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Acknowledgments

Acknowledgments should only be made to funding institutions and organizations and, if to persons, only to those
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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.

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Letter from the Editor:

Dear esteemed colleagues,

I hope this issue of your journal finds you all in good health. The last society meeting was a big success and the turn out from all over Egypt was great. Despite all the difficulties and uncertainties of the security in Cairo we had a large number of visiting professors who welcomed the opportunity to be in Egypt.

I would like to seize this chance to thank the President of the society **Prof. Gamal Serour** and Secretary General of the society **Prof. Amr El Shalakany** and all the secretarial staff for their effort during this very difficult time. However, whatever the attendance number was, it does not represent a fraction of practicing doctors of our specialty. To spread the benefits of the meetings to the vast majority of doctors we will start publishing the abstracts of important papers in the upcoming issues of the journal, and we would like to hear feedback from you about the conference and what you would like to be included in next years conference.

By the time this issue finds you, the parliamentary elections will be over (God willing) and whatever the results are, we should accept it and work together the good of our country. 2011 is finished and will always be remembered as the year the Egyptian people discovered themselves again. We may not all be of the same political opinions or religion or even have similar views in any of the issues discussed at present but above all we are all Egyptian. We have lived together for thousands of years, we have a common heritage, we all love this country and strive hard to make it a better place for all of us. May God help us all to make the right decisions for our country and make 2012 the first step towards democratic prosperity in Egypt.

Editor in Chief,
Prof. Mohamed Yehia

Electrosurgery In Laparoscopy

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'Electrosurgery' is the generation and delivery of radiofrequency current between an active and dispersive electrode to elevate the tissue temperature for the purpose of cutting, fulguration, and desiccation. 'Electrocautery' is not the correct synonym for electrosurgical units, although they are commonly used. Cautery refers to a direct heating process whereby the electricity does not flow through the tissue while in electrosurgery, the electric current actually passes through the tissues. Electrosurgical unit (ESU) is the correct term for modern units that utilize either monopolar or bipolar elements. The first modern, high-frequency electrosurgical generator was developed by William Bovie, a Harvard physicist, in 1926. This unit causes flow of electrons through the instruments and tissue, concentrated to small contact areas. This induces heat generation by creating molecular motion, rather like heating water in microwave. The transfer of heat from one object to another occurs through one or more of three basic mechanisms: conduction, convection and radiation. Electrosurgery when used properly, relies only on radiation which involves the transfer of thermal energy by electromagnetic waves.

Biological effects of electricity

Three biological effects can arise when electric currents flow through human tissue: electrolyte, faradic and thermal effects. The electrolytic effect is predominant with low-frequency alternating currents in which electrolysis induces flow of positively charged ions to the negative pole and the negatively charged ions to the positive pole. It is undesirable effect in electrosurgery as it can lead to chemical cauterisation and tissue damage. Currents with frequency range <20 KHz cause stimulation of nerves and muscle cells (faradic effect). This property is used in diagnosis of neuromuscular disorders, identification of the nerve trunks during surgery or used by anaesthetists to assess the neuromuscular blockade but is undesirable during electrosurgery as it could be dangerous especially when electrocuting is done near nerve trunk. The thermal effect is obtained with high-frequency currents and has its clinical application for cutting or coagulation. Above 40 0C, reversible cell damage occurs while irreversible cell changes occur above 50 0C. While coagulation and haemostasis (conversion of glucose to collagen and shrinkage of collagenous tissue) occur above 70 0C, desiccation (cellular fluid vaporization and shrinkage of coagulum) occurs above 100 0C and carbonization occur from 200 0C. The frequency of alternating current used for electrosurgery is between 500 and 4000 KHz so both the electrolyte and faradic effects are largely eliminated and allows utilization of only the thermal effect.

Therapeutic effects of electrosurgery

There are three distinct therapeutic effects that electrosurgical energy has been reduced to in practice; cutting, fulguration and desiccation. Electrosurgical cutting is non-contact mean of dissection that requires a high current (continuous uninterrupted flow) low voltage (small peak to peak difference) waveform to elevate rapidly the tissue temperature producing vaporization of the fluid contents and division. During pure electrosurgical cutting there is minimal effect on haemostasis on the walls of the incision. This pure cutting waveform is referred to as undampened or non-modulated current (Voyles and Tucker 1992). Advantages of this cutting technique over the traditional mechanical incision include reducing bleeding, preclusion of germ implantation, avoidance of mechanical damage to the tissue and being applicable endoscopically.

Electrosurgical fulguration (stray coagulation) is a non-contact modality of coagulation. It utilizes high voltage low current non-continuous waveform designed to coagulate by means of spraying long electrical sparks to the tissues. These bursts of increasing voltage are interrupted by intervals without current flow, resulting in cell heating and dehydration, with haemostasis and charring. Using the coagulation current, there is less rapid tissue heating as the pause between bursts of current allows the heat to dissipate with subsequent minimal or no cutting of the tissues. The commonest use of fulguration is to arrest bleeding emanating from capillary or arteriolar bed where a district bleeder cannot be identified. This is achieved by maintained raining effect of sparks until the electrode is withdrawn or the tissue is carbonized to the extent where sparking cease.

Desiccation is another form of coagulation that is achieved by electrode contact to the tissues. The high-voltage waveform of the coagulation mode causes tissue desiccation that is more penetrating and inflicts more damage to the surroundings than the cutting waveform of equal power. The commonest application of desiccation, in open surgery, is

occlusion of discrete bleeder caught by a haemostat; when energy is applied to the haemostat body. Coaptation of the vessels occurs by collagen chain reaction resulting in a fibrous bonding of the dehydrated denaturated cells of the endothelium.

With a ratio of modification of the cutting waveform, by interrupting the current and increasing the voltage, 'blend' waveform is established. It becomes non-continuous with a train of pockets of energy consisting of higher voltage and reduced current per-time to increase haemostasis during dissection. The blend waveforms will require a longer period of time to dissect the same length of incision as compared to the cutting waveform due to the interrupted delivery of current at the same power setting and consequently increase the coagulation of the small vessels. These blends are very valuable when needed to control bleeding during dissection but unfortunately, it increases the tissue necrosis and then risks of postoperative infection. The amount of smoke plume will be increased in laparoscopy on using high blends or coagulation modes.

Many variables determine the electrosurgical cutting and coagulation effects on the tissues. A definite correlation between peak voltage and coagulation zone was determined as the higher the voltage the broader the coagulation zone. The duration of the electrical energy application and the tissue impedance are other influencing factors. When the electrode is moved rapidly there will be little coagulation effect but slowing down the rate of movement increases it. Shape of the electrode is of decisive impact. A hook electrode, besides having a convenient geometry to handle the tissue, has a moderate electrode size to allow haemostatic dissection. Thin-needle electrodes are used in microsurgery as it provides finer and cleaner dissection but on the expense of coagulation. Ball electrodes will be good for electrodesiccation and fulguration of oozing tissue beds as the thicker the electrode the wider the coagulation zone.

Monopolar and bipolar systems

Electrosurgical units (ESU) can be divided into two major types: monopolar and bipolar. Monopolar system refers to current flow from one active electrode through the patient, who is entirely included in the circuit, and exists via dispersive electrode to the generator. Monopolar systems have proved advantages of being quick and effective in dissection. It's effective at haemostasis due to its greater penetrability so, it might be suitable to the deeply seated vessels.

Safety of monopolar system

Safety of the monopolar electrosurgical energy has been proved by 50 years during open laparotomy, however, the potential problems associated with its laparoscopic use need to be fully appreciated. Problems of the monopolar electrosurgery relate to unrecognised energy transfer or 'stray current' outside the view of the laparoscope. Mechanisms of these stray currents are found to be mainly insulation breaks, capacitative coupling or direct coupling. Laparoscopic images viewed on the monitors show about 10% only of the active electrode which is covered with insulating material. Any breakdown in insulation particularly along the shaft, out of the laparoscopic view, may produce severe burns to adjacent structures. Insulation failures can occur either due to normal wear, improper handling, sharp edges of the metal trocar or excessive heating by high voltage frequency. However, hazards of insulation failure depend largely on the location of the point of failure. Failures mostly occur in the distal portion of the instrument which is, fortunately, within view of the surgeon. Defects in the handle insulation usually pose little direct risk unless unexpected shock to the surgeon leads to patient injury. The shaft of the instrument is divided into two parts, intra- and extracannular. Failures in the part outside the cannula are the most dangerous being out of view and the most careful surgeon is unable to predict it. Insulation failure inside the cannula is not detectable if a plastic cannula is used, however, with metal ones the resulting arcing of currents create a lower frequency current which is usually dissipated harmlessly through the broad surface of contact at the abdominal wall. Some authors recommend the use of metal cannulas. Thorough visual examination of the instruments is mandatory preoperatively by the operating room staff to detect any defect in the insulation material. There are several recent commercial solutions to this problem including the use of disposable instruments or electroshields. The electroshield consists of the reusable sheath that surrounds the laparoscopic electrode and the electroshield monitor. It slides over the shaft of standard 5 mm instruments, dynamically monitors insulation failure and deactivates the electrosurgical generator should a fault develop. Although they are effective, two disadvantages are identified to these shields; the additional expenses and wider pore cannulas are mostly required (7-8 mm cannula for 5 mm instrument).

Capacitative coupling occurs when electrical energy is induced from the active electrode to a nearby conductive material despite intact insulation. A capacitor exists wherever an insulating material separates two conductors that have a different potential between them; a situation which may be created during the laparoscopic surgery. A classical example occurs when insulated monopolar electrode is passed through the operating channel of the laparoscope, 50% to 70% of the electrical energy passed through the well-insulated electrode will induce capacitative coupling to the surrounding laparoscope. The acquired energy can be transferred to surrounding skin then to the ground plate without causing injury if the covering sleeve of the scope is conductive. If the laparoscope is inserted through plastic cannula or conductive sleeve with non-conductive collar, the energy will be concentrated on the laparoscope and may cause an unintended injury to adjacent organ as bowel, omentum and blood vessels. This potential hazard can be avoided by using conductive trocar sleeves for the scope. In 1980, the Food and Drug Administration (FDA) advisory panel reported that the non-conductive trocar sleeves of the scope is potentially dangerous and should not be used with the operating laparoscope and recommended the use of all metal cannulas. Again the use of electroshields will collect measure and shunt all the capacitatively coupled currents. Direct coupling occur when an active electrode touches other metal instruments within the abdomen, transferring the energy to the second instrument and possibly injuring tissues with which it comes in contact. The only way to avoid such injuries is not to activate the electrode until the operative field is in full panoramic view and avoid contact of the activated electrode with other instruments or the scope during laparoscopic procedures. Many authors recommend the use of 300 forwards oblique scope instead of the zero degree as better and a more precise field of view is usually obtained. There had been controversy about the possibility of intestinal burns being the result of contact with hot recently cauterized tissues or with recently activated electrodes. DiGiovanni (1990) investigated this possibility in animal study and was unable to produce any histologic evidence of tissue injury.

High-frequency current leakage is a well known complication of monopolar units that may cause collateral injuries. This occurs when the patient's body comes in contact with another electrically conductive object e.g operating table or infusion stand, while the return electrode improperly placed, then high current density causes thermal necrosis at the point of contact. The burn usually occurs outside the surgeon's view while the patient is under anaesthesia unable to detect or respond to burn. Arcing or sparking to adjacent structures can also occur when high voltage coagulation current is used which may jump the gap between active electrode and unintended tissues. Under atmospheric condition, a driving pressure of 15000 V is required to push electrons more than one cm in the air which is, fortunately, well above the maximum volt-

age (1200 V) produced by the high-frequency electrogenerators currently recommended by the FDA for laparoscopic use. It has been found that electrons take 30% more power to spark or arc in CO2 than in room air; thus at the same electrosurgical power settings, less arcing occur in laparoscopy than laparotomy. Recent advancements have been introduced to promote the safety of the electrosurgical units such as the generators with return electrode monitoring (REM) that gives alarm and deactivates the unit when contact between the pad and skin is inadequate. A microprocessor-controlled generator is another new advancement. When applied to a tissue, this device automatically initiates and concludes the process when optimal coagulation has been achieved through detection of the vaporization which coincides end-point of coagulation. In case of cutting, the cutting effect and quality become unaffected by the variables of size and shape of the electrodes, type, speed of cutting and varying tissue characteristics.

Safety of bipolar systems

In bipolar units, both the active and return electrodes are housed within the same instrument so the current flows only through the tissue between the two blades of the electrode and return to the generator without passing through the whole body. No remote dispersive electrode is required. Reich first reported bipolar desiccation of large vessel coagulation in 1987. Bipolar forceps can use high frequency, low voltage cutting current to coagulate vessels as large as the cystic, ovarian and uterine arteries. Bipolar electrocoaptive desiccation seals arterial blood vessels immediately so that they can withstand the pulsating arterial pressure until permanent fusion of the collagen and elastic fibres is accomplished through the healing process. Cessation of flow is indicated by an ammeter or current flow monitor. Coagulation current is not preferred in bipolar systems as it may rapidly desiccate the outer layer of the tissue, producing superficial resistance that may prevent deeper penetration. Cessation of tissue bubbling is not a reliable indication of complete desiccation. It is always recommended to use an indicator system for complete haemostasis especially when the bipolar current is used for large vessel coagulation. Recently, microbipolar forceps has been introduced which contain a channel for irrigation. It allows irrigation of the bleeding sites to identify vessels before their coagulation and prevent sticking of the electrode to the eschar created.

Actually, bipolar electrosurgery is intrinsically safer than the monopolar systems as it uses low voltages that limit the depth and breadth of necrosis. It also has a more precise localised tissue effect. The current flows only to the site where coagulation is required and because each jaw of the forceps is the same surface area, the electrons only heat the tissue imposed between them.

This flow process is self-limiting because as the cells are charred, the current ceases to pass avoiding damage to the surrounding tissues. Standard bipolar technology eliminates the possibility of arcing or capacitative coupling as both electrodes are contained in the same instrument and their magnetic fields will cancel each other out. The possibility of inadvertent injury due to insulation failure is reduced in this system. Despite the advantages of the bipolar systems, however, they cannot fully respond to many challenges of using electrosurgery in the minimally invasive surgery due to some technical difficulties. It requires that the tissue being coagulated be surrounded by the forceps, therefore, it is more difficult to use with retracted blood vessels or with thick tissue. Until recently, only bipolar coagulation was possible but continuous developments have led to bipolar cutting and dissection. The power supplies on these cutting instruments adopt the same physical principles underlying the monopolar systems as the sparks occur between the tissue and one of the electrodes. Another characteristic of bipolar systems to be considered is its potential to create a 'mushrooming' coagulation effect inside and outside the actual space between the jaws of the instrument. This is created because of the magnetic field-like nature of the electrical conduction. This means that delicate structures such as the ureters are not immune from injury although the spread can go just for several millimetres around the tip of the device. Many cases of inadvertent ureteric injury have been reported after using bipolar electrosurgery.

In spite of this mushrooming, bipolar instruments provide much more control over the electric current than dose the monopolar devices. The standard bipolar systems have variable tissue effects due to variable tissue impedance. They have maximum efficiency output at impedance of 100 ohms which matches well with impedance encountered with vessels, blood and irrigants but the efficiency of coupled energy into desiccated vessels (impedance of 600-1500 ohms), fat and connective tissue (impedance of > 1000 ohms) is quite low. Another problem associated with a standard bipolar system is tissue carbonization that creates a thin layer of high impedance and subsequently decreases the operating efficiency, being an electrical barrier preventing the completion of circuit. When the temperature increases, tissue sticks to the paddle leading to vascular rebleeding as it peels off.

Procision bipolar electrosurgery system is a new modification introduced to overcome many of the disadvantages of the standard system. It uses Advanced Resonance Technology to deliver consistent power and repeatable tissue effects across a wide range of tissue impedance up to 10000 ohms, which is well in excess of the standard systems. This technology matches the output impedance of the Procision device to the patient's tissue impedance by varying the frequency at which the device operates. This means that the device is operating at optimum efficiency throughout the procedure allowing much lower power settings than conventional diathermy, which operate at fixed frequencies. Another problem that is solved is carbonization, which creates an electrical barrier.

Procision modulates the output waveform to induce carbonization but unlike conventional outputs, the waveform then changes to a high-energy sinusoidal output capable of breaking through the impedance wall and re-establishing electrical continuity with the tissue. It is claimed that Procision allows true electrosurgical cutting owing to the higher proportions of energy delivered to the patient's tissue at higher tissue impedances than the conventional systems. The early clinical experience with this technology is promising.

Prevention of electrosurgical complications

Burn injuries represent an important area of potential trauma during laparoscopy. Good surgeons perform good surgery with whatever tools they choose to use. The best surgeons make the effort to become very knowledgeable about the attributes of every technical tool from which they can choose. Wheeless (1978) has reported the overall incidence of thermal injury associated with laparoscopy at 2.2 per 1000 cases. The Royal College of Obstericians and Gynaecologists (RCOG) Confidential Enquiry into Gynaecological Laparoscopy (1978) reported one per 1000 cases of non-fatal burn complications and showed that sterilization by electrosurgery resulted in less complications than clips or rings. Peterson (1981) have reported two deaths resulting from thermal intestinal injury after monopolar surgery. More recent reports proved the same injury risks to the patients. The majority of injuries, mostly intestinal, go unrecognised at the time of insult and commonly present three to seven days afterwards. Grainger et al. (1990) reported many cases of ureteric injuries; most of them were following both monopolar and bipolar electrosurgical procedures of sterilization or ablation of endometriosis.

On using conventional electrosurgical equipments in laparoscopic surgery, many surgical techniques and guidelines have been suggested to prevent patient complications. It is recommended to avoid high power settings and high-voltage waveforms as spray coagulation and to use the lowest possible power setting that will deliver the desired tissue effect. There are three modes of coagulation: soft, forced and stray coagulation according to power and modulation of used current. Soft coagulation is the safest form of monopolar coagulation that employs relatively low unmodulated voltage (190 V) so; it is the mode preferred during minimal access surgery. It must be noted that coagulation can be accomplished adequately with low-voltage, low-power settings using a cutting waveform, as efficient but safer than coagulation waveform.

All-metal cannulas and no hybrid systems (trocars or cannulas that are comprised of metal and plastic components) are recommended so that stray currents can be dispersed harmlessly through the patient abdominal wall. Avoidance of touching the active electrodes to other metal instruments or open-circuit activation of the electrosurgical units is basic practical directions. Multiple short activations are preferable to prolonged activations to allow the normal surrounding tissues to remain cool. Using bipolar systems is to be recommended when appropriate. Recent technology, when available, as disposable instruments, microprocessor-controlled electrosurgery, Procision technique and active electrode monitor (AEM), undoubtedly, increase the margin of safety. There are suggested interventions for perioperative nurses to follow that can help to minimize electrosurgical complications. These directions include preoperative and postoperative examination of the instruments for insulation failure and discarding sharp edged cannulas, avoiding reuse single-use active electrodes, proper connection of equipments, ensuring that the power settings are in typically employed ranges and avoiding placing active electrodes on the patient drapes.

Further readings

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Dyslipidemia, Insulin Sensitivity Indices, MetabolicRisk Factors, And Anti Müllerian HormoneLevels In Egyptian Women With Polycystic Ovary Syndrome

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Abstract

Objective: To determine the levels and relationships between lipoproteins, triglycerides (TG), insulin sensitivity measures, and anti müllerian hormone (AMH) levels in polycystic ovary syndrome (PCOS) women as compared to healthy eumenorrheic controls.

Patients and method

We examined 110 PCOS and 36 controls aged 19-34 years old, body mass index (BMI), waist hip ratio (WHR), were determined, plasma levels of glucose, insulin, TG, total cholesterol(TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), lipoprotein a LP(a), Apolipoprotein B (Apo B); total testosterone, sex hormone binding globulin(SHBG) and AMH were measured, homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were calculated.

Results

According to BMI level 29.1%, 37.2% and 33.6% were lean, overweight, and obese in PCOS group which did not differ significantly from controls. High LP (a) levels were found in 68.2% followed by low HDL-C in 63.7% of PCOS women. At all BMI levels HDL-C, and QUICKI levels were significantly lower, whereas TG, FAI, HOMA-IR levels were significantly higher in PCOS than normal women. AMH levels were significantly higher in PCOS vs. control women, yet, significantly lower in obese vs. lean PCOS women. HDL-C, and FAI were independently associated with HOMA-IR, (p=0.037), and LP (a)(p=0.23) respectively.

Conclusion

Dyslipidemia is common in PCOS and is positively correlated to FAI, BMI, and HOMA-IR.
Keywords: Dyslipidemia, polycystic ovary syndrome, anti müllerian hormone, HOMA-IR.

Introduction

Polycystic ovary syndrome (PCOS), a condition of chronic anovulation and hyperandrogenism, constitutes the most frequently encountered reproductive endocrinopathy affecting 6-10% of all women (1). Women with PCOS are hyperinsulinemic (2) and fully 40% of all cases will develop type 2 diabetes by age of 50 (30). PCOS increases the risk for cardiovascular disease, a finding consistently reported across several geographic areas and ethnic groups (4). Up to 70% of women with PCOS have dyslipidemia (5), particularly increased levels of low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) levels (6).

Some studies, however, did not record any differences in lipid or lipoprotein profiles between PCOS patients, and weight matched controls (7), (8). Genetic environmental and ethnic factors may be responsible for the conflicting results. Polycystic ovaries are characterized by greater amounts of primary and preantral follicles than their normal counterparts (9), yet the antral follicle growth is arrested at the 4-7 mm stage due to production of local inhibitors as anti müllerian hormone (AMH) or inhibin (10).

AMH is produced in the granulosa cells of early developing follicles (11), and strongly correlates with the number of antral follicles (12). Women with PCOS display elevated levels of AMH when compared with age and body mass index (BMI) matched normally cycling women (13), (14). As asserted by a number of studies, AMH is a useful marker of ovarian responsiveness, embryo number or assisted reproductive technology outcomes (15), (16).

This study was conducted to assess the differences between Egyptian PCOS and healthy eumenorrheic women in plasma lipids and lipoproteins, insulin sensitivity index, metabolic risk factors, and AMH serum levels and to determine their relationship to each other.

Subjects and methods

The study group consisted of 110 PCOS patients recruited from women attending the obstetrics and gynecology outpatient clinic of Zagazig University affiliated hospitals from 2007 to 2009. The criteria for PCOS included the Rotterdam consensus (17) with the Androgen Excess Society (AES) modification (18) namely; oligo or anovulation with oligomenorrhea or amenorrhea and/or polycystic ovaries (> 12 follicles 2-9 mm, or ovarian volume >10 ml) on transvaginal ultrasound, clinical or biochemical evidence of hyperandrogenism (Ferriman-Gallway score > 8) (19) free androgen index >8, respectively.

Patients with thyroid dysfunction, hyper-prolactinemia , Cushing syndrome, late onset non –classical 21-hydroxylase deficiency, and androgen secreting tumours were excluded after appropriate testing.

Thirty six women with menstrual cycles in the normal range (25-34 days) for at least 12 months, who were age and body mass index (BMI)- matched with the PCOS group and having normal androgen and 17- hydroxyprogesterone levels were enrolled as controls. On admission, all subjects had a full medical examination including past medical history and use of medications. Exclusion criteria for all subjects were hormonal therapy including contraceptives, the presence of diabetes mellitus , renal or hepatic diseases , and hypolipidemic drugs. All subjects provided informed consent to participate in this study.

All subjects underwent anthropometric measurements, a blood draw within the first 4 days of menstrual cycle in the control group and after a spontaneous bleeding episode in the PCOS group. Height (without shoes) was measured to the nearest 0.5 cm with a vertical ruler. Weight (in light clothing) was measured to the nearest 0.2 kg with a portable scale. BMI was calculated as weight (kg)/ height (m2) and women were classified as; lean < 25 kg/m2, overweight ≥ 25 kg/m2, and obese > 30 kg/m2. Waist circumference (WC) was measured according to the American Heart Association/National Heart, Lung and Blood institute guidelines (20). At the narrowest circumference between the top of iliac crest and the lower margins of ribs, an inelastic tape was placed in a horizontal plane, around the abdomen. The tape must be snug, not compressing skin, and parallel to the floor. Measurement was made at the end of normal expiration. Hip circumference (HC) was measured at the widest circumference of the buttocks at the area of the greater trochanters, then the waist hip ratio (WHR) was calculated. Blood was collected early in the morning after a 12 hour overnight fast for lipid and hormone assays. Plasma total cholesterol and TG were measured on a Roche Modular System using commercial kits; (Roche Diagnostics, Rotkrenz, Switzerland) with a coefficient of variation of 2.3 and 2.4% respectively.

HDL-C, lipoprotein (a) [Lp(a)], and apolipoprotein B (apo B) were measured using commercial assays (Roche Diagnostics) with a coefficient of variation 4.1, 2.3, 1.2% respectively . LDL-C was calculated using the Friedwald formula (21). Plasma AMH was measured using an Immunotech immunoenzymatic assay (Beckman Coulter, Marseille, France). The detection limit of the assay was 0.1ng/ml. Fasting venous blood samples were taken for the assessment of plasma glucose, insulin, serum testosterone, and sex hormone binding globulin (SHBG). Glucose (mg/dl) was assessed by using enzymatic assay (Yellow Springs Glucose Analyzer), insulin (IU/ml) was measured by a solid-phase enzyme- amplified sensitivity immunoassay (INS-EASIA; Biosource Technologies, Nivelles, Belguim). Total testosterone (T) (ng/dl) was measured by testosterone enzyme immunoassay test kit (LI7603; Linear Chemicals). Dehydroepiandrosterone- sulfate (DHEA-S) (ng/ml) was measured by radioimmunoassay (Cisbio International, France).

Sex hormone binding globulin (SHBG) serum levels (nmol/L) were measured by ELISA (SHBG ELISA, MX 520 11;IBL, Hamburg, Germany). Glucose tolerance was determined by a 75- gram oral glucose tolerance test. The quantitative insulin sensitivity check index (QUICKI) (22), and homeostasis model assessment of insulin resistance (HOMA-IR) (23) were used as surrogate measures of insulin sensitivity. They were calculated as follows; QUICKI= 1/log fasting insulin (μ IU/ml) + log fasting glucose (mg/dl) [mg/d for glucose= mmol/L *18.182], HOMA-IR= fasting blood glucose (mg/dl) * fasting insulin (μIU/ml)/405.

Free androgen index (FAI) was calculated as T (nmol/L)*100/ SHBG (nmol/L). Dyslipidemia was diagnosed using the definitions proposed by the National Cholesterol Eductaion Program-Third Adult Treatment Panel (NCEP ATP III) for the metabolic syndrome (20), (25) viz; TG >1.7 mmol/L (150mg/dl), lowHDL-C: <1.29 mmol/L (50 mg/dl), high LDL-C: >4.1 mmol/L (155 mg/dl), elevated Lp (a) if > 30 mg/dl,and, elevated apo B if > 100 mg/dl.

Statistics

Statistical analysis was performed using SPSS (version 15.0) [SPSS, Chicago, IL, USA]. Continuous variables were presented as means ± SD, normalityl was assessed by Kolmogrov-Smirnov test. Data which are not normally distributed were log transformed and non-parametric tests were employed. Non-parametric Mann-Whitney u test was used for numeric variables' differences, and Chi-square test for categorical variables. Multiple regression was performed for plasma lipids and lipoproteins, as well as, AMH as dependent variables. Independent variables were the group (PCOS vs. control), FAI, BMI, QUICKI, and HOMA-IR. For all analyses two-tailed p≤0.05 was considered to indicate statistical significance.

Results

In this study we found that all PCOS women had menstrual irregularity, 79.7% of them had a Ferriman Gallway score > 8, and 75.8% of them had elevated serum total testosterone levels. As shown in table (1) PCOS women and healthy women had similar age, BMI, and WHR. AMH, total testosterone (TT), FAI; HOMA-IR levels were significantly higher; p<0.01 in PCOS women compared to controls. Levels of LDL-C were higher in the PCOS group than healthy women; however, this did not reach statistical significance. Unlike levels of Total Cholesterol TT and ApoB which did not differ between participant groups. HDL-C and Lp (a) levels were significantly lower in PCOS group p<0.05 than controls. In the PCOS group BMI categorical distribution was as follows; lean 29.1% (n=32), overweight 37.3% (n=41) and obese 33.6% (n=37). For the controls 30.6% (n=11) were lean 36.2% (n=13) were overweight and 33.3% (n=12) were obese. Both groups were not statistically different for BMI categories. Clinical, hormonal and insulin sensitivity indices according to BMI are listed in table (2).

Total Cholesterol and lipoprotein levels according to BMI are presented in table (3). No significant changes were noted with increasing body weight in total cholesterol (p=0.93), ApoB (p=0.182), LDL-C (p=0.231), DHEA-S (p=0.772). Lp(a),and HDL-C levels increased significantly at each level of increase in

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BMI; for Lp(a) obese vs lean; p=0.016, obese vs. overweight; p= 0.027 and overweight vs. lean p=0.033, for HDL-C levels obese vs. lean p=0.021 , obese vs. overweight p=0.043,and overweight vs. lean p= 0.047.

Total testosterone, SHGB, FAI, HOMA-IR were significantly higher in PCOS compared to control group and within the PCOS group they were significantly higher in overweight versus lean,and in obese versus lean women. Obese women in PCOS and control groups had significantly higher fasting blood glucose than overweight and lean women. QUICKI was significantly lower in obese women with PCOS compared to their lean and overweight counterparts. For AMH significantly higher levels were recorded in PCOS than control women for each BMI category. Obese women with PCOS, however, had significantly lower levels than lean PCOS women.

For the control group all variables were not significantly different in different BMI categories except for fasting insulin, HOMA-IR , TG levels which were significantly higher, HDL_C, QUICKI values which were significantly lower in obese vs. lean women p=0.032, p=0.032, p=0.047, p=0.041, p=o.029 and p=0.035 respectively. According to NECP III criteria (20),(25) 9 women of the control group (25%) had dyslipidemia. Five women of the dyslipidemic controls (56%) had low HDL-C levels, 4 women (45%) had elevated TG and 2 (22.2%) had high LDL-C levels. Of the dyslipidemic controls 3 women were obese and the remaining was overweight. All obese dyslipidemic control women had high values of TC, TG, LDL-C and low HDL-C levels, 4 of the overweight women had low HDL-C levels, 3 had high TG , 2 women had low LDL-C level.

Table (1) :
Anthropometric Characteristics, hormonal and metabolic profiles in polycystic ovary patients and controls

Study Group	POCS	Controls	
	n=110	n=36	
Age (years)	25.8±4.72	26.2±3.83	NS
BMI (kg/m2)	28.7±5.13	29.3±4.35	NS
WHR	0.78±0.15	0.76±0.13	NS
FG score	17.45±7.23	-	
TT (ng/dl)	111.32±25.83	43.29±3.61	<0.001
SHBG (nmol/L)	36.04±0.27	45.12±1.37	NS
DHEA-S (ng/ml)	2753.33±1128.41	2413.44±1215.45	NS
FAI	15.6±12.92	3.89±1.43	<0.001
AMH (ng/ml)	5.81±2.37	2.54±0.95	<0.001
Glucose (mg/dl)	98.41±6.52	85.74±1.89	<0.05
Insulin (µIU/ml)	17.43±6.82	7.41±4.21	<0.01
HOMA-IR	4.87±3.51	1.73±1.26	<0.001
QUICKI	0.333±0.005	0.371±0.003	<0.001
TC (mg/dl)	184.19±9.47	167.73±0.16	NS
LDL-C (mg/dl)	121.48±32.62	118.56±18.74	NS
HDL-C (mg/dl)	42.72±20.13	60.51±6.22	P<0.01
Lp (a) (mg/dl)	37.32±27.15	7.8±4.2	<0.0001
ApoB (mg/dl)	83.45±36.24	80.71±27.25	NS
TG	122.28±69.47	118.39±21.31	<0.05

- Data are mean ± SD, P<0.05 is statistically significant
- BMI= Body Mass Index; WHR= Waist Hip Ratio; FG score= Ferriman Gallway score; TT= Total Testosterone; SHBG= Sex Hormone Binding globulin; DHEA-S= Dehydroepiandrosterone- sulfate; FAI= Free Androgen Index; AMH=Anti Müllerian Hormone; HOMA-IR= Homeostatic Model Assessment Insulin Resistance; and QUICKI= Quantitative Insulin Sensitivity Check Index; TC=Total Cholesterol; LDL-C= Low Density Lipoprotein Cholesterol; HDL-C= High Density Lipoprotein Cholesterol; Lp(a)= Lipoprotein (a); ApoB=Apolipoprotein B; TG=Triglycerides.

In the PCOS group 71.8% (n=79) were dyslipidemic. Most frequent abnormalities were: high Lp (a) levels (n=75/110) ; 68.2% followed by low HDL-C levels (n=70/110); 63.7%, high TG levels (n=65/110); 59%,high LDL-C levels (n=64/110); 41% , then high total cholesterol levels (n=39/110); 35.4%.

For lean, overweight and obese women low HDL-C levels were reported in 18.5%, 70.7%, 94.6%, high Lp (a) levels in 28.1%, 80.4%, 86.5%; high LDL-C levels in 9.3%, 36.6%, 75.7% ;and high TC levels in 6.3%, 41.5%, 54.1% respectively. The following variables were positively related to BMI, Lp (a) (r = 0.575; p<0.001), TG (r = 0.419; p=0.001), LDL-C (r=0.296, p=0.021), HOMA-IR (r=0.319; p<0.01). HDL-C, and QUICKI levels were inversely correlated to BMI (r=0.389; p=0.021),and (r=-387; p<0.01) respectively. When HOMA-IR was correlated to individual variables it was positively related to Lp (a) (r =0.423; p<0.001), TG (r=0.252;p<0.05), AMH (r=0.268; p=0.034) and inversely correlated to QUICKI (r=-0.567; p<0.001), and HDL-C (r =-0.356; p =0.005). FAI was positively correlated to Lp (a), (r=0.427; p<0.001), AMH(r=0.553; p<0.001), HOMA-IR (r=0.453; p= 0.024) and inversely related to HDL-C (r= -0.448; p=0.019) and QUICKI (r= -0.511; p=0.017). The best predictor of Lp (a) was FAI (r2=0.244; p=0.023) the model was further improved by adding HOMA-IR (r2=0.361; p=0.017) and AMH (r2=0.519; p=0.002). For HDL-C best predictor is HOMA- IR (r2=0.231; p=0.037) adding FAI (r2=0.382; p=0.022) and AMH (r2=0.498; p=0.004) improved the model

Table (2):
Clinical, hormonal, insulin sensitivity indices in different body mass index categories in controls versus polycystic ovary patients

	Lean		Overweight		Obese	
	Control	PCOS	Control	PCOS	Control	PCOS
Variable	n=11	n=32	n=13	n=41	n=12	n=37
Age (years)	26.9±5.7	24.8±4.7	27.3±2.3	25.9±2.5	23.9±3.4	25.4±3.5
BMI (kg/m٢)	22.3±1.7	21.6±2.2	27.5±2.1	28.4±4.3b	32.8±1.8	34.9±3.8c,d
FG score	-	16.52±5.73	-	19.93±6.84	-	18.55±4.71
TT (ng/dl)	43.5±26.2	103.7±18.4a	45.7±22.4	110.8±25.4a,b	44.8±30.17	119.2±32.1a,c,d
SHBG (nmol/L)	47.5±0.9	35.2.7±1.3a	46.8±0.7	34.2±2.2a,b	45.3±1.1	33.6±1.9a,c,d
FAI	4.1±2.2	10.4±3.6a	3.8±1.7	12.8±9.5a,b	4.9±2.5	14.2±6.8a,c,d
DHEA-S (ng/ml)	2255±924	2371±1100	2334±1207	2501±1197	1989±1313	2421±1125
AMH (ng/ml)	2.3±0.90	6.4±2.5a	2.1±1.2	5.2±2.7a	1.8±1.3	4.7±1.1a,c
Glucose (mg/dl)	85.9±4.2	88.1±0.9	89.3±3.8	88.5±5.2	99.7±10.2	101.2±3.3a,c,d
Insulin (µIU/ml)	6.2±2.6	14.8±9.6a	10.8±2.7	19.8±12.9a,b	11.1±1.2	21.3±16.5a,c,d
HOMA-IR	2.1±1.5	3.7±2.1a	1.9±2.2	5.3±3.2a,b	2.9±0.6	6.2±2.8a,c,d
QUICKI	0.375±0.004	0.359±0.006	0.344±0.003	0.2275±0.007a,b	0.335±0.012	0.255±0.0.37a,c,d

- Values are mean ± SD, P<0.05 is statistically significant
- BMI= Body Mass Index; FG score= Ferriman Gallway score; TT= Total Testosterone; SHBG= Sex Hormone Binding globulin; FAI= Free Androgen Index; DHEA-S= Dehydroepiandrosterone- sulfate; AMH=Anti Müllerian Hormone; HOMA-IR= homeostatic Model Assessment Insulin Resistance; and QUICKI= Quantitative Insulin Sensitivity Check Index.

a P<0.05 PCOS vs. Controls.
b P<0.05 Overweight vs. lean
c P<0.05 Obese vs. lean
d P<0.05 Obese vs. overweight

Table (3):
Lipid profile according to body mass index categories in controls vs. polycystic ovary syndrome women

	Lean		Overweight		Obese	
	Control	**PCOS**	**Control**	**PCOS**	**Control**	**PCOS**
Variable	n=11	n=32	n=13	n=41	n=12	n=37
TC (mg/dl)	158.95±27.72	164.89±29.74	169.34±24.61	185.22±27.13	176.22±31.52	188.33±26.42
LDL-C (mg/dl)	97.39±32.06	106.61±25.44	105.28±19.91	111.39±23.05	113.47±18.78	116.99±22.23a,c,d
HDL-C(mg/DL)	65.37±14.26	50.48±10.33a	52.17±16.51	45.96±14.85a,b	49.56±11.26	38.92±12.14c,d
TG (mg/dl)	79.39±43.92	99.71±35.13a	122.74±33.65	131.22±37.19b	132.15±28.27	139.48±36.25a,c,d
Lp (a) (mg/dl)	7.3±6.2	18.8±31.1a	8.5±5.7	27.4±25.9a,b	7.5±5.3	41.2±85.2
Apo B (mg/dl)	82.35±19.7	80.82±20.3	82.62±38.6	86.5±29.4	81.7±42.2	87.3±44.6

- Data are mean ± SD P<0.05 is statistically significant
- TC= Total Cholesterol; LDL-C= Low Density Lipoprotein- cholesterol; HDL-C= High Density Lipoprotein- cholesterol; Lp(a)= Lipoprotein (a); APOB= Apolipoprotein B.

a P<0.05 PCOS vs. Controls.
b P<0.05 Overweight vs. lean
c P<0.05 Obese vs. lean
D P<0.05 Obese vs. overweight

Discussion

It is well-known that healthy women have hormonal protection against cardiovascular diseases delaying their onset by 10-15 years in comparison to men (26). In contrast young women may show increased cardiovascular risk when affected by PCOS (27). In PCOS hyperandrogenemia and chronic anovulation have been associated with metabolic aberrations and metabolic syndrome (28). FAI levels were significantly higher in obese than overweight or lean PCOS women analyzed in this study reflecting the fact that hyperandrogenemia can aggravate visceral obesity in women unlike men (29). Besides, androgen excess is known to lower circulatory HDL-C (30) that androgen excess is arthrogenic, a fact that was further substantiated in our study that demonstrated the inverse relationship between LDL-C and FAI. Moreover FAI was the best predictor of Lp (a) providing another link between androgen excess and cardiovascular risk disease.

Hyperandrogenemia can also perpetuate insulin resistance by inhibiting muscle glycogen synthase activity,and, by increasing the number of less insulin sensitive type IIb skeletal muscle fibers (31), (32), again here , FAI was positively correlated to HOMA-IR and negatively correlated to QUICKI measures of insulin sensitivity. Similar findings were reported in previous studies (33), (34). Lipid alterations are common in women with PCOS (5). Concerning the pattern of dyslipidemia in the PCOS the results of different studies are not entirely congruent in the present study a significant increase only in TG, Lp(a) and a significant decrease in HDL-C in PCOS women compared to healthy women was found , a pattern as that seen with insulin resistance and recorded in previous studies (33), (35), (36). Other authors reported only a single increase in TG (37), (38) or LDL-C (39) or decrease in HDL-C (40) in PCOS women.

When the effect of BMI was illustrated some studies showed decreased HDL-C levels in all PCOS women and an added increased TG levels only in obese PCOS women (41), (42). For all levels of BMI (lean, overweight, or obese) we found that the decrease in HDL-C and increase in TG was significant. Rizzo et al 2009 (4) pointed out that Italian PCOS women had significantly increased TG and LDL-C than healthy controls , however, their concentrations usually remained in the normal range. As we examined Egyptian women with PCOS 59% had TG levels >1.7 nmol/L and 41% had LDL-C levels >4mnol/L were reported which are significantly higher than those of Italian anovulatory women with PCOS 9%, 23% respectively (4). Lp(a) a marker of increased cardiovascular risk was noted to be significantly higher in Egyptian women with PCOS compared to controls with no alterations in ApoB ; in agreement with findings by other authors on Mediterranean PCOS women (4), (43), (44).
Nonetheless, contradictory results were shown for ApoB (45), Lp(a) (45) in PCOS women from different genetic backgrounds.

Obese women with PCOS from our locality had significantly higher levels of TG than overweight or lean PCOS women as stated in previous reports (41), (42). HDL-C levels decreased significantly with each level of increase in BMI, Rocha and coworkers (33), however, did not notice a difference between overweight and obese Brazilian women with PCOS. Insulin resistance was postulated to be the connecting factor between dyslipidemia

and PCOS (5),indeed, the best predictor for HDL-C was HOMA-IR in this study.

The present study confirmed previous results on increased serum AMH levels in women with PCOS (47), (48) which is due to increased number of preantral follicles and its increased production per granulosa cell in PCOS patients(49). As mentioned in previous studies (50),(51) a positive correlation between AMH and FAI was demonstrated in Egyptian PCOS women, as well. Our PCOS study women displayed a positive correlation between their AMH levels and HOMA-IR. Previous publications on the relationship between AMH levels and high surrogate measures were conflicting; some reported no association (14), (52) or positive correlation (50).

AMH levels were significantly lower in obese PCOS compared to lean PCOS sub group, this inverse relationship was consistently found in previous reports (54), (55). This can be explained by the fact that Insulin resistance can occur independent of obesity in PCOS women (53),and,surprisingly weight loss results in improvement in insulin resistance but not AMH probably because it has a greater effect on metabolic rather than gonadotropic presentation of PCOS and so far LH levels were found to be the most important independent determinant of AMH (48).

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Clinical Outcome Of Day 5 And Day 6 Blastocyst Vitrification

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Abstract

Objective: to compare survival, clinical pregnancy and ongoing pregnancy rates of blastocysts vitrified on day 5 and those which had one day delay and vitrified on day 6.
Materials & Methods: This was a retrospective study in a University based and a private practice setting. The study included 210 vitrified warmed cycles, 135 patients underwent vitrifivation at day 5 (group I) and 75 patients at day 6 (group II). The main outcome measures were blastocyst survival and clinical pregnancy/embryo transfer were primary outcomes. Ongoing pregnancy/embryo transfer was the secondary outcome.

Results: Blastocyst post-warming survival rates were comparable between both groups (92.9% (263/283) of day 5 versus 94.9% (166/175) of day 6 blastocysts). There was no statistically significant difference between the 2 groups regarding the mean number of transferred blastocysts. Clinical pregnancy rates were 40.6% (52/128) & 43.7% (31/71) in women who undergone vitrification at day 5and day 6 respectively with no significant differences. Similarly, ongoing pregnancy rate was comparable between the 2 groups, 37.5% (48/128) versus 39.4% (28/71) in groups I & II.
Conclusion(s): blastocysts vitrified on day 5 have the same survival, clinical and ongoing pregnancy rates of blastocysts which had one day delay and vitrified on day 6.
Key words: Blastocyst transfer, clinical pregnancy, vitrification.

Introduction

Cryopreservation has become an increasingly important therapeutic strategy in reproductive medicine, with the birth of many infants after use of this procedure. It is important for cryopreservation in general to establish consistent outcomes, especially in terms of embryo cryosurvival to allow high chances of success in performing a frozen embryo transfer (FET). However, standard cryopreservation technologies appear to illustrate their ultimate limitations in their lack of consistency in cryo-survival. Actually, interest has shifted to vitrification as an attractive alternative to slow-freezing methodology (1) and vitrification is now the preferred method of cryopreservation in many centers (2, 3).

It is as an ultra-rapid cooling technique that is simple, potentially faster, starting to become clinically established and seems to have the potential to be more reliable and consistent than conventional cryopreservation when carried out properly (4, 5). Further, the need for controlled-rate freezing equipment, which requires routine calibration and maintenance, is eliminated. The cells are placed into the cryoprotectant, then the cells are placed in a very small volume of cryoprotectant on a special carrier, and then they are cooled at extreme rates by plunging them directly into LN2. With this method, no ice crystals form with avoidance of damage to the cells or the tissues. Actually, Lack of ice crystallization and convenience of the procedure itself are two major advantages which changed entire cryopreservation program of many centers from conventional freezing to vitrification only (2, 3).

With the introduction of sequential culture media in ART, and driven by the large increase in the rate of multiple pregnancies arising from earlier-stage ET, extended culture to the blastocyst stage has become more common. The best available evidence suggests that the probability of pregnancy, implantation and live birth rates after fresh IVF is significantly higher after blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer (6, 7). However, possibility of some embryos not developing into blastocysts in vitro and as a result cancellation of embryo transfer should be considered. So, blastocyst transfer policy should be applied in good prognosis patients (6, 7). With this concept, many centers have shifted to blastocyst transfer. Consequently, the need to cryopreserve human blastocysts is also increasing. Although the results achieved by conventional slow freezing seem successful (8-10), clinical results with blastocyst cryopreservation have not necessarily been consistent, owing to the higher potential for damaging ice crystal formation in traditional slow-freezing protocols. So, there have been an increasing number of reports of successful human blastocyst vitrification (11-15).

Generally, if there is failure in achieving pregnancy after initial transfer of fresh blastocysts, surplus vitrified blastocysts would be transferred in a subsequent cycle.
Moreover, there have been suggestions that, fresh BT cycles might be canceled for patients who have exhibited poor endometrial receptivity or ovarian hyperstimulation syn-

drome. Under such circumstances, all available fresh blastocysts would be vitrified for transfer in a subsequent cycle (16). Importantly, previous investigators have found superior implantation rates with fresh transfers occurring at day 5 as compared with day 6. They reported an almost doubled clinical pregnancy and implantation for fresh day 5 blastocyst compared with fresh day 6 blastocysts (17).The one-day delay in expansion was considered in itself an indication of inferior viability.

A pertinent question is whether extra blastocysts which were vitrified on day 5 or the ones which had required 6 days to reach expanded blastocyst and vitrified on day 6 have the same or different embryonic developmental potential upon warming. So, the objective of the current study is to compare survival, clinical pregnancy and ongoing pregnancy rates of blastocysts vitrified on day 5 and those which had one day delay and vitrified on day 6.

Materials & Methods

From October 2007 to November 2010, 210 vitrified-warmed BET cycles were evaluated. 135 women had undergone blastocyst vitrification on day 5 and 75 on day 6. All patients included used standard long protocol for controlled ovarian stimulation (COS) and underwent ICSI. In our program, women who have ≥ 4 grade one embryos (i.e. regular symmetrical blastomeres with no fragmentation) on day 3 after retrieval (18) are counseled for extended culture and BET. Ovarian stimulation was performed as previously reported (19).

Embryo Scoring

Embryos reaching the blastocyst stage, whether on day 5 or day 6, were graded by using the system of Gardner and Schoolcraft (20). Blastocysts were given a number based on the degree of expansion and hatching status (from 1 to 6): 1 = early blastocyst: the blastocele accounts for less than one-half of the volume of the embryo; 2 = blastocyst: the blastocoel occupies more than one-half of the volume of the embryo; 3 = full blastocyst: the blastocoel fills the embryo completely; 4 = expanded blastocyst: the blastocoel is now larger than the early embryo, and the zona pellucida has begun to thin; 5 = hatching blastocyst: trophectoderm (TE) cells have begun to herniate through the zona pellucida; and 6 = hatched blastocyst: the blastocyst has completely escaped the zona pellucida. For blastocysts regarded to be full blastocysts and onward (grades 3–6), a second scoring step was performed under an inverted microscope to assess the inner cell mass (ICM) and the TE. For the ICM, the following descriptions are used: A = tightly packed with many cells; B = loosely grouped with several cells; and C = very few cells. For the TE, the following grading is used: A = many cells forming a cohesive epithelium; B = few cells forming a loose epithelium; and C = very few large cells. Extra blastocysts were only considered for vitrification if they were regarded to be full blastocysts and onward (grades 3–6), Inner cell mass (ICM) scored A-B and trophoectoderm (TE) scored A-B.

Protocol for Vitrification and Warming

Vitrification of blastocysts was undertaken using the Cryoloop carrier system (Vitrolife, Sweeden) after a two-step loading with cryoprotectant agents at 24°C. Briefly, blastocysts were placed in equilibration solution, which is the base medium (HEPES-buffered solution with 20% serum supplement; Irvine Scientific, USA) containing 7.5% Ethylene glycol (EG) and 7.5% DMSO. After 8–13 minutes, the blastocysts were washed quickly in vitrification solution, which is the base medium containing 15% DMSO, 15% EG, and 0.5 mol/L sucrose. These 2 solutions were to be used in sequence according to the step-wise microdrop vitrification protocol. Importantly, blastocysts were exposed to the vitrification solution ≤30 seconds. From last microdrop, 1-3 blastocysts in < 1uL media was loaded into the Cryoloop carrier, capped under the LN2 with the cryovial immerged in the LN2 till final storage.

Patients not achieving a clinical pregnancy returned for a frozen blastocyst transfer cycle. All women received letrozole (femara, Novartis), one tablet (2.5 mg)/day, starting from day 3 of the cycle for 5 days. When dominant follicle reached ≥18mm and endometrium thickness≥8mm, 10000 IU of HCG were given (day 0). Vaginal administration of progesterone (cyclogest, Florham Park, NJ) was initiated on day HCG+3 (usually 4 days before the frozen blastocyst transfer was scheduled).

On day of vitrified blastocyst transfer, to remove the cryoprotectants, blastocysts were warmed and diluted in a two-step process. With the Cryoloop submerged in LN2, the protective cap was removed and placed directly into a pre-warmed (approximately 30°C) organ culture dish containing thawing solution (HEPES buffered solution containing gentamycin sulphate, 1.0 mol/L sucrose and 20% serum supplement).After 1 minute, blastocysts were transferred to dilution solution (HEPES buffered solution containing gentamycin sulphate, 0.5 mol/L sucrose and 20% serum supplement) for 4 minutes. Then, blastocysts were transferred to the washing solution (HEPES buffered solution containing gentamycin sulphate and 20% serum supplement) for 9 minutes and then returned to the culture medium (Sage Blastocyst Medium) until transfer. Whether vitrification was performed on day 5 or day 6, one to three blastocysts were transferred into the patient's uterus on day HCG+7.

Serum β-hCG tests were performed two weeks after ET and transvaginal ultrasound
(US) were scheduled three weeks later to confirm a clinical pregnancy. Spontaneous abortion was defined as the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age (21). Clinical pregnancy rate was defined as the number of clinical pregnancies expressed per 100 embryo transfer cycles (21). On-going pregnancy rate was defined as the number of clinical pregnancies, continuing beyond 20 weeks of gestation and expressed per 100 initiated embryo transfer cycles.
Outcome measures

Blastocyst survival and clinical pregnancy/embryo transfer were primary outcomes. Ongoing pregnancy/embryo transfer was the secondary outcome.

Data were statistically described in terms of mean ± standard deviation (SD), frequencies (number of cases) and relative frequencies (percentages) when appropriate. Analysis was carried out by means of a X2 test using computer programs Excel version 7 (Microsoft Corporation, NY, USA). Statistical significance was defined as $P<0.05$.

Results

The study included 210 vitrified warmed cycles, 135 patients underwent vitrification at day 5 (group I) and 75 patients at day 6 (group II).Table 1 shows the mean age and clinical outcome of patients who completed the vitrified blastocyst transfer program. No significant differences could be observed regarding age in the two groups. Of 135 women who had vitrification at day 5, 128 women underwent warmed BET (94.8%, 128/135). Meanwhile, 71 of the 75 women who had vitrification at day 6 had undergone warmed BET (94.7%, 71/75) with no significant differences between the 2 groups. Regarding the blastocyst post-warming survival rates, 92.9% (263/283) of day 5 blastocysts and 94.9% (166/175) of day 6 blastocysts survived after warming and this difference was not significant. 260 blastocysts were transferred in first group, while 145 blastocysts were transferred in second group with no statistically significant difference between the 2 groups regarding the mean number of transferred blastocysts. Clinical pregnancy rate was 40.6% (52/128) in women who undergone vitrification at day 5 and was 43.7% (31/71) among those who had vitrification at day 6 with no significant differences. Similarly, ongoing pregnancy rate was comparable between the 2 groups, 37.5% (48/128) versus 39.4% (28/71) respectively in groups I & II.

Discussion

Data from the present study suggest that, blastocysts which had shown one day delay and vitrified on day 6, results in similar survival, clinical and ongoing pregnancy rates when transferred in subsequent cycles compared to transfer of blastocysts vitrified on day 5.

Previous studies have demonstrated that fresh embryos reaching the blastocyst stage and transferred on day 5 had a significantly higher pregnancy rate than those blastocyst embryos transferred on day 6 (17). We recently performed a study (submitted for publication) upon174 patients who had undergone BET on day 5 and 22 participants who did not have expanded blastocysts on day 5 and were left for one day, and all developed expanded blastocysts and had undergone BET on day 6. Blastocysts transferred on day 5 implanted at nearly twice the rate of blastocysts transferred on day 6 (40% vs. 19%, $P < 0.05$). Pregnancy rates were also almost twice as high in day 5BET {106/174 (60.9%)} than those undergoing day 6BET {7/22(31.8%)}. Similarly, ongoing pregnancy/live-birth rates were also higher in first group{91/174(52.3%)} than in those undergoing day 6BETgroup{6/22(27.3%)} Actually, Shapiro et al. present provocative retrospective data suggesting that synchrony of embryo and endometrial development may be an important factor in pregnancy rates following blastocyst transfer (17).

So, the transfer of blastocysts which had shown one day delay in expansion and transferred on day 6 might result in embryo-endometrial dyssynchrony. Moreover, it might be suggested that, the more slowly developing blastocysts could be innately compromised to some extent. Importantly, Embryos that were vitrified on day 6 were required to be expanded blastocysts and, before they were transferred, must have survived the warming process. These requirements may have selected better-quality embryos than day 6 blastocysts transferred in the fresh cycle. It appears that, there is profound clinical value in knowing they can be vitrified as late as day 6, successfully warmed and result in ongoing pregnancy. Additionally, it is plausible that a more synchronous transfer of these warmed blastocysts contributed to the good outcome.

In accordance with current study findings, Richter et al, suggested that blastocysts cryopreserved on day 6 resulted in similar pregnancy rates when transferred to artificially prepared endometrium in cryopreserved cycles or in donor egg cycles, compared to transfer of blastocysts cryopreserved on day 5 (22). So, with the reported high clinical and ongoing pregnancy rates following vitrified-warmed transfer of day 6 blastocysts, it might be a good policy to encourage vitrification of supernumerary embryos reaching the blastocyst stage beyond day 5. In the meantime, this study should stimulate further investigation in this field in the ongoing quest to improve outcomes from in vitro fertilization and ICSI.

There are other issues with vitrification that need further discussion. Concerns about introduction of high concentrations of cryoprotectant, which are necessary to prevent mechanical damage from ice, exist with vitrification. The problem of cryoprotectant toxicity is an immediate and practical one, just as it is to a lesser extent in classic slow- cooling procedures. Extremely rapid cooling allows a decrease to be made in the concentration of the cryoprotectant and thereby a reduction in potential toxicity (23).
The greatest advantages of vitrification have been seen in chill-sensitive cells such as oocytes and blastocysts (24). The main characteristic of the blastocyst is its fluid-filled cavity, the blastocele. It has been reported that, with increasing volume of the blastocelic cavity, the survival rate drops with vitrification. This is thought to be due to insufficient permeation of cryoprotectant into the blastocelic cavity, such that residual water may promote ice crystallization during the vitrification process. Several articles report that survival rates in cryopreserved expanded blastocysts could be improved by artificial reduction of the blastocelic cavity (12-14, 25-26). In our protocol and others (5, 16), we proceeded without any opening in the zona pellucida before vitrification independent of the size of the blastocelic cavity. The previous concern appears theoretical rather than practical and proceeding without blastocele collapse spares extra-procedure with a comparable survival and PR (5, 16).

Another concern has been made that fungi, bacteria, and viruses are able to survive in LN2 (27-29). Given that with vitrification the cells are directly plunged into LN2, they therefore have direct contact with LN2 and so the question arises as to whether the LN2 has to be sterilized because it may be a possible source of contamination. Use of clean LN2 for the initial vitrification step, followed by sealing of the carrier, seems to address the concern of potential contamination during cryostorage. To further reduce fears of contamination, it is possible to store material from potentially infectious patients separately from seemingly noninfectious samples. Therefore, it is important to perform routine screening tests for viral infections, including hepatitis B and C, on all couples undergoing infertility treatment. In the event that a couple screens positive, we offer vitrification of blastocysts. Even though we consider the risk of cross contamination during storage to be almost infinitesimal, in such cases we nevertheless recommend placing embryos in specially designated tanks, or shipping them off-site. It is worth noting that to date no viral, fungal, or bacterial contamination event has been described from many publications related to vitrification since 1985.

So, concerns about vitrification are well defined, limited in number, and easily surmountable. In general, with much shorter protocols, vitrification [1] is able to be undertaken on a more flexible basis by laboratory staff, [2] allows for the potential reduction in personnel time needed during the entire vitrification process, [3] simplifies laboratory techniques for cryopreservation in human ART, and [4] may enable more optimal timing of embryo cryopreservation, e.g., individual blastocysts may be cryopreserved at their optimal stage of development and expansion. Interest levels will inevitably rise, given the potential benefits of vitrification. This in turn will drive its development to higher levels of clinical efficiency and utilization (1, 31-32).

In conclusion, blastocysts vitrified on day 5 have the same survival, clinical and ongoing pregnancy rates of blastocysts which had one day delay and vitrified on day 6.

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Table I:
Vitrification/warming data of blastocysts vitrified on day 5 and day 6.

	Day 5(n=135)	Day 6(n=75)	P
Age (years)	32 ± 3.1	31.8 ± 3.9	0.84
Transfer cycles	128 (94.8%)	71 (94.7%)	1
Survived blastocysts	263/283 (92.9%)	166/175 (94.9%)	0.41
Blastocysts transfer	2.03 ± 0.5	2.04 ± 0.4	0.88
CP/ET	52(40.6%)	31(43.7%)	0.67
OP/ET	48(37.5)	28(39.4%)	0.78

CP=clinical pregnancy, ET=embryo transfer, OP= ongoing pregnancy. Data presented as mean ± SD unless otherwise specified. P > 0.05 non-significant

Prenatal Diagnosis In Low Resource Setting: Is It Acceptable?

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Abstract

Objective: This study aimed to explore knowledge and acceptability of prenatal procedures both non invasive prenatal screening tests and invasive procedures among Egyptian women in child bearing age and to assess their attitude towards such procedures. Also to examine confounding factors affecting women's attitude towards prenatal procedures.
Study design:
A cross-sectional study on a representative sample of women in childbearing age attending Obstetrics & Gynecology outpatient clinic at Mansoura University Hospital, Egypt. An anonymous questionnaire was supplemented by voluntary interviewers for women in childbearing age.

Results:

465 women were included in the study. The mean age ± SD was 27 ± 6 years. About 44% of women were knowledgeable about non invasive prenatal screening procedure and only 25.5% heard about invasive prenatal procedures. 88.8% express positive attitude regarding performance of the screening tests. Forty one percent of the group agreed to perform invasive prenatal procedures during their pregnancy. Educational level and family history of congenital anomalies significantly affected attitudes towards testing during future pregnancy. The cost of procedure affected the decision to perform it in 56.7% of women.

Conclusion:

Egyptian women express positive attitude towards non invasive prenatal screening but showed poor knowledge. Their attitude towards invasive procedure is guarded by the risk of abortion. Education and family history of fetal anomalies are the factors that affect attitudes. The cost of the prenatal test affects the decision made by the women to participate in testing.

Key words: prenatal procedures, attitudes, knowledge, pregnancy termination.

Introduction

Prenatal screening was first introduced nearly four decades ago, yet gaps still exist in public knowledge about the screening program (1). Prenatal screening procedures are options available to women in both, the first (11-13 weeks) and second (14-18 weeks) trimesters of pregnancy, aimed at identifying those at increased risk of birth defects and/or hereditary conditions, such as Down syndrome, neural tube defects (NTD) and some other fetal anomalies. Diagnostic procedures are invasive tests that carry a risk of miscarriage and can confirm, with 99% accuracy, the presence of a chromosomal abnormality (2).

The general population is familiar with Down syndrome (trisomy 21), but they are not aware of more uncommon conditions such as Patau syndrome (trisomy 13) and Edward's syndrome (trisomy 18). They are aware of diagnostic testing from friends, TV/press, or because of family history (1). Recent guidelines from the American College of Obstetricians and Gynecologists, and the American Society of Medical Genetics recommend that all pregnant women have to be offered prenatal screening for the most common aneuploidies (3). No simple correlation has been found between the change in technology to the changes in values and beliefs towards genetic testing and prenatal procedures. Some think that genetic testing is a great advance while others think it will cause troubles (4).

The availability of information about prenatal screening and diagnostic procedures affects the choice of women of whether or not to undergo testing (5). Limited information is available on how knowledge of prenatal screening, education level and former experience of disability affect the decision to participate in prenatal screening (6).

Some modern Islamic opinion and rulings have accepted prenatal diagnosis and approved severe congenital anomalies and malformations, per se, as a reason for termination of pregnancy before ensoulment (7, 8).
This study aimed to explore knowledge, attitude, and acceptability of prenatal procedures (non invasive prenatal screening tests and invasive procedures) among Egyptian women in child-bearing age and to examine confounding factors affecting women attitude towards prenatal procedures.

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Patients and Methods

A cross-sectional study on a representative sample of women attending Obstetrics and Gynecology outpatient clinic in Mansoura University Hospital between January 2011 and April 2011.

An anonymous questionnaire was supplemented by voluntary interviewers for the women in childbearing age after their consent to participate in the study. The structured questionnaire included information about the socioeconomic variables and risk factors for fetal anomalies (maternal diseases, personal or family history of a child with anomalies).

The questionnaire included items about knowledge and attitude towards non invasive prenatal screening (18 items) and invasive prenatal diagnostic procedure (8 items).

Statistical analysis:

Statistical analysis was carried out using the statistical package SPSS 16.0 for Windows (SPSS, Chicago, IL, USA). The means and standard deviations (SD) were calculated for continuous variables. An independent sample t-test was used to evaluate the associations between continuous variables. Two-sided p-value was considered statistically significant at $p < 0.005$.

Results

A total of 465 women of child bearing age were interviewed during the period from January 2011 to April 2011. Table (1) represents demographic data of the studied population, where mean age ± SD was 27 ± 6 years and 8 % of the women were ≥35 years. Eight percent of the studied women had previous history of infertility and 11.4 % had history of congenital anomalies either in their sibling or their family (table 2). About 44 % of women were knowledgeable about non invasive prenatal screening procedure and 88.8 % express positive attitude regarding performance of the tests (table 3).

Forty one percent of the group agreed to perform invasive prenatal procedures during their pregnancy. This figure declined to 31.6 % after explaining the procedure related risk of miscarriage (table 4). Only 25.5 % of women heard about invasive prenatal procedures.

Nineteen percent of the interviewed women chose to terminate pregnancy after positive screening test results and 90 % refused, while 72.6 % of them chose termination if there is evident fetal anomaly that may result in handicapping. This decision was influenced by educational level and history of congenital anomalies (p value = .001 and .000 respectively). Presence of maternal disease, infertility, previous abortions and occupation didn't significantly affect women decision to terminate an affected pregnancy. The cost of the prenatal procedure affected the decision of performing it by 56.7% of the women, whereas in 43.3%, the cost did not affect their decisions.

When we studied the factors that may influence knowledge and attitude towards prenatal procedures, we found that higher education, employed and urban women were more knowledgeable (tables 5&7) whereas maternal ages, number of pregnancies, presence of congenital anomalies didn't, significantly, affect women's knowledge.

Urban women accepted the idea of prenatal procedures more than women living in rural areas (table 6). Education significantly affected women attitude towards non invasive tests, while no significant difference in attitude was found towards invasive procedure between educated and non educated women. Presence of congenital anomalies either in their families or previous child significantly affected women's attitude to undergo prenatal procedure in their future pregnancy (100 %, p = 0.001).

Discussion

Women favor prenatal examinations, but the choice of participation does not seem to be based on insight to enable fully informed consent. More than 90% of the pregnant women expressed a positive attitude toward screening procedures in pregnancy. About 96 % were found knowledgeable about the procedural and practical aspects (9).

Unfortunately, the present study showed that all surveyed women had positive attitudes, but poor knowledge about prenatal screening. About 88% of the studied group accepted prenatal screening and 87% found it valuable for the outcome of pregnancy. Knowledge about prenatal screening was found in 43.4 % to 48.3 % of the studied group, whereas only 25.5 % were found knowledgeable about invasive procedures.

Forty one percent of the studied group accepted to perform invasive prenatal diagnosis but this declined to 31.6 % after explaining the risk of miscarriage. Willruth et al reported a higher acceptance, where only 21.5 % of his surveyed group refused the procedures (10).

Factors such as education, maternal age, and religion affect the acceptability of prenatal diagnosis. In our study educational level significantly influence women knowledge and their acceptance of prenatal screening but doesn't affect their acceptance of invasive procedures. Brajenovic et al reported a statistically significant difference in knowledge scores with respect to educational level. In contrast, no difference regarding their attitudes toward amniocentesis (11). Julian-Reynier et al. reported that, educational level had no effect on acceptance of invasive diagnostic procedures (12).

Regarding residence we found women living in urban areas more knowledgeable and expressed positive attitude toward prenatal procedures than women living in rural areas. This difference may be explained by a higher educational level and the more availability of medical services in urban areas (13).

In our study employment significantly affect knowledge about prenatal procedures and this may be attributed to higher educational level.

Although Rostant et al found an association between increasing women age and number of pregnancies with knowledge and attitude toward prenatal tests (14), our study found those variables not significantly affecting both knowledge and attitude.

One of the important factors that may influence knowledge and attitude toward prenatal tests was history of congenital anomalies. Such women expressed positive attitude (100 %) to perform testing in their future pregnancy, although they have poor knowledge. these findings were reported by different authors (15, 8) .

In different countries, prenatal care is free of charge and a part of general health care. However, there is a fee for first trimester prenatal screening as this is considered an optional service (6). In our hospital only ultrasonic prenatal screening is free of charge and when discussing the cost of biochemical markers and invasive procedure with the interviewed women 56.7% found the procedures expensive and their cost affect the decision to perform them during pregnancy.

Acceptance of termination of pregnancy for severe clinical conditions was comparable to that reported from European countries (72.6 %) (16). In our study, this decision was significantly affected by educational level and history of congenital anomalies. Japer et al (2000) reported high level of opposition to termination of pregnancy in the event of severely affected fetus (61.8 %) but he found educated women willing pregnancy termination more than less educated if they faced an affected fetus (17). The majority of our groups were Muslim and this doesn't influence their attitude towards pregnancy termination for severe fetal anomaly.

Table (1):
Demographic data of the study group

	Number (465)	Percentage (%)
Age (years)		
Mean ± SD	27 ± 6	
≥35 years	37	8 %
Occupation		
Employed	188	40.4 %
Not employed	277	59.6 %
Education		
Not educated	87	18.7 %
1ry & 2ry school	184	39.5 %
Higher	194	41.7 %
Residence		
Urban	192	41.3 %
Rural	273	58.7 %
Religion		
Muslim	434	93.3 %
Christian	31	6.7 %
Consanguinity		
Present	61	13.1 %
Absent	404	86.9 %

Table (2):
Obstetric and medical history of the participants

	Number	Percentage
Previous infertility		
Present	37	8 %
Absent	428	92 %
Abortions		
Present	153	32.9 %
Absent	312	67.1 %
Congenital anomalies		
Present	53	11.4 %
Absent	412	88.6 %
Maternal disease		
Present	81	17.4 %
Absent	384	82.6 %

Table (3):
Knowledge & attitude towards non invasive procedures

	Biochemical markers		Ultrasonic diagnosis	
Knowledge				
Yes	202	(43.44%)	225	(48.38 %)
No	263	(56.56%)	240	(51.62 %)
Agree to perform				
Yes	367	(78.92%)	413	(88.81%)
No	98	(21.08%)	52	(11.19%)
Valuable				
Yes	364	(78.28%)	405	(87.09%)
No	101	(21.72%)	60	(12.91 %)

Table (4):
Knowledge & attitude towards Invasive diagnostic procedures

	Number	Percentage
Knowledge		
Yes	119	25.59 %
No	346	74.41 %
Agree to perform		
Yes	192	41.29 %
No	273	58.71 %
Carry risk (agree)		
Yes	147	31.61 %
No	318	68.39 %

Table (5):
Effect of occupation on knowledge

	Biochemical markers		US procedures		Invasive procedures	
	Yes	No	Yes	No	Yes	No
Employed (188)	102	86	130	58	87	91
Not employed (277)	100	177	95	182	66	211
Total	202	263	225	240	153	312
P value	.000		.001		. 003	

Table (6):
Effect of residence on attitude

	Biochemical markers		US procedures		Invasive procedures	
	Yes	No	Yes	No	Yes	No
Urban (192)	103	86	131	58	88	91
Rural (273)	101	177	96	182	67	211
Total	203	263	226	240	154	312
P value	.000		.001		. 003	

Table (7):
Effect of education on knowledge

	Biochemical markers		US procedures		Invasive procedures	
	Yes	No	Yes	No	Yes	No
Non-educated (87)	17	70	13	80	10	77
Lower education (184)	67	117	70	114	49	135
Higher education (194)	118	76	142	52	94	100
P value	.000		.001		. 000	

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Comparison Of Pregnancy Outcomes Between Day 2 And Day 3 Embryo Transfer

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Abstract

Objective: To test if the extended embryo culture and embryo selection methods have a positive effect on pregnancy outcome or not.
Materials & Methods: This study had been carried out in infertility unit at C-PLAS hospital at Sana'a, Yemen during the period from July 2007 to July 2009, it includes 305 intracytoplasmic sperm injection cycles had fresh embryo transfer either on day 2, (220 patients) or on day 3, (85 patients). It retrospectively analyzes the pregnancy rates for both groups.

Results: The mean age was similar in both groups. Pregnancy rates were slightly higher in day 3 embryo transfer (43.52 %) versus day 2 embryo transfer (40 %) but not statistically significant. There was no statistical significant difference in pregnancy rates based on the number of embryo transferred in both groups, However there was significant difference in the quality and cleavage stage of embryo, day 2 embryo transfer (grad A 68.14 %, grad B 25.62 % and grade AB 6.23 %, 4 cell 98.19 % and 8 cell 1.8 %) versus day 3 (grad A 85.71 %, grad B 11.84 % and grade AB 2.43 %, 4 cell 49.13 % and 8 cell 50.87 %).

Conclusion: Extending embryo culture period from day 2 to day 3 have positive effect on cleavage stage and quality of embryo, but had no adverse effect on pregnancy rate .Embryo transfer could be done on day 2 or day 3 according to quality and cleavage stage of embryo.

Key word: Embryo quality, cleavage stage, pregnancy rate, day2 and day3 ET

Introduction

Many studies have compare day 2 versus day 3 embryo transfer outcome in intracytoplasmic sperm injection "ICSI, using extended embryo culture together with selection of good quality embryos. Transfer of day 3 embryos should be associated with higher implantation rate and pregnancy rate than transfer of day 2 embryos .Since the start of IVF embryo has been transferred 2 days at 4 cell stage due to lack of suitable culture media able to sustain embryonic development for several days. The timing of arrival of the embryo in the uterus however is premature compared with the situation in vivo where the embryo enters the uterus at morula stage "4-5" days after ovulation (1).

Transfer of embryo to the uterus on day 3 after oocyte retrieval may be closer to physiological time of uterine entry than transfer on day 2. Delaying embryo transfer would allow the selection of the most vital embryos for transfer (2). A retrospective study showed that pregnancy rates were similar between day 2 and day 3 transfers but implantation rate in day 3 groups was higher, (3). The pregnancy and implantation rates were found to be increased after transfer on day 3, (4). Aboulghar et al. 2003, found no significant difference in pregnancy rate between day 2 day 3 embryo transfer (5). As shown in previous studies on day 2 versus day 3 embryos transfer remains controversial. It is useful to perform this study to compare implantation and pregnancy rate between day 2 and day 3 embryo transfers.

Patients and Methods

This study had been carried out in infertility unit at C-PLAS hospital at Sana'a, Yemen during the period from July 2007 to July 2009. It includes 305 patients undergoing infertility treatment by ICSI due to either male or female factor, They were classified into two groups, group one day 2 embryo transfer, group two day 3 embryo transfer.

Both groups were subjected full history taking, general examination, vaginal ultrasound, basal hormonal profile (most normally and some ICSI due to tubal factor and pco) and seminal analysis (All by ejaculate either normal or oligo-athenospermia with normal morphology). All patients were treated using short protocol (as routine for our center in this period) gonadotrophin–releasing hormone "GnRH" decapeptyl 0.1 from first day of menses, then starting human menopausal gonadotrophin "HMG" (150IU TO 300IU) according to age ,weight and response to stimulation and folliculometry follow up then injection of 10000 IU human chorionic gonadotrophin " HCG" was given for oocyte maturation. At 34-36 hours ovum pickup through transvaginal ultrasound guided was prepared.

Embryo culture and embryo transfer procedure Oocytes were identified in the laboratory and briefly rinsed free of follicular fluid and blood in handling Gamete medium (K-SIGB-50; Cook IVF, Australia). Freshly ejaculated semen was washed in Sperm media (K-SISM-50; Cook IVF, Australia) by centrifugation at 1600 rpm for 5min after 30 min liquefaction period. The pellet was further processed by the side migration technique for ICSI as described by (Dozortsev et al., 1996). Oocytes for injection were denuded of cumulus cells following brief exposure to hyaluronidase for >1min (K-SIHY-1-5; Cook IVF, Australia) and then assessed for maturity. MetaphaseII oocytes were injected using the method described by (Dozortsev et al., 1996). Microinjection was carried out on the heated stage of an inverted microscope (Nikon-TE2000-U, Japan). The injected Oocytes were incubated in 50µl drop of fertilization media (K-SIFM-20; Cook IVF, Australia) in culture dish (REF-353004; Falcon, USA) under mineral Oil (K-SICO-200; Cook IVF, Australia) in an incubator containing 6% CO2 in air at 37°C. 16–20 hours post-insemination, oocytes were assessed for fertilization. Those oocytes exhibiting two pronuclei and two polar bodies were placed in one or two groups in <l micro drops of a ready to use Cleavage media (K-SICM-20; Cook IVF, Australia) under mineral oil (K-SICO-200; Cook IVF, Australia) in culture Dish (REF-353004; Falcon, USA). In the day 2 transfer group, embryonic development was assessed under the inverted microscope 42¬¬- 44 hours after ICSI. In the day 3 transfer group, embryos were first evaluated 42-44 hours after ICSI and then for a second time 24 hours after the first evaluation. Embryos were classified based on morphological criteria as described by (Laverge et al., 1997). Briefly, embryos without a nucleated fragments and with equally-sized blastomeres were graded as type I. Embryos with some a nucleated fragments (>10%) and/or with unequally-sized blastomeres were graded as type I-II. Embryos with unequally-sized blastomeres with either =20%, up to 50% or >50% a nucleated fragments were classified as type II, II-III, and III respectively. Embryos of grade I and I-II were classified as excellent quality embryos, embryos of grade II as god quality embryos and embryos of Grade II-III and III as moderate to poor quality embryos. The number for transfer being determine by the availability of embryos for transfer and the patient's age and previous clinical history. If the patient aged 35 years or had failed determine by the availability of embryos for transfer and the patient's age and previous clinical history. If the patient aged 35 years or had failed to achieve a pregnancy after three or more previous IVF cycles then consideration was given to transferring three or four Embryos, if Available, rather than only two embryos, which would be the usual recommendation to patients of a younger age or with a limited IVF history. One to four Embryos were transferred to each patient. Supernumerary embryos up to type II were cryopreserved with a dimethyl sulphoxide (DMS) rapid-freezing protocol. Pregnancy was defined as positive if the ßHCG measured in venous blood was >20 mIU/ml. Clinical pregnancy was defined as a positive pregnancy test followed by the presence of a fetal sac on transvaginal ultrasound 4 weeks after transfer.

Statistical analysis

Data were analyzed using the Minitab software (version 12.1, Minitab Inc.).The Paired t test was used to compare the mean differences in number of embryos transferred in both day 2 and day 3. The Chi-square test was used to compare Pregnancy rate after day 2 and day 3 embryo transfers and quality of embryos transferred. A P value less than 0.05 were considered significant.

Results

The mean age was similar in both groups. Pregnancy rates were slightly higher in day 3 embryo transfer (43.52 %) versus day 2 embryo transfer (40 %) but not statistically significant (Table-2).

There was no statistical significant difference in pregnancy rates based on the number of embryo transferred in both groups (Table-3, 4), however there was significantly difference in the quality and cleavage stage of embryo, day 2 embryo transfer (grad A 68.14 %, grad B 25.62 % and grade AB 6.23 %, 4 cell 98.19 % and 8 cell 1.8 %) versus day 3 (grad A 85.71%, grad B 11.84 % and grade AB 2.43 %, 4 cell 49.13 % and 8 cell 50.87 %) (Table-5).

Table 1:
Show the pregnancy rate in day 2 and day 3 according to patient age.

P value	Pregnancy Rate (%)			Age (Yr)
	Total	Day 3	Day 2	
0.109	26/57 (45.61)	5/17 (29.41)	21/40 (52.05)	18-25
0.336	49/110 (44.54)	17/33 (51.51)	32/77 (41.55)	26-30
0.252	29/77 (37.66)	8/16 (50)	21/61 (34.42)	31-35
0.568	14/35 (40.00)	6/13 (46.15)	8/22 (36.36)	36-39
0.518	7/26 (26.92)	1/6 (16.66)	6/20 (30)	≥ 40

Fig 1:
Show the pregnancy rate in day 2 And day 3 according to patient age

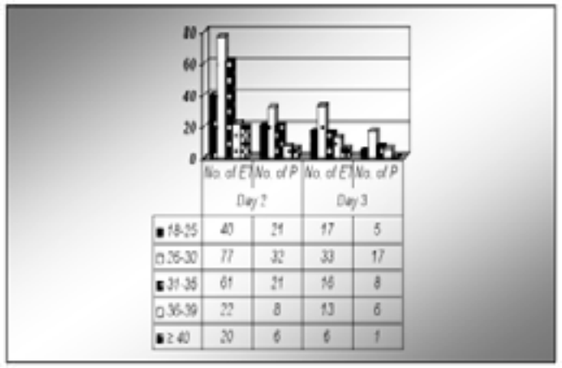


Table 2:
Comparison of pregnancy rate (PR) after Day 2 and Day 3 embryo transfer.

Transfer day	No. of Transfers	No. of Pregnancies	PR (%)	P-value
Day 2	220	88	40.00	0.574
Day 3	85	37	43.52	
Total	305	125	40.98	

Fig 2:
Comparison of pregnancy rate (PR) after Day 2 and day 3 embryo transfer.

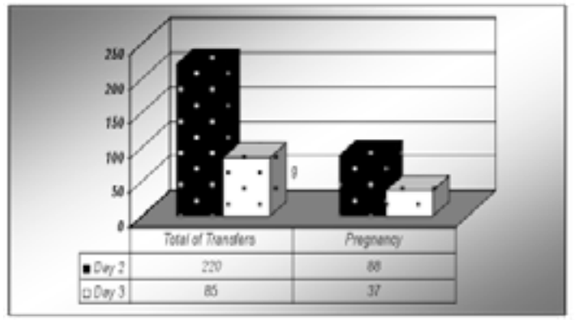


Table 3:
Comparison between the means of the transferred embryos in day 2 and day 3.

Number of Transferred Embryos	Day 2	Day 3	P value
Total No. of ET	722	287	
Mean of Embryos Transferred	3.50±1.32	3.44±1.34	(0.70)

Table 4:
Pregnancy rate (PR) based on the number of embryos transferred (ET).

No. of ET	PR (%)		Total	P value
	Day 2	Day 3		
1-2	16/49 (32.65)	11/22 (50.00)	27/71 (38.02)	0.164
3	12/46 (26.08)	5/12 (41.66)	17/58 (29.31)	0.291
4	34/75 (45.33)	13/34 (38.23)	47/109 (43.11)	0.488
≥5	26/50 (52.00)	8/17 (47.05)	34/67 (50.74)	0.725

Fig 3:
Pregnancy rate (PR) based on the number of embryos transferred (ET).

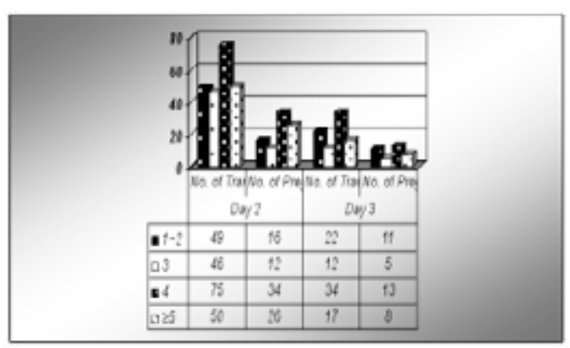
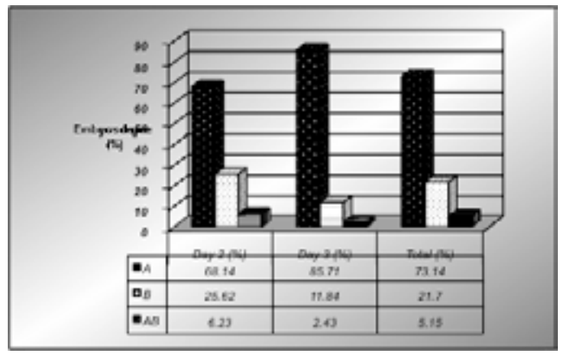


Table 5:
Comparison Embryos quality in day 2 and day 3 based on degree of fragmentation.

Embryo Degree	Day2 (%)	Day3 (%)	Total (%)	P-value
Excellent quality embryos (grade A)	492/722 (68.14)	246/287 (85.71)	738/1009 (73.14)	>0.0001
Good quality embryos (grade B)	185/722 (25.62)	34/287 (11.84)	219/1009 (21.70)	>0.0001
Moderate quality embryo (grade AB)	45/722 (06.23)	7/287 (02.43)	52/1009 (05.15)	0.014
Cleavage stages				
Second stage (>=4 cell)	709/722 (98.19)	141/287 (49.13)	850/1009 (84.24)	>0.0001
Third stage (>=8 cell)	13/722 (01.80)	146/287 (50.87)	159/1009 (15.75)	>0.0001

Fig 4:
Distribution of embryo grades on day 2 and day 3



Discussion

This prospective study compares day 2 and day 3 embryo transfer after oocyte retrieval in 305 patients, who were compared for age and number of embryo transfer but fixed for treatment protocol, all were short protocol. The pregnancy and embryo implantation rates were comparable in day 2 and day 3, although the pregnancy rate was slightly higher after transfer in day 3 than on day 2 (table 2), this difference was not statistically significant. This result is in agreement with results of Edward et al., 1984, Dawson et al., 1995, Oatway et al., 2004 and Ashrafi et al., 2007 (3,6-8).

A number of studies addressing the same issue as Huisman et al., 1994 (9) and Aboulghar et al., 2003 (5) in large retrospective study compared results after day 2 and day 3 embryo transfer, the pregnancy and implantation rates were similar in both groups. However some studies have reported positive effects of embryo transfer on day 3 than day 2 (10,11). Transfer of embryos to the uterus on day 3 after oocyte retrieval may be closer to the physiological time of arrival of embryo to the uterine cavity than transfer on day 2. Moreover delaying embryo transfer would allow the selection of the most vital embryos for transfer (12) and these factors may have

had positive effects. There was no significant difference in number of embryo transfer which was in agreement with (8).

In our study, the selection of excellent quality embryo grade A for transfer is one the most important factors with other factors (as endometrial receptivity, ovarian response and oocyte maturity) for successful ICSI program, this selection based on morphological criteria to select embryo showed better significant difference in day 3 than day 2, these results were in agreement with (13). Also the third cleavage stage was more in day 3 than in day 2. Dawson et al., 1987, reported that there is no difference in embryo quality between day 2 and day 3 in distribution of embryo grads which was in disagreement with our study which may be due to a delay of one day may be too short for use to better differentiate the quality of embryos (3). Delaying embryo transfer until day 3 provides an opportunity to observe the embryos for a further 24 hours in culture. Any morphologically normal embryos on day 2 which subsequently arrest or degenerate can be identified and their transfer avoided .This might have appositive effect on implantation rates and further successful pregnancy outcome.

Some authors believe that some suboptimal quality embryos may be rescued in uterine environment and that extended culture might be a cause of arrest for further development of such embryos (14).

So a large proportion of human embryos will arrest in vitro between the 4 and 8-cell stage (2). The percentage of second cleavage stage on day 2 embryo transfer was (98.19%) as compared to the (50.8%) third cleavage stage on day 3 (table 5). Yet this difference did not improve the pregnancy rate in day 3 over day 2 after we had the opportunity to exclude arrested embryos at 4 and 8 stage (table 5). The human cleavage stage embryo normally resides in the oviduct and does not enter the uterus until after compaction (15). The oviduct and uterus provide different nutrition environment for the embryo (16). In recent years, there for, several investigators (3) have tried amore extended delay of embryo transfer, up to blastocyst stage. Extending the culture period to beyond the time of expected activation of the embryonic genome might optimize the selection of viable embryos for transfer (17).

In addition, by delaying the embryo culture, embryos with limited and any abnormal development potential may be identified and avoided (2). Some chromosomally abnormal embryos fail to develop in culture (18). At present study, the embryo transfer with one day delay not only have no adverse effect on embryo quality and embryo transfer, but also it showed positive effects (non statistically significant) on pregnancy rate. Extending embryo culture period from day 2 to day 3 have positive effect on cleavage stage and quality of embryo, but had no adverse effect on pregnancy rate .Embryo transfer could be done on day 2 or day 3 according to Moreover these finding indicate that embryo transfer can be safely scheduled at the convenience of the patient and the center.

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Evaluation Of Tubal Patency Using Hysterosal pingography Following Salpingostomy And Medical Treatment Of Tubal Pregnancy

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Abstract

Objective: Ectopic pregnancy adversely affects the patency of the fallopian tube & consequently the future pregnancy. There are different modalities of conservative treatment of tubal pregnancy as methotrexate and salpingostomy1. There is little information about the success rate of maintenance of tubal patency after methotrexate & salpingostomy2. Therefore, the aim of this study is to evaluate the ipsilateral tubal patency by using hysterosalpingography, after treatment of tubal pregnancy by methotrexate therapy versus salpingostomy.

Patients and Methods

This was a case-series study that was done at Ain Shams & Al-Azhar University Maternity Hospitals to evaluate the ipsilateral tubal patency using hysterosalpingography (HSG) following salpingostomy and medical treatment of tubal pregnancy. This study included patients who were admitted at Ain Shams & Al-Azhar University Maternity Hospitals, for having tubal pregnancy & were treated either by methotrexate or salpingostomy, over a 3-year period, between January 2007 and December 2009. The patients seeking fertility were re-evaluated for fallopian tubes patency by hysterosalpingogram 3 months after discharge. The study included 2 groups of women: group I [n=200]: women who were treated by methotrexate, and group II [n=140]: women who were treated by salpingostomy.

Results

HSG was done in 200 cases of tubal pregnancy that were treated by methotrexate and 140 cases who were treated by salpingostomy. The patency of the ipsilateral tube was 85% after methotrexate treatment and 84.2% after salpingostomy. There was no statistically significant difference between the two groups.

Conclusion

The findings suggest similar success rate in maintaining the patency of the fallopian tube with either methotrexate & salpingostomy.
Key Words: Ectopic pregnancy – hysterosalpingography – methotrexate – infertility – salpingostomy.

Introduction

Ectopic pregnancy is a potentially life threatening condition3. The rate of ectopic pregnancy is increasing tremendously in the last decade4. On the other hand, the early diagnosis of ectopic pregnancy is being available due to the more sensitive ways of diagnosis as sensitive assays of β subunit of human chorionic gonadotrophins and high resolution transvaginal ultrasonography. Now, advances in treatment of ectopic pregnancy allowed conservative modalities for saving the tubes for future pregnancy as methotrexate & salpingostomy 5.

Methotrexate is a chemotherapeutic agent that acts by inhibiting DNA synthesis. Both systemic and local treatment can be useful in treatment of early unruptured ectopics6. Some women are not good candidates for medical treatment & need surgical treatment including females with the following characters7.8: 1) hemodynamically unstable patient 2) impending tubal rupture (severe abdominal pain) or > 300 ml of free fluid in pelvic cavity 3) breast feeding 4) hypersensitivity to methotrexate 5) Immunodeficiency. 6) Unable to complaint with postmedical treatment follow up. 7) Clinically important abnormality in baseline hematologic renal or hepatic laboratory values.

Hysterosalpingography is an important modality for diagnosing the patency of fallopian tube. If the results demonstrate obstruction of both fallopian tubes, the spontaneous pregnancy is impossible9. The equipment was a Legend CR9 (General Electrics, Hungary).

There are little information about the tubal patency among patients who had been treated by medical treatment and salpingostomy, so the results of subsequent HSG are viewed in the present study.

In this study, the ipsilateral tubal patency is evaluated by hysterosalpingography following treatment of ectopic pregnancy by methotrexate and salpingostomy.

Patients and Methods

This was a case-series study that was done at Ain Shams & Al-Azhar University Maternity Hospitals. The study included women who were admitted at Ain Shams University & Al-Azhar University Maternity Hospitals, for having tubal pregnancy & were treated either by methotrexate or salpingostomy, over a 3-year period, between January 2007 and December 2009. The patients seeking fertility were re-evaluated for fallopian tubes patency by HSG 3 months after discharge. The study included 2 groups of women: group I [n=200]: women who were treated by methotrexate, and group II [n=140]: women who treated by salpingostomy.

Inclusion criteria: patients diagnosed as tubal pregnancy & treated by single modality either by methotrexate or salpingostomy, no laparotomy or history of pelvic infections in the 3 months period after treatment. HSG was done under fluoroscopic observation with a balloon tipped catheters. An abnormality was documented if the dye was not seen to spill from the tubal end.

Technique: Hysterosalpingography is carried out within the first ten days after the last menstrual period and when menstrual flow has ceased. The patient is advised to abstain from sexual intercourse in the days after her menses and prior to the procedure, to ensure that she is not pregnant during the procedure. Using an aseptic technique, a speculum is used to distend the vagina and a Leech-Wilkinson is inserted into the uterine cavity. Diluted, water soluble, hyperosmolar iodinated contrast agent (urograffin) is then hand injected into the uterine cavity via the Foley catheter. A normal hysterosalpingogram depicts a smooth triangular uterine outline with opacification of both fallopian tubes and free spillage of contrast into the peritoneum.

Statistical analysis: All retrieved data were recorded on an investigative report form. These data were analyzed with SPSS® for Windows®, version 15.0 (SPSS, Inc, USA). Description of quantitative (numerical) variables was performed in the form of mean, standard deviation (SD) and range. Description of qualitative (categorical) data was performed in the form of number of cases and percent. Analysis of numerical variables was performed by using student's unpaired t-test (for two groups) or ANOVA (for more than two groups).

Results

A total 340 cases were recruited from January 2007 till December 2009 in 3 year interval. 200 cases were treated by methotrexate (group I) while 140 were managed surgically by salpingostomy (group II).

Table (1):
shows the demographic characteristics of the patients under the study.

	Group I (N=200) Mean	SD	Group II (N=140) Mean	SD	t	P
Age	26.8	4.6	27.1	3.8	0.7	0.4 (not significant)
Parity	2.03	0.8	2.1	0.9	0.9	0.3 (not significant)

Table (2):
shows the clinical characteristics of the patients under the study

	Group I (N=200) No. (%)	Group II (N=140) No (%)	X2	P
Previous PID	34 (17.0)	30 (21.4)	0.7	0.3 (not significant)
Previous laparotomy	18 (9.0)	12 (8.6)	0.02	0.9 (not significant)
History of infertility	31 (15.5)	25 (17.9)	0.001	0.9 (not significant)

Table (3):
shows a comparison between the two studied groups as regards the tubal patency (ipsilateral and contralateral tubes) after treatment (outcome).

	Group I No. (%)	Group II No. (%)	X2	P
Ipsilateral tubal patency	170 (85.0)	118 (84.2)	0.001	0.9 (not significant)
Contralateral tubal patency	163 (81.5)	125 (89.3)		

Table (4):
comparison between rates of occlusion among cases in group I as regards the number of doses of methotrexate

	Single dose (N=170) No. %	Two doses (N=30) No. %	X2	P
Patency	149 87.6	21 70.0	4.9	0.02 (significant)
Occulsion	21 12.3	9 30.0		

Table (5):
comparison between rate of patency in the ipsilateral and contralteral tubes in group II as regards the method of salpingiostomy

	Laparoscopy N=80 No. %	Laparotomy N=60 No. %	X2	P
Ipsilateral tubal Patency	68 85.0	50 83.3	0.001	0.9 (not significant)
Contralateral tubal patency	71 88.7	54 90.0		

Discussion

More than 25 years have past since the use of methotrexate as the accepted management of early cases of ectopic pregnancy in many hospitals. The patient characters were compared between the two groups. The mean age of group I, who were treated by methotrexate, was 26.8 years (± 4.6 SD), and mean parity was 2.03 (± 0.8 SD), while the mean age of group II, who was treated by surgical treatment, was 27.1 years (± 3.8 SD) and mean parity was 2.1 (± 0.9 SD). There is no significant difference statistically between the two studied groups as regards the mean age and mean parity. Table (1)

The risk factors of ectopic pregnancy were compared between the two groups. The two groups were compared as regards the presence of previous PID or laparotomy. There is a higher percentage of PID among group II compared to group I but the difference is not significant statistically. There is a higher percentage of previous laparotomy among group I compared to group II but the difference is not significant statistically. The two groups were compared as regards the previous history of infertility. Higher percentage of infertility among group II compared to group I but the difference is not significant statistically. Table (2)

The patency of the ipsilateral tube was compared between the two groups. There is no statistically significant difference between the two studied groups as regards the rates of tubal patency and occlusion after treatment. Systematic reviews involving 400 cases treated by parenteral methotrexate show tubal patency rates to be as high as 92%. Some regimens are treated by single dose protocol of methotrexate therapy at 50 mg/ M2 of surface area5. Further, Stoval6 stated 82.6% of ipsilateral tubal patency rate in patients received methotrexate. In a small randomized study of an ultrasound guided intratubal methotrexate injection versus linear salpingostomy, HCG levels decreased rapidly after surgery, with similar success rates & tubal patency. Based on this study, medical treatment of early ectopic pregnancy may carry a viable option of treatment and yields tubal patency rate comparable to surgical treatment7. Hajenius8 et al reported similar tubal patency 90% & 92% respectively in patients receiving single dose of methotrexate and cases treated by linear salpingostomy.

Also, similar studies as Guven11 2007 showed similar rates of tubal patency of 83.9%, Elito10 2006 stated 84% patency rate. In accordance, Fujishite12 2004 study reported 90% tubal patency after salpingostomy without tubal suturing and 94% in salpingostomy with tubal suturing. In a more recent study by same author 2008 reported 63.4% tubal patency after laparoscopic salpingostomy13
Colacurci14 1998, reported bilateral tubal patency in 90% of cases if HCG < 10.000 U/L, and 60% if HCG > 10.000 U/L. In studies done by Spalding15 1997, Keckstein16 1990, Vermesh17 1989, the rates of ipsilateral tubal patency after salpingostomy were 68%, 64%, 80%, respectively. In another study done by, Mordechai Pansky18 1989, in which tubal patency was examined by hysterosalpingogram after successful treatment by methotrexate, the rate of patency was 85.7%. Tolaymat19 1999 stated that the rate of tubal patency after methotrexate treatment was 72% & 81% after treatment by salpingostomy and he found no statistically significant difference between the two groups. Olofsson21 2001 in a similar study reported a similar patency rates after methotrexate and surgical treatment.

The contralateral tube was assessed in the two groups and there is a higher rate of occlusion of contralateral tube among medical treatment but the difference is not significant statistically. In the study of Guven11 2007, the rate of patency of the contralateral tube was 56.7%. Similar results of 81.5% was produced by Langer21 1990. In a study done in Sao Paolo by Elito10 2006, the contralateral tubal patency after methotrexate was 97% and 83% after salpingostomy with statistically no signifant differences between the two lines of treatment. Table (3)

The rate of occlusion among cases in group I as regards the number of doses of methotrexate was assessed. Higher percentage of patent tubes among cases with single dose and the difference is significant statistically this in agreement with Guven11 2007 who stated that tubal patency after single dose of methotrexate was 83.9% and 56.7% after multiple doses of methotrexate and he stated that multiple doses of methotrexate carry a negative effect on tubal patency. Table (4)

The rate of occlusion in the affected & contralateral tubes in group II is assessed as regards the route of salpingostomy. Higher rate of occlusion among cases done with laparotomy but the difference is not significant statistically. This is in agreement with Vermesh17 1989, who reported tubal patency 89% after laparoscopy and 80% after laparotomy with no significant difference between the two groups. In contrast, Lundorff22 1991, demonstrated that laparoscopic treatment of ectopic pregnancy results in less impairment of the pelvic status compared with conventional conservative surgery. Table (5)

In haemodynamically stable patients with unruptured tubal pregnancy, systemic methotrexate and laparoscopic salpingostomy were successful in treating the majority of cases. We found no significant difference between the treatments in the homolateral patency rate. Subsequent fertility outcome has to be awaited to show which treatment yields better fertility prospects.

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Aromastase Inhibitor Therapy For Symptomatic Uterine Leimyomata In Premenopausal Women

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Abstract

Objective: To examine the effect of letrozole on symptomatic leiomyomata.
Patients and Methods: In a prospective, intervention study , forty six premenopausal women with fifty two leiomyomata were invited to take 5 mg letrozole daily for 3 treatment cycles of 28 days each. Leiomyoma, uterine and ovarian volumes, endometrial thickness, gonadotrophins, and the scores of menstrual pictograms and Uterine Fibroid Symptom and Health Related Quality of Life (UFS – HRQoL) were recorded at the start and conclusion of this study.
Results: Letrozole resulted in 53.29% mean reduction in original leiomyomata volumes ($P < 0.01$) without significant reduction in myoma free uterine tissue. Estradiol levels dropped significantly from baseline levels (64.32 + 16.77) (mean + SD) to (40.83 + 12.032) pg/ml ($p<0.001$) at the end of this study.
Conclusion: Adding to its significant shrinkage of leiomyomta, letrozole improved leiomyoma related symptomology, quality of life
and menstrual pattern significantly.

Introduction

Uterine leiomyomata are the most common benign tumors in the female reproductive tract with an incidence ranging from 5.4 % to 77% (1), and are responsible for 33% of hysterectomies performed in the United States (2)

Ovarian steroids particularly estrogen are important factors for fibroid growth. This fact is supported by the increased incidence of symptoms from these tumors among women in their 30s and 40s (3), the rare occurance of myomas before puberty, their regression after menopause (4) and the higher expression of estrogen and progesterone receptors in uterine myomas compared to the normal myometrium (5), (6). Besides, deprivation of ovarian estrogen as seen during gonadotropin releasing hormone – agonist (GnRH a) therapy causes leiomyomata to shrink significantly (7).

Symptomatic leiomyomata treatment is mainly surgical; however, numerous hormonal agents have been used for their management (8). Aromatase, a member of cytochrome P450 superfamily is a microsomal enzyme that catalyzes the conversion of androstenedione and testosterone by hydroxylation to estrone and estradial respectively (9). Bulun and co-workers (10) in 1994 reported that aromatase m RNA concentrations are 1.5-25 times higher in leiomyomas than in the surrounding myometrium, which illustrates their ability to synthesize estrogen in situ and allows their independence from ovarian estrogen (11). Letrozole is a potent and highly specific nonsteroidal aromatase inhibitor that was approved initially for use in postmenopausal women with breast cancer to block estrogen production (12). This study was done to examine the efficacy of letrozole on symptomatic uterine leiomyomata in premenopausal women regarding reduction of volume and control of symptoms

Patients and methods

This prospective study was held in Obstetrics and Gynecology Department of Zagazig university hospital between July 2007 and April 2009.Forty six Egyptian women attending the outpatient clinic were enrolled for this study. All of the study participants were symptomatic premenopausal women with ovulatory menstrual cycles ranging from 26-30 days, they were presented with no more than two leiomyomata of at least 35 mm in diameter. Women with neoplastic, metabolic endocrine, renal, liver, hematologic and infectious disease, history of acute recurrent or past thromboembolic disease, body mass index (BMI) > 30 kg/m^2, history of osteopenia or osteoporosis or presence of hypoechoic or calcified leiomyomata detected at ultrasonography were excluded from this trial. Endometrial abnormalities as ovarian cysts detected by transvaginal ultrasound were among exclusion criteria. Women who smoke and women trying to become pregnant; requiring or requesting immediate surgical treatment were not included, as well. Patients were required to have a washout period of at least 3 months for hormonal medication before screening. Women fulfilling inclusion criteria were prescribed letrozole (Femara, Novartis Pharm A G, Basle Switzerland) 5 mg i.e 2 tables daily for 12 weeks. All women signed an informed consent after explaining aim and potential risks of treatment, and all of them agreed to use barrier contraception during study period.

Sonographic assessment

The examination included an intial 2 dimensional ultrasound assessment of the uterus using 7.5 MHz endovaginal probe and a 2.5 MHz abdominal probe of a Voluson Epert 730 TM (GE Medical systems, Zipf, Austria). Number and size of leiomyomas, uterine size, and ovarian size were assessed at the start of the study and at the end of first and third treatment cycles. The position of the fibroids within the uterus was not specifically recorded further to previous evidence that symptom of heavy menstrual bleeding do not appear to correlate with fibroid location (13). All dimension measurements (D1, D2, D3) were done twice and the mean was recorded (where D1= length, D2= anteroposterior diameter and D3= transverse diameter). Leiomyoma and uterine volumes were calculated by applying the ellipsoid formula; D1 × D2 × D3× 0.52 (14), using the integrated machine software. An arithmetic mean of the sizes was used in the presence of two leimyomas (15). All women were asked to describe their menstrual patterns and keep a menstrual calendar over the three cycles of treatment.

Menstrual pictogram (MP)

The severity of uterine bleeding was carefully recorded by each woman using the menstrual pictogram (MP) introduced by Higham et al (16) and modified and validated by Wyatt et al (17), the score is calculated in milliliters and is equivilant to the actual volume of blood lost.

UFS-Qol

Before treatment cycles and at the end of them, patients completed the Uterine Fibroid Symptom and Health Related Quality of life HRQL questionnaire (UFS-Qol) (18), with HRQL and its 6 subscales of: concern, effect on activities, energy mood, control, self consciousness, sexual function score and the score of symptom severity.

Laboratory study

Laboratory analyses included hematological, serum follicle stimulating hormone FSH, Luteinizing hormone (LH) and estradiol measurements at trial entry and at the end of the first and third treatment cycles. FSH and LH were measured with immunoradiometric assay (IRMA) using commercial kits (^{125} I – h FSH and ^{125} I- h LH IRMA Kits. Institute of Isotopes Company Ltd, Budapest, Hungary). Estradiol levels were obtained with radioimmunoassay (RIA) using commercial kits Ultra-Senstive, Estradiol RIA DSL 4800, (Diagnostic Systems laboratories, Webster TX).

Outcome measures

The change in liomyoma volume was set as the primary outcome measure in this study. A minimal required sample size of 33 leiomyomata was calculated to achieve 85% power at an alpha level of 1% in detecting a 50% change in the volume of leiomyomata with an anticipated volume of 160 ml and a standard deviation of 120 ml. The selected secondary outcome measures in this trial were changes in the uterine volume, difference between uterine and leiomyomata volumes, endometrial thickness, heamatocrit, gonadotropins, estradiol, menstrual pictograms and self-reported symptom and health related quality of life HRQL score. Adverse drug reactions were also secondary outcome measures among. Statistical analysis and sample size were done with SPSS 15.0 (SPSS Inc, Chicago, IL)

Results

Forty six women with fifty two leiomyomata were recruited for present study. Demographic characteristics of study population at the inception of this study are pointed out in table (1).
All women pulled through the study period despite the presence of side effects viz ; follicular cysts (13 patients; 27.7%), hot flushes (10 patients; 21.3%), Dyspareunia (8 patients, 17%), vaginal dryness (6 patients, 13%), hair thinning (5 patients, 10.9%) and headache (4 patients, 8%). These side effects were not serious and were well tolerated by affected women. The mean volume of uterine leiomyomata decreased significantly by the end of first treatment cycle (P< 0.001) and again after the last 2 cycles (P < 0.05) . table (2).

Table (1): Demographic characteristics of study population

Age (yr)	37.3 ± 5.6
BMI (kg/m²)	23.6 ± 2.3
Parity (n)	2.68 ± 1.1

*Values are reported as mean ± standard deviation

At study conclusion letrozole resulted in a mean 53.29% reduction of the original leiomyoma volume mean reduction ± SD was 57.72 ml ± 49.813. No change in leiomyoma volume by end of treatment was noted in 4 patients. For women at 40 years of age or older, there was significant leiomyoma size reduction, the reduction was non-significant for women forty years of age or younger. Fig. (1).

Table (2):
Variables studied at entry and throughout study period

Variable	Baseline	First cycle	Third cycle	Pvalue
Uterine size (cm3)	298.82± 163.62	219.23± 98.75*	199.88± 78.53	P<0.001
Leiomyoma size (cm3)	105.49± 112.31	73.32±72.16*	56.22± 61.04*	P<0.001
Δ Size *	159.31 + 46.2	152.6 ± 41.3	146.9±49.4	NS
Endometrial thickness (mm)	8.17 ± 4.21	8.02± 4.8	7.6± 3.8	NS
Ovarian size (cm)	9.71±1.75	10.94± 9.312	19.63 ± 15.174	P<0.01
Length of uterine bleediag(d)	4.9 ± 1.2	2.1±2.6	1.7±1.9	P<0.001
Hematocrit %	33.41±4.521	35.02±2.632	37.14±4.023	<0.001
FSH IU/L	7.31± 2.746	13.04±10.131*	15.62±11892	<0.01
LH IU/L	6.22±1.784	10.24±10.042*	11.46±11.173	<0.01
E2 Pg/mL	64.32±16.775	43.47±10.412*	40.83±12.032	<0.001

- Data are expressed as mean ± standard deviation.
- Δ Size : Difference between uterine size and leiomyoma size.
- Significantly different from previous result.

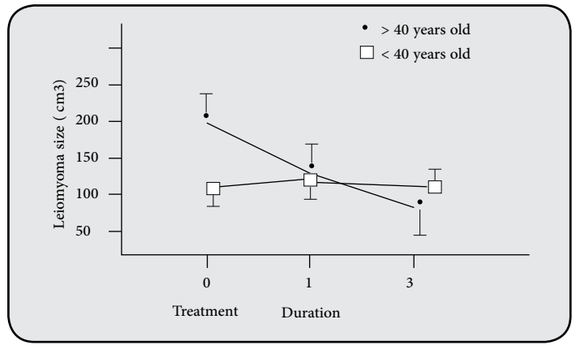


Fig (1) :
Changes in leiomyoma volume mean± standard deviation below and above age of 40 years

• P< 0.05 compared with previous volume.

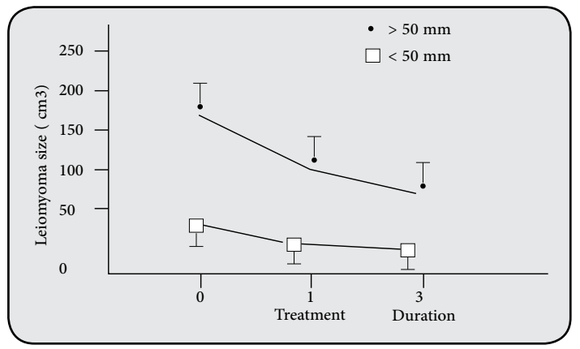
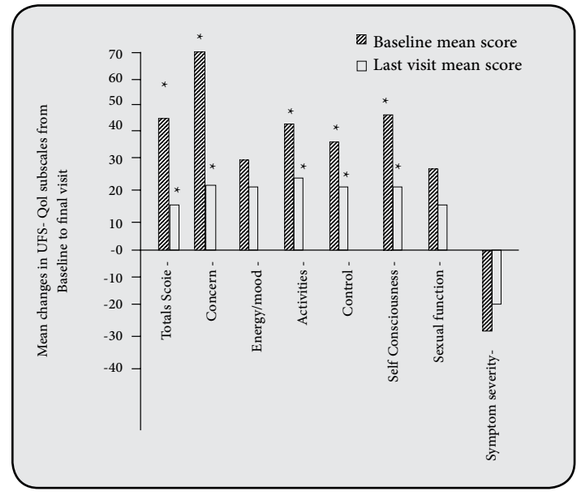


Fig (2) Changes in leiomyoma volume (mean± SD)

Concerning leiomyoma size, it was appeared that tumors > 50 mm in diameter showed significant reduction in size after the first ($p < 0.01$) and the last 2 cycles ($p < 0.05$) of letrozole therapy fig. (2). Reduction was not significant in small (50 mm or less) liomyomata. Mean uterine size decreased significantly by the end of the trial (mean reduction ± SD; 96.83 ± 86.15 ml) $p < 0.001$. The mean reduction was 31.12% of the original volume unlike leiomyoma, there was no relation between age af 40 years or older and the decrease in uterine volume. Letrozole was not found to have a significant effect on the normal myometrium (the difference between uterine and myoma volumes) as presented in table (2). Mean ovarian volume increased significantly at the end of 3rd treatment cycle compared to the end of first one, mean increase ± ± SD; 9.89± 14.42 ml, the mean increase was 46.38% above initial ovarian volume. Hematocrit was significantly increased at termination of this study ($p < 0.01$).

The mean increase in hematocrit value was 13.67% of the mean at the beginning. No interaction between hematocrit and age > 40 years was present. Levels of gonadotrophins studied showed a significant elevation after the first 28 days of therapy, levels continued to rise over the remaining period however it was not significant.



*Statistically significant at <0.05 level compared to baseline.

Fig (3):
UFS- Qol questionnaire analysis of mean changes in total score and subscales from baseline to final visit for symptom severity, a lower score = lower severity, for other scales higher = better quality of life.

Alternatively, estrodiol (E2) levels plumped significantly at the end of the first 28 days of letrozole intake. Levels continued to fall but the decrease was not significant. Mean estradiol (E2) level decreased by 36.52% below levels at study entry. The briefings collected from patients demonstrated a significant decrease in the self-reported scores of symptom severity i.e. vaginal bleeding, pelvic pain pressure, fatigue ($p< 0.001$), however there was no change in urinary frequency. The menstrual pattern improved significantly and women with regular cycles increased from 21% at baseline to 67% at the end ($p< 0.001$). Besides, HRQol scores and its 6 subscales showed significant improvement at final visit compared to the start of study fig. (3). At trial end, menstrual pictogram (MPs) calculated scores decreased significantly below those reported before treatment. Mean MP scores ∓ ∓ SD at baseline was 215.8 ∓ ± 107.6 ml and dropped to 59.3 ∓ ± 18.7 ml at final observation. Mean ∓± SD of difference = 126.5 ∓± 88.9 ml ($p < 0.05$)

Discussion

Letrozole (an an aromatase inhibitor), treatment in this study was associated with a significant (53.29 %) reduction , of leiomyoma size in premenopausal women after a three cycle-treatment ($p < 0.01$). These results are in concordance with those of GnRHa used alone in five randomized controlled trials (RCTs) (19). However, the significant reduction in uterine size 35 – 65 % (20), and the significantly increased proportion of woman with amenorrhea after 3 months of GnRHa treatment compared to placebo (51 % with nafarelin vs 8% with placebo $p < 0.05$) (21), clouded their benefits. Conversely, letrozole treatment as magnified in this study was not associated with amenorrhea despite significant reduction in (MP) scores.

Add – back regimens to overcome hypoestrogenic effect of GnRHa and to permit extension of treatment have been tried using medroxyprogesterone acetate (22), tibolone, (23) estrogen

alone (24) combined with progestogen (25), or raloxifene (15)

Raloxifene has been used alone in high dose to inhibit the growth of leiomyomata in premenopausal women but growth of new leiomyomata has been observed even during low dose treatment (15).There is limited evidence that add – back therapy can reduce menopausal symptoms and/or loss of bone with GnRHa .Results regarding uterine or leiomyoma size were inconsistent and even insignificant and it may be useful in older premenopausal women with lower background estradiol concentration (15).
Ru 486 (mifepristone); a high progesterone receptor affinity antiprogestin was tried as well, but it appears to have unopposed estrogenic effect with high rate of endometrial hyperplasia (26).

In this study there was no change in endometrial thickness throughout and after 3 months of letrozole treatment. More recently a selective progesterone receptor modulator (SPRMs) Asoprisnil has been tested but the uterine and fibroid volumes were reduced in a dose dependent manner. The largest 25 mg dose reduced uterine volume by 36% but caused a reversible suppression of menstruation, and variable effects on ovulation (27). So far, no RCTs evaluated the levonorgestrel releasing intra-uterine system in women with fibroids (28). Dopamine receptor agonists were recently shown to shrink myomas as an adjunct to surgical management (29). Rapid resumption of pretreatment uterine volume was remarkable with GnRHa. The only drug known to have a "carry-over effect" in which the size of the uterus continues to decrease after treatment discontinuation is gestrinone, however, androgenic side effects viz. weight gain, seborrhea / acne are limitations to its use (30).

Whether the volume reduction observed with letrozole is sustained after treatment discontinuation or not needs to be evaluated. The same applies to anastrazole (another aromatase inhibitor). Nevertheless, previous studies (31), (32) pointed out that the half life of both letrozole and anastrazole is between 40 and 50 hours and within 4 weeks of stopping them little drug remains.

The non myoma uterine volume was significantly reduced in many studies addressing GnRHa effects on uterine size (33). This was not the case in this study where the difference in non myoma uterine volume from baseline to the end of trial was insignificant. Other studies employing letrozole (34), (35) or anastrazole (36), (37) agreed with findings of this study. This could be explained by the fact that levels of aromatase mRNA are 1.5 – 25 times higher in the leiomyoma than in surrounding myometrium (10). Shrinkage of myomas in postmenopausal women treated with aromatase inhibitors is a proof of the above – mentioned explanation since ovarian estrogen production in these women is nearly absent (38). The significant reduction of leiomyoma volume in women over 40 years was noted in this study and other studies using aromatase inhibitors (37) declares a remarkable effect on this age group.

In this study, the location of myomas was not specified because previous studies with (37) or without (13) aromatase inhibitors showed no effect of leiomyoma location on tumor volume reduction or menstrual loss. Estradiol levels decreased significantly $p < 0.01$ by the end of the first treatment cycle, the reduction continued till the end however it was not significant. Same results were replicated in previous studies (39) which pointed out the significant drop in estradiol levels in premenopausal women 4 to 5 weeks after the start of letrozole treatment.

It is conceivable that letrozole a competitive inhibitor of aromatase which is the rate limiting step in conversion of the androstenedione and testerone to the estrone and estradiol respectively (9), so aromatase inhibitors inhibit both gonadal and ovarian estrogen production unlike the majority of medical therapies that impact ovarian estrogen production only. Indeed, aromatase inhibitors have been shown to suppress estrogen levels to 95% of their pretreatment values in postmenopausal women where estrogen production is derived from a non gonadal source (40). More recently Bedaiwy and co-workers (41) manifested that letrozole prevented the estrogen associated flare effect known to happen 1 day after the injection of GnRHa in premenopausal women with endometriosis. Consequently, estrogen serum levels start to decline as early as the first day after aromatase inhibitors administration (42) indicating the superiority of aromatase inhibitors over GnRHa in medical treatment of leiomyomata. Using another potent aromatase inhibitor, anastrazole. Varelas and colleagues could not detect any significant change in estradiol levels throughout their three (28 days) cycles of treatment (36).

Previous reports demonstating that letrozole suppresses plasma estradiol and estrone sulphate more completely than anastrazole in postmenopausal women with breast cancer (43) could provide an explanation of this dichotomy. Besides, women in this study received 2 tablets 5 mg versus 2.5 mg/day in the previous (43) study, the anastrazole dose however was the same in both studies, so the increased estrogen suppression of letrazole may be dose dependent. Letrozole is known to be the most potent agent of the third – generation aromatase inhibitors in vitro (44). The answer to whether the greater estrogen suppression with letrozole is clinically relevant will have to await further trials.

Gonadotropins (FSH, LH) increased exponentially after treatment $p < 0.01$. Conversely earlier studies (39) reported their reduction 4-8 weeks after treatment using letrozole. Again using anastrazole Veralas et al (37) failed to find any change in gonadotropins. In this study, Follicular Cysts developed in 11 patients (23.9%).

Ovarian size increased remarkably for each treatment cycle $p < 0.01$, same was noted by Gurates et al (34). These findings were expected as letrozole is used to induce ovulation (45). Oral contraceptive were not allowed in this study to get the probable almost benefit of estrogen suppression induced by letrozole. It is pertinent to mention that estrogen lowering drugs may promote osteoporosis or decrease bone mineral density (BMD), increasing the number of bone fractures in patients (46). Even though, further studies are required to confirm this. Earlier experimental studies (47), actually, recorded that letrazole did not affect BMD as well as recent clinical studies (34). Finally, significant improvement of quality of life and hematocrit values was noted in this study. Similarly improvement was reported in previous studies using other agents (20), (27).

In conclusion, this study is not blinded, controlled or randomized which is the stigma of studies devised to demonstrate effectiveness of medical therapies in uterine leiomyomata. This underscores the difficulty of making definitive conclusions above the safety of drugs manipulating hormones in premenopausal women with fibroids. High-quality RCTs are urgently needed for this common problem.

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Ovarian Volume Assessment In Relation To Histologic Findings And Sex Hormone Levels In Women With Postmenopausal Bleeding And Thickened Endometrium

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Abstract

Objectives: The aim of the present study was to verify the association of ovarian volumes with histologic findings and sex hormones levels in women with postmenopausal bleeding and thickened endometrium.

Patients and Methods

A prospective observational study was done on 90 women with postmenopausal bleeding and thickened endometrium (≥ 5mm). They underwent vaginal sonography (7.5 MHz probe) for ovarian volumes measurement. Blood samples were collected for sex steroid hormones assay. In addition, endometrial sampling was done for definitive histologic diagnosis.

Results

According to histologic results, 18 cases (20%) had endometrial adenocarcinoma, 24 cases (26.7%) had endometrial hyperplasia with or without atypia and 48 cases (53.3%) had benign histologic findings. Large ovaries were significantly associated with BMI≥ 30 (p= 0.002) and endometrial adenocarcinoma (p< 0.001). High mean ovarian volume in adenocarcinoma was positively associated with high serum level of estradiol (p< 0.001), serum testosterone (p= 0.04) and serum free testosterone (p< 0.01) compared with other histologic findings.

Conclusion

Large ovaries among women with postmenopausal bleeding and thick endometrium were associated with elevated serum sex steroid hormones and represent a marker of risk for endometrial adenocarcinoma. The present results call for larger studies to further elucidate ovarian volume associated with serum sex steroids as screening tools in predicting endometrial carcinoma in obese asymptomatic, bleeding-free postmenopausal women.

Keyword: Postmenopausal bleeding; Thickened endometrium; Endometrial adenocarcinoma; Ovarian volume; Sex steroid hormones

Introduction

Postmenopausal endometrial thickening is a nonspecific finding that may be caused by a variety of conditions, such as carcinoma, polyps, hyperplasia, endometritis, or cystic atrophy. However postmenopausal bleeding is usually the first symptom, only 10-15% of women with postmenopausal bleeding will actually have an endometrial cancer and the risk become low when double-layer endometrial thickness is < 5 mm [1, 2]. Postmenopausal women with high levels of circulating estrogens or androgens are at increased risk for developing breast and endometrial cancer [3, 4]. Recognition that aromatization of androgens to estrogens in peripheral adipose tissue represents the main source of circulating estrogens among postmenopausal women, thereby linking obesity, elevated circulating estrogen levels, and increased endometrial carcinoma risk [5]. Postmenopausal ovaries consist largely of stroma, which includes hormone synthesizing cells. Larger ovaries were more likely to contain luteinized cells and hilar cells, overall suggesting a link between size and potential for hormone synthesis [6]. Ovarian stromal hyperplasia and endometrial cancer are often identified concurrently, suggesting that ovarian morphology may represent a marker of cancer risk among older women [7]. This association may reflect increased production of androgen, the main hormone product of the postmenopausal ovary. Our aim to analyze the relationships between ovarian volumes and endometrial histologic findings, serum sex hormones levels in women with postmenopausal bleeding and thickened endometrium.

Materials and methods

This study was carried out between March 2008 and February 2010 in the department of Obstetrics and Gynecology, Ohoud hospital, one of the Taibah university hospitals, Al-Madinah Al-Munawarah province, Saudi Arabia. A series of women with one or more episodes of postmenopausal vaginal bleeding were participated in this study. The inclusion criteria were (1) postmenopausal bleeding, defined as vaginal bleeding after 12 months of menopause in women older than 45 years and (2) double layer endometrial thickness of ≥ 5 mm as measured by baseline transvaginal sonography. Exclusion criteria were (1) endometrial thickness < 5mm, (2) use of any kind of hormone replacement therapy in the 6 months prior to the study and (3) both ovaries cannot be visualized by transvaginal sonography.

Diagnostic work-up included, complete medical history, physical examination and transvaginal ultrasound examination (TVU) using (Toshiba SSA 270A/ HG Tokyo Japan, vaginal probe 7.5 MHz). Maximal endometrial thickness (double layer) was measured in the longitudinal plane. As stated earlier, only patients with endometrial thickness of ≥ 5 mm were included. Written informed consent was obtained from all patients.

To estimate the ovarian volumes, the following ovarian dimensions were measured; maximum longitudinal (D1), anteroposterior (D2), and transversal (D3) diameters. Then, ovarian volumes were calculated as: D1 ×D2 ×D3 ×0.523 [8]. Mean ovarian volume was calculated when both right and left ovaries could be measured by ultrasound, when only one ovary could be measured by ultrasound, its measurement was considered to be the patient's ovarian volume.

All studied women had donated a blood sample at time of ultrasound evaluation that was assayed for estradiol, estrone, sex hormone-binding globulin (SHBG), androstenedione, testosterone and free testosterone using ELISA (GenWay Biotech, Inc, San Diego, California, USA). In addition, the participants underwent endometrial sampling within few days by hysteroscopy or dilatation and curettage (D and C). Definitive histologic diagnosis was obtained in all cases that were included in this study.

Body mass index (BMI) was calculated by dividing weight in kilograms by height squared (m2), and categorized as <25.0, 25.0–29.9, and ≥30.0 kg/m2 [9]. Data related to age at menopause and parity (0 vs. 1+) based on the number of vaginal deliveries and/or C-section performed was obtained through women interviews.

The statistical analysis was made using the Statistical Package for the Social Sciences (SPSS) Version 13 for Windows (SPSS, Chicago, IL). Values are given as mean± SD or number (percentage). Paired t- test was used for quantitative variables and the $\chi 2$ or Fisher exact test for qualitative variables. Levels of SHBG and sex steroids were log transformed to normalize their distribution. We assessed the mean ovarian volume in each histologic group to log-transformed hormones and SHBG levels using similar methods. P≤0.05 was considered significant with a 95% confidence interval (CI).

Results

During the study period, 103 women with postmenopausal bleeding and thickened endometrium (≥ 5mm) were evaluated. 13 patients were excluded. The following findings led to exclusion: no definitive histopathologic diagnosis, 4 patients; ovarian cyst, 3 patients and impossibility to measure any ovary, 6 patients.
Only 90 women were included ultimately. Five women underwent hysterectomy due to recurrent postmenopausal bleeding, but they were included in our study after they had definitive histologic diagnosis. According to histologic results, 18 cases (20%) had endometrial adenocarcinoma, 24 cases (26.7%) had endometrial hyperplasia with or without atypia and 48 cases (53.3%) had benign histologic findings (cystic atrophy, endometrial polyp and submucous myoma).

Epidemiologic and medical characteristics of the sample are shown in Table (1), adenocarcinoma showed a significant higher age at menopause and higher BMI (p=0.033), (p<0.001) respectively. Table (2) showed that mean ovarian volume decreased from 2.03 cm3 among women aged 50 years or less to 1.89 cm3 among women aged 70 years or older but there was no significant difference (p= 0.071). Increased ovarian volume was associated significantly with both higher BMI ≥ 30 (p= 0.002) and endometrial adenocarcinoma (p< 0.001).

Among the studied sample, the women presented with endometrial adenocarcinoma and high mean ovarian volume had significantly higher serum levels of estradiol (p< 0.001), testosterone (p= 0.04) and free testosterone (p< 0.01) compared with the other two histologic findings, Table (3).

Table (1):
Epidemiologic and medical characteristics in women with postmenopausal bleeding according to histologic results of thickend endometriuma. a

Characteristics	Benign[b] N=48	Hyperplasia N=24	Adenocarcinoma N = 18	P-value
Age (y)	58.8 ± 4.2	59.3 ± 3.6	61.2 ± 4.0	0.081
Parity (%)				
-Nulliparous	18.50	14.32	20.75	0.17
-Parous	81.45	85.70	79.25	
BMI (kg/m2)	24.3±2.3	25.6±4.2	28.7±7.4	<0.001
Age at menopause (y)	46.2 ± 1.3	48.2 ± 4.2	54.1 ± 2.1	0.033
Diabetes (%)	14.8	16.2	18.75	0.70
Hypertension (%)	25.9	28.5	31.2	0.30

a Values are given as mean ±SD or number (percentage).
b Benign endometrial histology included (cystic atrophy, endometrial polyp, and submucous myoma)

Table (2):
Mean ovarian volume (cm3) in relation to age, parity, BMI (kg/m2) and histologic results of thickened endometrium in women with postmenopausal bleeding.

Variable	N	MOV (cm^3) (95% CI)	P-value
Age(y)			
≤ 50	13	2.03 (1.91-2.14)	
51-57	22	1.97 (1.86-2.05)	
58-64	38	1.96 (1.84-1.99)	0.071
≥ 70	17	1.89 (1.80-1.94)	
Parity			
-Nulliparous	18	1.81 (1.77-1.89)	0.18
-Parous	72	1.83 (1.75-1.90)	
BMI (kg/m2)			
- < 25	18	1.73 (1.69-1.87)	
- 25-29.9	30	1.85 (1.80-1.96)	0.002
- ≥ 30	42	2.08 (1.94-2.12)	
Histologic results			
-Adenocarcinoma	18	2.10 (1.99-2.13)	
-Hyperplasia	24	1.91 (1.87-1.98)	<0.001
-Begnin histology	48	1.80 (1.74-1.84)	

MOV: mean ovarian volume

Table (3):
Mean (95% confidence interval) of serum sex hormones by ovarian volume among studied women.

Steroid hormone	Benign MOV (1.80cm3)	Hyperplasia MOV (1.91cm3)	Adenocarcinoma MOV (2.10 cm3)	P-value
Estradiol (pg/ml)	5.1 (2.6-7.3)	6.3 (3.1-8.1)	10.8 (8.2-13.4)	<0.001
Estrone (pg/ml)	32 (27-39)	33 (26-42)	35 (29-45)	0.25
SHBG (nmol/l)	26.8 (22.1-36.2)	25.6 (23.2-38.4)	26.1 (20.1-35.2)	0.70
Androstenedione (ng/ml)	52 (40-61)	54 (42-65)	53 (42-60)	0.31
Testosterone (ng/ml)	0.43 (0.20-0.51)	0.52 (0.32-0.62)	0.61 (0.48-0.59)	0.04
Free Testosterone (ng/ml)	2.1 (1.6-2.8)	3.2 (2.4-3.7)	6.4 (3.8-8.7)	<0.01

SHBG: sex hormone binding globulin.
MOV: mean ovarian volume

Discussion

The main finding in this study was that, ovarian volume measurement associated with serum sex steroids are good diagnostic tools in predicting endometrial carcinoma in patient with postmenopausal bleeding and thick endometrium. Previous analysis had considered large postmenopausal ovaries as a marker of risk for endometrial carcinoma [7, 10]. Ovarian enlargement in women presented with postmenopausal bleeding and thick endometrium may represent a marker of hormonal imbalance mostly higher androgen level (current, past or at both times) indicating greater availability of substrate for estrogen synthesis in peripheral adipose tissue which is a factor that could increase risk for endometrial cancer [11]. Transvaginal sonography is currently considered as first step to rule out endometrial carcinoma in women with postmenopausal bleeding when endometrial thickness is < 5mm [1, 12]. However, a thick endometrium is a non specific finding; most current protocols include use of hysteroscopy or endometrial office biopsy for histologic diagnosis [13, 14]. For purpose of this study, we included only women with thick endometrium (≥ 5 mm) because they have a high risk for endometrial cancer [15]. Due to this selection and small sample size, our incidence for endometrial adenocarcinoma was higher (20%). Ovarian assessment in this study was based on transvaginal ultrasound precluding assessment of characteristics such as ovarian stromal hyperplasia, however in non cystic postmenopausal ovaries, stroma accounts for great majority of volume [16]. The present study showed that obesity was associated with increased endometrial cancer risk in postmenopausal women as was established previously [17]. The prevailing hypothesis is that this association can be explained by increases in the amount of bioavailable estrogens in the circulation and endometrial tissue via peripheral conversion of adrenal and ovarian androgens mostly within adipose tissue [18]. In this analysis, the ovarian volume declined from 2.03 cm3 to 1.89 cm3 among women aged 50 years or less and women aged 70 years or more respectively but the magnitude of change was small and not significant.

Previous studies that were done on asymptomatic, bleeding-free postmenopausal women reported inverse associations between ovarian volumes determined by ultrasound and age [10, 19]. In our study, the non significant decline in ovarian volume with age might be due the presence of 20 % women with postmenopausal vaginal bleeding, diagnosed as endometrial adenocarcinoma and had significantly large sized ovaries. There is elevated risk of endometrial cancer among women with late age at menopause [20], this was observed in our study; the women with endometrial adenocarcinoma had a significantly higher menopausal age compared with other histologic groups. The finding that obesity is associated with increased endometrial cancer well established [20]. The present results revealed significant association between large ovaries and higher BMI; this was in accordance with others [10, 21]. Obese women (BMI ≥ 30) well known to have insulin resistance and compensatory hyperinsulinemia which play a role in ovarian enlargement observed in these women [21, 22].

The larger ovarian volume among postmenopausal women was associated with increased risk of endometrial cancer and it was greatest for women with largest ovarian volumes [10, 23]. This was in consistent with our findings that endometrial adenocarcinoma was significantly associated with larger sized ovaries relative to other histologic groups. Indeed increased ovarian volume and relatively high serum concentration of estrogens and free testosterone in postmenopausal women were associated with an increased risk of endometrial cancer [20,23], this observation was confirmed by our findings that large ovaries in postmenopausal women with endometrial adenocarcinoma was associated with significant increase in serum levels of estradiol, free testosterone and testosterone. A large recent prospective study showed that circulating blood levels of estrogens, free testosterone and to a lesser extent total testosterone are positively associated with an increased risk of endometrial cancer in postmenopausal women, also they suggested that free testosterone may be an important determinant of endometrial cancer risk in postmenopausal women and this association could be a result of peripheral conversion of these androgen to estradiol [20]. In conclusion, our analysis suggested that enlarged ovaries in women with postmenopausal bleeding and thickened endometrium is associated with endometrial adenocarcinoma risk and represent a marker of the availability of the androgens for peripheral estrogen synthesis, whereas, obesity affects the degree of conversion. Our work call for larger studies regarding the use of ovarian volume assessment associated with serum sex steroids as screening tools in predicting endometrial carcinoma in obese asymptomatic, bleeding-free postmenopausal women.

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Uterine Receptivity Assessed By Three Dimensional Ultrasound and Power Doppler in Women with Polycystic Ovary Syndrome Treated By Clomiphene Citrate Alone or Combined To Metformin

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Abstract

Objective: A prospective randomized single blinded study was carried out to examine whether metformin when combined with clomiphene citrate (CC) had an added effect, if any, on uterine receptivity.
Patients and Methods: Seventy seven egyptian women with polycystic ovary syndrome (PCOS) aging between 20-35 years were randomized to 2 groups received either 100 mg cc for 5 days alone, or with metformin 850mg bid throughout menstrual cycle. Three dimensional (3D) ultrasound with power Doppler examination was done on day 2 after human chorionic gonadotropin (hCG) injection for both groups and; endometrial volume, vascularization index (VI), flow index (FI), and vascularization flow index (VFI) of endometrial and subendometrial regions were measured.
Results: Patients taking metformin, and those destined to be pregnant had significantly lower VI/FI/VFI in endometrial region. Corresponding decreases of values in subendometrial region were insignificant. Serum estradiol (E2) levels on day of hCG injection were significantly lower in cycles, where metformin was added.
Conclusion: Metformin had significantly lowered VI/FI/VFI in early luteal phase in PCOS affected women.

Key Words: Uterine Receptivity, Three Dimensional Ultrasound, Power Doppler, Polycystic Ovary Syndrome, Clomiphene Citrate, Metformin

Introduction

Polycystic ovary syndrome (PCOS) affects 4-12% of women of reproductive age (1). In May 2003 a joint consensus meeting of the European Society of Human Reproduction and Embryology (ESHRE), and the American Society of Reproductive Medicine (ASRM) held in Rotterdam defined PCOS as the presence of 2 of the following 3 criteria: (a) Oligo or anovulation (b) polycystic ovaries on US with 12 or more follicles measuring 2-9mm in diameter and/or increased ovarian volume >10cm3. (c) hyperandro-genism clinical and/or biochemical with the exclusion of other etiologies (2).

The pregnancy rate among PCOS women is only 40-50% even when other factors are excluded and ovulation induction is successful (3), (4). Moreover PCOS is associated with an increased rate of early pregnancy loss of 30-40% (5), due to luteal phase defect (3). The realization that features of PCOS appear to be caused by insulin resistance led to an explosion of interest in the use of insulin sensitizing drugs like metformin. In 1998 a randomized controlled trial (RCT) which reported high rates of ovulation in women treated with metformin and clomiphene fuelled this interest (6).

In addition recent RCTs have shown that women with PCOS who have reported ovulation after metformin exhibited surprisingly high reproductive potential with lower than expected rate of spontaneous miscarriage (7). Subsequent papers; however, have not shown metformin to be as effective as some of the early reports had suggested (8)(9). Being invasive histological analysis of endometrial biopsy (10), endometrial cytokines in uterine flushing (11), the genomic study of a timed endometrial biopsy (12) as methods of predicting uterine receptivity are impossible to be done in treatment cycles.

As a non-invasive method, Ultrasound examination of uterine receptivity has been widely used in spontaneous or stimulated cycles (13). Unfortunately, when using two dimensional (2D), endometrial thickness, and pattern have low positive predictive value and specificity for IVF outcome (14), (15). On the other hand, endometrial blood flow can be non-invasively evaluated by 2D or 3D ultrasound with color and power Doppler. Power Doppler is more sensitive than color Doppler imaging at detecting low velocity flow and hence improves visualization of small vessels.

Blood flow and vessel patterns are demonstrated by encoding the power in the Doppler signal rather than its mean frequency shift (16), (17). This prospective study was carried out to study whether metformin when combined to clomiphene citrate had an effect – if any – on uterine receptivity using 3D ultrasound and 3D power Doppler study of the endometrium and subendometrial region.

Patients and Methods

This prospective single blinded study was conducted on egyptian women attending the infertility clinic in Obstetrics and Gynecology department, Zagazig university from 1/6/2008 till 1/9/2009. The women's age range was 20-35 years. Of all the women attending the infertility clinic; eighty seven women satisfying the ESHRE and ASRM criteria for PCOS were included in this study (2). Ten women failed to ovulate during both arms of the study and were discontinued.

All had normal serum prolactin concentrations and thyroid function tests. Levels of 17 α hydroxy-progesterone were examined to rule out late onset congenital adrenal hyperplasia, all patients had normal values of < 2ng/ml. Waist to hip ratio to detect presence of android obesity was measured in all patients (waist to hip ratio > 0.85) (18). Free androgen index (FAI) was calculated for all women in the study. Women who intended to start a diet or specific physical activity program, affected by organic pelvic diseases, previous pelvic surgery, suspected peritoneal factor infertility, tubal or male factor infertility were excluded.

All participants had normal baseline FSH values and estradiol values on cycle day 2. Women were then monitored in stimulated cycles during which they were randomized to receive either 100mg of clomiphene citrate (Clomid; Merrell Dow, Middlesex, UK) daily for 5 days and metformin (850mg twice daily) with meals throughout menstrual cycle; or clomiphene citrate (CC) alone.
The women were blinded to metformin and placebo which was packaged by US and provided to patients. 3D ultrasound and 3D power Doppler indices on day 2 after hCG injection (hCG +2), and 7 days later serum progesterone levels were obtained. Serum estradiol (E2) values were measured on the day of hCG injection. All ultrasound measurements were performed 2 days after injection of hCG using Volusan 730 PROV (GE, Kretz, Zipf, Austria). Once a longitudinal view of a satisfactory grey scale image of the uterus had been obtained, the uterus was centralized within the 3D sector on the screen. The ultrasound machine was then switched to the 3D mode with power Doppler. The setting conditions were pulse repetition frequency 1, control frequency mid, colour gain 38.4, dynamic set: 2, balance: G>140, wall motion filter 75, frame rate 4-6, rise 0.2 persistance 0.8. These settings were found to offer the best compromise between small vessel detection and Doppler artefact (19). The setting condition for the subpower Doppler mode was gain – 6.0; balance: 140; wall motion filter: low 1.

When the power Doppler mode was switched on, the power Doppler box was positioned to cover the whole uterus and volume mode was then switched on. The resultant truncated sector covering the endometrial cavity in a longitudinal plane of the uterus was adjusted and moved and the sweep angle was set to 90o to ensure that a complete uterine volume encompassing the subendometrium was obtained. The patient and the 3D 7.5 MHz transvaginal probes remained as still as possible during volume acquisition; the volume of the endometrium was determined by manually drawing the endometrial outlines. The integrated VOCAL version 4.0 (Virtual Organ Computer Aided Analysis) – the imaging program for the 3D power Doppler histogram – was employed to calculate the endometrial volume and indices of blood flow within the endometrium. Vascularization index (VI) which is the ratio of the number of colour voxels to the number of all the voxels expressed as a percentage (%) of endometrium volume was used to represent the presence of endometrial vascularity. Flow index (FI) the mean power Doppler signal intensity inside the endometrium was used to express the intensity of flow, then vascularization flow index (VFI) multiplying VI and FI was calculated to combine the aforementioned indices (18). These parameters are unitless with the exception of the VI hat is expressed as a percentage.

The manual mode of the VOCAL contour editor was used throughout analysis and calculation with a 15o rotation step. As a result 12 contour planes were defined for the endometrium of each patient to cover 180o. The shell imaging technique which allows the user to generate a variable contour that parallels the original defined surface contour was used to examine the subendometrium. Subendometrial region was considered to be within 2 mm of the originally defined myometrial – endometrial contour. This is an arbitrary distance but one that reflects the inner third of the myometrium and the region supplied by radial arteries (20). VI, FI and VFI of the subendometrial region were then measured. The bias due to inter-observer error was avoided as ultrasonographic assessments were done by single operator.

To test intraobserver reliability of measures, the intraclass correlation coeffecients with 95% confidence interval was determined using one – way random effects model (21). It was calculated by scanning 20 patients twice and analyzing each 3D dataset twice to assess consistency of 3D scanning and data acquisition. For the endometrium, the mean intraobserver correlation coefficient (95% CI) for volume, VI, FI, VFI were as follows; 0.95 (0.86, 0.98), 0.99 (0.97, 0.996), 0.89 (0.72, 0.96) and 0.99 (0.98, 0.997) respectively. The mean (95% CI) intraobserver correlation coefficient and the intraobserver variation was not statistically significant.

A urine pregnancy test was done 18 days after hCG injection. If it was positive, ultrasound examination was performed 10-14 days later to confirm intrauterine pregnancy and to determine the number of gestational sacs present.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS release 15.0, SPSS. Inc., USA). The primary outcome measures were endometrial volume, VI, FI, VFI for endometrial and subendometrial region as well as occurance of clinical pregnancy. As measures were not normally distributed a non parametric test Wilcoxon signed ranks test or Fisher exact test and X2-test were employed to test association between measures of CC stimulated cycles and cycles when metformin was added. Two-tailed $P \leq 0.05$ was taken as significant.

Results

Of the 87 women, who entered the study, 10 failed to ovulate in response to the first CC induction. This left 77 eligible women, 39 of whom were randomized to metformin, and 38 to placebo. All the 77 women ovulated in response to the second CC induction. Baseline characteristics and early follicular (day 3) serum FSH, estradiol levels differences between both groups were insignificant as demonstrated in table (1). Endometrial subendometrial blood flow was totally absent in 3 patients (8%) in CC alone arm versus one patient (3%) in CC –metformin arm. Table (2) illustrates differences in ultrasound parameters on day 2 after hCG injection, estradiol level on day of hCG injection, as well as, pregnancy rate between study groups. Pregnancy rate was significantly higher in metformin receiving PCOS women. Ultrasound parameters between those who became pregnant and those, who failed to conceive on day 2 after hCG, and estradiol levels on hCG injection day are presented in table (3).

Table (1):
Baseline characteristics of participant groups.

Parameter	CC n=38	CC + metformin n=39	P value
Age (yr)	25.8 ± 2.8	26 ± 1.6	0.82
Duration of infertility (yr)	4.2 ± 2.3	3.9 ± 2.5	0.91
History of EPL* (%)	7/38 (19%	7/39 (18%)	0.97
BMI (kg/m^2)	26.7 ± 2.3	27.1 ±1.8	0.76
Fasting glucose (mmol/L)	5.3 ± 0.2	4.8 ± 0.7	0.25
Fasting insulin (pmol/L)	308.2 ±52.1	240.3 ±67.2	0.33
Free Testosterone (pmol/L)	32.8 ± 7.0	29.8 ± 3.1	0.77
Free Androgen index (%)	13.2 ± 4.8	14.8 ± 4.2	0.63
FSH (IU/L)	7.3 ± 1.7	7.2 ± 2.0	0.97
E_2 (pmol/L)	359.1 ± 51.1	340.6 ± 54.3	0.87

Values are mean ± SE.
* EPL = Early pregnancy loss.
P<0.05 is considered significant.

Table (2):
Ultrasound parameters, estradiol levels, pregnancy rates in studied patients.

Parameter	CC alone n=38	CC - metformin n=39	P value
Estradiol on day* of hCGinjection (pmol/L)	1897.93±1396	1674.72±1238	NS
Endometrial volume+ (cm^3)	4.42 (1.27-9.52)	5.08 (1.42-11.72)	NS
Endometrial (VI) (%)	0.942 (0-8.421)	0.583 (0-6.321)	P<0.01
Endometrial FI (0-100)	24.463 (0-29.271)	22.613 (0-26.352)	P<0.01
Endometrial VFI (0-100)	0.314 (0 – 3.152)	0.117 (0-2.252)	P<0.01
Subendometrial VI (%)	2.734 (0- 20.871)	2.231 (0-19.789)	NS
Subendometrial FI (0-100)	25.027 (0-37.137)	24.871 (0-32.763)	NS
Subendometrial VFI (0-100)	0.583 (0-4.972)	0.462 (0-3.241)	NS
Pregnancy rate (%)	9/38 (24%)	14/39 (36%)	P<0.05

* Values are mean ± SD.

+ values are median (range)

Table (3):
Comparison of ultrasound parameters and estradiol values between pregnant and non pregnant women.

Parameter	Non pregnant n=54	Pregnant n=23	P value
Estradiol (pmol/L)	1836±1229	1544 ± 1035	P<0.05
Endometrial volume (cm^3)	4.96 ±1.9	4.62 ± 1.3	NS
Endometrial VI (%)	0.89 ±3.4	0.56 ± 1.9	P<0.01
Endometrial FI	22.4 ± 1.2	22.5 ±2.5	NS
Endometrial VFI	0.23 ± 1.8	0.34 ± 2.6	P<0.05
Subendometrial VI (%)	2.73 ± 5.88	2.187 ± 6.73	NS
Subendometrial FI	24.312 ±10.83	25.75 ± 11.96	NS
Subendometrial VFI	0.468 ± 1.68	0.581 ± 2.31	NS

Values are mean ± SD.

Discussion

Hyperinsulinemia contributes to the increased rate of early pregnancy loss in PCOS (3) (22) (23). Metformin treatment significantly decreased both serum insulin and glucose concentrations, and simultaneously decreased serum androgens and increased serum hormone binding globulin concentrations (24). On the other hand, the overall effectiveness of CC in inducing ovulation is 70% but this rate is reduced in the obese and in those with PCOS (25), and it is generally accepted that CC reduced uterine receptivity and thus the chances of conception (26), with further reduction in conception rate after the 3rd cycle of CC administration (9). We recorded significant reduction in endometrial volume and pregnancy rate in CC alone compared to CC-metformin arm of the study which substantiated previous studies.

In 2005, a randomized controlled trial, CC – metformin administration was associated with a higher improvement of fertility and reproductive outcome (9). In the current study, we hypothesized a specific effect of metformin on uterine receptivity. Blood flow in uterine blood vessels assessed by colour Doppler ultrasound is usually expressed as downstream impedance to flow and assumed by many studies to reflect actual blood flow to the endometrium, although the major compartment of the uterus is the myometrium and there is collateral circulation between uterine and ovarian vessels (27).

The 3D ultrasound and power Doppler provides a unique tool to examine blood supply towards the whole endometrium and the subendometrial region (28). In the present study, it was found that endometrial VI, VFI decreased significantly on the day of hCG injection in CC- metformin cycles compared to CC cycles. In the subendometrial region, there was a reduction in same indices in CC- Metformin group; however, it was not significant. This could be explained by the fact that the period of menstrual cycle when endometrial vascularization is at its lowest i.e. 1 to 5 days after ovulation is the period when morphological changes to prepare the endometrium for blastocyst implantation occur (29), and it is also the period when endometrial receptivity is thought to be at its maximum (30).
Decreased endometrial vascul-arity days after ovulation may lead to endometrial hypoxia. It has been shown in animal studies that near atmospheric oxygen concentration reduce embryo viability comprmises embryo development (31), and that oxygen tension in the uterus is lowest during the implantation period (32). Earlier two-dimensional Doppler studies reported a decline in blood flow velocity and pulsalitity index in the uterine arteries 2 days before ovulation and an increase in blood flow velocity and a decline in resistance during the mid-luteal phase (33).

Endometrial hypoxia stimulates vascular endometrial growth factor (VEGF) in endometrial stromal cells. VEGf could explain the increase of VI and VFI values in endometrium and subendometrium 2 days after hCG injection (34)(35). A positive correlation between serum VEGF levels and levels of estradiol (E2) and progesterone has been demonstrated (36). The aforementioned fact agrees with the result of this study, which showed a significantly lower estradiol and endometrial VI 2 days later in women who became pregnant compared to these who were not.

Previous studies noted a nadir of VI and VFI in the endometrium 2 days after ovulation followed by an increase again during luteal phase and that these changes mirror the changes in plasma estradiol during menstrual cycle (37). In The present study, pregnancy rate in CC – metformin group was significantly higher than CC alone group. Metformin could act directly or indirectly on uterine vascularity.

Despite the fact that insulin resistance does not play a key role in reducing uterine perfusion in PCOs (38), it is possible to suppose that metformin acts on uterine perfusion by reducing androgen levels (39) and their vasoconstrictive effect on vascular tissues (38). Besides, metformin was found to increase mid-luteal concentration of serum glycodelin and insulin like growth factor 1 IGF-1, two putative biomarkers of endometrial receptivity of 3-fold and 4-fold respectively (40).

In this study, endometrial volumes were not significantly different between pregnant and non-pregnant women. Similar findings were found in other studies employing 3D ultrasound (41)(42). There is disagreement among studies about the definition of subendometrial region. In the studies of Kupesic et al (2001)(43) and Wu et al (2003)(44), 3D power Doppler indices of the endometrial region were not given and the subendometrial region included 5mm of the myometrial-endometrial interface.

In this study, we recorded endometrial and subendometrial region was defined as a shell within 2mm of the myometrial-endometrial so that we may study the most vascularized area of the subendometrium, the 2-mm shell was also chosen by Jakubkein et al (2006)(37), because only the myometrium immediately under-lying the endometrium exhibits a cyclic pattern of steroid receptor expression like that of the endometrium (45).

Other authors (26)(44)(46) considered 5mm shell as the subendometrial region, however if 5mm was taken the subendometrial region may extend beyond the uterine contour especially in the corneal region, and fibroids may be included within that shell. Even larger 10mm- shell was defined as subendometrial region by Chien and coworkers 2002 (47). Conversely, Ng et at (2006)(48) used a small 1mm shell from myometrial endometrial interface.

It was found that subend-ometrial VI, FI, and VFI values were not statistically different between CC alone and CC- metformin treatment groups on the day of hCG injection. Similarly, no significant difference in subendometrial 3D power Doppler values between pregnant women and women who failed to conceive. Other studies measuring subendometrial blood flow on the day of hCG injection (44)(48), reported a significantly higher VFI values in those who became pregnant but, they were excluding PCOS patients, using a long protocol of GnRH agonist and gonadotropin stimulation and the difference in the selection of subendometrial region could explain the difference.

In conclusion, women receiving CC- metformin had significantly lower endometrial VI, FI and VFI than women taking CC alone on the day of hCG injection. Patients in the pregnant group have significantly lower endometrial VI, VFI than those in the non pregnant group. In contrast E2 levels on hCG injection day were significantly higher in women who failed to conceive. Further studies using same; inclusion criteria of patients, ovulation stimulation regimen, the day of ultrasound examination and subendometrial shell selection are required to clarify the role of 3D power Doppler in the prediction of pregnancy in PCOS patients.

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News & Views

This section with compiled by
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1.FDA approves drug to prevent preterm labor

Hydroxyprogesterone caproate (Makena) has been approved to reduce the risk for preterm delivery, the FDA announced. The drug is intended for women with a singleton pregnancy who have had at least one spontaneous preterm delivery. It is not approved for women with a multiple pregnancy or other risk factors for preterm delivery. Makena is injected into the hip once a week, beginning at 16 weeks of pregnancy and up to 21 weeks. In a trial of some 460 women, rates of delivery before 37 weeks were 37% in women randomized to Makena and 55% in controls. A follow-up study showed no developmental differences between children born to mothers in the two groups. Reported side effects include pain, swelling, and itching at the injection site and hives, nausea, and diarrhea. Serious adverse reactions were uncommon: one case each of infection at the injection site and pulmonary embolism

2. Real family planning

The Dutch are amongst the most diligent family planners in the world. Recent data reveal most deliveries occur in 25 – 35 year olds with the mean age at first delivery being 29 years old. They also have far fewer women having babies over the age of 40 than half a century ago (Sheldon BMJ 2008; 337:134).

This circumspect reproduction has now taken a further brave new step forward with the recommendation that women in the Netherlands can have their oocytes frozen for non-medical reasons. This means a woman choosing to delay a pregnancy can have her young oocytes preserved for postponed assisted reproduction thus increasing her chances of successful in vitro fertilization later on. Sheldon (BMJ 2010;341:c4823) reports that the decision should not open the door to delayed conception without "considerable reasons" and the upper age limit of embryo transfers will remain at 45 years. This can be viewed as expensive or a logical extension of a woman's right to controlling her reproduction. You decide. Offering and promoting such service in our country needs plenty of aspects to be studied, religious, social financial, and counselling.

3.Risk after a caesarean section

Does a caesarean section prejudice the next pregnancy? There are increased risks of uterine rupture and placenta praevia but it is unclear as to whether the subsequent pregnancy outcome is compromised or not. Wallin et al (BJOG 2010;117:1088-97) looked at perinatal mortality rates and low Apgar scores in infants whose predecessor had been delivered abdominally and found that they were more at risk when compared with those whose predecessors had been delivered vaginally. On closer inspection the reasons for the greater risk was found to be related to the medical condition associated with the caesarean section per se and not the fact that the uterus was scared.

4. Transdermal HRT and stroke

It appears that the mode of delivery and the dosage strengths of hormone replacement therapy (HRT) do make a difference to stroke risk. The UK General Practice Research database is a rich source of information about women who visit their GPs regularly and provides data on over 800 000 postmenopausal women who have or have not been taking HRT and who were followed up for 7 years. The researchers (Renoux et al BMJ 2010;340:C2519) found the overall risk rate for stroke was 3 per 1000 per year and carried out a nested case-control study to assess the influence of transdermal or oral HRT on stroke risk. They found that low dose HRT delivered via a transdermal patch did not increase the risk of stroke whereas high dose patches and oral HRT did.

Estrogen plus progesterone or estrogens alone to women without a uterus have fallen from favour since the Women's Health Initiative publications but informed opinion has been sceptical about extrapolating the results to all forms of HRT. The data in this latest paper show that oral HRT or high-dose patches are associated with a 1 per 1000 per year increase in stroke risk but not low-dose patches. More and more it seems that low doses of HRT given transdermally starting soon after the menopause do not carry stroke and

cardiovascular risk anything like the WHI findings and the outcomes of this study support this more optimistic view and it may well turn out that there is actually an advantage.

Transdermal HRT is too expensive to be taken for years for average income Egyptian woman. Let us hope it may manufactured in our country with a lower price.

Another study suggests that losing weight can improve menopausal symptoms. Huang et al (Arch Intern Med 2010;170:1161-7) conducted a study linking weight loss and urinary incontinence but also noted an improvement on hot flushes in those who lost weight. The controls did not find a decrease in bothersome flushes to the same extent so women should be encouraged to try lifestyle changes to assist at this difficult time.

5. Genetics and cancer risk

The human genome has massive potential for defining which people are susceptible to certain diseases and which people are not. The problem is that specific abnormalities in the structure of genes seldom indicate more than a predisposition towards a condition. Even combining abnormalities known to be associated with a condition has not prospectively yielded "at risk" groups or individuals who go on to develop the condition.

For example there are at least 12 genetic markers (specific nucleotide polymorphisms or SNPs) linked to breast cancer but tracking them individually or in combination does not yield useful clinical data (Travis et al Lancet 2010; 375:2143.51). But other approaches may be more fruitful so Willet et al (JAMA 2010; 304:69-75) looked at a group of individual's risk of cancer and the telomere length of their cells. Telomeres are the nucleoprotein complexes at the ends of chromosomes. As cells reproduce themselves the DNA of each chromosome is faithfully replicated except for the telomeres which shorten with each cell cycle. By measuring the telomere length it is possible to gauge the cells age or its senescence.

Short telomere length may also indicate chromosomal instability or malignant potential so the researchers measured nearly 800 individual's telomere lengths (in leucocytes) and followed them up for 10 years to see if they developed cancers and recorded their mortality. They found the shorter the telomere length the higher the person's risk of suffering from cancer and dying from his/her disease. The greater the degree of telomere shortening – the higher the risk. The findings are not near clinical application yet but do indicate the direction of future research.

6.Smoking in pregnancy

The following can be negatively affected by smoking in pregnancy: growth restriction, preterm labour, later academic performance, deep vein thrombosis, stroke, pulmonary embolism, myocardial infarction, influenza, pneumonia, asthma, gastro-intestinal ulcers – and now it seems pelvic pain. Biering et al (BJOG 2010; 117:1019-26) point out that pelvic pain is the most frequent reason for sick-leave during pregnancy in Denmark. Its aetiology is unknown but it has been linked to smoking via vasoconstriction and local ischaemia so the authors tested the possible connections using their National Birth Cohort data. Linking smoking to pelvic pain in over 100 000 women they found an association that persisted after adjustments for background and lifestyle factors. There was a dose-related pattern in those who gave up in early pregnancy and those who continued to smoke.

It is still considered shameful for an Egyptian woman to smoke in public in both rural and urban areas.. This probably limited the number of pregnant smokers in our country to less than 4% according to a personal communication with a ministry of health official. The number of primiparous Danish women who smoke is relatively low at 15% whereas the statistics for the entire population of Europe (male and female) are 50% have never smoked, 20% have given up and 30% smoke(BMJ 2010:340:C2908).

7.Weight and diabetes

Anyone wondering when the global obesity epidemic would turn into a diabetic epidemic need wonder no longer. It is here. A whole issue of the Lancet in June/July traces its ravages through not only the developed world but China "the diabetic capital of the world" to its hold in sub-Saharan Africa (Mbanga et al Lancet 2010;375:2254-66). Its effects on fertility, pregnancy, oncology and incontinence mean that our speciality will be much involved in weight and blood sugar control for generations to come.

There is a strong association between being overweight and the risk of diabetes. With whole populations increasing their BMI it is not surprising that the incidence of type 2 diabetes is increasing. As overweight or obese people enter old age they raise their fat mass, lose muscle mass, redistribute adipose tissue and their height reduces.

The consequences of overweight and aging conspire to cause concerns about trends in health and diabetic control. Failing to control weight in middle and old age has clearly been shown to raise the risk of diabetes (Biggs et al JAMA 2010; 303: 2504-12) with its dangers of metabolic, cardiovascular, urinary, cancer and other health consequences.

8.Diet and cancer risk

You are what you eat. Everything in moderation. Eat your greens. These homilies may seem unscientific but large studies suggest that a balanced diet does decrease your risk of metabolic disorders, cardiovascular disease and cancer. The European Prospective Investigation into Cancer and Nutrition (EPIC) study followed up half a million people from 12 countries for nearly a decade to see if a diet rich in fruit and vegetables really does reduce the risk of cancer and found that indeed it does – to a modest extent (Bofetta et al J Natl Cancer Inst 2010; 102: 529-37).

They showed that increasing fruit and vegetables intake by 200g per day was associated with a 3% reduction of cancer risk. The result was less than anticipated. Experts weighing-in on these findings are quick to reiterate that diet is only part of a healthy lifestyle with not smoking, maintaining a healthy weight and exercising regularly being as important as a sensible diet. A medium size apple weighs 300g so maybe "an apple a day" does keep the doctor away.

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1- Schultz-Zehden B, Borschitsch E. User Experience with an Oral Contraceptive Containing Ethinylestradiol 30 µg and Drospirenone 3 mg (Yasmin®) in Clinical Practice. Treatments in Endocrinology 2006; 5 (4): 251-256.
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