapse in the Women's Health
4. Initiative: gravity and gravidity. Am J Obstet Gynecol 2002;
5. 186:1160–6.
6. Swift S, Woodman P, O'Boyle A, Kahn M, Valley M, Bland D,et al. Pelvic Organ Support Study (POSST): the distribution, clinical definition, and epidemiologic condition of pelvic organ
7. support defects. Am J Obstet Gynecol 2005; 192:795–806.
8. Dietz HP, Shek C, Clarke B. Biometry of the pubovisceral
9. muscle and levator hiatus by three-dimensional pelvic floor
10. ultrasound. Ultrasound Obstet Gynecol 2005; 25:580–5.
11. Dietz HP, Shek C, Clarke B. Biometry of the pubovisceral muscle and levator hiatus by three-dimensional pelvic floor ultrasound. UltrasoundO bstet Gynecol 2005;25:580–5.
12. Svabı´k K, Shek KL, Dietz HP. How much does the levator hiatus have to stretch during childbirth? BJOG 2009;116:1657–62.
13. DeLancey JOL, Hurd WW. Size of urogenital hiatus in the levator ani muscles in normal women and women with pelvic organ prolapse. Obstet Gynecol 1998;91:364–8.
14. DeLancey JO, Morgan DM, Fenner DE, Kearney R, Guire K, MillerJM, et al. Comparison of levator muscle defects and function in women with and without pelvic organ prolapse. Obstet Gynecol2007; 109:295–302.
15. Gainey HL. Post-partum observation of pelvic tissue damage.Am J Obstet Gynecol 1943; 46: 457–466.
16. MacLennan AH, Taylor AW, Wilson DH, Wilson PD. The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of delivery. Br J Obstet Gynaecol 2000; 107:1460–1470.
17. Mant J, Painter R, Vessey M. Epidemiology of genital prolapse: observations from the Oxford Family Planning Association Study. Br J Obstet Gynaecol1997; 104:579–585.
18. Tegerstedt G, Miedel A, Maehle-Schmidt M, et al. Obstetric risk factors for symptomatic prolapse: a population-based approach. Am J Obstet Gynecol2006; 194:75–81.
19. DeLancey JO, Kearney R, Chou Q, Speights S, Binno S. Theappearance of levator ani muscle abnormalities in magneticresonance images after vaginal delivery. Obstet Gynecol 2003;101:46–53.
20. Dietz HP, Simpson JM. Levator trauma is associated withpelvic organ prolapse. BJOG 2008;115:979–84.
21. Dietz HP, Lanzarone V. Levator trauma after vaginal delivery.Obstet Gynecol 2005;106:707–12.
22. Dietz HP, Steensma AB. The prevalence of major abnormalities of the levator ani in urogynaecological patients. BJOG2006; 113:225–30.
23. Dietz HP, Simpson JM. Does delayed child-bearing increase the risk of levator injury in labour? Aust N Z J Obstet Gynaecol2007;47:491–5.
24. Dietz H, Hyland G, Hay Smith E. A blinded comparison ofpalpation and 3D/4D ultrasound imaging of the pubovisceral muscle. Int Urogynecol J 2005; 16(S2): S75.
25. Kearney R, Miller JM, Delancey JO. Interrater reliability and physical examination of the pubovisceral portion of the levator ani muscle, validity comparisons using MR imaging. NeurourolUrodynam 2006; 25: 50–54.
26. Sandridge DA, Thorp JM. Vaginal endosonography in the assessment of the anorectum. Obstet Gynecol 1995; 86:
27. 1007–9.
28. Sultan AH, Kamm MA, Hudson CN, Thomas JM, BartramCI. Anal-sphincter disruption during vaginal delivery.
29. N Engl J Med 1993; 329 (26): 1905–11.
30. Kamm MA. Obstetric damage and faecal incontinence.
31. Lancet 1994; 344 (8924): 730–3.
32. Dietz H. Ultrasound imaging of the pelvic floor: 3D aspects.Ultrasound Obstet Gynecol 2004; 23: 615–625.
33. Dietz H, Steensma A. The prevalence of major abnormalities of the levator ani in urogynaecological patients. BJOG 2006; 113:225–230.
34. Dietz HP, Lanzarone V. Levator trauma after vaginal delivery.Obstet Gynecol 2005; 106: 707–712.
35. Kearney R, Miller J, Ashton-Miller J, Delancey J. Obstetric factors associated with levator ani muscle injury after vaginal birth. Obstet Gynecol 2006; 107: 144–149.
36. nygaard I. urinary incontinence: is cesarean delivery protective? Semin Perinatol. 2006 oct;30(5):267-71.
37. Wijma J, Weis Potters aE, van der Mark tW, tinga dJ,
38. aarnoudse JG. displacement and recovery of the vesical
39. neck position during pregnancy and after childbirth. Neuro urolurodyn. 2007;26(3):372-6.
40. Dietz HP, Shek C, Clarke B. Biometry of the pubovisceral muscle and levator hiatus by three-dimensional pelvic floor ultrasound. Ultrasound Obstet Gynecol 2005;25:580–5.
41. Yang J, Yang S, Huang W. Biometry of the pubovisceral muscle and levator hiatus in nulliparous Chinese women. Ultraosund Obstet Gynecol 2006;28:710–6.
42. Hoff Braekken I, Majida M, Ellstrom Engh M, Dietz HP, Umek W, BoK. Test–retest and intra-observer repeatability of two, three and fou rdimensional perineal ultrasound of pelvic floor muscle anatomy and function. Int Urogynecol J 2007;19:227–35.
43. 32- Dietz HP, Shek KL. Tomographic ultrasound imaging of the pelvic floor: which levels matter most? Ultrasound Obstet Gynecol 2009;33:698–703.
44. Albrich S, Laterza R, Skala C, Salvatore S, Koelbl H, Naumann G. Impact of mode of delivery on levator morphology: a prospective observational study with three-dimensional ultrasound early in the postpartum period. BJOG 2011;118:
45. Blasi I, Fuchs I, D'Amico R, Vinci V, La Sala GB, Mazza V, et al. Intrapartum translabial 3D ultrasound visualization of levator trauma. Ultrasound Obstet Gynecol 2011;37:88–92.
46. Valsky DV, Lipschuetz M, Bord A, Eldar I, Messing B, Hochner-CelnikierD, et al. Fetal head circumference and length of second stage of labour are risk factors for levator ani muscle injury, diagnosed by3-dimensional transperineal ultrasound in primiparous women. Am J Obstet Gynecol 2009;201:91.e1–7.
47. Kruger JA, Heap SW, Murphy BA, Dietz HP. How best to measure the levator hiatus: evidence for the non-Euclidean nature of the 'plane of minimal dimensions'. Ultrasound Obstet Gynecol 2010;36:755–8.
48. Dietz HP, Bernardo MJ, Kirby A, Shek KL. Minimal criteria for the diagnosis of avulsion of the puborectalis muscle by tomographic ultrasound. Int Urogynecol J 2011;22:699–704.
49. altman d, Ekström a, Forsgren c, nordenstam J,
50. Zetterström J. Symptoms of anal and urinary incontinence
51. following cesarean section or spontaneous vaginal
52. delivery. am J obstet Gynecol. 2007 nov;197(5):512.e1-7.
53. Boyles SH, li H, Mori t, osterweil P, Guise JM. Effect
54. of Mode of delivery on the Incidence of urinary Incontinence in Primiparous Women. obstet Gynecol. 2009Jan;113(1):134-141.
55. Novellas S, Chassang M, Verger S, Bafghi A, Bongain A, Chevallier P.MR features of the levator ani muscle in the immediate postpartum following caesarean delivery. Int Urogynecol J, 2010;21:563–8.
56. Toozs-Hobson P, Balmforth J, Cardozo L, Khullar V, Athanasoiu S. The effect of mode of delivery on pelvic floor functional anatomy. Int Urogynecol J 2008;19:407–16.
57. Kearney R, Miller JM, Ashton-Miller JA, DeLancey JOL. Obstetric factors associated with levator ani muscle injury after vaginal birth. Obstet Gynecol 2006;107:144–9.
58. Krofta L, Otccenasek M, Kasikova E, Feyereisl J. Pubococcygeus puborectalis trauma after forceps delivery: evaluation of the levator ani muscle with 3D/4D ultrasound. Int Urogynecol J 2009;20:1175–
59. 81.
60. Dietz H, Abbu A, Shek K. The levator urethral gap measurement: amore objective means of determining levator avulsion? Ultrasound Obstet Gynecol 2008;32:941–5

THE OUTCOME OF INTRACYTOPLASMIC SPERM INJECTION AFTER LAPAROSCOPIC OVARIAN DRILLING

[1]Abdel Samie Abdel Momeim Abdel Samie, [1]Mohammed A.Mohammed, [2]Mohammed Atef Behery, [2]Mosaad M.Ibrahim, [2]Eman Ahmed Ali,
[1]Department of Obstetrics & Gynecology, Faculty of Medicine, Fayoum University,
[2]International Islamic Center for Population Studies and Research, Al-Azhar University

Introduction

The polycystic ovary syndrome (PCOS) is the most common cause of oligoovulation and anovulation both in general population and among women presenting with infertility. The association of the clinical features of obesity, oligomenorrhoea or amenorrhoea and hirsutism with biochemical evidence of hyperandrogenemia, and characteristic morphology on ultrasound has formed the basis of the diagnosis of the PCOS. Rotterdam consensus conference recently defined PCOS as a clinical syndrome comprising two of the three features: amenorrhoea or oligomenorrhoea, clinical or biochemical hyperandrogenism, and bilateral polycystic ovaries on ultrasound (The Rotterdam ESHRE/ASRM Sponsored PCOS Consensus workshop group, 2004).

The drug of choice for inducing ovulation in patients with PCO is clomiphene citrate, taken orally, although 20% of women given clomiphene citrate fail to ovulate. Ovulation induction with gonadotrophin is well established in patients resistant to clomiphene citrate, but extensive monitoring is necessary because of high sensitivity of polycystic ovaries to exogenous gonadotrophins, with the risk of multiple follicle development leading to termination of the cycle, ovarian hyperstimulation syndrome, or multip4le pregnancy (Homburg, 1993). Laparoscopic ovarian drilling is an alternative to gonadotrophin therapy for clomiphene citrate resistant PCOS and damaging technique of ovarian wedge resection. Ovarian drilling appear to be effective as routine gonadotropin therapy in the treatment of clomiphene citrate resistant PCOS, and the Cochrane database concluded that, while there is insufficient evidence to demonstrate a difference between 6 and 12 months follow up after LOD and 3 and 6 cycles of ovulation induction with gonadotrophins, multiple pregnancy rates are considerably reduced with LOD. Also, LOD is free of the risk of OHSS and does not require intensive ultrasound monitoring (Farquhar et al., 2007).

IVF is not the first line of treatment for PCOS, but many patients with the syndrome may be referred for IVF, because they fail to conceive despite ovulation, that is their infertility remains unexplained. Furthermore, approximately 30% of women have PCOS, may present for assisted conception treatment because of other reasons as tubal factor or male factor (Homburg et al., 1993).

However, few data exist, regarding the effect of LOD on subsequent ovarian stimulation for IVF (Mac Dougall et al., 1993).

Patients and Methods

This retrospective study compare ovarian stimulation and outcome of ICSI in 25 patients with clomiphene citrate resistant polycystic ovarian syndrome (PCOS), who had previously treated with laparoscopic ovarian drilling (LOD) with 25 control patients with clomiphene citrate resistant PCOS, who had not undergone such treatment before ICSI. The study was carried out in the ART unit, international Islamic Center for Population Studies and Research (IICPSR), Al-Azhar University, we reviewed the ART registered data during the period from January 2007 to December 2009.

Abdel Samie Abdel Momeim
Abdel Samie
Department of Obstetrics & Gynecology,
Faculty of Medicine, Fayoum University

All cases will be submitted to the following:

- Detailed history excluding the patient with previous or recent hepatic, renal, metabolic or gastrointestinal diseases which might affect the parameters to be investigated and they are non-smokers.
- Complete examination (general and local) and laboratory investigations to confirm the history and to exclude the presence of any autoimmune diseases, hypertension, diabetes mellitus and other endomcrinological diseases.
- The following biochemical estimations were performed for every patient included in the study:

- CBC
- Complete liver function tests (ALT, AST, Billirubin (total & direct), Total Protein, Albumin, ALP, GGT)
- Serum Urea, Creatinine.
- Fasting & Post-prandial blood sugar levels.
- Antimullerian hormone levels using ELISA (Enzyme Linked immunosorbant assay) (Teco diagnostics).
- Basal (FSH, LH, PRL, E2) , and E2 level repeated at day 6 of stimulation and according to ovarian response, T3, T4, TSH using ELFA technique (Enzyme linked fluorescent assay) (Vidas Biomerieux).

The diagnosis of PCOS in the selected group of women was based on the Rotterdam criteria, including the association of at least two of the following three criteria:

1. Ovulatory disturbance, mainly oligomenorrhoea or amenorrhoea,
2. Hyperadrogenism defined only clinically in our study by hirsutism as there is no available data in the files regarding the serum testosterone level.
3. Ultrasonographic picture of PCOS. All the studied women were <40 years of age with history of anovulatory primary infertility and with normal male factor.

All underwent COH for ICSI using the long midluteal GnRH agonist protocol. GnRH agonist leuprolide acetate (Lucrine 10 IU) or treptoreline (Decapeptyle 0.1mg) was given subcutaneously daily starting from day 21 of the preceding cycle. Adequate pituitary desensitization is obtained when (E2<50 pg/mI).

The starting dose of gonadotrophins depend on, patients age, BMI, endocrinological status of the patients, number of antral follicles on cycle day 3, ovarian volume and previous ovarian response. The starting dose was fixed for the 1st 5 days, and then the dose of gonadotrophins was adjusted according to individual ovarian response as indicated by transvaginal u/s every other day and serum E2 level as indicated. Human chorionic gonadotropins 10.000 IU given intramuscular when at least half number of follicle approach diameter 18-20mm.Ovum pick-up was performed 34-36 hrs after hCG injection. Retrieved oocytes were subjected to ICSI. Embryo transferred transcervically guided by u/s 2-3 days after retrieval. Number of transferred embryos varied with age of the patient, embryo quality and previous cycle outcome. Luteal phase was supported by either cyclogest 200 mg vaginal suppository every 12 hrs or prontogest 100 mg ampoule IM every 24 hrs. Serum βhCG was estimated 2wks after embryo transfer. Clinical pregnancy was defined by the demonstration of a gestational sac on u/s.

Studied women were achieved 61 cycle, 31 cycles for women in (Group A) and 30 cycles for women in (Group B) eight cycles were cancelled.

Statistical Analysis

The study analysis was done by using Statistical Analysis System Software package (SAS, version 9.0). Descriptive analysis (X2 test and Fisher exact tests, as appropriate, for the categorical variables and t test for the continuous variables) were prepared to examine the difference between the studied group of women with clomiphene citrate resistant polycystic ovary syndrome (PCOS) who had previously treated with laparoscopic ovarian drilling (LOD) (Group A) with those who had not undergone such treatment before ICSI (Group B).The two groups were compared according to their characteristics (age, BMI, infertility factors, etc...), cancellation, and results of ovarian stimulation, fertilization rate, and pregnancy outcome using appropriate statistical tests. The significant levels in all analysis were accepted if P value is less than 0.05.

Results

Table (1)
Characteristics of women with polycystic ovary syndrome in the studied group (g roup A) compared with the control group (group B)

Characteristics *	Group A (n=25)	Group B (n=25)	P Value
Age in years (mean + SD)	29.0 + 4.1	28.7 + 4.5	0.77
BMI kg/m2 (mean + SD)	29.0 + 4.1	28.5 + 3.5	0.43
BMI kg/m2			0.48
< 25	5 (20%)	3 (12%)	
25-<30	6 (24 %)	10 (40%)	
> 30	14(65%)	12 (48%)	

* Data are presented by mean+ SD or by n (%).

Table (2)
Additional causes and duration of infertility in the studied group (group A) compared with the control group (group B)

Infertility characteristics* and number of cycle trial	Group A (n=25)		Group B (n=25)		P Value
	No	%	No	%	
Additional cause of infertility					0.68
Tubal	6	24.0	6	24.0	
Pelvic adhesion	4	16.0	2	8.0	
Unexplained	15	60.0	17	68.0	
Infertility duration in years (mean + SD)	6.0 + 3.4		6.1 + 3.2		0.90
Number of cycle trial					0.45
One trial	22	88.0	21	84.0	
Two trials	1	4.0	3	12.0	
Three trials	1	4.0	1	4.0	
Four trials	1	4.0	0	0.0	

* Data are presented by number and percent or by mean+ SD

Table (3)
Basal hormonal profiles in the studied group (group A) compared with the control group (group B)

Basal hormone profiles	Group A (n=25) (cycle = 31)	Group B (n=25) (cycle = 30)	P Value
FSH (mean + SD)	4.5 + 1.8	5.1 + 1.7	0.10
LH (mean + SD)	10.7 + 4.3	12.1 + 3.1	0.04*
PRL (mean + SD)	15.0 + 4.1	13.0 + 4.2	0.11
E2 (mean + SD)	57.7 + 34.8	49.8 + 39.7	0.46

* Significant

Table (4)
Total number of ampoules of HMG administrated, the day HCG injection and E2 level at the day of HCG injection in the studied group (group A) compared with the control group (group B)

Treatment factor	Group A (n=25) (cycle = 31)	Group B (n=25) (cycle = 30)	P Value
Total number of HMG ampoules (mean + SD)	38.5 + 10.1	33.1 + 8.7	0.35
Day of hCG injection (mean + SD)	14.3 + 5.5	13.9 + 4.2	0.18
E2 level at the day of hCG injection (mean + SD)	1672 + 954	1688 + 980	0.87

Table (5)
Comparison between follicle number >14mm, oocyte retrieved, endometrial thickness, mature and fertilized oocytes in the studied group (group A) compared with the control group (group B)

Follicle and oocyte factors	Group A (n=25) (cycle = 31)	Group B (n=25) (cycle = 30)	P Value
Follicle >14 mm (mean + SD)	13.6 + 6.8	15.6 + 6.2	0.04*
Oocyte retrieved (mean + SD)	9.1 + 3.7	10.2 + 4.1	0.26
Mature oocyte (mean + SD)	5.8 + 3.7	8.5 + 4.8	0.01*
Fertilized oocyte (mean + SD)	4.0 + 2.5	5.8 + 3.6	0.36*
Endometrial thickness (mean + SD)	10.8 + 1.5	11.4 + 2.1	0.27

* Significant

Table (6)
Comparison between fertilization rate in the studied group (group A) compared with the control group (group B)

Fertilization	Group A (n=25) (cycle = 31)	Group B (n=25) (cycle = 30)	P Value
Fertilization % per ovum	195/282=69.0%	312/820=74.0%	0.16
Fertilization % per cycle*	27/27 =100%	26/26=100%	1.00

* Excluding cancelled cycles in both groups (four cycles were cancelled from each group)

Table (7)
Comparison between total embryo obtained and embryo grading in the studied group (group A) compared with the control group (group B)

Embryo factor	Group A (n=25) (cycle = 31)	Group B (n=25) (cycle = 30)	P Value
Total embryo number (mean + SD)	5.0 + 3.9	6.2 + 4.1	0.04*
Embryo Grade A (mean + SD)	3.8 + 3.2	4.7 + 3.5	0.04*
Embryo Grade B (mean + SD)	1.9 + 1.1	2.2 + 0.9	1.00
Embryo Grade C (mean + SD)	1.4 + 0.7	1.5 + 0.8	0.98
No. of transferred embryos (mean + SD)	2.5 + 0.7	2.8 + 0.8	0.58

* Significant

Table (8)
Comparison between pregnancy rate in the studied group (group A) compared with the control group (group B)

Pregnancy outcome	Group A (n=25) (cycle = 31)	Group B (n=25) (cycle = 30)	P Value
Pregnancy rate per cycle	29% (9/31)	33% (10/30)	0.40
Pregnancy rate per embryo transfer	33% (9/27)	38% (10/26)	0.18

* Significant

Table (9)
Causes of cycle cancellation in the studied group (group A) compared with the control group (group B)

Causes of cycle cancellation	Group A (n=25) (cycle = 31)	Group B (n=25) (cycle = 30)	P Value
Ovarian hyperstimulation syndrome (OHSS)	1/31 (3.2%)	2/30 (6.7%)	0.23
Empty follicles	1/31 (3.2%)	1/31 (3.3%)	0.85
Poor responders	2/31 (6.5%)	1/31 (3.3%)	0.22
Total	4/31 = 12.9	4/30=13.3	0.90

* Data are presented by n (%).

Discussion

One of the main causes of female infertility is chronic anovulation associated with polycystic ovarian syndrome (PCOS). PCOS is thought to occur in 5 to 10% of reproductive-age women, with anovulatory ovarian dysfunction accounting for 30% of female infertility (Seow et al., 2008).

Laparoscopic ovarian drilling (LOD) is an alternative approach in treating clomiphene citrate resistant patients with PCOS. LOD is currently recommended by the European Society of Human Reproduction as well as the PCOS Consensus Workshop Group sponsored by the American Society of Reproductive Medicine as a second-line intervention for Clomiphene citrate resistant PCOS. There is evidence that LOD may improve endocrine status in addition to fertility outcome in patients with PCOS (Thessaloniki EHSRE-ASRM-Sponsored PCOS Consensus Workshop Group, 2008).

LOD is increasingly being recommended as early treatment option for women with clomiphene resistant PCOS. Few data exist, regarding the effects of LOD on subsequent ovarian stimulation for IVF. So, the aim of our present study was to evaluate the effect of LOD done for management of clomiphene citrate resistant patients with PCOS on the outcome of ICSI.

Comparison of the basal hormone profiles between the studied groups had shown no significant differences between the two groups with regard to all studied mean levels of basal hormonal profiles except for the mean level of LH (p=0.04) with the highest mean level was among group B (12.1 + 3.1) compared with (10.7 + 4.3) in group A. Our results compare favorably with previously reported results of (Greenblatt and Casper, 1987; Rossmanith et al., 1991) and(Naether et al., 1994).

The reduction in the serum LH concentration following LOD is reported to be the main mechanism by which reproductive outcome is improved, with elevated concentrations being associated with a reduction in oocyte quality, fertilization rates and embryo quality (Stanger and Yovich, 1985; Urman et al., 1992), and higher miscarriage rates (Homburg et al., 1988; Regan et al., 1990).

The study done by Amer et al., in 2002, included 16 anovulatory women with polycystic ovary syndrome (PCOS) who underwent LOD between 1991 and 1999 (study group) and 34 anovulatory PCOS women diagnosed during the same period, who had not undergone LOD (control group).The LH/ FSH ratio, mean serum concentrations of LH and testosterone and free androgen index decreased significantly after LOD and

remained low during the medium-and long-term follow-up periods.

In the study done by Malkawi et al., 2003, patients were allocated into two groups; group 1 includes 64 women who received metformin, 850 mg twice daily throughout the cycle, and group 2 which included 97 women who underwent laparoscopic ovarian drilling. A significant improvement in the regularity of menstrual cycles (P<0.05) and a significant reduction in the serum levels of testosterone (P<0.01), and rostenedione (P<0.01), DHEAS (P<0.05), LH (P<0.01) and LH: FSH ratio (P<0.05) were noted after the treatment.

In two similar studies done by Wu et al. in 2004 and Api et al. in 2005, they found significant decreases in the free androgen index, and total testosterone, luteinizing hormone (LH), and LH/FSH ratio, and a significant increase in sex hormone binding globulin (SHBG) concentration in the 3 months after the operation.

The results of the above mentioned studies cannot be directly compared with our study because we don't have enough data regarding the androgen levels. However, those results are consistent with ours in that it shows similar reduction in the levels of LH concentrations following LOD.

Comparison between number of follicles >14 mm, oocytes retrieved, mature and fertilized oocytes and the endometrial thickness in the studied women (group A) compared with the control women (group B) had revealed that the mean number of follicle >14 mm, oocytes are higher in group B compared with group A, with statistically significant differences were observed between the two groups as regarding the total follicle count (15.6 + 6.2 versus 13.6 + 6.8), mature oocyte (8.5 + 4.8 versus 5.8 + 3.7) and fertilized oocytes (5.8+ 3.6 versus 4.6 + 2.5). The mean endometrial thickness of group B is slightly higher in group A with no statistically significant difference.

In contrast to our results, in a study done by Colacurci et al. in 1997, in which IVF embryo transfer outcome was assessed in 23 women with PCOS following LOD within 9 months of surgery. In this report, no significant differences were identified in the fertilization rate between women who had undergone ovarian diathermy before IVF and those who had not.

A possible explanation for the difference in results between our study and those of Colacurci et al. in 1997 is that in the present study, the higher fertilization rate observed in group B women may be explained by the greater number of follicles > 14mm in diameter, which have yielded more mature oocytes. However, despite more embryos being available in group B, two or three good grade embryos were available for transfer in both groups. The increased number of follicles > 14mm in diameter in group B compared with group A (15.6 + 6.2 versus 13.6 + 6.8) may be an indication of subtle decrease in ovarian responsiveness following LOD.
Comparison between fertilization rate per cycle and per ovum in the studied women (group A) compared with the control women (group B) had declared that the total fertilization rate per ovum in all studied women was 72% (507/702). The fertilization rate per ovum was 69% in group A and 74% in group B with no statistically significant difference (p=0.16). The fertilization rate per cycle among the studied group A and B, excluding cancelled cycles before injection, is calculated to be 100% (p=1.0).

Comparison between total embryo number obtained and embryo grading in the studied women (group A) compared with the control women (group B) had shown that the mean level of total embryo number obtained (5.0 + 3.9 versus 6.2 + 4.1) as well as the mean number of the obtained embryo grade A (3.8 + 3.2 versus 4.7 + 3.5) were found to be higher in the (group B) compared with (group A) with statistically significant difference (p=0.04). The mean level of grade B and C embryos as well as the number of transferred embryo was nearly the same in both groups with no statistically significant difference.

Comparison between pregnancy rate in the studied women (group A) compared with the control women (group B) revealed no significant difference between the two groups with regard to pregnancy rate per cycle (p=0.40). Although the pregnancy rate was higher in group B compared with group A (33% [10/30] versus 29% [9/31]) . Also, the pregnancy rate per embryo transfer was higher in group B (38% [10/26] versus 33% [9/27])in group A, but with no statistically significant difference between the two groups.

The pregnancy rate varies between different studies, and the possible reasons may be any of the following; (1) the small sample size of the studies. (2) The number and depth of the punctures are different in studies. Further, the thermal power also varies, and it thus produced different results. (3) Any additional infertility features will influence these pregnancy rates (Seow et al., 2008).

Previously, a reduced trend for OHSS following LOD has been observed (Colacurci et al., 1997). In the present study we observed a significant difference in multiple follicular development, with a greater percentage of the follicles > 14mm in diameter in (group B) or the untreated group (15.6 + 6.2 versus 13.6+ 6.8; p=0.04) which may reflect an increased risk for OHSS. This reduced potential for the development of OHSS in women who have undergone LOD has also been described by Gadir et al. in 1992.

Also in a similar study done by Rimington et al. in 1997, LOD was performed on 25 out of 50 women with PCOS following pituitary down regulation just before receiving gonadotrophins for ovarian stimulation in an IVF cycle. These authors found the occurrence of moderate or severe OHSS to be higher in the untreated group, and proposed that LOD would be a potentially useful treatment in women who have previously had an IVF cycle cancelled due to risk of OHSS, or who have suffered OHSS.

Egbase et al. in 1998, reported three women in whom unilateral LOD was performed 3-5 weeks before IVF-embryo transfer and who had previously developed moderate to severe OHSS as a consequence of ovarian stimulation. All three women were asymptomatic following ovarian stimulation, despite having received similar doses of gonadotrophins.

Conclusion

The study has not shown any significant differences between the two patient groups in terms of ICSI outcome with respect to the pregnancy rate per cycle or per embryo transfer. LOD may be associated with lower miscarriage rates and higher ongoing pregnancy rates per cycle and per embryo transfer, as suggested previously by Colacurci et al., 1997. LOD may also have benefits for women undergoing ICSI by possibly reducing the incidence of OHSS. Meanwhile, it is reassuring that the judicious use of ovarian diathermy does not appear to alter the outcome of ICSI.

References

1. Amer SA, Banu Z, Li TC and Cooke ID: Long-term follow-up of patients with polycystic ovary syndrome after laparoscopic ovarian drilling: endocrine and ultrasonographic outcomes. Hum. Reprod. 2002; Vol.17, No 11, 2851-2857.
2. Colacurci N, Zallo F and DeFraciscis P: In vitro fertilization following laparoscopic ovarian diathermy in patients with patients with Polycystic Ovarian Syndrome. Acta. Obstet. Gynecol. Scand. 1997; 76:555-558.
3. Egbase P, Al-Adawi, Al-Sharan M and Grudzinskas JG: Unilateral ovarian diathermy prior to successful in-vitro fertilization: a strategy to prevent recurrence of ovarian hyperstimulation syndrome? J. Obstet. Gynecol. 1998; 18(2):171-173.
4. Farquhar CM, Lilford RJ and Marjoribanks J: Laparoscopic ovarian diathermy versus gonadotrophin therapy for women with clomiphene citrate resistant polycystic syndrome. Hum. Reprod. 2007; 19:1110-1115.
5. Gadir AA, Alnaser HM, Mowafi RS and Shaw RW: The response of patients with polycystic ovarian syndrome to human menopausal gonadotrophin therapy after electrocautery of luteinizing hormone releasing hormone agonist. Fertil. Steril. 1992; 57(2):309-301.
6. Greenblatt E and Casper RF: Endocrine changes after laparoscopic ovarian cautery in polycystic ovarian syndrome. Am. J. Obstet. Gynecol. 1987; 156:279-285.
7. Homburg R, Berkowitz D and Levy T: In-vitro fertilization and embryo transfer for the treatment of infertility associated with polycystic ovary syndrome. Fertil. Steril. 1993; 60:858-868.
8. MacDougall MJ, Tan SL, Balen AH and Jacobs HS: A controlled study comparing patients with and without polycystic ovaries undergoing in-vitro fertilization and the ovarian hyperstimulation syndrome. Hum. Reprod. 1993; 8:233-237.
9. Malkawi HY, Qublan HS and Hamaideh AH: Medical vs. surgical treatment for clomiphene citrate resistant women with polycystic ovary syndrome. J. Obstet. Gynaecol. 2003 May; 23(3):289-293.
10. Naether OG, Baukloh V, Ficher R and Kowalczyk T: Long term follow up in 206 infertility patients with polycystic ovary syndrome after laparscopicelectrocautery of the ovarian surface. Hum. Reprod. 1994; 9(12):2342-2349.
11. Regan L, Owen EJ and Jacobs HS: Hypersecretion of Luteinizing hormone, infertility, and miscarriage. Lancet 1990; 336(8724):1141-1144.
12. Rimington MR, Walker SM and Shaw RW: The

use of laparoscopic ovarian electrocautery in preventing cancellation of in-vitro fertilization treatment cycles due to risk of ovarian hyperstimulation syndrome in women with polycystic ovaries. Hum. Reprod. 1997; 12(7):1443-1447.
13. Rossmanith WE, Keckstein J., Spatzier K and Lauritzen C: The impact of ovarian laser surgery on gonadotrophin secretion in women with polycystic ovarian syndrome. Clin. Endocrinol. 1991; 34(3):223-230.
14. Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group: Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PPCOS). Hum. Reprod. 2004; 19(1):41-47.
15. Seow KM, Juan CC, Hwang JL and Ho LT: Laparoscopic surgery in polycystic ovary syndrome: reproductive and metabolic effects. Semin. Reprod. Med. 2008; 26(1):101-110.
16. Stanger J and Yovich J: Reduced in vitro fertilization of human oocytes from the patients with raised basal luteinizing hormone levels during the follicular phase. Br. J. Obstet. Gynecol. 1985; 92:385-393.
17. The Thessaloniki/ASRM-sponsored PCOS Consensus Workshop Group: Thessaloniki 54603. Greece. Hum. Reprod. 2008' 23:462-477.
18. Urman B, Fluker M and Yuen BH: The outcome of in vitro fertilization and embryo transfer in women with polycystic ovary syndrome failing to conceive after ovulation induction with exogenous gonadotrophins. Fertil. Steril. 1992; 57:1269-1273.

MORPHOLOGICAL AND MATHEMATICAL ANALYSIS OF ICSI EMBRYO SHAPES AND DEVELOPMENTAL FATE: PRELIMINARY OBSERVATIONS

Aragona C[1], Galli L[3], Passaro D[3], Linari A[1], Bezerra Espinola MS[1], Micara G[1], Tranquilli D[1], Boninsegna C[1] and Bizzarri M[2]

Infertility and Assisted Reproduction Unit, Department of Obstetrics and Gynecology[1],"Sapienza" University of Rome, Rome, Italy. Department of Experimental Medicine[2], "Sapienza" University of Rome, Rome, Italy, Advanced Computer Systems S.p.a. Rome, Italy[3]

Abstract:

Main and most common methods of embryo analysis and morphological classification currently used in ART medical practice evaluate a multiplicity of morphological parameters, e.g. blastomeres number and symmetry, internal structures homogeneity, presence of corpuscles, cytoplasmatic features and characterization of embryo external shape (regularity, thickness and thickness variation of zona pellucida, ZP). Growing evidences are suggesting that cell shape should be considered as the most critical determinant of embryo competence, therefore quantitative shape descriptors could provide an insight on complex systems being observed.

In this study it is presented an original method for calculating a shape form factor (algorithm) linked to local morphological variability of embryo ZP, called Local ZP Variation (LZPV), instead of its macroscopic characteristics. This method is based on harmonic analysis techniques application in frequency-domain representation of ZP inner and outer boundaries, to verify whether harmonic measures could be observationally associated to better results in terms of pregnancy outcome.

Preliminary observations show that our method, whether confirmed in a larger series of patients for its validity and reproducibility, may represent a valuable tool for specialists to distinguish embryos of good quality with higher ability to implant and reach term pregnancy.

Introduction

In vitro fertilization is an important tool in infertility treatment: technical sophisticated approaches have been developed but final goal remains to achieve embryos of "good" quality leading to healthy born babies. To maximize pregnancy rate in Assisted Reproduction Technology (ART) it is essential to identify viable embryos with high implantation potential, in order to transfer a limited number of embryos, reducing multiple pregnancy risk without decreasing the chance of pregnancy (1-2).

A large amount of literature has been produced to identify noninvasive methods of embryo selection as morphology evaluation (1-8), in vitro metabolic activity determination (9-15) or invasive techniques as preimplantation genetic diagnosis (PGD) of the first and/or second polar body, and embryo blastomeres at cleavage or blastocyst stage (16-29).

Main and most common methods of analysis and morphological classification of embryos currently used in ART are based on evaluation of many morphological characteristics, e.g. cell symmetry and number, internal structures homogeneity, presence of corpuscles, specific cytoplasmatic characteristics, etc. Furthermore extensive ultrastructural studies of human zona pellucida during in vitro fertilization have been reported (30-33).

Such evaluations are based on high-level practical guide-lines shared within scientific community and applied through observer experience and skill. As a consequence, they are subjective parameters leading to several disadvantages. In particular, experimental evidences indicate quite a weak corre-

Cesare Aragona MD,
Infertility and Assisted Reproduction Unit,
Department of Obstetrics and Gynecology, "Sapienza" University of Rome,
Rome, Italy
Fax:+39 0649972548
E-mail: cesare.aragona@uniroma1.it

lation between assessment of the above-mentioned set of parameters and ART procedures success. Alternative embryo assessment methods have been proposed in literature, e.g. characterization of embryo external shape (zona pellucida regularity, thickness and variability). According to some authors zona pellucida thickness (ZPT) and zona pellucida thickness variation (ZPTV) seem to influence clinical ART pregnancy outcome (34-38).

Although more quantitative, these methods also present sub-optimization for intra- operators variability, as well as other factors like embryo orientation in the image or even images size and resolution. As a result, today there is no conclusive evidence of an effective advantage for ART. Growing evidences are suggesting that cell shape (for example in cancer cells) should be considered as the most critical determinant of embryo competence (39), therefore quantitative shape descriptors could provide an insight on complex systems being observed (40-41). On the other hand it has been suggested that Day 3 embryo shape is weakly associated with IVF outcome and it is unlike to be a useful additional marker for embryo selection (42).

Furthermore, automatic analysis of human embryo microscope images was reported (43, 44) and reviewed by Santos Filho in 2010 (45). More recently the same author described a semi-automatic grading method for human blastocyst microscope images: trophectoderm layer, inner cell mass and inner and outer boundary of zona pellucida (46).

ZP inner boundary is well defined and therefore its reliable automatic segmentation can be easily obtained using different methods: intensity-based thresholding methods, direct least square fitting of ellipses (47). Conversely, ZP outer boundary is not well defined because ZP is relatively transparent and does not have a high-contrast outer boundary. In addition, ZP outer boundary may present several artifacts such as granular cell fragments and spermatozoa, which should be carefully masked-out in order to obtain an accurate segmentation.

Thus automatic segmentation of ZP outer boundary is a difficult task and a fully automatic segmentation method is currently under development. As a result, a quantitative and reproducible automatic method of embryo analysis is still lacking.

Aim of the study

In this study it is presented an original method for calculating a shape form factor (algorithm) linked to local morphological variability of embryo ZP, called Local Zona Pellucida Variation (LZPV), instead of its macroscopic characteristics.

Material and methods

This study was reviewed and approved by Institutional Review Board and all patients gave informed written consent. We reported a retrospective study using a data set of 75 images from 30 ICSI treatments performed at Infertility and Assisted Reproduction Unit, Department of Obstetrics and Gynecology[1],"Sapienza" University of Rome, Rome, Italy.
Female patients age ranged from 26 to 42 (mean 35.92 ±3.6 years). Male patients age ranged from 30 to 51 (mean 39.68 ±4.8 years).

All patients included in this study had a normal karyotype, normal hormonal assessments, negative vaginal or urethral cultures and had no malignancy or systemic diseases.

All patients who underwent a standard infertility evaluation were nulliparous with previous failed IVF cycles ranging from 0 to 4 and none of them showed basal FSH >10 mIU/ml or E2 > 40 pg/ml on cycle Day 3. No difference was found between the two groups.

Ovarian stimulation and oocyte retrieval

ICSI cycle management consisted of down regulation with a long protocol starting from day 21 of the pretreatment cycle with a GnRH agonist (Decapeptyl 0.1 ml, daily subcutaneous (s.c.), IPSEN/BIOTECH, Paris, France). Once ovarian suppression was assessed by E2 profiles and ovarian ultrasound scan (US), daily subcutaneous administration of 150 IU urinary or recombinant FSH was commenced.

From the seventh day of stimulation, daily monitoring of follicles size by US was performed and plasma levels of E2 and progesterone were measured. From this stage, the dose of FSH was adjusted depending on the individual response of each patient.
Criteria used for triggering ovulation with 10.000 IU hCG (Gonasi HP 5000® IBSA) s.c. were plasma E2 between 1000 and 3000 pg/ml and at least four follicles >18 mm mean diameter (two perpendicular measurements) with plasma Progesterone < 1.5 ng/ml.
Oocyte retrieval was performed 36 hours after hCG administration, by transvaginal US-guided follicular aspiration under i.v. sedation.

Semen preparation and insemination

Preparation of semen samples collected by masturbation was performed following the World Health Organization (WHO) standard protocol (48). ICSI was performed according to procedure reported by Palermo (49) and exclusively on metaphase II oocytes.

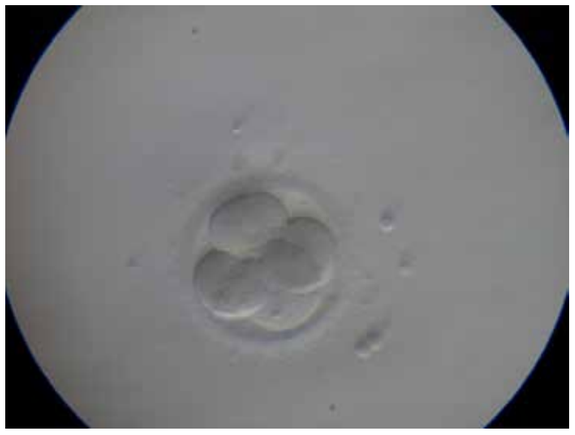
Fertilization assessment

Oocyte fertilization was assessed 16-18 hours (Day 1) after ICSI to confirm two pronuclei presence.

Embryo Quality Assessment

In our laboratory embryos are routinely scored at 40-42 hours (Day 2) after ICSI according to Veeck classification. A morphologic grade (I, II, III, IV, and V) is assigned according to blastomeres number and fragmentation percentage (50). Embryos were observed by an inverted microscope with Hoffman modulation contrast at magnification 400x (TE 2000 U, Nikon Corporation, Tokio, Japan) and images were captured by a computerized system (X-ProTM). Photographic acquisition format was jpg at resolution 2560x1920.

Figure : microscope embryo image acquired on second day after fertilization with Hoffman modulation contrast system at a magnification of 400x.



Embryo image analysis process consists in determining ZP region. This is obtained by defining ZP outer and inner boundary as illustrated in figure 2. In order to obtain very high accurate results, we followed a semi-automatic approach. ZP boundary is designed by an expert embryologist, then segmentation refining is performed automatically.

Figure : Example of Day 2 embryo outer and inner ZP manual segmentation (400x).

After ZP inner and outer boundaries detection, ZP is analysed by computing the Local Zona Pellucida Variation – LZPV. LZPV method is based on an innovative harmonic analysis technique application in the frequency-domain representation of the ZP inner and outer boundaries, which takes into account local variation of ZP borders. The overall methodology has been developed by Advanced Computer System S.p.a. Rome, Italy (www.acsys.it) and it is protected by a patent.
Main advantage of LZPV is to calculate ZP local characteristics with greater accuracy compared to traditional morphological analysis currently used. Indeed, while routinely used methods (e.g. VSZP) focus only on high-level average values of ZP thickness variability (considering only 4/8 ZP thickness measurements), this new approach analyzes and synthesizes a more precise and granular set of local shape factors, measured in proximity of ZP borders. It is important to point out that this proposed shape factor is not affected by variability linked to orientation of biological structure in the image or scale/resolution of image itself. Consequently, this method guarantees a total reproducibility, which potentially may translate into fully standardized approaches with high reliability of use in ART practice.

Embryo Transfer

75 embryos (I-III scoring grade) were transferred in 30 patients (mean 2.5 embryos/patient). Embryo transfer was performed at Day 2 after oocytes retrieval under US guidance, with Wallace embryo transfer catheter (HG Wallace, Hythe, Kent, UK). All transfer procedures were performed by the same physician to avoid interoperator variability. All patients undergoing embryo transfer received supplemental progesterone intramuscular (50 mg/day; Prontogest; Amsa, Milano, Italy) from the day of replacement.

Establishment of Pregnancy

Chemical pregnancy was initially determined 14 days after embryo transfer by a positive qualitative serum β-hCG assay (>50 mIU), followed by a repeated quantitative β-hCG levels. A clinical pregnancy was defined as at least one foetus with a positive heartbeat revealed by transvaginal sonography 4 to 5 weeks after embryo transfer. Implantation rate was defined as the number of gestational sacs on ultrasound as a percentage of the embryos transferred.

Statistical analysis

Data were retrospectively collected and analyzed by computing test statistics figures: estimated population midpoints and 95% confidence interval. Confidence intervals are calculated according to the efficient-score method (corrected for continuity) described by Newcombe(51).

Results

In this study 75 images relative to 30 patients were blinded analyzed and statistically evaluated by using a “test data set”. “Test data set” is used in order to find out local ZP variability threshold value for classifying embryos as “+” and “-”: an embryo is classified “+”if the LZPV value is superior or”-“if is inferior to the determined threshold. All 30 patients are split in positive and negative clusters: positive patient if at least one embryo is “+”, negative patient if the previous condition is not verified. In patients analyzed by “test data set” 13 pregnancies were observed, including 3 abortions and 10 term pregnancies. Overall, births prevalence is equal to 33.3% (10 out of 30 cycles), while pregnancies prevalence is equal to 43.3% (13 pregnancies out of 30 cycles).

Table (1)
clinical trial results

Test -			18
	No pregnancy	14	
	Abortion	3	
	Birth	1	
Test +			12
	No pregnancy	3	
	Abortion	0	
	Birth	9	

The method detects as positive 12 patients (see Table 1), of which:

- 9 patients term delivered (out of a total of 10 deliveries);
- 0 patients aborted (out of a total of 3 abortions);
- 3 patients did not present pregnancy (out of a total of 17 cases of non-pregnancy).

The same method identifies as negative 18 patients (see Table 1), of which:

- 1 patients term delivered (out of a total of 10 deliveries);
- 3 patients aborted (out of a total of 3 abortions);
- 14 patients did not present pregnancy (out of a total of 17 cases of non-pregnancy).

Main results are reported in Table 2 and Table 3. Definition of computed statistical figures are reported in Table 4. Results includes also 95% of confidence interval, in line with standards used in clinical practice.

Table (2)
Birth vs. No Birth (= No pregnancy + Abortion) statistical analysis

	Birth	No Birth	TOT
Test +	9	3	12
Test -	1	17	18
TOT	10	20	30

Prevalence	33,3	95% confidence interval	
Sensitivity	90.0	54.1	99.5
Specificity	85.0	61.1	96.0
PPV	75.0	42.8	93.3
NPV	94.4	70.6	99.7

Table (3)
Pregnancy (= Birth + Abortion) vs. No Pregnancy statistical analysis

	Pregnancy	No Pregnancy	TOT
Test +	9	3	12
Test -	4	14	18
TOT	13	17	30

Prevalence	43,3	95% confidence interval	
Sensitivity	69.2	38.9	89.6
Specificity	82.3	55.8	95.3
PPV	75.0	42.8	93.3
NPV	77.8	51.9	92.6

Main outcomes are here reported:

1. Probability that a patient testing positive leads to term pregnancy is 75% ([42.8% - 93.3%] - 95% CI), versus 33.3% without test.
2. Probability that a patient testing positive presents a pregnancy is 75 % ([42.8% - 93.3%] - 95% CI) versus 43.3% without test.

Figure 3

	Outcome (+)	**Outcome (-)**	**TOT**
Test +	A	B	A + B
Test -	C	D	C +D
TOT	A + C	B + D	

Sensitivity = A/(A+C); Specificity = D/(B+D); PPV (Positive Predictive Value) = A/(A+B); VPN (Negative Predictive Value) = D/(C+D); Prevalence = (A+C)/(A+B+C+D).

Discussion

ART is nowadays a growing reality with a number of born babies larger than 5 million and an actual increase rate of 340000 yearly (ESHRE data, 2010), being ART pregnancies 2% of all births in industrialized countries. In assisted reproduction multiple pregnancy rate associated with number of embryos transferred is a major problem, stimulating our attention regarding standardized scoring systems helpful in selecting embryos with higher reproductive potential.

Although several morphological scoring system have been proposed in the literature and culture conditions have been improved, implantation and pregnancy rate remains relatively low. Actual embryo scoring and selection methods may be misleading because performed on single observation at a static point. Furthermore recent time lapse embryo imaging systems enabling continuous embryo monitoring appears promising in order to dynamically define optimal embryo development.
If most scoring system proposed are subjective reflecting embryologist personal experience semiautomatic or automatic image analysis have been proposed by developing algorithms derived from multiple images relative to zygote, cleaving embryo or blastocyst and considering external shape, blastomers, blastocyst features, and zona pellucida.

In this study we focused our attention on Day 2 embryo ZP, after preliminary observations on Day 1 and 3 (data not shown). Indeed zona pellucida represents the “shape” of the oocyte, end point between internal and external influences that in different systems (cancer cells) has been defined as the most critical determinant of cell function capable to govern how individual cells will respond to chemical signals in their local microenvironment. In this paper it is proposed an innovative and original mathematical method aimed to supply embryo morphological measurements as a reproducible and useful tool.

Specifically, this method is useful in calculating a “form factor” linked to embryo ZP local morphological variability, rather than to its macroscopic characteristics. Indeed, while similar methods (e.g. ZPSV), commonly used, focus only on ZP shape values high-level average, our new approach analyzes and synthesizes a more precise and granular set of local shape factors measured in ZP border proximity. This approach is based on harmonic analysis techniques application in frequency-domain representation of ZP inner and outer boundaries, to verify whether harmonic measures could be observationally associated to better results in terms of pregnancy outcome.

This mathematical system allows local morphological ZP variability evaluation on Day 2 embryos by a single numerical value and independently from quality embryo grading. In conclusion embryos with high local ZP variability value have better implantation competence and higher probability to develop to term pregnancy.

Described method has several advantages including:

- Greater accuracy in measuring ZP local characteristics compared to traditional morphological methodologies currently used;
- Analysis of the “form factor” not affected by intra-operators variability as well as other factors like embryo orientation in the image or even images size and resolution.

Consequently, this method allows better robustness and total reproducibility, which translate into a fully standardized approach with high reliability of use in ART practice.

It is important to stress however that many other “external” factors not related to the embryo quality contribute to final successful results.

Conclusions

Our preliminary observations, whether prospectively confirmed in larger series of patients, appear suitable to provide an additional source of information in ART practice, to distinguish among embryos of good quality with intrinsic similar morphological appearance from those with superior quality and higher capacity to implant and proceed to term.

References

1. Elster N. Less is more: the risks of multiple births. The Institute for Science, Law, and Technology Working Group on Reproductive Technology. Fertil Steril. 2000; 74: 617-23.
2. Bergh T, Ericson A, Hillensjö T, Nygren KG, Wennerholm UB. Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: a retrospective cohort study. Lancet. 1999; 354: 1579-85.
3. Cummins J.M., Breen T.M., Harrison K.L., Shaw J.M., Wilson L.M. and Hennessey J.F., A formula for scoring human embryo growth rates in in vitro fertilization: its value in predicting pregnancy and in comparison with visual estimates of embryo quality. J in Vitro Fertil Embr Trans 3 (1986), pp. 284-295.
4. Steer C., Mills C., Tan S., The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer

in an in-vitro fertilization and embryo transfer programme, Hum Reprod, Volume: 7, (1992), pp. 117-119
5. Giorgetti C., Terriou P., Aupier P., Hans E., Spach J.L., Salzmann J., Roulier R., Embryoscore to predict implantation after in vitro fertilization: based on 957 single embryo transfers. Hum Reprod, Volume: 10(9), 1995, pp. 2427-2431
6. Puissant F., Van Rysselberge M., Barlow P., Deweze J. and Leroy F., Embryo scoring as a prognostic tool in IVF treatment. Hum Reprod 2 (1987), pp. 705–708.
7. Roux C., Joanne C., Agnani G., Fromm M., Clavequin M.C., Bresson J.L., Morphometric parameters of living human in-vitro fertilization embryos; importance of the asynchronous division process. Hum Reprod , Volume 10(5),1995, pp. 1201-1207.
8. Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Andersen AN. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro ertilization. Hum Reprod. 1997 Jul;12(7):1545-9.
9. Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, Eestermans W, Gerris J. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod. 1999 Sep;14(9):2345-9.
10. Shoukir Y., Campana A., Farley T. andSakkas D., Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability. Hum Reprod 12 (1997), pp. 1531–1536.
11. Barbehenn EK, Wales RG, Lowry OH. Measurement of metabolites in single preimplantation embryos; a new means to study metabolic control in early embryos. J Embryol Exp Morphol. 1978 Feb;43:29-46.
12. Hardy K, Hooper MA, Handyside AH, Rutherford AJ, Winston RM, Leese HJ. Non-invasive measurement of glucose and pyruvate uptake by individual human oocytes and preimplantation embryos. Hum Reprod. 1989 Feb;4(2):188-91.
13. Leese HJ, Conaghan J, Martin KL, Hardy K. Early human embryo metabolism. Bioessays. 1993 Apr;15(4):259-64.
14. Leese HJ. Metabolism of the preimplantation mammalian embryo. Oxf Rev Reprod Biol. 1991;13:35-72.
15. Barnett DK, Bavister BD. What is the relationship between the metabolism of preimplantation embryos and their developmental competence? Mol Reprod Dev. 1996 Jan;43(1):105-33.
16. Gardner DK, Lane M, Calderon I, Leeton J. Environment of the preimplantation human embryo in vivo: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. Fertil Steril. 1996 Feb;65(2):349-53.
17. Gregory L. and Leese H.J., Determinant of oocyte and preimplantation embryo quality: metabolic requirements and the potential role cumulus cells. JBFS 1(2), Hum Reprod Natl Suppl, 1996, 11, 96-102
18. Handyside AH, Pattinson Jk, Penketh Rj et al. (1989) Biopsy of human preimplantation embryos and sexing by DNA amplification. Lancet 1 :347-9.
19. Verlinsky Y, Ginsberg N, Lifchez A, Valle J, Moise J, Strom CM. (1990) Analysis of the first polar body: preconception genetic diagnosis. Hum Reprod 5:826-9.
20. Verlinsky Y and Kuliev A. (1996) Preimplantation polar body diagnosis. Biochem Mol Med 58(1):13-7.
21. Gianaroli L., Magli M.C., Ferraretti A.P., Fiorentino A., Garrisi J. andMunne S., Preimplantation genetic diagnosis increases the implantation rate in human in vitro fertilization by avoiding the transfer of chromosomally abnormal embryos. Fertil Steril 68 (1997), pp. 1128–1131.
22. Magli MC, Gianaroli L, Ferraretti P, Toschi M, Esposito F, Fasolino C. (2004) The combination of polar body and embryo byopsy does not affect embryo viability. Human Reprod 19(5): 1163-1169.
23. Twisk M, Mastenbroek S, Van Wely M, Heineman MJ, Van der Veen F, Repping S. (2006) Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilization or intracytoplasmic sperm injection. Cochrane Database Syst rev 25(1):CD005291.
24. Pagidas K, ying Y, Keefe D. (2008) Predictive value of pre implantation genetic diagnosis for aneuploidy screening in repeated IVF-ET cycles among women with recurrent implantation failure. J Assist Reprod Genet 25: 103 -1 06.
25. Lalioti MD. (2008) Can preimplantation genetic diagnosis overcome recurrent pregnancy failure? Curr Opin Obste Gynecol 20(3): 199-204.
26. Montag M,van der Ven K, Rosing B, Van der Ven H. (2009) Polar body biopsy: a viable alternative to preimplantation genetic diagnosis and screening. Reprod Biomed Online 18 Suppl1:6-ll.
27. Basile C, Frydman R, El Aly A, Hesters L, Fanchin R, Tachdjian G, Steffann J, LeLorc'h M, Achour-Frydman N. (2009) Preimplantation genetic diagnosis: state of the art. Eur J Obstet Gynecol Reprod BioI 145(1):9-13.
28. Altarescu G, Eldar-Geva T, Brooks B, Zylber-Haran E, Varshaver I, Margalioth EJ, Levy-Lahad E, Renbaum P. (2009) Preimplantation genetic diagnosis (pGD) for nonsyndromic deafness by polar body and blastomere biopsy. J Assist Reprod genet 26(7): 3 91- 7.
29. Griesinger G, Biindgen N, Salmen D, Schwinger E, Gillessen-Kaesbach G, Diedrich K. (2009) Polar Body Biopsy in the diagnosis of monogenic diseases. DtschArztebl Int 106(33):533-8.
30. Familiari G, Nottola SA, Micara G, Aragona C, Motta PM. Is the sperm-binding capability of the zona pellucida linked to its surface structure? A scanning electron microscopic study of human in vitro fertilization. J In Vitro Fert Embryo Transf. 1988 Jun;5(3):134-43.
31. Familiari G, Nottola SA, Micara G, Aragona C, Motta P. Human in vitro fertilization: the fine three-dimensional architecture of the zona pellucida. Prog Clin Biol Res. 1989;296:335-44.
32. Familiari G, Nottola SA, Macchiarelli G, Micara G, Aragona C, Motta PM. Human zona pellucida during in vitro fertilization: an ultrastructural study using saponin, ruthenium red, and osmium-thiocarbohydrazide. Mol Reprod Dev. 1992 May;32(1):51-61.
33. Nottola SA, Makabe S, Stallone T, Familiari G, Correr S, Macchiarelli G. Surface morphology of the zona pellucida surrounding human blastocysts obtained after in vitro fertilization. Arch Histol Cytol. 2005 Jun;68(2):133-41.
34. Cohen J, Inge KL, Suzman M, Wiker SR, Wright G. Videocinematography of fresh and cryopreserved embryos: a retrospective analysis of embryonic morphology and implantation. Fertil Steril. 1989 May;51(5):820-7.
35. Bertrand E, Van den Bergh M, Englert Y. Does zona pellucida thickness influence the fertilization rate? Hum Reprod. 1995 May;10(5):1189-93.
36. Garside WT, Loret de Mola JR, Bucci JA, Tureck RW, Heyner S. Sequential analysis of zona thickness during in vitro culture of human zygotes: correlation with embryo quality, age, and implantation. Mol Reprod Dev. 1997 May;47(1):99-104.
37. Palmstierna M, Murkes D, Csemiczky G, Anderson O, Wramsby H. Zona pellucida thickness variation and occurrence of visible mononucleated blastomers in preembryos are associated with a high pregnancy rate in IVF treatment. J Assist Reprod Genet. 1998 Feb;15(2):70-5.
38. Gabrielsen A, Lindenberg S, Petersen K. The impact of the zona pellucida thickness variation of human embryos on pregnancy outcome in relation to suboptimal embryo development. A prospective randomized controlled study. Hum Reprod. 2001 Oct;16(10):2166-70.
39. Ingber D.How cells (might) sense microgravity. FASEB J.1999;13 Suppl:S3-15.
40. Bizzarri M, Pasqualato A, Cucina A, Pasta V. Physical forces and non linear dynamics mould fractal cell shape. Quantitative morphological parameters and cell phenotype. Histol Histopathol. 2013 Feb;28(2):155-74.
41. D'Anselmi F, Valerio M, Cucina A, Galli L, Proietti S, Dinicola S, Pasqualato A, Manetti C, Ricci G, Giuliani A, Bizzarri M. Metabolism and cell shape in cancer: a fractal analysis. Int J Biochem Cell Biol. 2011 Jul;43(7):1052-8. doi: 10.1016/j.biocel.2010.05.002. Epub 2010 May 10.
42. Kamran SC, Reichman DE, Missmer SA, Correia KF, Karaca N, Romano A, Racowsky C. Day 3 embryo shape as a morphologic selection parameter in in vitro fertilization. J Assist Reprod Genet. 2012 Oct;29(10):1135-9. doi: 10.1007/s10815-012-9842-7. Epub 2012 Aug 14.
43. Morales DA, Bengoetxea E, Larranaga P. Automatic Segmentation of Zona Pellucida in Human Embryo Images Applying an Active Contour Model. Proc. Of WIAU. 2008
44. Giusti A, Corani G, Gambardella LM, Magli MC, Gianaroli L. Segmentation of Human Zygotes in Hoffman Modulation Contrast Images. 2010 IEEE International Symposium on Biomedical Imaging. 2010 Rotterdam, The Netherlands.
45. Filho ES, Noble JA, Wells D. A review on automatic analysis of human embryo microscope images. Open Biomed Eng J. 2010 Oct 11;4:170-7.
46. Santos Filho E, Noble JA, Poli M, Griffiths T, Emerson G, Wells D.A method for semi-automatic grading of human blastocyst microscope images. Hum Reprod. 2012 Sep;27(9):2641-8
47. Fitzgibbon A, Pilu M, Fisher RB Direct least square fitting of ellipses. IEEE Trans. Pattern Anal Mach Intel 1999; 21:476-480.
48. World Health Organization (WHO), Laboratory manual for the examination of human semes and sperm-cervical mucus interaction, 4th ed (1999), Cambridge University Press, New York.
49. Palermo GD, Cohen J, Alikani M, Adler A, Rosenwaks Z. Development and implementation of intracytoplasmic sperm injection (ICSI). Reprod Fertil Dev. 1995;7(2):211-7; discussion 217-8.
50. Veeck LL "Preembryo grading and degree of cytoplasmic fragmentation" In: An Atlas of Human Gametes and Conceptuses (1999). The Parthenon Publishing Group Cap 6, 46-51.
51. Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," Statistics in Medicine, 17, 857-872 (1998).

- **Management of infertility in women over 40's**

Published in **Maturitas, 03/15/2014**
Cabry R, et al. – Women's fertility potential is declining with age because of multiples intrinsic and extrinsic factors such as life style, oxidative stress and/or endocrine disruptors and is affecting the ability of these women to conceive naturally. This declining fertility potential and the late age of motherhood is increasing significantly the number of patients consulting infertility specialists. Different strategies of investigation and management are proposed to patients over 40 in order to overcome their infertility and improve the live birth rate in these patients. This manuscript reports the results of the own experience from patients older than 40 in the light of the published data and discusses the different therapeutic alternatives which can be proposed to patients over 40 consulting ART centres.

- **Serum progesterone concentration on day of embryo transfer in donor oocyte cycles**

Published in **Journal of Assisted Reproduction and Genetics, 03/14/2014**
Brady PC, et al. – The study aims to evaluate the association between serum progesterone (P) levels on the day of embryo transfer (ET) and pregnancy rates in fresh donor IVF/ICSI cycles. Serum P levels on the day of ET in fresh donor IVF/ICSI cycles were positively correlated with clinical pregnancy and live birth rates. An increase in P dose after ET was insufficient to rescue pregnancy rates. Overweight and obese recipients may require higher initial doses of P supplementation.

- **Reproductive prognosis in endometriosis. A national cohort study**

Published in **Acta Obstetricia et Gynecologica Scandinavica, 03/14/2014**
Hansen MVH, et al. – The study aims to assess the reproductive long–term prognosis of women with and without endometriosis, explore changes over time, and quantify the contribution of artificial reproductive techniques. Women with endometriosis have slightly fewer children, but this lessened over time due to artificially conceived pregnancies. The risk for miscarriages and ectopic pregnancies was increased compared to women without the disease.

- **Value of antimullerian hormone as a prognostic indicator of in vitro fertilization outcome**

Published in **Fertility and Sterility, 02/18/2014**
Reichman DE, et al. – This study aims to determine the predictive attributes of antimüllerian hormone (AMH) in terms of oocyte yield, cycle cancellation, and pregnancy outcomes. Antimüllerian hormone is a fairly robust metric for the prediction of cancellation and how many oocytes may be retrieved after stimulation but is a relatively poor test for prediction of pregnancy after any given treatment cycle. Patients with extremely low levels of AMH still can achieve reasonable treatment outcomes and should not be precluded from attempting IVF solely on the basis of an AMH value.

- **Anti-Mullerian hormone (AMH): A reliable biomarker of oocyte quality in IVF**

Published in **Journal of Assisted Reproduction and Genetics, 02/28/2014**
Lehmann P, et al. – This study aims to evaluate the impact of serum AMH levels on stimulated IVF implantation and clinical pregnancy rates. Patients with AMH < 0.47 ng/ml should be advised before starting a stimulated IVF cycle of the poorer prognosis compared to the reference population independently of their age, total exogenous FSH dosage and number of eggs retrieved. Therefore, AMH could enable a more individualized number of embryo transfer policy based on oocyte quality.

- **Assisted oocyte activation following ICSI fertilization failure**

Published in **Reproductive BioMedicine Online, 02/18/2014**
Meerschaut FV, et al. – This review tackles the mechanism of human oocyte activation and the relatively rare phenomenon of fertilization failure after ICSI. Next, authors describe the current diagnostic approaches and focus on the application, efficiency and safety of AOA in human assisted reproduction.

- **Reproductive outcomes after operative laparoscopy of patients with tubal infertility with or without hydrosalpinx**

Published in **Chinese Medical Journal, 02/14/2014**
Li X, et al. – The study demonstrates the feasibility and effectiveness of laparoscopic treatment for tubal infertile patients without hydrosalpinx. However, there is a lower pregnancy rate and a high risk of ectopic pregnancy in hydrosalpinx patients after laparoscopy. Further investigations are required for determining whether reconstructive surgery should be abandoned in severe hydrosalpinx.

- **Reproductive performance after conservative surgical treatment of postpartum hemorrhage**

Published in **International Journal of Gynecology & Obstetrics, 01/09/2014**
Rasheed SM, et al. – This study aims to evaluate the impact of bilateral internal iliac artery ligation (BIL), bilateral uterine artery ligation (BUAL), step–wise uterine devascularization (SWUD), and B–Lynch on infertility, ovarian reserve, and pregnancy outcome. Of the 4 procedures, BIL had the least deleterious effect on reproductive performance; SWUD increased the risk of premature ovarian failure, and B–Lynch increased the risks of endometriosis, intrauterine adhesions, placenta previa, and preterm labor. The study included 168 infertile or pregnant patients–recruited at outpatient clinics in Egypt–who had previously undergone uterine–sparing surgery (BIL [group I], n=59; SWUD [group II], n=65); BUAL [group III], n=2; and B–Lynch [group IV], n=42). Groups II and IV had the highest prevalences of infertility. The ovarian reserve was significantly lower in group II. Unexplained infertility was the predominant cause of infertility in group I, anovulation and premature ovarian failure in group II, and endometriosis and intrauterine adhesions in group IV. The frequency of obstetric complications, particularly placenta previa and preterm labor, was high in group IV.

Compiled By Prof. Ahmed Badawy, MD FRCOG PhD
Mansoura University

Egypt.J.Fertil.Steril. Volume 18, Number 1, January 2014