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Acknowledgments

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

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

2- Books:

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

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

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

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Contents :

Letter from the Editor:

Dear esteemed colleagues,

Warm greetings

We welcome your comments as well as the scientific activity to be incorporated in the upcoming issues. Very important subjects are included in this issue. Not all cases of vaginal atresia cause primary amenorrhea but may present later with sexual problems. 3D rectal ultrasound is an excellent aid in diagnosis and treatment aid. Follicular fluid E2 concentration had fair predictive value in oocyte maturation, fertilization, embryo quality, chemical and clinical pregnancy. But it was an independent predictor of MII-grade oocytes production. Hysteroscopy (diagnostic, therapeutic) should be performed in women with unexplained infertility. Azithromycin can be used in place of erythromycin for the expectant management of PPROM. Azithromycin only benefits from its availability and reduced gastrointestinal side effects. Corticosteroids were highly effective in reducing the severity of vomiting in hyper emesis gravidarum patients after 48 hours from the start of the treatment using PUQE score and improving their QOL score compared with the standard treatment in the control group. The uterocervical angle is a potential novel screening tool for predicting preterm birth better than cervical length. Adding chromium picolinate to metformin in PCOS patients caused further improvement of Hirsutism score and regularity of menses, and further decrease in BMI. Estrogen priming did not increase the number of MII retrieved ova in POR. Double blastocyst transfer (DBT) even of good quality worsens the outcomes of ICSI for PCOS women. DBT is significantly associated with small birth weight, high incidence of preterm labour, need for operative delivery and NICU admission with subsequent reduction of live birth rate.

Best regards.

Aboubakr Elnashar
MD
Chief Editor of EFSSJ
Prof. obs Gyn. Benha university, Egypt
elnashar53@hotmail.com

Third-time recurrence of CS scar endometriosis after one cesarean section: A case report and Review of literature

Ahmed Sherif Abdel Hamid [1]*,
Maged Mahmoud El Shourbagy [1]
[1]Assistant professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University Cairo, Egypt

Abstract

Background: Recurrent scar endometriosis was thought to be caused by implantation theory; this is a rare case to have recurrent CS scar endometriosis for the third time in different sites of scar of one CS, which could oppose this theory; we are presenting 34 years of patient complaint of recurrent painful swelling related to menses, previously excised twice and proved to be endometriosis by histopathology. Surgical excision of this swelling was done and proved to be endometrioma for the third time.

Conclusion: Implantation theory as a cause of scar endometriosis should be revised as it cannot support the recurrence of endometrioma for a third time.

Keywords: Caesarean section scar; Recurrent endometrioma

Scar endometriosis incidence has been reported to range from 0.03% to 1.7%. The most frequent symptom is cyclical or non-cyclical painful swelling presented in the abdomen at the site of previous obstetric and gynecological operations [1]. The occurrence of scar endometriosis is supported to be caused by the iatrogenic implantation theory. Secure-free margins must be obtained in surgical excision of scar endometriosis to prevent recurrence [2].

CASE PRESENTATION

A 34-year-old patient previously presented complaining of painful lower abdominal swelling at the left side of the CS scar with a typical presentation of endometriosis as it began before menses, increased in size and pain during menses, then disappeared after menses. The patient gave a history that she had one CS 6 years ago and a history of previous CS scar endometriomas twice, both excised and proved by histopathology to be endometriomas. On examination, left tender well-circumscribed swelling 5 x 5 cm in size at the site of scar of previous CS. The patient refused to have MRI as she had no medical insurance stating she had done it twice before, and it proved to be endometriosis. After routine preoperative preparation, we opened the scar and excision two swellings located subcutaneously not related to the rectus sheath (DD of desmoid tumor of rectus sheath); the first was 6 x 6 cm (Fig. 1.) and the second was 4 x 4 cm (Fig. 2.), and care was taken to remove any small swellings, ensuring hemostasis then closure of the wound.

Corresponding author:
Ahmed Sherif Abdel Hamid *
Assistant Professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University Cairo, Egypt
Address: Faculty of Medicine, Ain Shams University, Department of Obstetrics and Gynecology, Abbaseyya Square, Cairo, Egypt.
e-mail: ahmedsherif@med.asu.edu.eg
phone/fax: +966564820942, +20 122 7960980
ORCID number: 0000-0002-8495-5909

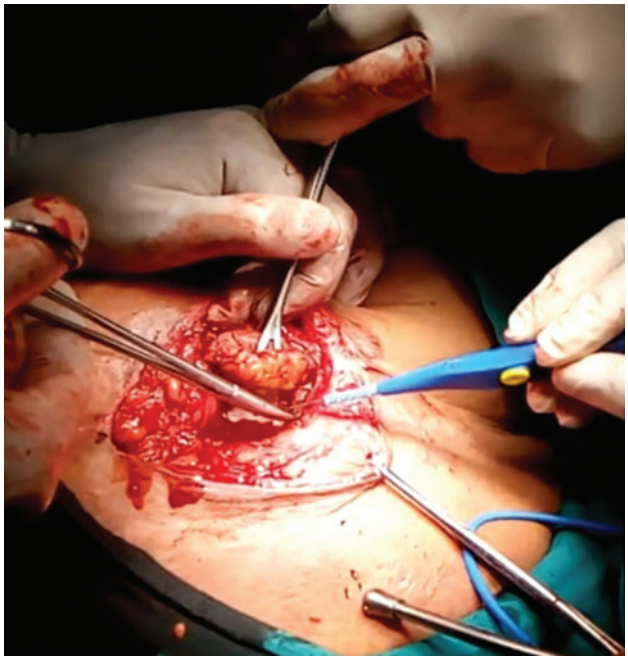

Fig. 1. Dissection of endometriotic mass with good surgical margin.

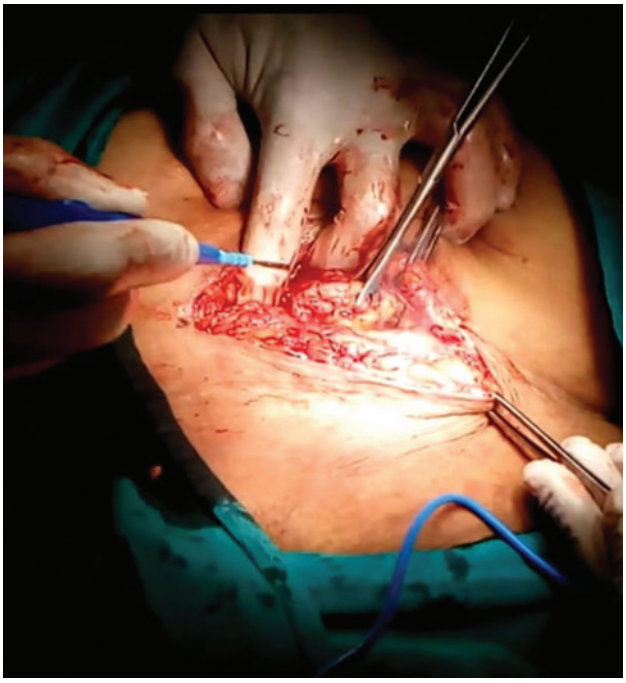

Fig. 2. Dissection of 2nd endometriotic mass with good surgical margin.

Both specimens were sent to histopathology and proved to be external endometriosis with free surgical margins (Fig. 3. is the pathology report to show free surgical margins). The patient follows up till the removal of stitches.

The pathology report revealed:

1. Fibrofatty tissue piece measured 7 x 6 x 5 cm, sectioning revealed circumscribed area, measuring 2.5 x 2.5 cm with a rubbery greyish-pink cut section.
2. Fibrofatty tissue piece measured 4 x 4 x 3 cm; sectioning revealed a circumscribed area, measured 2 x 2 cm with the same cut section as the previous specimen.

Microscopic: Sections examined from the BOTH specimens received revealed fibrofatty tissue showing endometrial glands with columnar non-secreting lining surrounded by stromal cells. There are foci showing excess hemosiderin. There is surrounding excess fatty tissue. Surgical margins are free. No evidence of malignancy in the sections examined (Fig. 4.).

PATHOLOGY REPORT

Gross:

Two undesignated containers were received:

1. Fibrofatty tissue piece measured 7x6x5 cm, sectioning revealed circumsribed area, measured 2.5x2.5 cm a with rubbery graish pink cut section, showing focal reddish areas and cystification.
2. Fibrofatty tissue piece measured 4x4x3 cm, sectioning revealed circumsribed area, measured 2x2 cm having the same cut section of the previous specimen.

Microscopic:

Sections examined from the BOTH specimens received revealed fibrofatty tissue showing
endometrial glands with columnar non-secreting lining surrounded by stromal cells. There are foci showing excess haemosidrin. There is surrounding excess fatty tissue.
Surgical margins are free.
No evidence of malignancy in sections examined.

Fig. 3. Pathology report stating free surgical margins.

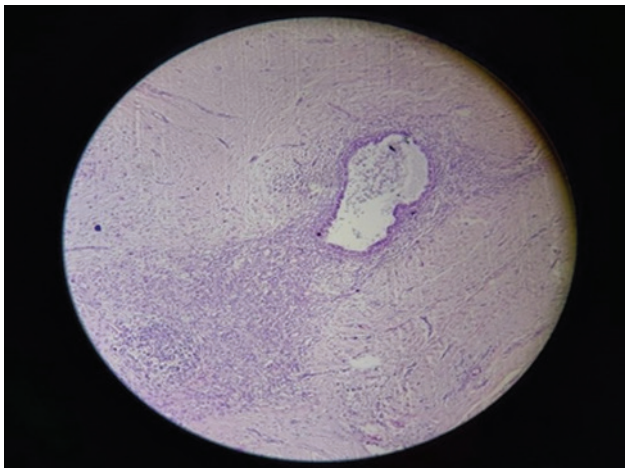

Fig. 4. Microscopic picture.

On contacting the patient to get her consent for publishing the case, she reported recurrence for the fourth time. No one can tell her the cause of recurrence; the cause of recurrence is unknown, especially since the pathology report proved free surgical margins.

Discussion

Cs scars endometriosis, although rare [1], is a distressing condition, especially if recurrent. In a retrospective study done by Yildirim et al. [3] on 29 patients in a period of 60 months with preoperative diagnoses of scar endometriosis, all were confirmed by histopathological diagnosis; they concluded that surgical excision of scar endometriosis is the gold standard treatment option but must be done with at least 1 cm surgical margin. In this case, although the patient had surgery three times for this condition, last time there was more than a 2 cm free surgical margin; when the patient was conducted to have consent for case publication, she said she had a recurrence for the fourth time again.

We cannot find a cause of recurrence. This case report sends the message that incomplete excision is not the only cause of recurrence, and further research must be done to investigate other causes to prevent recurrence. And to investigate the efficacy of other treatment modalities than surgery, especially for recurrent cases.

Review of literature

1. Epidemiology of Endometriosis and Abdominal wall endometriosis

Endometriosis is characterized by endometrial epithelial and stromal cells in extra-uterine locations. Endometriosis is associated with chronic pelvic pain and infertility and affects 10% of women in their reproductive age, depending on the site of endometriosis. [4]. For instance, Pelvic endometriotic tissue's most common locations are the ovary and pelvic peritoneum. Sites of extra-pelvic localization include the gastrointestinal tract, the urinary tract, the respiratory system, and abdominal wall endometriosis (AWE), as those cesarean scar endometriosis (CSE). [4,5,6]

CSE has increased due to the increased rates of cesarean sections worldwide. This condition is only partially understood, and the diagnosis is often delayed or missed [7,8,9]. The effects of estrogen exposure after CS and endometrial seeding during the section are enhanced by altered immunity, chronic inflammation, and local growth factors [7,8,10]. This issue has many challenges since the preoperative diagnostic rate is low with no particular clinical risk factors, and the histological report remains the final diagnostic confirmation [9,10].

Abdominal Wall Endometriosis occurs after many obstetrical and gynecological surgeries as hysterectomy and laparoscopic surgeries, performed for non-surgical endometriosis as in the study of Akbarzadeh-Jahromi et al., where they reported trocar port site endometriosis in 18 patients [11,12]

Sumathy et al. reported CSE endometriosis in 18.9% in a case series of 16 women, while Tatli et al. reported no synchronous pelvic endometriotic lesions in 18 patients [13,14]. In 2 different case series studies, the mean age at diagnosis was 35 years, and the time from surgery to diagnosis of endometriosis varied from 3 months to 20 years [14,15]. The

reported incidence of CSE is 0.03-0.45%; however, this figure needs to be estimated due to the non-existence of consistent epidemiological data and the rarity of CSE [16,17]. Andolf et al. reported an incidence of 1.8% risk for developing endometriosis after CS. [18].

2. Pathogenesis and possible genetic role

Although many researchers describe AWE as a subtype of iatrogenic endometriosis, this did not explain why CSE happens. The pathogenesis is multifactorial, including immune, endocrine, and inflammatory pathways. Theories of cell migration in association with direct seeding or metaplasia have been proposed to explain this enigma [18]. Other theories of endometriosis as Sampson's theory (the retrograde menstruation hypothesis), cannot explain CSE but can explain pelvic endometriosis. Intra-operative implantation is certainly not relevant to non-surgical endometriosis (or "endogenous" endometriosis) [19-21]. Sumathy et al; Tatli et al. have identified pelvic endometriosis in cases of CSE [14,15]. In these endometriotic local implants [21]. Vascular Endothelial Growth factor abnormalities may be associated with this condition [22].

Genetic/epigenetic theory may explain the heterogeneity of endometriosis with a hereditary profile. Some Genome-wide studies have identified 12 nucleotide polymorphisms (single) at ten independent genetic foci associated with endometriosis. Two chromosomal areas with a significant linkage were observed on 7p13-15 and 10q26 (harboring genes such as INHBA, CYP2C19, HOXA10, and SFRP4). These changes include DNA methylation, de-methylation, and histone code modifications [23,24].

PPAR-γ is a nuclear receptor with neuroprotective and anti-inflammatory roles and is highly expressed in post-operative lesions [25]. Molecular biology studies of endometriosis have shown that Estrogen Receptors activation is a hallmark of the local changes occurring in CSE. Endometriotic lesions have estrogen and progesterone receptors. Hyperestrogenemia can occur secondary to defects of Methylation genes encoding transcription factors (steroidogenic factor-1, GATA6) and causing secondary inhibition of progesterone receptor. [26, 27].

Overall, CSE is developed only in some females. The postulated mechanisms involve the local environment at the implant site, including metalloproteinase activation due to local growth factors and inflammation, increased estrogen production through stimulation of estrogen receptors, and potential epigenetic changes. [28]

3. Diagnosis

The most common complaint is pain at the site of the CS scar during menstruation. Chronic pain may be experienced unrelated to the menstrual cycle, including the pelvic, lumbar, and abdominal regions [29,30]. Rarely, the patient may present with skin changes, ecchymosis, or hyperpigmentation of the scar during menstruation [13]. A palpable lump may be felt at the abdominal wall during menses [30]. The clinical triad includes cyclical pain, a mass at or near the level of the CS scar, and a history of CS. [3,11]. In the study of Zhang et al., 98.5% of the patients presented with abdominal swelling, followed by cyclic pain in 86.9%of patients. Khan et al. performed a case-control study at Mayo Clinic, in which 2539 women had endometriosis-surgery were enrolled, showing that 1.34% of the patients had Abdominal wall endometriosis; CSE was recorded in 59% of cases with AWE. [31,32].

4. Pre-operative investigations

If EEE is suspected, we can use ultrasound, magnetic resonance imaging (MRI) of the abdomen, and computed tomography (CT) to examine the abdominal wall. (28)

MRI is better for discovering small lesions,

while CT is better in cases with subcutaneous layer and muscle involvement. Ultrasound is still the best screening method. By ultrasound, the lesions of CSE have a hyperechoic or isoechoic pattern (46.7%), with peripheral vascularization (61.5%), and are hyper-vascular or homogenous on CT scan [33]. MRI is the most common method for preoperative endometriosis staging [34].

Wozniak et al. have concluded that sonoelastography significantly improved ultrasound accuracy in evaluating the depth of infiltration of CSE, even in women with high. [35]. Fawzy and Amer evaluated transabdominal sonoelastography in 34 patients with CSE. They found that it is particularly useful in endometriomas [36]. Fine-needle aspiration (FNA) has been used for ultrasound-guided aspiration of superficial lesions [37,38]. FNA is a non-invasive and simple procedure. Lopez-Soto et al., in their theses of 33 patients, used FNA in 72% of patients [32]. Fine-needle aspiration is useful in the diagnosis and for differential diagnosis. The differential diagnosis of CSE includes hernia (incisional or inguinal), hematomas, lipomas, granulomas, and desmoid tumors [28].

5. Pathological report

The definitive diagnosis is the final histological report, where grossly, the mass is well-defined and manifests as endometriomas. The endometrial cells are implanted in the dermis and rectus abdominis muscle. [28]

6. Therapy

CSE may need a multidisciplinary approach. Usually, endometriosis is treated by hormonal drugs in addition to painkillers and, finally, surgery, depending on the pain management and/or desire for fertility. surgery is the only curative therapy in cases of CSE to resolve chronic pain. During surgery of endometriotic nodules, A wide incision is recommended to decrease the risk of recurrence, which is described in 5-9% of cases [28]. Sclerotherapy with ultra-sound guided ethanol injection into the lesion of scar endometriosis has been reported to be effective in isolated cases to prevent abdominal wall defects after wide excision [35]. High-intensity focused ultrasound ablation (HIFA) has been used as an alternative to surgery, with a recurrence rate of 3.9%. The study of Lee JS et al. showed that HIFA had fewer side effects, such as blood loss and parietal defects after surgery [38]. Combined oral contraceptives, progestins, and gonadotrophin-releasing hormone (GnRH) analogs are used in the postoperative period to delay new growth and reduce the risk of recurrence. [28]

Conclusion

CSE represents a dynamic multidisciplinary topic with an increasing incidence due to the increasing CS. The clinical manifestations range from a mass to local pain at the cesarean scar. Ultrasound and MRI may help diagnose, but the definitive diagnosis remains the histological report. The best management is the surgical removal of the implant.

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Distal vaginal atresia presented with apareunia with regular menses and not primary amenorrhea diagnosed by 3D ultrasound and managed by vaginal pull-through aided by 3D ultrasound: A case report and review of literature

Ahmed Sherif Abdel Hamid[1]*, Mounir Mohamed Fawzy el Hao[1], Suzy Abdelaziz Abdel Hamid 2
[1]Department of Obstetrics and Gynecology, Ain Shams University, Cairo, Egypt
[2]Department of Obstetrics and Gynecology, Cairo University, Cairo, Egypt
Ahmed Sherif Abdel Hamid: Assistant professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University Cairo, Egypt
Mounir Mohamed Fawzy el Hao: Professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
Suzy Abdelaziz Abdel Hamid: Assistant professor of Obstetrics & Gynecology, Faculty of Medicine, Cairo University Cairo, Egypt

Abstract

Introduction: We report a rare case of distal vaginal atresia not presented by primary amenorrhea but with the inability to have the first sexual intercourse after marriage.

Case: The patient had normal menarche and regular cycles. Thus, the challenge was in diagnosing such cases with vaginal atresia with normal menses. The menstrual flow was small in amount. The complaint came after her marriage when her husband felt strong resistance. On examination, no vaginal opening was observed. When examined again during menses, a very small opening was observed, from which the menses flow was discharged: it admitted only a pediatric Foley catheter (denoting a small fistulous tract). 3D ultrasound revealed complete uterus, cervix, and vaginal pouch development. Vaginal atresia was identified: an operation was done under a 3D rectal ultrasound guide to identifying the dissection plane. Perineal dissection was done, and pull-through of the vaginal pouch, then suturing the lower end of the vagina to the perineum.

Conclusion: not all cases of vaginal atresia cause primary amenorrhea but may present later with sexual problems. 3D rectal ultrasound is an excellent aid in diagnosis and treatment aid.

Keywords: Apareunia; Ultrasound; Distal vaginal atresia

Corresponding author:
Ahmed Sherif Abdel Hamid *
Assistant Professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University Cairo, Egypt
Address: Faculty of Medicine, Ain Shams University, Department of Obstetrics and Gynecology, Abbaseyya Square, Cairo, Egypt.
e-mail: ahmedsherif@med.asu.edu.eg
phone/fax: +966564820942, +20 122 7960980
ORCID number: 0000-0002-8495-5909

INTRODUCTION

Vaginal atresia is a rare congenital defect of the female genital tract due to canalization failure in the urogenital sinus [1]. During embryogenesis, the uterus, tubes, and upper 2/3 of the vagina arise from the Müllerian ducts, and the urogenital sinus gives the lower 1/3 of the vagina. Complete vaginal atresia is considered a part of Mullerian agenesis, as described by the American Society for Fertility Medicine [2]. Most reported cases of the imperforate hymen or vaginal atresia presented in the literature were presented by primary amenorrhea at the time of puberty by cryptomenorrhea, hematocolpus, or hematometra, unlike

this case which presented after marriage with a history of regular menses and no Vagina.

CASE PRESENTATION

The patient's primary concern was to have her first intercourse after her husband complained that he felt that there is no vagina, they thought that she might have a thick hymen, and she came asking for a hymenotomy. There was no relevant family history of a similar condition, no past surgical interventions or circumcision, and the patient before marriage has never complained. Physical examination (PE) during the first visit revealed no hymen, and per rectal examination, the uterus and cervix were felt with no hematocolpus. When the patient insisted that she had regular menses, we asked her to come during menses; on the second visit, the patient was menstruating, and a small dimple was seen, admitting a small pediatric foley catheter to a distance of 5 cm denoting a small fistulous tract.

Three Dimensional US was done through an endorectal probe revealing a normal uterus, cervix, and vagina was 6 cm (upper part 3.5cm x 1 cm dilatation). The lower part is 2.5 cm long and constricted, suggesting atresia rather than stenosis **(Fig. 1.)**

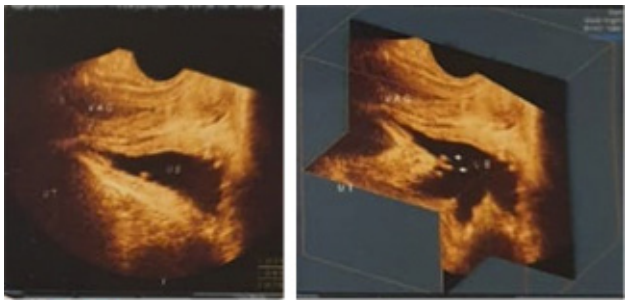

Fig. 1. a,b showing upper and lower part of vagina.

The patient was counseled for the operation about her future fertility. After preparation of the patient and routine preoperative assessment, surgery was performed; No vagina was seen even the dimple seen before was not identified; 3D endorectal probe was placed, identifying the uterus, then cervix, then the upper vaginal, perineal dissection began under 3D guidance for about 3 cm till we reached the upper vaginal pouch, then freeing of the vagina from all sides and pull through of the vaginal pouch then suturing lower end of the vagina to the perineum **(Fig. 2.)**. The patient was discharged on regular analgesics and was instructed that no sexual intercourse for three weeks.

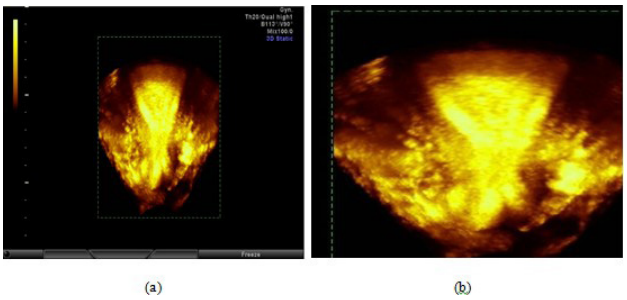

Fig. 2. a & b showed uterine cav- ity triangular which is completely normal which encouraged us to proceed for the surgery.

The patient was assessed in the outpatient clinic after the operation by one week, and a pelvic examination revealed good vaginal length; after 3 weeks, the patient had her first intercourse, and after one week of regular intercourse, she came for a second follow-up and she was happy by the procedure.

Discussion

In most cases described in the literature of distal vaginal atresia, patients were presented before the age of 16 and with primary amenorrhea [3-6]; their diagnosis is confirmed by haematocolpus and hematometra as in cases of imperforate hymen and distal vaginal atresia. In cases described distal vaginal atresia in the literature as in the case report of Awad and El-agwan [7], where the patient was a 13 years old girl who presented with abdominal swelling with a radiological finding of hematocolpus and haematometra; this case reported lower vaginal agenesis which was managed by dissecting the lower part of vagina and pull-through vaginoplasty.

The rare presentation of this case is that it describes atresia of the lower part only of the vagina with a normal upper part presenting with normal menstruation; the presence of a small fistulous opening allowed the menstrual flow. We recommend using 3D ultrasound intraoperatively in cases of vaginal atresia to identify the dissection plane.

Review of Literature

Embryology

Vaginal atresia is defined as an anomaly with failure of the natural development of the lower part of the vagina. This congenital anomaly reflects the incomplete canalization of the Mullerien ducts. Beginning from the 6th week, the embryo develops the Mullerian ducts. The ducts give origin to the Fallopian tubes and the urogenital sinus and then give the Muller tuberculum, from which epithelium grows up to give the Mullerian ducts. This contributes to the obliteration and forming of the vaginal canal and a rigid vaginal epithelial plate. In the 17th week, vaginal canalization takes place. (8)

The vagina is completely canalized by the 20th week. Vaginal anomalies result as a failure of canalization or fusion in the vertical plane and are presented clinically with a vaginal septum, atresia, or agenesis. Vaginal atresia is a Mullerian duct anomaly where fibrous tissue replaces the missing part of the vagina. (9)

Epidemiology

Female genital tract anomalies occur in 2–3% of women; The most common abnormality is the imperforate hymen (10). Isolated vaginal atresia occurs in 1:5000 women, while that uterine didelphys in 16:1000. (11). Women with impaired fertility have higher Müllerian duct anomalies (8% of women having uterus didelphys). (12)

Classifications

Mullerian anomalies are rare congenital anomalies of the female genital tract. Many classification systems for Mullerian anomalies have been proposed. The American Fertility Society Classification 1988 has been the most utilized and recognized (13).

However, the American Fertility Society classification has been criticized for focusing on uterine anomalies and excluding vaginal and cervical anomalies. This classification lacks clear diagnostic criteria and is unable to classify complex anomalies. (14)

THE European Society for Gynecological Endoscopy classification focused primarily on the uterine anatomy and classified the cervical and vaginal anomalies as independent subclasses to identify each anomaly precisely. (15)

The European Society for Gynecological Endoscopy classifies the vaginal anomalies inti 5 subclasses (V0-V4):

Sub-class V0; which includes all cases of normal vagina and normal vaginal development.

Sub-class V1; non-obstructing longitudinal vaginal septum. The included anomaly here is clear and allows classifying different variants of bicorporal or septate uterus and double or septate cervices.

Sub-class V2; non-obstructing longitudinal vaginal septum. The included anomaly here is clear and allows classifying different variants of obstructing vaginal defects.

Sub-class V3; imperforate hymen and/or transverse vaginal septum. They usually present with the same clinical presentation.

Sub-class V4; all cases of partial or complete vaginal aplasia. (16).

ESHRE/ESGE classification Female genital tract anomalies

	Uterine anomaly		Cervical/vaginal anomaly	
	Main class	**Sub-class**	**Co-existent class**	
U0	Normal uterus		C0	Normal cervix
U1	Dysmorphic uterus	a. T-shaped b. Infantilis c. Others	C1	Septate cervix
			C2	Double "normal" cervix
U2	Septate uterus	a. Partial b. Complete	C3	Unilateral cervical aplasia
			C4	Cervical aplasia
U3	Bicorporeal uterus	a. Partial b. Complete c. Bicorporeal septate		
			V0	Normal vagina
U4	Hemi-uterus	a. With rudimentary cavity (communicating or not horn) b. Without rudimentary cavity (horn without cavity/no horn)	V1	Longitudunal non-obstructing vaginal septum
			V2	Longitudunal obstructing vaginal septum
U5	Aplastic	a. With rudimentary cavity (bi-or unilateral horn) Without rudimentary cavity (bi-or unilateral uterine remnants/aplasia)	V3	Transverse vaginal septum and/or imperforate hymen
			V4	Vaginal aplasia
U6	Unclassified malformations			
U			**C**	**V**

Table 1 classification of uterine and vaginal anomalies (ESHRE/ESGE) classification system (16)

The demand for a new classification was needed so the ASRM Task Force made a new classification (17), where the categories were modified, including three additional groups; transverse vaginal septum, longitudinal vaginal septum, and finally, the complex anomalies.

In the ASRM classification, the categories are described by different terminologies:

- 1-Mullerian agenesis
- 2-Cervical agenesis
- 3-Unicornuate uterus
- 4-Uterus didelphys
- 5-Bicornuate uterus
- 6-Septate uterus
- 7-Longitudinal vaginal septum
- 8-Transverse vaginal septum
- 9-Complex anomalies (17)

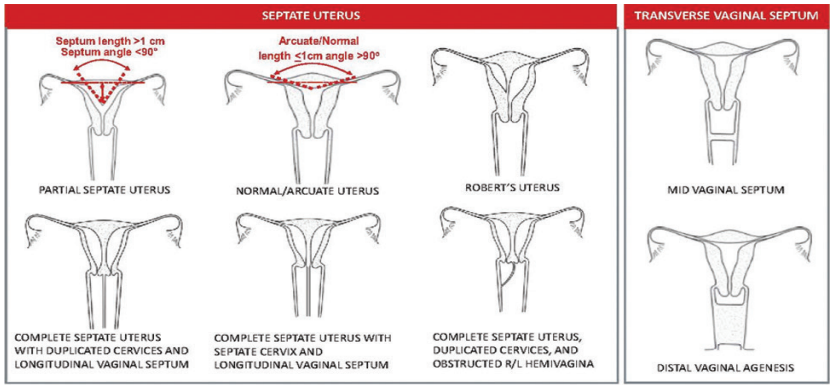

Fig (3) showing some uterine and vaginal anomalies including distal vaginal atresia according to ASRM classification

Clinical Presentations

It is reported that 39 patients with vaginal atresia are at an age average of 16 years. Primary amenorrhea was the most common presentation (71%), periodic abdominal pain (41%), lower abdominal pain (36%), dyspareunia (10%), menstrual irregularities (5%), and pelvic swellings (5%) (18). In another study on seven neonates, six had vaginal malformations combined with anorectal anomalies. The majority of infants show fewer abdominal masses and intestinal or urinary obstruction. These are caused by hydrometrocolpos (19).

Other malformations may accompany congenital vaginal atresia. In a report, two 14 aged adolescent girls with congenital cervical agenesis combined vaginal atresia, representing a rare type of obstructive mullerian anomaly (20). In a study of Troiano et al. of 24 children with imperforate hymen and vaginal atresia, show urinary tract anomalies (21).

In a report of 39 cases, 10 patients had vaginal atresia in addition to cervical agenesis. One patient had a bicornuate uterus and double cervix, and three had imperforate hymen. However, the patients were spinal malformations free (22).

Distal vaginal atresia is characterized by the presence of an atretic lower segment of vagina and presence of the upper vagina, cervix and uterus. Patients usually present by lower abdominal pain and a pelviabdominal mass, and no vaginal opening. Endorectal and abdominal ultrasound, abdominal and endorectal can confirm the diagnosis (23).

Pelvic MRI can ascertain the presence of a vagina, cervix, and uterus. MRI can diagnose the urinary tract anomalies associated with atresia or other Mullerian anomalies. MRI can also help in planning surgery by measuring the distance from the perineum to the vaginal bulge. (24).

Management of Distal Vaginal Atresia

A Pull-through vaginoplasty is usually performed for distal vaginal atresia. There is no agreement on the optimal surgical method in the literature. This is explained by different patient presentations, the relative rarity of the condition, and associations of different anomalies. Postoperatively, patients may experience infection, graft failure, vaginal restenosis, fistula formation, and injury to surrounding structures. (24)

Several techniques were described of pull-through vaginoplasty that ensure the anastomosing the vaginal mucosa to the perineum. The commonly used method is the perineal approach; where a crescentic incision at the hymen or on the introitus, with pediculus dissection of planes between the bladder and the rectum, creating a space

in the vagina till reaching to the cervix. This is followed by simple anastomosis to the perineum. An additional graft may be needed using the bowel or skin for higher atresia. (25,26) Kresowik et al. reported using ultrasound assistance in the dissection and using a posterior. (27)

Nikolaev and Bizhanova reported dissection in high atresias with an incision on the perineum (H-shaped incision) with a posterior and anterior U-shaped flap. (28)

The combined abdominoperineal approach can be done either by laparotomy or laparoscopy. These approaches help dissect the proximal vagina or push an instrument through the vagina from the abdomen to help in vaginal dissection. To prevent recurrence, postoperative therapies, vaginal stents, or dilators are used, with no agreement on their duration of use. (29,30)

In a retrospective Study of Sixteen patients with pull-through vaginoplasty done in one center with the same operative technique; The average distance from the perineum to the highest point of the atretic vagina was 1.84 ± 1.2 cm. In this study, 2 patients with atretic length greater than 3 cm, had postoperative vaginal restenosis, 4 patients had had postoperative vaginitis. Only 1 patient experienced UTI. (25).

ETHICS APPROVAL

Not required.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

COMPETING INTERESTS

The authors report there are no competing interests to declare.

AVAILABILITY OF DATA AND MATERI- ALS

All Data are available from the corresponding author on reasonable request.

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The value of subcutaneous Fucidic acid instillation during elective cesarean delivery in prevention of surgical site infection

Asmaa I Ogila [1,2] MD (drasmaaibrahim@hotmail.com)
Yossra Lasheen [1] MD (yossralasheen@gmail.com)
Ahmed H Attia [1] MD (a.hamdi15@yahoo.com)
[1] Department of Obstetrics and Gynecology, Cairo University, Egypt

Keywords: Surgical site infection; elective cesarean delivery; fucidic acid

Abstract

Background: Surgical site infections (SSI) in cesarean delivery (CD) can delay wound healing, impair cosmetic outcome and increase healthcare costs. Topical antibiotics are sometimes used to reduce microbial contaminant exposure following cesarean section.

Objective: The current study will investigate the role of subcutaneous Fusidic acid instillation for prophylaxis against surgical site infection in cesarean delivery.

Methods: This is a single blind randomized controlled trial that involved 200 females who underwent elective CD at the department of obstetrics and gynecology of Kasr Alainy medical school, Cairo university between February 2022 and August 2022

Results : Our study showed that with the use of subcutaneous fusidic acid before closing the skin in absorbable stitches, the infection rate was almost 6 times lower as compared to no fusidic acid before closing the skin.

Conclusion: Therefore, the use of subcutaneous fusidic acid instillation can be safely recommended for the prevention of wound infection (surgical site infection).

Key words: (Fucidic Acid – Surgical Site Infection – Subcutaneous Instillation – Elective Cesarean Delivery)

INTRODUCTION

Worldwide, the most common major operation is Cesarean delivery (CD) is. CD rates are continuously rising especially in developing countries. The rate of CD reaches more than 50% of deliveries in countries like Brazil and Egypt.(1)

Surgical site infections (SSI) is defined as any infection at the site of surgery that occurs up to 30 days of any operative intervention. It is classified to three main types: superficial and deep incisional (both have primary and secondary subcategories), and organ/space type (defined

Corresponding author:
Asmaa Ibrahim Ogila
52 ElManial Street, Cairo, Egypt
Tel +201001937908
Postcode 12111
Email drasmaaibrahim@hotmail.com

when the infection involve structures deeper than the muscle and the fascial spaces). [2]

SSI accounts for 25 % of hospital acquired infections despite the use of routine prophylactic antibiotics and the improvement in surgical techniques. SSI accounts for significant increase in morbidity, mortality and financial burdens. SSI occurs in 9% of women undergoing CD. Although most of these are superficial ,yet it is associated with prolonged hospital stay, increased hospital costs, maternal dissatisfaction .[3]

The main cause of postoperative wound infection is the bacterial contamination that may occur during or after the intervention [4].

Risk factors for SSI include preexisting morbidities as age, obesity, smoking, malnutrition, blood transfusion, lowered immunity, impaired glucose tolerance, immunosuppressive treatment, longer preoperative hospitalization [5], and factors related to pregnancy and delivery as history of prior CD, improper prenatal care, multifetal gestation, prolonged labor, chorioamnionitis, prolonged prelabor rupture of membranes, , emergency procedure, and obstetric services provided [6]

One of the major challenges that faces surgeons is wound infection. It represents a main complication for both surgery and trauma. Contamination with patient's own microorganisms during the procedure is the most common cause of wound infections. [7].

A global guideline for prevention of surgical site infection has been published by the world health organization. These included a list of 29 concrete recommendations on 23 topics based on 28 systematic reviews of the evidence that should be followed before, during, and after the operation. The guidelines were updated in 2018 to include an additional eight anesthesiology experts.[8]

Interventions advocated to reduce SSI include preoperative optimization of underlying morbidities as diabetes mellitus, the follow of aseptic surgical technique and the use of systemic prophylactic antibiotics [9].

Preoperative intravenous antibiotic prophylaxis has been thoroughly researched and has been demonstrated to be beneficial among the several therapies recommended to avoid SSI. [10].

To reduce post-operative surgical infections, particularly SSI, topical or local antimicrobial medicines are frequently used in surgical practise. Topical or local antibiotic distribution provides numerous potential benefits over systemic antibiotic therapy, as well as some drawbacks. [11].

High and persistent concentrations at the infection site, where local physiological changes may reduce systemic antibiotics' effectiveness, are some advantages of local administration. There are also fewer risks of systemic absorption and toxicity, lower doses of antibiotic usage, and perhaps a lower risk of the emergence of antibiotic resistance. [11].

An antibiotic known as fusidic acid is a member of the fusidanes family. Despite having a steroid-like structure, the molecule has no steroid-like properties. Fusidic acid's antibacterial activity is particularly targeted at the most prevalent skin pathogens, such as staphylococcus aureus, for which it is one of the most effective antibiotics. Treatment of minor to moderate skin and soft tissue infections with fusidic acid is successful. [12].

The study was carried on to assess the rate and type of wound infection after elective CD with and without the use of subcutaneous fucidic acid.

Material and methods

This is a single blind randomized controlled trial that involved 200 females who underwent elective CD at the department of obstetrics and gynecology of Kasr Alainy medical school, Cairo university between February 2022 and August 2022

An informed written consent was signed by all participating women after explanation of the

aim, procedure, risks and benefits of the trial. kasr Alainy ethical committee approved the study on 20/2/2022 with number MS-672-2021.

Inclusion criteria included women between 19 and 35 years of age, and 18 and 29.9 kg/m2 body mass index who were pregnant in their 3rd trimester and have history of prior CD and candidate for elective CD in the present pregnancy. Exclusion criteria were women with previous SSI after previous surgery, skin infections or skin diseases that may affect wound healing, or systemic diseases as diabetes or anemia (defined as hemoglobin < 10 mg/dL), those under steroid or immunosuppressive therapy within the last six months prior to surgery. Women also were excluded if they had a prolonged rupture of membranes, allergy to suture material and those who underwent midline incision.

All participants were evaluated through full history and examination then examined through obstetric transabdominal ultrasound to assess proper timing for the procedure. Preoperative laboratory investigations including complete blood count, kidney and liver functions, blood glucose assessment and coagulation status evaluation were done for all participants.

Participants were randomized in the morning of surgery using a computer generated system into two groups. Group F (Fucidic acid group) included 100 women who received 5 drops of subcutaneous fucidic acid 10mg (O fucidic , Orchidia, Egypt) instilled before closing the skin followed by dry dressing. and groups C (control group) included 100 women who received no fucidic acid instillation.

Allocation concealment was done through sequentially sealed opaque envelopes. The letter F was located in half of the envelopes, while the letter N was located in the other half. The corresponding letter that represents the assigned group was placed in each envelope in accordance with the randomization table, and all envelopes were then sealed and placed in a single box. The first envelope was opened when the first patient showed up, and the patient was assigned based on the note inside. Our study comprised pregnant women who had elective caesarean procedures.

CD was done under spinal anaesthesia and were carried out through surgeon with at least 5 years' experience in obstetric surgery and the same technique was used in all procedures.

All the procedures were done under complete aseptic measures. First by surgical hand scrub using standard 5 minutes surgical scrub using Iodophor, hair at operative site was clipped short with scissors if interfering with the operative procedure, then cleaning the operative site with povidone iodine scrub solution 7%, blotted with dry sterile towels and then painted by aqueous povidone iodine solution 10%.[13]

All of them were given preoperative antibiotics prophylaxis. Cefazolin (a first-generation cephalosporin) is classified as category- B that provides good and modest coverage for gram positive and negative organisms, respectively. Its recommended dose is 1-2 grams given through intravenous injection within 30 minutes of skin incision [14]

The dressing of all women was removed on the 3rd postoperative day and regularly followed up every week for four weeks for any wound infection. If infection was detected, swab and culture will be done to diagnose type of infection.

Any SSI within the thirty days following surgery was documented and classified following to Southampton Wound Grading system.

Southampton Wound Grading system includes 6 grades. Grade 0 indicates normal healing, Grade I indicates mild bruising or erythema (subdivided to a, b and c if some, considerable bruising and mild erythema respectively) , Grade II indicates erythema with signs of inflammation (subdivided to a, b, c and d if occurs at one point, around sutures, along wound and around wound respectively), Grade

III indicates clear or hemoserous discharge (subdivided to a, b, c and d if occurs at one point ≤ 2cm, along wound > 2cm, large volume and prolonged more than 3 days respectively), grade IV indicates presence of pus (subdivided to a and b if occurs at one point ≤ 2cm and along wound > 2cm, respectively) and grade V if it involves deep and/or severe infection or hematoma that requires aspiration regardless of the presence of tissue breakdown or not [15].

Primary outcomes

1. The role of fuidic acid as a prophylactic against SSI.
2. Assessment of infection rate and type of wound infection after elective CD with and without fucidic acid.

- Secondary outcome parameters

1. Overcome infection and better healing of wounds.
2. Enhance cosmetic outcome.
3. Decrease the incidence of SSI

Sample size calculation:

sample size calculation was done by comparing the incidence of surgical site infection (SSI) between women undergoing elective CD treated with subcutaneous fusidic acid installation and those treated with the standard care. Fisher Exact test was used in a prospective study to compare two proportions from separate samples; the 0.05 -error level was fixed, the power was set at 80%, and the intervention group ratio was set at 1. As previously published (16), the incidence of SSI among women treated with fusidic acid was 2.8% while it was 17.1% in control women. As a result, 68 individuals in each group should make up the minimum sample size. The PS Power and Sample Size Calculations software, version 3.0.11 for MS Windows, was used to calculate the sample size (William D. Dupont and Walton D., Vanderbilt University, Nashville, Tennessee, USA). • We recruited 100 women in each group.

statistical methods used in analysis

Mean and standard deviation (SD) were used to statistically describe numerical data that is normally distributed, whereas median and range or the IQR (interquartile range) were used to statistically describe data that is not normally distributed. Frequencies (number of cases) and percentages were used to describe qualitative (categorical) data. A Kolmogorov-Smirnov test was used to determine whether numerical data supports the normal assumption and to compare numerical variables between the research groups. When comparing regularly distributed data, the Student t test was used for independent samples, and when the data are not normally distributed, the Mann Whitney U test was used for independent samples. categorical data were compared using the Chi-square (2) test,. When the anticipated frequency is less than 5, an exact test was be employed instead. Statistical significance is defined as a probability value (p value) less than 0.05. Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows will be used for all statistical calculations.

Results

Figure 1 describes the consort flow chart of the study

RESULTS

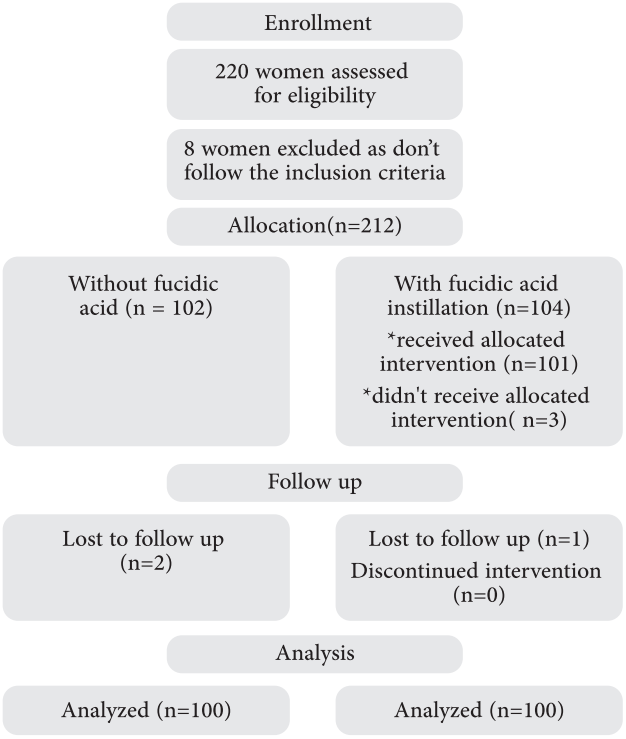

Fig (1): Consort Flow chart

Table 2 Demographic criteria of the study participants

	Group F	Group C	All	t-test	P value
Age (years)	29.8±4.59	29.79±4.31	29.8±4.44	0.016	0.987
Parity	2.53±1.37	2.54±1.37	2.53±1.37	-0.052	0.959
BMI (kg/m^2)	27.97±2.01	28.09±1.91	28.03±1.95	-0.433	0.665
GA (weeks)	38.02±0.98	38.05±0.93	38.03±0.96	-0.211	0.833

Table 3 SSI distribution among participants

		Group F	Group C	All	X^2	P value
SSI		4	17	21 (10.5)	8.992	0.003
1st week	0	78	63	141 (70.5)	16.662	0.046
	Ia	14	10	24 (12)		
	Ib	4	6	10 (5)		
	Ic	0	5	5 (2.5)		
	IIa	0	0	0		
	IIb	0	2	2 (1)		
	IIc	0	2	2 (1)		
	IId	1	2	3 (1.5)		
	IIIa	1	5	6 (3)		
	IIIb	0	1	1 (0.5)		
	IIIc	0	0	0		
	IIId	0	0	0		
	IVa	1	1	2 (1)		
	IVb	1	3	4 (2)		
	V	0	0	0		
2nd week	0	83	62	145 (72.5)	20.470	0.015
	Ia	9	12	21 (10.5)		
	Ib	4	4	8 (4)		
	Ic	0	5	5 (2.5)		
	IIa	0	0	0		
	IIb	0	3	3 (1.5)		
	IIc	0	0	0		
	IId	0	2	2 (1)		
	IIIa	1	1	2 (1)		
	IIIb	1	3	4(2)		
	IIIc	0	1	1 (0.5)		
	IIId	0	0	0		
	IVa	0	3	3 (1.5)		
	IVb	2	2	4 (2)		
	V	0	2	2 (1)		

3rd week	0	89	76	165 (82.5)	12.220	0.092
	Ia	**7**	10	17 (8.5)		
	Ib	0	0	0		
	Ic	0	0	0		
	IIa	0	2	2 (1)		
	IIb	0	1	1 (0.5)		
	IIc	0	1	1 (0.5)		
	IId	0	0	0		
	IIIa	1	0	1 (0.5)		
	IIIb	0	0	0		
	IIIc	0	0	0		
	IIId	0	0	0		
	IVa	0	3	3 (1.5)		
	IVb	1	5	6 (3)		
	V	2	2	4 (2)		
4th week	0	92	81	173 (86.5)	11.033	0.163
	Ia	5	5	10 (5)		
	Ib	0	0	0		
	Ic	0	0	0		
	IIa	0	2	2 (1)		
	IIb	0	2	2 (1)		
	IIc	0	1	1 (0.5)		
	IId	0	0	0		
	IIIa	0	1	1 (0.5)		
	IIIb	0	0	0		
	IIIc	0	0	0		
	IIId	0	0	0		
	IVa	0	4	4 (2)		
	IVb	1	2	3 (1.5)		
	V	2	2	4 (2)		

Table 2 show that there is statistically insignificant difference between women received fucidic acid and those who didn't receive fucidic acid regarding demographic and characteristics of the included cases.

Table 3 shows that there was statistically significant increase in the Southampton Wound Grading Scale in cases who did not receive fucidic acid at first week than who received fucidic acid with p value =0.046. Also the second week showed statistically significant increase in the Southampton grading scale in cases who did not receive fucidic acid than cases who received fucidic acid with p value = 0.015. However, there was no statistically significant difference in the Suthampton Wound Grading Scale between cases in third week with p value =0.092 and fourth week with p value =0.163.

DISCUSSION

This study confirmed that the administration of subcutaneous fusidic acid before closing the skin with absorbable sutures was associated with 6 times lower infection rate when compared to non fusidic acid administration.

Both Fucidic acid and mupirocin are recommended for treatment of acute skin pathology caused by staphylococci (17). However, their prolonged use (> 10 days) is associated with development of resistance [18].

Regarding surgical site infection after first week, there was statistically significant difference between the two groups regarding the incidence of surgical site infection favoring the fucidic acid group (4.0% in the C group vs. 2.0 % in the F group).

According to Southampton wound grading system, most cases of SSI in the C group were graded as (1A) followed by (1B). While all cases of SSI in F group were graded as (1A).

As for second week, there was significant statistical difference regarding the incidence of SSI between the two groups favoring the fucidic acid group (7.0% in the N group vs. 2.0% in the F group). Most of cases in the N group were graded as (1A) while in the F group were graded as (1A).

As for the third and fourth week, there was no statistically significant difference regarding the occurrence of SSI among the studied groups.

An antibiotic known as fusidic acid is a member of the fusidanes family. Although the molecule has a steroid-like structure, it lacks steroid-like action. Fucidic acid's antibacterial activity is particularly targeted at the most prevalent skin pathogens, such as staphylococcus aureus, for which it is one of the most effective antibiotics. Treatment of minor to moderate skin and soft tissue infections with fucidic acid is successful. [12].

The results of our study correspond to the study made by Pradhan and Agrawal, (16) which compared the rate of postoperative wound infection after emergency CD with and without the use of topical fucidic acid. They reported wound infection at the surgical site in 6/35 (17.1%) versus 1/35 (2.8%) in women with povidone iodine dressing versus women with fucidic acid respectively. They concluded that the use of fucidic acid is associated with 6 times reduction in wound infection rate.

Our results were against the results of the study made by Gupta et al., (19) which observe the efficacy of sodium fucidate and ethanol spray compared to conventional methods as savlon & spirit, povidone iodine and povidone iodine with metronidazole for skin preparation for the prevention of SSI. In clean contaminated wounds, the incidence of surgical site infection was 16.52%; the group treated with povidone iodine and metronidazole had the lowest SSI rate (13.04%). This finding can be explained by the fact that in clean contaminated surgeries, the source of infection is primarily endogenous from the genito-urinary or alimentary tract.

They reported the highest infection rate with the use of Savlon and spirit group (23.07%) compared to (16.28%) that was associated with fucidic acid spray use. However, this difference was not statistically significant (P = 0.295).

This is the first study in the literature to discuss the efficacy of Fucidic acid instillation in the subcutaneous space before closing the skin in cases of elective caesarean section. The two groups were similar regarding demographic data including age, parity, BMI. Another strength of our study is its proper randomization and allocation concealment.

The mail limitations of our study were the relatively small sample size, non - blinding of operator (although blinding of participants and outcome assessors was achieved) and the limited follow up duration.

We recommend many studies to be achieved on subcutaneous fucidic acid instillation before closing the skin as there are few studies in the literature about fucidic acid to investigate the role of fucidic acid in SSI prophylaxis and the adverse outcomes of it.

CONCLUSION

This study confirmed that the administration of subcutaneous fusidic acid before closing the skin in absorbable sutures was associated with 6 times reduced risk of infection when compared to non-use of fusidic acid before closing the skin.

Therefore, the subcutaneous instillation of fusidic acid is a safe method that could be used for for the prevention of SSI.

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Association between Follicular Fluid Estradiol and Clinical Pregnancy Outcome in Intracytoplasmic Sperm Injection Cycles

Hussein M[1]., Al-Kady M[1]., Abdel Wahab H[1]., Rushdy E[2]., Refaat N[2]., Dina Y. Mansour [1].
[1]Department of Obstetrics and Gynecology - Ain Shams University Maternity Hospitals
[2]Department of Clinical Pathology - Faculty of Medicine - Ain Shams University

Abstract

Background: The outcome of in vitro fertilization (IVF) is influenced by a number of factors. One of the most important factors is oocyte quality. The microenvironment of the follicular fluid is important for oocyte development.

Objectives: Assessment of the accuracy of follicular fluid estradiol level in predicting clinical pregnancy outcome, oocyte quality and fertilization rate in women undergoing ICSI.

Methodology: The current study was prospective study that included 180 women. Follicular fluid concentrations of 17β-estradiol were determined using the enzyme immunosorbent assays. Upon retrieval, oocytes were analyzed for hallmarks of maturity and classified as GV, MI, or MII based on appearance. Fertilization status observed at 24 h and the nutrient solution renewed, morphology of the dividing embryo was observed and 'embryo grading' done.

Results: Serum E2 concentration ranged from 2361±1583 (100 to 7589) pg\ml. The mean of total number oocytes was 10 with 53% of MII of good quality. And (47%) were of bad quality. All cases had normal fertilization. Number of transferred Embryos ranged from one to three embryos (good quality was of 63.9%), (bad quality was of 36.1) and 103 of cases had embryo transfer on day 5 ,77 had transfer on day 3.

Conclusion: Follicular fluid E2 concentration had fair predictive value in oocyte maturation, fertilization, embryo quality, chemical and clinical pregnancy. But it was an independent predictor of MII-grade oocytes production.

Keywords: Follicular Fluid Estradiol; clinical pregnancy; Intracytoplasmic Sperm Injection

INTRODUCTION

Artificial reproductive technologies (ARTs), which have been utilized since 1978, and in vitro fertilization (IVF) techniques are frequently employed to treat infertility in humans [1].

Corresponding author:
Dina Yahia Mansour
Email: Dinayahiamansour@hotmail.com

In the physiology of follicular development, oocyte maturation, and ovulation, follicular fluid (FF) is crucial [2]. Numerous factors that mediate the formation of follicles and oocytes are present in follicular fluid [3].

In order to prevent the overproduction of embryos during human in vitro fertilization, oocyte quality evaluation is a key objective. Oocyte morphology was the first step in the process of determining the equality of oocytes, which then moved on to the identification of genetic and biochemical components [4].

Controlled ovarian hyperstimulation that produces a multi-follicular response is essential for the success of in vitro fertilization (IVF). Granulosa cells seen in the follicles secrete the hormone estradiol (E2). E2 is crucial for the development of oocytes and follicles as well as the uterus's preparation for implantation [5].

Regular metrics used to track follicular growth and oocyte maturation include serum estradiol levels and follidular size [6].

But in order to reach the objective and induce gestation, every cycle of ovarian stimulation turns into a series of challenges that must be surmounted. Finding additional elements that contribute to the overall process's optimization is crucial in this scenario. It has been proposed that certain elements of the follicular fluid medium, in which the oocyte is submerged, are biochemical indicators of the quality of the oocyte and, consequently, of the likelihood of successful fertilization and embryonic development [7].

The development of oocytes depends critically on the milieu provided by follicular fluid (FF). FF results from both the secretory activity of granulosa and thecal cells as well as the transfer of blood plasma elements that pass across the blood follicular barrier [8].

It is commonly known that healthy follicular growth and anti-atresia effects are linked to an environment that is mostly intra-follicular and estrogenic. In addition. Through direct, non-genomic action at the plasma membrane level, E2 promotes the cytoplasmic maturation of oocytes. This, in turn, causes extracellular calcium influx into the cell and a particular pattern of Ca2+ oscillations [5].

A higher risk of becoming pregnant has consistently been linked to elevated E2 in FF, which indicates a more advanced stage of oocyte maturation. Up until the fertilization stage, the role of estradiol in in vitro fertilization (IVF) is well established; however, its continued use after that point is still debatable. While some organizations report no effect of estradiol in these processes, others link high levels of the hormone to substantial oocyte production, which is accompanied with suppression of implantation and endometrial receptivity and lower pregnancy rates [9].

Follicles from which oocytes with higher rates of fertilization were retrieved were found to contain higher levels of estradiol [10]. Elevated levels of progesterone and estradiol in follicular fluid were linked to a higher likelihood of conception in terms of pregnancy rates [7].

Because exogenous steroids could be added to the culture media to improve the conditions, it is crucial to gather comprehensive information about the oocyte environment, particularly regarding the makeup and impact of FF on the maturation process. Important factors that affect the success of in vitro fertilization are the caliber of the eggs and the embryos. A study on the influence of serum estradiol on these measures may be found in [11].

It is commonly known that healthy follicular growth and anti-atresia effects are linked to an environment that is mostly intra-follicular and estrogenic. Furthermore, by directly acting non-genomically at the plasma membrane level, E2 promotes the cytoplasmic maturation of oocytes, which in turn causes extracellular calcium to enter

the cell and a particular pattern of Ca2+ oscillations [5].

A higher risk of becoming pregnant has consistently been linked to elevated E2 and E2/P ratios in FF, which signify a more advanced stage of oocyte maturation. Up until the fertilization stage, the role of estradiol in in vitro fertilization (IVF) is well established; however, its continued use after that point is still debatable.

Patients and methods

Patients who attended the infertility outpatient clinic and were candidates for ICSI at the Ain Shams University Maternity Hospital were the subjects of this prospective observational study. 180 women participated in this trial, which lasted more than two years.

All women between the ages of 18 and 38 who met the following criteria were included in the study: they had to have a BMI between 18.5 and 25 kg/m2, be free of medical conditions, have normal cervical and uterine morphology, have tubal factors other than untreated hydrosalpnix, and have good ovarian reserve as determined by the anti-mullarian hormone level and antral follicule count.

Polycystic ovarian syndrome, patient age greater than 38 years, BMI greater than 25 kg/m2, endometriosis diagnosis, hydrosalpinx, FSH and LH levels greater than 15 IU/ml, and women exhibiting poor ovarian response (POR), which is defined by two or three of the following three factors, were among the exclusion criteria: 1) The mother is older than 40. 2) prior pregnancy loss, and 3) an abnormal ovarian reserve test (i.e., low anti-mullarian hormone (AMH) less than 0.9 or antral follicular count (AFC) <5-7 follicles), women complicated by ovarian hyperstimulation syndrome (OHSS), or women who refuse to consent to the use of their data in the study.

Methods

The study involved women who underwent a comprehensive history, including personal, menstrual, obstetric, medical, surgical, sexual, and husband histories. A physical examination was conducted, including general, abdominal, and gynecological examinations. Infertility tests were conducted, including semen analysis and hormonal profiles on the third day of menstruation. A flexible antagonist protocol was used to stimulate ovulation, starting on the second day of the menstrual cycle with recombinant human rFSH. The dose was adjusted based on BMI, age, AMH, previous induction history, and expected ovarian response. A subcutaneous daily dose of GnRH antagonist was started when the leading follicle size was 14mm or more. Intramuscular human chorionic gonadotrophin (hCG) was administered when folliculometry showed an average diameter of 3 or more preovulatory follicles approaching 18-20mm.

Oocyte retrieval and semen processing

Using a single lumen needle guided by vaginal ultrasonography, oocyte retrieval was carried out under general anesthesia (intravenous administration of Propofol) 34–36 hours after hCG administration. Selection and immobilization of sperm. A dish with an electrostatic coating is used to store the garnets for ICSI. Placing a 10-milliliter microdroplet of polyvinylpyrrolidone (PVP) in the middle of the dish [12].

During oocyte retrieval, mature follicles (>17 mm) are aspirated and collected individually. The retrived fluid is linked to each cultured oocyte, labeled with patient information and identification number. Blood-stained follicular fluid samples are discharded, and the cumulus oophorus's corona radiata is removed. Oocytes are classified as GV, MI, or MII, with MII based on oocyte morphology abnormalities such as large perivitelline space, dark zona pellucida, dark incorporations, spots, vacuoles, refractile bodies, and irregular shape. Abnormal morphological criteria are observed in sperms, including amorphous, round, large,

small, vacillated or tapered head, neck, midpiece defects, excess residual cytoplasm, and coiled, broken multi and short tail [13].

These morphologic categories correlated roughly with phases of meiotic progression. Oocytes that had not progressed through meiosis to Mil were immature and not able to be successfully fertilized [14].

Steps of FFE2 Measurement

The quantitative determination of Follicular Estradiol was performed using an ELISA kit supplied by Immunospec Corporation's Monocent Inc Estradiol. The kit was precoated with an anti-Estradiol monoclonal antibody epitope (Biotin reagent). Standard solutions, follicular fluid, and controls were dispensed into wells, followed by 50pl of Estradiol Biotin Reagent and 100pl of Estradiol Enzyme Reagent. The liquid was then washed three times with IX wash buffer and blotted on absorbance paper or paper towel. The blue color changed to yellow after adding 50pi of Stop Solution. Absorption values were read at 450 nm within 15 minutes, and the mean absorbance values were calculated for each set of reference standards, controls, and samples. A standard curve was constructed by plotting the mean absorbance against its concentration in ng/ml on a linear-linear graph paper. The mean absorbance values were used to determine the corresponding concentration of Estradiol in ng/ml from the standard curve.

Injection of oocytes and fertiliation

The oocytes were placed in a special culture (PVP) by the embryologist, who then used a microscope and a tiny needle to inject one sperm into each oocyte. The state of fertilization was monitored 16–19 hours following ICSI. The presence of two polar corpuscles and two pronuclei (2PN) indicated that fertilization was normal. Patients with no or abnormal fertilization were removed, and those who met the same inclusion and exclusion criteria were substituted.

Embryo grading and transfer

In our study we classified embryos Based on the several morphological criteria are considered in embryo classification

- Cell number: embryos should be 2 to 4 cells at 48 hours after egg retrieval and 7 to 10 cells by 72 hours [15].
- Cell regularity or degree of blastomere size equality (uneven blastomere cleavage): if individual cells are similar in size, the embryos have the best cell regularity. If they are approximately the same size, it is better to be compared with a different size.
- Degree of fragmentation: although the fragmentation phenomenon is totally common in human embryos, those with great than 25% fragmentation, have a low implantation potential.
- Presence of multinucleation: if there is more than one nucleus in each blastomere on either days 2 or 3, the embryo is multinucleated. After day 3, it is highly difficult to identify multinucleation. Additional factors to be considered for grading and selection for transfers includes the presence of vacuoles, granularity and thickness of the zona pellucida.

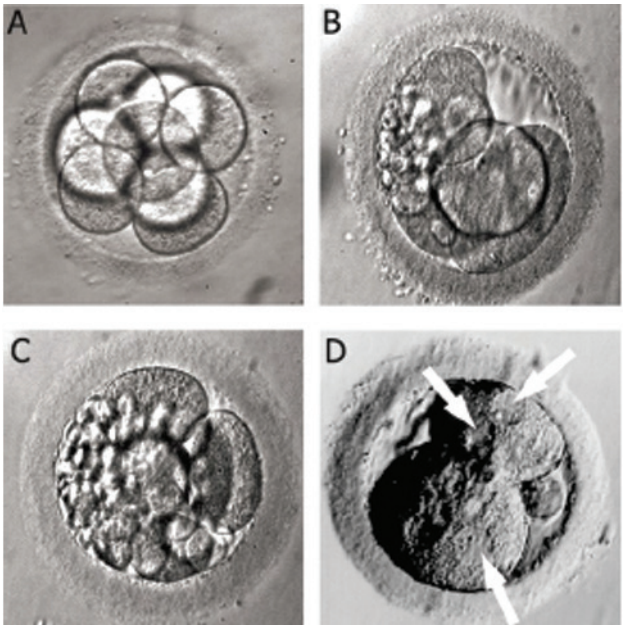

Figure (): Embryo grading by Advanced Fertility Center of Chicago

Embryo scoring based on blastocyst expansion grade according to Gardner et al, 2000 [16].

Expansion grade	Description
1	Blastocyst development and stage status
2	Blastocoel cavity more than half the volume of the embryo
3	Full blastocyst, cavity completely filling the embryo
4	Expanded blastocyst cavity larger than the embryo, with thinning of the shell
5	Hatching out of the shell
6	Hatched out of the shell

These three scores add up to the total score that is provided to each blastocyst. Consequently, the expansion score—a value between 1-6 depending on the degree of expansion and the hatching status—is the first log.

Embryo Transfer

Under ultrasound guidance, embryos were put onto a soft catheter (Labotect) and inserted into the uterine cavity through the cervix to reach the maximal implantation potential while maintaining complete bladder function. Three embryos at most were transferred during the cleavage stage (day three following oocyte retrieval) or the blastocyst stage (day five following oocyte retrieval) in our investigation. Before transfer, patients with fragile endometrium, fluid in the uterus, and high serum progesterone levels had their embryos frozen

Luteal Support

Cyclogest 400mg (Alpharma, UK) BD, acetylsalicylic acid (aspocid, CID, Egypt) 75 mg and hostacortin 5 mg (prednisolone, Sanofi Aventis, Egypt) once daily. Chemical pregnancy was defined as positive serum B HCG at 12 days post 5th day embryo transfer or 14 days post 3rd day embryo transfer. Clinical pregnancy demonstrated by embryonic cardiac pulsation by vaginal ultrasound 2 weeks after positive S. BHCG.

Study outcome:

Primary outcome was to predict association between Follicular Fluid Estradiol levels and pregnancy outcome in ICSI cycles. Secondary outcomes were to predict association between Follicular Fluid Estradiol levels and oocyte quality, fertilization rate in ICSI cycles.

Ethical Considerations:

The study was approved by the institutional review boards and ethics. Informed written consent was obtained from all the participants.

Ethical considerations:

The study was approved by the institutional review boards and ethics. Informed written consent was obtained from all the participants.

Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Results

The mean duration of infertility (5 years) ± 3 years (2 to 13 years). Primary infertility in 62.8 % of cases 113 cases and secondary infertility in 67 cases 37.8%. The mean cause of infertility was tubal cases 81 cases (45%) then male factor 67 of cases (37.2%), then unexplained causes 26 cases (14.4%) **(Table I).**

This table shows that Follicular fluid E2 concentration ranged from 247±199 (0 to 700) (ng\ ml). Serum E2 concentration ranged from 2361±1583 (100 to 7589) pg\ ml. Percentage of grade 1 Mil was 70.6% and grade 2,3 Mil was 29.4% with Fertilization rate 100 of cases. The mean of total number oocytes was 10 with 53% of Mil of good

quality and 47% of Mil of bad quality. Number of transferred embryos ranged from one to three embryos(good quality was 63.9% and of bad quality 36.1%) ,46 cases transferred single embryo,69 cases transferred 2 embryos and 65 cases transferred 3 embryos(57.2% of cases was transferred on day 5 and 42.8% of them on day 3) and 11 cases had frozen embryo transfer. Chemical pregnancy was positive in 90 cases (50%) and the clinical pregnancy positive in 66 cases (36.7%) **(Table II)**.

This table shows that the median follicular fluid E2 concentration was 281 ranged from (165 to 440)(ng\ml) in oocyte with good quality MII(gradel) while when the concentration of follicular fluid E2 ranged from (20 to 130) (ng\ml) median concentration 45 in oocyte with bad quality(grade 2,3) MII. The median serum E2 concentration was 2822 ranged from (1800 to 3798) (pg\ml) in oocyte with good quality MII (gradel) while when the concentration of serum E2 ranged from (390 to 1655) (pg\ml) median concentration 800 in oocyte with bad quality (grade 2,3) MII **(Table III)**.

This table shows that in follicular fluid E2 concentration ranged from (180 to 420 ng\ ml) had good embryo quality ajnd in this ranges, from (25 to 170 ng\ml) had bad embryo quality. As regard serum E2 Ranges from (1800 to 3877pg\ml) had good quality embryos and ranges from (430 to 1908pg\^nl) had bad quality embryos **(Table IV)**.

This table show's that in patient with follicular fluid E2 concentration (200 to 440 ng\ml) (median value 300) had a positive chemical pregnancy (50% of cases) and in patient with concentrations ranged from (30 to 260 ng\ml) had negative chemical pregnancy median 80 (52.8% of cases). As regard serum E2, patients with serum E2 ranging from (1898 to 3765pg\ml) had positive chemical pregnancy and in patients with serum E2 concentrations ranging from (540 to 2859pg\ ml) (median 1079) had negative chemical pregnancy **(Table V)**.

This table shows that in patient with follicular fluid E2 concentrations ranging from (220 to 476 ng\ml) (median 315) had clinical pregnancy and patients with Follicular Fluid E2 concentrations ranging from (36 to 320 ng\ml) (median 111) had no clinical pregnancy. As regard serum E2, patients with serum E2 concentrations ranging from (2388 to 3899pg\ml) (median 3241) had clinical pregnancy and patients with serum E2 concentrations ranging from (630 to 2859pg\ ml) (median 1561) had no clinical pregnancy **(Table VI)**.

Receiver-operating characteristic (ROC) curves show that follicular fluid E2 had fair predictive value in predictio^ of Mil maturity (AUC = 0.837 and 0.838, respectively). Follicular fluid E2 concentration >170 ng/ ml had sensitivity of 74.8% and specificity of 83%in prediction of Mil maturity.) Follicular fluid had good predictive value (AUC=0.880) in prediction of embryo quality. Follicular fluid E2 concentration 130ng/ ml had sensitivity of 80.9% and specificity of 72.3%. Follicular fluid E2 had fair predictive value (AUC=0.757) in prediction of chemical pregnancy. Follicular fluid E2 concentration > 144 ng/ml had sensitivity of 83.3% and specificity of 61.1%. As regard clinical pregnancy follicular fluid E2 had fair predictive value (AUC=0.749). Follicular fluid E2 concentration >160ng/ml had sensitivity of 86.4% and specificity of 57% **(Table VII)**.

Receiver-operating characteristic (ROC) curves show that serum E2 had good predictive value in prediction of Mil maturity (AUC = 0.819). Serum E2 concentration >1768 pg/ml had sensitivity of 77.2% and specificity of 81.1%in prediction of Mil maturity. Serum E2 had good predictive value (AUC=0.814) in prediction of embryo quality. Serum E2 concentration 1099pg/ ml had sensitivity of 91.3% and specificity of 64.6%. Serum E2 had fair predictive value (AUC=0.731) in prediction of chemical pregnancy. Serum E2 concentration > 1059

pg/ml had sensitivity of 94.4% and specificity of 50%. As regard clinical pregnancy serum E2 had fair predictive value (AUC=0.768). Serum E2 concentration >2134 pg/ml had sensitivity of 80.3% and specificity of 65.8% **(Table VIII)**.

Discussion

According to WHO estimates, infertility is the third most serious condition globally. Even with ICSI being used all around the world, the birth rate is still just about 30%. Exogenous gonadotropins are utilized for ovarian stimulation to produce numerous follicles in order to maximize its success. Nevertheless, it has been demonstrated that these gonadotropins negatively impact the quality of oocytes and embryos. It has been shown that exposure to gonadotropin concentrations above the normal range disrupts oocyte maturation and meiosis, resulting in chromosomal aneuploid oocytes [17].

Furthermore, since not all oocytes produce healthy embryos, in reality, retrieving more oocytes is associated with lower oocyte quality [10]. This is caused by low oocyte developmental competence, often known as "oocyte quality," which up until this point has lacked a true measurement. It is now commonly acknowledged that the quality of the oocyte influences the quality of the embryo since the oocyte provides the majority of the embryo's cytoplasm, which aids in early embryogenesis and embryonic genome activation [18]. Since the overall number of high-quality embryos has been demonstrated to be less indicative of the success of ICSI, the conventional criterion for embryo selection—which is solely focused on morphology—is really insufficient to reflect "Embryo Quality*" [19].

Predicting "oocyte quality" thus becomes essential in ICSI so that the doctor can tailor the stimulation regimen and the embryologist can choose which "best embiyo" to transfer.

Ovarian follicle development culminates in the oocyte gaining the ability to proceed with meiosis ("Nuclear maturation") and develop into an embiyo ("Cytoplasmic maturation"). A unique humoral milieu known as "The Follicular Fluid" surrounds the egg while it is in this stage of development in the antral follicle. Given that the follicular fluid is the result of blood plasma passing over the blood-follicle barrier and follicle cell secretions, a potential relationship between it and the oocyte is anticipated. Its constituents may function in a paracrine or autocrine manner, so affecting the quality of the egg and its subsequent capacity to become fertilized and grow into a healthy embryo [7].

When added to in vitro maturation media, oocytes with high follicular E2 are linked to healthy follicles that contain oocytes capable of resuming meiosis and resulting in a healthy pregnancy. These oocytes also develop to the blastocyst stage and have a direct, non-genomic effect on the oocyte surface, changing its calcium release mechanisms, which are thought to be involved in oocyte cytoplasmic maturation [20].

Follicles containing developed nucleus oocytes, typically fertilized oocytes, and oocytes resulting in pregnancy were found to have high E2 levels in their follicular fluid [20].

The 180 infertility patients who had ICSI for tubal, male, or unexplained cases of infertility provided 180 follicular fluid samples for the current prospective observational study. The induction protocol that was employed was the flexible antagonist protocol.

The objective of the research was to evaluate the relationship between the level of E2 in follicular fluid and clinical pregnancy, oocyte maturation, fertilization, and embryo quality.

The results of this study demonstrated that the development of MH-grade oocytes, fertilization, embryo quality, chemical pregnancy, and clinical pregnancy may all be independently predicted by the follicular fluid E2.

An observational study that demonstrated the relationship between the follicular fluid E2, mature and immature, fertilized and non-fertilized oocytes, and the grades of embryos formed from these oocytes was the first to challenge the theory. It demonstrates that the biochemical prediction of the ICSI outcome came from follicular fluid E2 [21].

This study discovered that follicles producing mature (Mil) oocytes had higher E2 levels in their follicular fluid than follicles producing immature oocytes, in accordance with oocyte nuclear maturation.In actuality, the follicle with the highest concentration of E2 is the one that selects other follicles by stimulating FSH receptors on granulosa cells, which promotes follicle development even in the presence of low FSH levels brought on by E2's negative feedback inhibition. Our findings show that elevated E2 may not only indicate follicular maturation but also oocyte nuclear maturation. It seems sense to assume that a healthy follicle has a big number of granulosa cells that can produce a significant amount of E2, which will result in a healthy oocyte.

Several research have reported on this finding [22–23]. On the other hand, Costa et al., 2004 discovered that follicles with mature oocytes had much lower E2 levels than follicles with immature oocytes [24].

E2 might stop premature nuclear maturation. allowing enough time for appropriate cytoplasmic maturation consequently synchronizes ovulation of a completely developmentally competent egg with meiotic maturation [25]. It has been suggested that the poorly known relationship between E2 and nuclear maturation may be due to steroid synthesis in response to LH-induced meiosis resumption. By binding on estrogen receptors (ERs) on the oocyte surface, E2 may mediate LH-induced resumption of meiosis [26–27]. This will change the oocyte's Ca2+ oscillations, which will activate the meiosis promoting factor (MPF) [28].

This study indicates that high E2 levels are associated with proper fertilization and high-quality embryos, which is relevant to oocyte developmental competence. The maturation of both the nuclear and cytoplasmic ovaries occurs during the last stage of follicle development. Since normal fertilization and blastocyst development are all dependent on the degree of cytoplasmic maturation, high E2 may be important for proper oocyte cytoplasmic maturation. During this phase, intrafollicular E2 concentrations undergo significant changes, suggesting its contribution in this crucial final stage of oocyte maturation and demonstrating that E2 in the follicular fluid is distinctly different among follicles yielding oocytes with different developmental potentials. Others have also discovered these results [10]. Gilshrest et al. [29] established a direct correlation between E2 and oocytes, observing that oocytes stimulate E2 production by cumulus cells via oocyte secreting factors (OSFs). Furthermore, they found a direct, non-genomic effect of E2 on ER on the oocyte surface, which leads to Ca2+ oscillations thought to impact cytoplasmic maturation [26]. Thus, we might conjecture that E2 may have a direct effect in the developmental potential of oocytes.

Although even MII oocytes have varying developmental potentials, exogenous gonadotropins are used in ART to enhance the number of oocytes retrieved in practice. Thus, it is possible that follicle development can continue even in the absence of oocyte maturation. Although exogenously administered gonadotropins lead to follicle development, they may interfere with steroidogenesis, which could account for variations in oocyte quality, since appropriate steroid sequence and pattern has been linked to oocyte maturation and its acquisition of die molecular programming for proper fertilization and embryo development [30].

Follicle E2 has a positive and significant relationship with oocyte developmental potential. It also plays a role in follicle

development and enhances oocyte cytoplasmic maturation [31].

Unlike a study published in 2002 by Mendoza et al., it was found that the greatest amounts of estradiol in follicular fluid may be used to identify the quality of the embryo. In all situations, the ratio of estradiol to progesterone was higher in embryos of A and B grade quality compared to those of C quality, and the ratio of estradiol to testosterone was higher in embryos of B quality compared to those of C quality [7].

However, with gestation being the ultimate objective, there were higher amounts of estradiol in follicular fluid in cases of pregnancy when looking at total pregnancy rates. This was consistent with a 2008 study by Asimakopoulos et al. that found no variations in the E2 products in the follicular fluid between follicles that produce a healthy egg and those that do not fertilize. By accounting for nuclear maturation during fertilization, we were able to precisely examine the connection between intrafollicular E2 levels and the particular fertilization result [32].

On the other hand, follicular fluid E2 levels were associated with oocyte competence, which is the capacity to carry out a typical fertilization process. It should come as no surprise that patients whose follicle produced a MII oocyte that developed into a 2PN also had higher serum E2 levels. Additionally, serum E2 levels were higher for each follicle and egg that was recovered, indicating a possible rise in granulosa cell capacity worldwide.

As this study's results showed, there was a known positive correlation between estradiol and follicular volume as well as a positive correlation between estradiol and follicular diameter in plasma during the follicular phase in both spontaneous and induced cycles [33].

The current data indicate that mature oocytes are linked to lower estradiol levels in FF fluid following hCG. It appears that the process of oocyte maturation and this inversion of the steroidogenic pattern are both impacted by the ovulatory LH surge (Moor et al., 1980). Follicle diameter and estrogen levels in FF did indeed correlate negatively. Estradiol and testosterone levels significantly decrease with oocyte maturation, and there is a positive correlation between them. As androgens, and testosterone in particular, are direct precursors of estradiol, this could not be any different [34].

Additionally, this was refuted by a study (Frederick et al., 1991) that showed concurrent observations of follicular fluid estrogen synthesis and oocyte development. The relationship between steroids seemed to be the most important aspect of the oocyte maturation process. Using the steroid ratios in FF, some researchers hypothesized that the progesterone/estradiol ratio would be the most accurate measure of maturity. Progesterone/estradiol, progesterone/testosterone, and estradiol/testosterone ratios were higher in FF from follicles of mature oocytes, according to preovulatory follicular fluid steroid levels in stimulated and unstimulated cycles triggered with human chorionic). The metabolism of C21 (progestogens) to C19 (androgens) decreases with an increase in the progesterone/testosterone ratio. It may be possible to use these indices to predict oocyte maturity based on the levels of these steroids in FF, as evidenced by the positive relationship between estradiol and testosterone and the rise in the estradiol/testosterone ratio, which suggests that the fall in estradiol levels may be caused by a reduction in testosterone rather than a reduction in aromatase activity [35].

Endometrial receptivity [9], which is a function of embryo quality and implantation potential, is crucial for the success of IVF programs in achieving pregnancy. Up until the fertilization stage, estradiol has a beneficial effect on in vitro fertilization (IVF); however, its effects during the implantation and pregnancy stages are debatable [36].

Conclusion

It appears that the E2 content in fibrous fluid was fairly predictive of oocyte maturation, fertilization, embryo quality, chemical pregnancy, and clinical pregnancy. However, it was a separate predictor of the generation of oocytes of MH grade. Therefore, in an effort to enhance pregnancy outcomes for patients having ICSI, it is advised to conduct additional research to evaluate various markers in the follicular fluid that may be utilized to predict the quality of oocytes recovered and embryo grading. Additional research to link follicular fluid E2 for each eash follicle and the likelihood of conception for each chosen embryo.

Conflict of interest

None

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Table I: Characteristics of the whole study population: Numerical variables

Variable	Mean	SD	Maximum	25th Percentile	Median	75th Percentile
Age (yr)	31	5	38	28	32	35
Weight (kg)	66	5	76	63	66	70
Height (cm)	164	3	177	163	164	167
BMI (kg/m²)	24.4	1.4	26.3	23.7	24.8	25.6
Duration of infertility	5	3	12	3	4	6
Parity				P0	113	62.8%
				P1	50	27.8%
				P2	16	8.9%
				P3	1	0.6%
Cause of infertility				Unexplained	26	14.4%
				Tubal factor	81	45.0%
				Male factor	67	37.2%
				Tubal and male factors	6	3.3%

Table II: Characteristics of the whole study population: Categorical variables

Variable	Mean	SD	Minimum	Maximum	25th Percentile	Median	75th Percentile
Follicuar fluid E2 (ng/ml)	247	199	0	700	60	230	385
Serum E2 (pg/ml)	2361	1583	100	7589	940	2276	3486
Total number of oocytes	10	6	1	26	5	10	15
Total number of MII oocytes	6	4	1	20	3	6	9
Number of good-quality MII occytes	4	3	0	17	1	3	6
Number of poor-quality MII occytes	2	1	0	7	1	2	3
Percentage of good-quality MII occytes	53.0	28.3	0.0	100.0	36.7	59.2	75.0
Percentage of poor-quality MII occytes	47.0	28.3	0.0	100.0	25.0	40.8	63.3

Varialble		Count	%
MII oocyte maturation	Grade 2 or 3	53	29.4%
	Grade 1	127	70.6%
Fertilization	Negative	0	0%
	Positive	180	100%
Day of embryo transfer	D3	77	42.8%
	D5	103	57.2%
Number of transferred embryo	Single embryo	46	25.6%
	2 Embryo	69	38.3%
	3 Embryo	65	36.1%
Quality of transferred embryo	Poor quality	65	36.1%
	Good quality	115	63.9%
Frozen embryo transfer			
	Positive	11	6.1%
Chemical pregnancy	Negative	90	50.0%
	Positive	90	50.0%
Clinical pregnancy	Negative	114	63.3%
	Positive	66	36.7%

Table III: Relation between follicular fluid or serum E2 concentration and MII oocyte quality

	MII oocyte maturation Grade 2 or 3 (N=53)		Grade 1 (N=172)				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value†**
Follicuar fluid E2 (ng/ml)	45	20 - 130	281	165 - 440	1094.5	-7.128	<0.001
Serum E2 (pg/ml)	800	390 - 1655	2822	1800 - 3798	1219.5	-6.735	<0.001

Table IV: Relation between follicular fluid or serum E2 and embryo quality

Quality of transferred embryo							
	Poor quality (N=65)		**Good quality (N=115)**				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value†**
Follicuar fluid E2 (ng/ml)	55	25 - 170	290	180 - 420	1494.5	-6.681	<0.001
Serum E2 (pg/ml)	800	430 - 1908	2871	1800 - 3887	1393.5	-6.981	<0.001

Table V: Relation between follicular fluid or serum E2 concentration and chemical pregnancy

Chemical pregnancy							
	Negative (N=90)		**Positive (N=90)**				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value†**
Follicuar fluid E2 (ng/ml)	80	30 - 260	300	200 - 440	1971.5	-5.947	<0.001
Serum E2 (pg/ml)	1079	540 - 2859	2874	1898 - 3765	2179.5	-5.351	<0.001

Table VI: Relation between follicular fluid or serum E2 concentration and clinical pregnancy

Clinical pregnancy							
	Negative (N=114)		Positive (N=66)				
Variable	Median	IQR	Median	IQR	U	Z	P-value†
Follicuar fluid E2 (ng/ml)	111	36 - 320	315	220 - 476	1887.5	-5.565	<0.001
Serum E2 (pg/ml)	1561	630 - 2859	3241	2388 - 3988	1748.5	-5.977	<0.001

Table VII: Receiver-operating characteristic (ROC) curve analysis for predictive value of follicular fluid E2

Outcome				
ROC curve parameter	MII maturity	Embryo quality	Chemical pregnancy	Clinical pregnancy
AUC	0.837	0.800	0.757	0.749
SE	0.034	0.038	0.037	0.036
95% CI	.0775 to 0.888	0.734 to 0.856	0.687 to 0.817	0.679 to 0.811
z statistic	9.839	7.892	7.002	6.995
P-value (AUC_0=0.5)	<0.0001	<0.0001	<0.0001	<0.0001
Youden index J	0.58	.053	0.44	0.43
Associated criterion	>170	>130	>144	>160
Sensitivity	74.8	80.9	83.3	86.4
Specificity	83.0	72.3	61.1	57.0

Table VIII: Receiver-operating characteristic (ROC) curve analysis for predictive value of serum E2

Outcome				
ROC curve parameter	MII maturity	Embryo quality	Chemical pregnancy	Clinical pregnancy
AUC	0.819	0.814	0.731	0.768
SE	0.037	0.036	0.039	0.035
95% CI	0.755 to 0.872	0.749 to 0.868	0660 to 0.794	0699 to 0827
z statistic	8.635	8.732	5.988	7.752
P-value (AUC_0=0.5)	<0.0001	<0.0001	<0.0001	<0.0001
Youden index J	0.58	0.56	0.44	0.46
Associated criterion	>1768	>1099	>1059	>2134
Sensitivity	77.2	91.3	94.4	80.3
Specificity	81.1	64.6	50.0	65.8

Role of Routine Hysteroscopy in Management of Women with Unexplained Infertility

Amr M. El Helaly, Khaled S. Mohamed, Hanan H. ElKhateeb, Dina Y. Mansour [1]
[1]Department of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University

Abstract

Background: The gold standard for diagnosing intrauterine anomalies is now hysteroscopy. 10–15% of women undergoing subfertility treatment have intrauterine lesions such adhesions, uterine septum polyps, or submucous myomas, which are significantly more accurately detected via hysteroscopy.

Objectives: Assessment of the value of hysteroscopy in cases with unexplained infertility.

Methodology: This study was conducted in early cancer detection unit, Ain Shams Maternity University Hospital where 75 women with unexplained infertility from 21-35 years were included in the study from Jan 2018 to Feb 2021. All women were subjected to hysteroscopy to diagnose and treat any uterine lesions undetected by the conventional means. Hysteroscopic examination was performed in the proliferative phase of the menstrual cycle.

Results: There was no difference between cases with and without hysteroscopic finding as regard personal and medical characteristics. There was no significant difference between cases with and without hysteroscopic finding as regard occurrence of complications. However, a highly significant difference was found between cases with and without hysteroscopic finding as regard occurrence of pregnancy, as 56% of cases with positive findings got pregnant compared to 2% only of cases without hysteroscopic finding.

Conclusion: So, hysteroscopy (diagnostic, therapeutic) should be performed in women with unexplained infertility.

Keywords: Hysteroscopy; Unexplained infertility

INTRODUCTION

There is a wide range of proposed definitions in the literature. The term "infertility" was initially defined by Duffy et al. as the inability to conceive following three years of unprotected sexual activity in the face of standard baseline investigations [1].

Corresponding author:
Dina Yahia Mansour
Dinayahiamansour@hotmail.com

When an infertility evaluation is unable to identify abnormalities, the diagnosis of unexplained infertility is one of exclusion. Regarding which tests should be run prior to reaching this diagnosis, there is no agreement. Standard diagnostic tests for the assessment of infertility have been recommended by the European Society for Human and Embryology (ESHRE). Semen analysis, hystosalpingography (HSG) or laparoscopy-demonstrated tubal patency, and laboratory evaluation of ovulation are among these techniques [2].

Furthermore, some authors have stated that a post-coital test is a necessary prerequisite for the diagnosis of unexplained infertility, while other authors have concluded that it is not. Nevertheless, managing women with unexplained infertility, particularly in older couples, may benefit from further investigation and treatment of any abnormalities found. Transvaginalsonography (TVS) with or without the addition of saline or gel as a contrast medium is the fundamental work-up for uterine cavity evaluation. This may be followed by either HSG or hysteroscopy to directly assess the uterine cavity [3].

Treatment of minor uterine pathologies in the same setting is made possible by hysteroscopy. It is commonly referred to as the "golden standard" as a result. Although it is debatable whether these subtle lesions are the cause of infertility, many studies have concluded that whenever laparoscopy is performed, it should be combined with hysteroscopy to complete the assessment before beginning the infertility treatment [4].

Hysteroscopic assessment and treatment of any abnormalities detected has improved the clinical pregnancy rate, live birth, and considered cost effective before IVF cycles [5].

Hysteroscopy is used in the examination of infertility to identify potential intrauterine changes that may obstruct the conceptus's growth or implantation, or both, and to assess the efficacy of direct treatment techniques in reestablishing a normal endometrial environment [6].

Hysteroscopy should be a part of every patient's infertility workup before receiving IVF treatment, according to several studies that have also shown that hysteroscopic treatment of intrauterine pathologies lowers the failure rate of VFET. These studies also suggest screening the uterus with a hysteroscopy before pursuing IVF/CS in order to reduce implantation failure [7].

Patients and methods

This prospective study was conducted on 75 patients with unexplained infertility admitted to Ain Shams University Maternity Hospital. This study was done from January 2018 to February 2021 after the permission of the hospital ethical committee and informed consent was obtained from all patients before participation.

Inclusion criteria included all women aged (20-35) years old with unexplained infertility. Exclusion criteria included presence of male factor of infertility, active pelvic infection, known uterine or tubal factor for infertility.

After counseling and explanation of all aspects of the procedure to the participants, a written consent was taken prior to the participation.

Methods

This prospective study was carried out on 75 women who met the pre-established requirements. All women were subjected to full history taking including personal history including age, Duration of marriage, address, occupation, special habits, past history including medical, surgical, blood transfusion, allergy, family history including chronic disease as diabetes mellitus or hypertension, gynecological history including galactorrhea, hirsutism, excess acne, history of previous conditions suggestive of tubal factor infertility e.g., previous salpingitis, previous appendicitis, also ovulatory disorders, breast masses, vaginal infections

or vulvitis and pelvic masses, menstrual history including age of menarche, duration and number of menses, inter menstrual signs of ovulation as ovulatory pain, ovulatory spotting and primary spasmodic dysmenorrhea, obstetric history including (in cases of 2ry infertility) previous pregnancies outcome, mode of termination, postpartum complications, previous abortions; (timing-cause), previous evacuation and curettage and pregnancy intervals, sexual history including frequency of sexual intercourse and dyspareunia, husband history including age, occupation, history of testicular trauma, history of any previous marriage and the existence of offspring in other female partner and medical disorders like diabetes mellitus, hypertension, chronic liver disease or drug intake as anti-psychotic drugs and surgical history as operations done at groin region.

In addition to a laboratory investigation that included semen analysis and a hormonal assay specific to the case (such as FSH-LH-serum prolactin level-thyroid function tests), a general, abdominal, and local examination was conducted. Additionally, an HSG and a pelvic U/S examination were carried out.

In order to identify and treat any mild uterine lesions that were missed by traditional methods, hysteroscopy was performed on each woman. Small uterine flaws that may not be easily recognized by HSG or U/S are referred to as subtle uterine abnormalities.

Hysteroscopic examination was performed in the proliferation phase of the menstrual cycle by 5mm rigid sheath hysteroscope (Karl Storz Endoscopy) without anesthesia (continuous flow, 30 degree forward oblique view)

Illumination: High intensity cold light source and fiberoptic cable D stent on medium: solution of 0.9% normal saline with pressure at 100-120 mmHg.

All procedures were performed with a vaginoscopic approach without utilizing a speculum and applying tract on to the cervix with a tenaculum.

During hysteroscopy, the routine evaluation included assessment of cervical canal, intrauterine lesions, the endometrium, and the uterotubal junction.

When hysteroscopy revealed a lesion, its type, site, size, locationwas determined. Also, any morbidity was recorded including failure of procedure.

Any detected uterine abnonnalty was treated under general anesthesia using operative hysteroscopy. Biopsy was taken when there was any doubt about the pathology of these lesions.

Every finding and outcome, including any intraoperative or postoperative problems like bleeding, infection, or even uterine perforation, was documented.

Every woman underwent a 6-month follow-up period in order to determine whether clinical pregnancy was detected through ultrasound (6 weeks) and to identify any issues related to hysteroscopic interference or lesion recurrence.

Ethical considerations:

The investigator kept a list of sub-investigators and other suitably competent individuals to whom substantial trial-related activities were assigned, and made sure that everyone helping with the trial was properly informed about the protocol and the trial-related duties were explained.

Patient information and informed consent:

Before being admitted to the clinical trial the patients had informed about the nature, scope, and possible consequences of the clinical trial in a form understandable to her.

Confidentiality:

In the case report form, the patients' numbers and initials were the only information entered. The investigators maintained patient privacy whenever the patient's name appeared on any other document (such as a pathology report or reservation note). In order to identify

records and facilitate communication with patients, the investigator initially retained the patients' identifying information, including their numbers, names, and contact details.

Protocol approval:

Before beginning of the trial and in accordance with the local regulations followed, the protocol and all related documents were declared for ethical and research approval by the Council of Obstetrics and Gynecology Department, Ain Shams university.

Statistical analysis:

Using the Statistical Package for Social Science ((BM Corp. Released 2011), the gathered data was updated, coded, tabulated, and brought onto a PC. Armonk, NY: BM Corp., BM SPSS Statistics for Windows, Version 20.0. Data were shown, and appropriate analysis was carried out based on the kind of data found for each parameter.

Results

The study was carried out on 75 patients with unexplained infertility between January 2018 till February 2021. The age ranged from 20 to 35 years with a mean of 28 years (Table I).

There was no significant difference between cases with and without hysteroscopicfinding as regard personal and medical characteristics (Table II).

There was no significant difference between cases with and without hysteroscopicfinding as regard occurrence of complication in the form of infection the 1st case was vaginitis (vaginal discharge and itching) and the 2nd case was presented by dysuria (UTI). The two cases presented by these manifestations within the 2 weeks after hysteroscopy. Clinical evaluation was done and the 1stcase was treated by metronidazole and the2nd case was treated by 3ra generation cephalosporins. And however, a highly significant difference was found between cases with and without hysteroscopic finding as regard occurrence of pregnancy, as 56 of cases with Positive findings got pregnant compared to 12 only of cases without hysteroscopicfinding (Table III).

There was no significant difference between cases with different types of hysteroscopic finding as regard personal and medical characteristics (Table IV).

There was no significant difference between cases with different types of hysteroscopic finding as regard rate of complication or pregnancy (Table V).

Discussion

Because these women have a higher incidence of hysteroscopic pathological findings, published studies assessing the impact of hysteroscopy on reproductive outcome in IVF patients have suggested that hysteroscopy should be performed as a routine infertility examination in all cases. However, this trial found no evidence that hysteroscopy performed prior to IVF-embryo transfer improved pregnancy outcomes [8].

In a prospectively enrolled IVF group, Knynlcin et al. reported on a total of 2500 consecutive office-based diagnostic hysteroscopies conducted before treatment. In 22.9% of cases, endometrial pathology on hysteroscopy was found, which may have compromised the success of IVF [9].

Knraynlin et al. attempted to determine the effect of scheduling office hysteroscopy prior to embryo transfer on pregnancy rate by enrolling 1258 individuals attending an IVF clinic within normal hysteroscopic findings. Significant rates of implantation, pregnancy, and clinical pregnancy were observed [10].

Numerous recent studies have demonstrated that utilizing assisted reproductive techniques prior to treating patients with infertility that cannot be explained by other means significantly increases the likelihood of becoming pregnant [11].

Three theories serve as the foundation for

the use of endometrial scraping in infertile women. The first, based on research on animals, is that endometrial damage could encourage endometrial decidualization, which would increase the endometrium's receptivity to the embryo [12].

Diagnostic office hysteroscopy revealed that in 33.3% of cases with infertility that could not be explained, abnormal uterine findings were found, while the uterine cavity was normal in 66.7% of cases. Intrauterine adhesions accounted for 17.3% of all hysteroscopic findings, followed by endometrial polyps (9.3%), small submucousmyomas (4%), and endocervical polyps (2.7%).

Our findings corroborated those of Elbareg et al., who examined infertile women in whom routine infertility examinations found no anomalies; their findings showed that 33% of patients had aberrant uterine findings, the majority of which were minor adhesions, tiny submucousmyomas, and polyps [13].

Transvaginal sonography (TVS) and hysterosalpingography (HSG) were used by Bakas et al. to evaluate infertile women first. If no aberrant intrauterine findings were seen, a diagnostic hysteroscopy was also carried out. Hysteroscopy results were normal in 68.2% of cases, while intrauterine lesions (polyps, septa, submucosalleiomyomas, or synechiaes) were found in 31.8% of cases [14].

According to the results of studies published by Sahuet al and El-Sheikh et al [15, 16], there was no statistically significant difference between cases with and without hysteroscopic findings in the current study with regard to personal and medical characteristics, such as age P value = 0.122, duration of infertility P value = 0.54, and type of infertility P value = 0.72.

Additionally, the current study showed that there was no significant difference in the occurrence of a complication (P value = 0.1) between cases with and without hysteroscopic findings, which was in line with study [17].

However, research by Shokeir et al. concluded that local endometrial injury for the natural cycle concept in women with Us was not justified and that there were no statistically significant differences in cumulative pregnancy rates between women with and without local endometrial injury (16.7% and 11.7%, respectively; OR, 2.83; 95%C: 1.07-7.48; P = 0.4) [18].

The following is a definition of intrauterine adhesion severity: Moderate: 25-75% of the uterine cavity has adhesions that partially obstruct the ostium and upper fundus; severe: more than 75% of the cavity has thick bands or wall agglutination. Mild: Less than 25% of the cavity has thin or filmy adhesions [19].

Conclusion

Finally, the present study concluded that as a cause of unexplained infertility, subtle uterine abnormalities is diagnosed during hysteroscopy; the r correct on seems to be necessary to get pregnant.

Conflict of interest

None

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Table I: Description of personal and medical characteristics of study participants

		Mean	±SD	No.	%	Mini.	Maxi.|
Age		27.95	3.74			20.00	35.00
Duration of infertility		4.06	2.01			2.00	9.00
Age group	20-24			13	17.3		
	25-29			39	52.0		
	30-35			23	30.7		
Parity	P0			50	66.7		
	PI			13	17.3		
	P2			10	13.3		
	P3			2	2.7		
Abortion	No			72	96.0		
	Yes			3	4.0		
Type of infertility	Primary			50	66.7		
	Secondary			25	33.3		
Procedure	Diagnostic hysteroscopy			50	66.7		
	Diagnostic & therapeutichysteroscopy			25	33.3		
Hysteroscopy Findings	Negative			50	66.7		
	Positive			25	33.3		
Hysteroscopic Findings	None			50	66.7		
	intra uterine adhesion			13	17.3		
	Endometrial polyp			7	9.3		
	Small submucousmyoma			3	4.0		
	Endoccrvical polyp			2	2.7		
Type of Finding (n=25)	intra uterine adhesion			13	52.0		
	Endometrial polyp			7	28.0		
	Small submucousmyoma			3	12.0		
	Endoccrvical polyp			2	8.0		
Complications	No			73	97.3		
	Yes			2	2.7		
Pregnancy	No			55	73.3		
	Yes			20	26.7		

Table II: Comparisons between cases with and without hysteroscopic finding as regard personal and medical characteristics

		Hysteroscopic Finding								P	Sig.
		Negative (n=50)				Positive (n=25					
		Mean	±SD	No.	%	Mean	±SD	No.	%		
Age		28.42	3.60			27.00	3.89			0.122§	NS
Duration of infertility		3.96	1.92			4.26	2.20			0.545§	NS
Age group	20-24			6	12.0			7	28.0	0.21*	NS
	25-29			27	54.0			12	48.0		
	30-35			17	34.0			6	24.0		
Parity	P0			37	68.0			16	64.0	0.507**	NS
	PI			7	14.0			6	24.0		
	P2-3			9	18.0			3	12.0		
Abortion	No			47	94.0			25	100.0	0.546**	NS
	Yes			3	6.0			0	0,0		
Type of infertility	Primary			34	68.0			16	64.0	0.729*	NS
	Second-ary			16	32.0			9	36.0		

§JANOVA test *Ch -Square test **Fisher exact test

Table III: Comparison between cases with and without hysteroscopic finding as regard procedure outcome (complication)

		Hysteroscopic Finding				P	Sig.
		Negative (n=50)		Positive (n=25)			
		No.	%	No.	%		
Complications	No	50	100.0	23	92.0%	0.108*	NS
	Yes	0	0.0	2	8.0%		
Pregnancy	No	44	88.0	11	44.0%	0.001**	HS
	Yes	6	12.0	14	56.0%		

*Fisher exact test
**Ch -Square test

Table IV: Relation between type of hysteroscopic Finding and personal and medical characteristics

		Type of Finding																P	Sg
		U adhesion (n=13)				Endometrial p. (N=7)				Submucousmyoma (N=3)				Endocervical p. <N=2)					
		Mean	±SD	No.		Mean	±SD	No.		Mean	±SD	No.		Mean	±SD	No.			
Age		27.38	3.01			25.29	4.72			31.00	3.61			24.50	3.54			0.13*	NS
Duration of infertility		4.35	1.84			3.43	2.15			6.67	3.21			3.00	1.41			0.15*	NS
Age group	20-24			3	23.1			3	42.9			0	0.0			1	50.0	0.46**	NS
	25-29			8	61.5			2	28.6			1	333			1	50.0		
	30-35			2	15.4			2	28.6			2	66.7			0	0.0		
Party	P0			7	53.8			6	85.7			1	33.3			2	100.0	0.27**	NS
	PI			5	38.5			0	0.0			1	33.3			0	0.0		
	P2-3			1	7.7			1	14.3			1	33.3			0	0.0		
Type of infertility	Primary			7	53.8			6	85.7			1	33.3			2	100.0	0.273**	NS
	Secondary			6	46.2			1	14.3			2	66.7			0	0.0		

§JANOVA test *Ch -Square test **Fisher exact test

Table V: Relation between type of hysteroscopic finding and procedure outcome (complication and pregnancy)

		Type of findings								P	Sig.
		U adhesion (n=13)		Endometrial P. (N=7)		Submucousmyoma (N=3)		Endocervical			
Complications	No	11	84.6	7	100.0	3	100.0	2	100.0	0.69**	NS
	Yes	2	15.4	0	0.0	0	0.0	0	0.0		
Complications	No	5	38.5	4	57.1	2	66.7	0	0.0	0.54**	NS
	Yes	8	61.5	3	42.9	1	33.3	2	100.0		

Azithromycin versus Erythromycin in Preterm Premature Rupture of Membranes: Mansoura Experience

Hamsa Gomaa Ramadan, Hesham Mahmoud Shalan, Abd Elhady Abd Elhady Zayed and Ahmed Abdelhamid El-Zayadi
Obstetrics and Gynecology Department, Faculty of Medicine, Mansoura University.

Abstract

Background: Fetal membrane rupture prior to the 37th week of gestation is a condition termed preterm premature rupture of membranes (PPROM). Many institutions now recommend azithromycin over erythromycin in management of PPROM Due to national erythromycin shortages, azithromycin's better side effect profile, and ease of administration.

Aim of work: Assess effectiveness, side effects, and cost of azithromycin versus erythromycin in management of PPROM.

Patients and methods: this research involved women with PPROM who were distributed into two groups; group A (Azithromycin treated group) and group B (Erythromycin treated group). All cases underwent full history taking, clinical examination, laboratory analysis and obstetric ultrasound (including AFI and FHR). Different outcomes were determined including the duration of latency, chorioamnionitis, neonatal death, neonatal respiratory distress, drug prices and effects.

Results: Differences between groups have been shown to be statistically significant as regard incidence of nausea, vomiting and diarrhea. Higher incidence of side effects was detected among erythromycin than Azithromycin group. The cost of azithromycin was higher compared to the erythromycin regimen.

Conclusion: Azithromycin can be used in place of erythromycin for the expectant management of **PPROM**. Azithromycin only benefits from its availability and reduced gastrointestinal side effects..

Keywords: PROM, PPROM, Azithromycin, Erythromycin

INTRODUCTION

Preterm premature rupture of membranes (PPROM) is A term used to describe the occurrence of membrane rupture prior to the onset of labor in pregnancies that are less than 37 weeks along. About 30% of all births are premature because of this issue, and it affects 1-3% of all pregnancies. [1].

Corresponding author:
Hamsa Gomaa Ramadan
Mobile: (+20)01069535327 ,
E-mail:hamsaelkasaby5@gmail.com

It may cause devastating maternal, foetal, and neonatal outcomes. Chorioamnionitis, cord compression, abruptio placenta, neonatal sepsis, respiratory distress syndrome, intraventricular bleeding, and even neonatal death are some of the severe perinatal complications that can occur after PPROM. [2].

The infectious process seems to be one of the most important causes of PPROM and this seems to lead to an inflammatory reaction, which alters the tissue structure of the membrane, weakening it and, thus, allowing its rupture [3]. The main agents involved in this pathophysiology are Gardnerella vaginalis, Neisseria gonorrhoeae, Streptococcus agalactiae, Escherichia coli, and Bacteroides sp. [4]

There are many debates about the best course of medical action to take when a woman's ovular membranes rupture before 37 weeks of pregnancy, which is a common occurrence in obstetric practice.[5].

Disagreements include expectant management based on diagnosis, hospitalization, tocolysis, and corticosteroids. In addition, there are the methods used to diagnose infection, the ideal delivery time, and antibiotics usage both for prophylaxis of infection by Group B streptococcus, as well as to increase the period of latency [6].

The most common ACOG-approved PPROM regimen is intravenous erythromycin and ampicillin for two days, followed by oral erythromycin and amoxicillin for five days. This treatment plan reduced chorioamnionitis and other fetal/neonatal complications and prolonged latency delivery..[7].

Nowadays, azithromycin is used instead of erythromycin because it is simpler to administer, has fewer side effects, and is more readily available than erythromycin.[8].

PATIENTS AND METHODS

A prospective, randomized, and controlled clinical research was performed at the Obstetrics and Gynecology Department's inpatient and outpatient clinics at Mansoura University Hospitals in Mansoura, Egypt. The study was carried out from January 2021 to December 2021 over a period of a year.

This research included female cases with PPROM into two groups; Group A (that included 135 patients with were treated by azithromycin and group B (that included 134 patients who were treated with erythromycin)

We included the patients diagnosed with PPROM in the age between 18 and 38 years who are pregnant with gestational age before 37 weeks. We excluded patients with the following characters: PROM <24 or >37 weeks, patients in active labor, presence of any placental insufficiency or abnormality and signs of chorioamnionitis e.g. fetal tachycardia.

The study follows the 2013 Helsinki Standards.[9]. After receiving approval from Mansoura University's Faculty of Medicine's regional ethics committee and written or verbal informed consent from the included cases, the study was conducted.

The cases had a full physical examination, history (including demographics, general medical history, and comorbidities), and menstrual history (to confirm the date and ensure she had an LMP) (General and abdominal examinations focused on uterine contractions).

To confirm gestational age, measure the amount of amniotic fluid (AFI and FHR), and document the viability of the pregnancy, obstetric ultrasound (trans-abdominal) was performed. Total leucocyte count and CRP titer were two laboratory tests that were performed..

Women who had PPROM before 37 weeks of pregnancy, who were not in active labour, who had no clinical signs of chorioamnionitis or placental abruption, and who were admitted for expectant management according to the departmental protocol; women were treated in accordance with this protocol as follows: Every 12 hours, four intramuscular injections of 6-mg dexamethasone are to be given. [10].

The cases were split into two categories.:

- Group A (azithromycin treated group): comprised 135 cases who were given 500 mg of azithromycin orally once every 12 hours for 5 days (Zithromax 500, Pfizer, USA).
- Group B (erythromycin treated group): included 134 patients who were given 500 mg of erythromycin orally every eight hours for five days (Erythromycin 500, Amirya, Egypt).

The outcome measures involved assessment of the latency period as the primary outcome. The latency period is the interval between the beginning of membrane rupture and delivery.

Neonatal respiratory distress requiring oxygen supplementation, neonatal death, and secondary outcomes of chorioamnionitis were also reviewed. Additionally, in a post-treatment patient survey, the cost of medications and their respective side-effect profiles were evaluated.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 26 for Windows® was used to code, process, and analyse the collected data (IBM, SPSS Inc, Chicago, IL, USA). Number (frequency) and percent-based qualitative data were displayed. The Chi-Square test (or Fisher's exact test) made the comparison between groups. The Kolmogorov-Smirnov test tested quantitative data for normality. Parametric data were shown as median ± SD while non-parametric data were expressed as median (range).

Using independent samples (student's) t-test, two groups with normally distributed quantitative variables were compared, Additionally, the Mann-Whitney U-test was applied if the data had an abnormal distribution.For all tests, P values <0.05 are considered significant.

RESULTS

The current research initially included 278 female patients who were assessed for eligibility. Among them 6 females were excluded. The remaining 272 cases were randomly allocated into two groups using randomly generated computer tables; group A and group B .

One female only lost follow up in group A while two females lost follow up in group B. So, the final analyzed number was 135 cases in group A and 134 cases in group B.

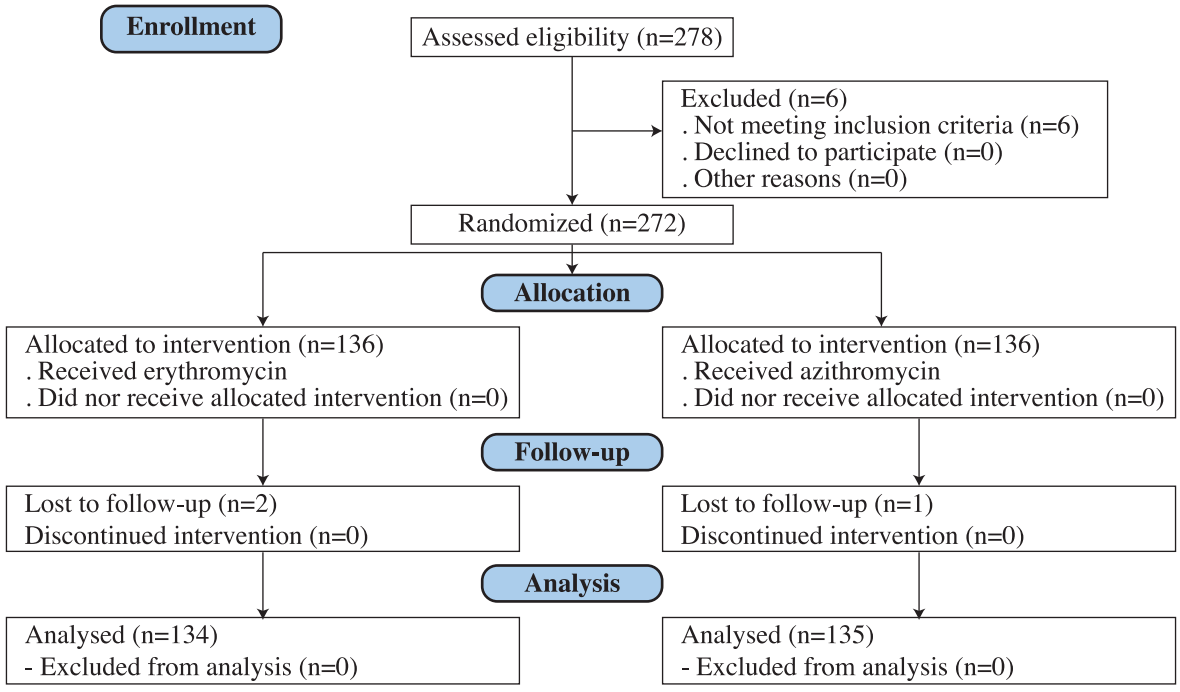

Figure (1): CONSORT Flow chart showing study design

Table (1): shows that there is no statistically significant difference between studied groups as regard age of the cases, gestational age, body mass index ,parity and number of CS .Mean age of erythromycin group is 25.81 years and 25.22 years for azithromycin group .Mean gestational age is 31.07 weeks versus 30.99 weeks for Erythromycin & Azithromycin group , respectively. For erythromycin group; 39.6% ≥2nd para, 32.1% primi-para and 28.4% nullipara and for Azithromycin group; 41.5% ≥2nd para, 30.4% primi-para and 28.1% nullipara.

Table (1): Comparison of sociodemographic data between the studied groups

	Erythromycin group N=134	**Azithromycin group N=135**	**test of significance**
Age in years mean±SD	25.81±5.31	25.22±4.75	t=0.938 p=0.349
Gestational age in weeks mean±SD	31.07±1.87	30.99±1.91	t=0.388 p=0.698
Body mass index (kg/m^2) mean±SD	29.0±1.97	28.85±2.01	t=0.395 p=0.693
Parity n(%) Nullipara Primi para ≥2nd para	38(28.4%) 43(32.1%) 53(39.6%)	38(28.1%) 41(30.4%) 56(41.5%)	χ^2=0.126 P=0.939
Number of CS n(%) NO 1-2 >2	56(41.8%) 66(49.3%) 12(9.0%)	61(45.2%) 60(44.4%) 14(10.4%)	χ^2=0.650 P=0.723

Table (2): Mean temperature among erythromycin group is 37.3 versus 37.28 for azithromycin group without statistically significant difference between them .Mean heart rate illustrates non statistically significant difference between groups with mean heart rate among erythromycin group is 90.68 versus 90.32 for Azithromycin group.

Table (2): comparison of general examination between the studied groups

	Erythromycin group N=134	**Azithromycin group N=135**	**test of significance**
Temperature (°C) mean±SD	37.30±0.59	37.28±0.57	t=0.273 p=0.785
Heart rate (bpm) mean±SD	90.68±8.89	90.32±8.59	t=0.345 p=0.730

Table (3): A non-statistically significant difference between groups is detected for CRP , total leukocytic count , AFI & fetal heart rate .Mean CRP is 7.43 versus 6.96 ,mean total leukocytic count is 9.09 versus 8.23 , mean AFI is 4.62 versus 4.79 , mean fetal heart rate is 145.39 versus 144.19 , for Erythromycin & Azithromycin groups , respectively.

Table (3): comparison of laboratory and ultrasound examination between the studied groups

	Erythromycin group N=134	Azithromycin group N=135	test of significance
CRP mean±SD	7.43±4.07	6.96±4.57	t=0.88 p=0.379
Total leucocytic count mean±SD	9.09±4.26	8.23±3.03	t=1.90 p=0.06
AFI mean±SD	4.62±1.45	4.79±0.89	t=1.17 p=0.245
Fetal heart rate (bpm) mean±SD	145.39±14.11	144.19±14.99	t=0.682 p=0.496

Table (4) : A non-statistically significant difference is detected between studied groups as regard latency , mode of delivery , chorioamnionitis and maternal sepsis. Mean latency is higher among azithromycin than erythromycin group (11.48 versus 10.95) , 55.2% and 52.6% of erythromycin versus azithromycin groups , 9% & 7.4% of the erythromycin versus azithromycin groups, 6%&6.7% of erythromycin versus azithromycin groups have maternal sepsis.

Table (4): comparison of maternal outcome between the studied groups.

Maternal outcome	Erythromycin group N=134	Azithromycin group N=135	test of significance
Latency (days) mean±SD	10.95±5.18	11.48±4.56	t=0.921 p=0.358
Mode of delivery n(%)			
Vaginal	60(44.8%)	64(47.4%)	χ^2=0.187
CS	74(55.2%)	71(52.6%)	p=0.665
Chorioamnionitis n(%)			
-ve	122(91%)	125(92.6%)	χ^2=0.215
+ve	12(9%)	10(7.4%)	p=0.643
Maternal sepsis n(%)			
-ve	126(94%)	126(93.3%)	χ^2=0.055
+ve	8(6%)	9(6.7%)	p=1.0

Table (5) : A non-statistically significant difference is detected between studied groups as regard gestational age , birth weight , APGAR score , respiratory distress , neonatal sepsis & death . Mean gestational age is 32.7 versus 32.65 weeks for Erythromycin and azithromycin groups, respectively. Mean APGAR score is 6.41 and 6.31 for azithromycin and Erythromycin groups, respectively. Mean birth weight is 1809.37 and 1797.63 for Erythromycin and azithromycin groups, respectively. Respiratory distress was detected among 23.9%, 13.4% versus 31.9%&9.6% for azithromycin & Erythromycin groups and neonatal death was the same for both groups (3%).

Table (5): comparison of fetal outcome between the studied groups

fetal outcome	Erythromycin group N=134	Azithromycin group N=135	test of significance
Gestational age at birth in weeks mean±SD	32.70±1.42	32.65±1.49	t=0.280 p=0.780
Birth Weight in gram mean±SD	1809.37±313.93	1797.63±330.45	t=0.299 p=0.765
APGAR score mean±SD	6.31±0.87	6.41±0.97	t=0.831 p=0.407
Respiratory distress n(%)	32(23.9%)	43(31.9%)	χ^2=2.13 p=0.145
Neonatal sepsis n(%)	18(13.4%)	13(9.6%)	χ^2=0.954 p=0.329
Neonatal death n(%)	4(3.0%)	4(3.0%)	FET=0.0 P=1.0

Table (6) shows that there is statistically significant difference between studied groups as regard incidence of nausea , vomiting and diarrhea. Higher incidence of side effects were detected among erythromycin than Azithromycin group. of the studied cases ; 23.1% versus 10.4%, 18.7%versus 6.7% &11.2% versus 2.2% have nausea , vomiting and diarrhea ,respectively for Erythromycin & Azithromycin groups.

Table (6): comparison of drug side effects between the studied groups.

Drug side effects	Erythromycin group N=134	Azithromycin group N=135	test of significance
Nausea n(%)	31(23.1%)	14(10.4%)	χ^2=7.87 P=0.005*
Vomiting n(%)	25(18.7%)	9(6.7%)	χ^2=8.76 P=0.003*
Diarrhea n(%)	15(11.2%)	3(2.2%)	χ^2=8.67 P=0.003*

Table (7) demonstrates that dose of erythromycin treatment is 1*3*5 with total course is 15 tablets with 500 mg concentration and net cost is 13 LE. Dose of Azithromycin treatment is 1*2*5 with total course is 10 Caps with 500 mg concentration and net cost is 283 LE.

Table (5): comparison of fetal outcome between the studied groups

	Erythromycin group N=134	Azithromycin group N=135
Dose:	1x3x5	1x2x5
Total	15 tab.	10 caps.
Concentration:	500 mg	500 mg
Net Cost/ patient: (LE)	13	283

Table (8) shows that alternative treatment companies for Zithrokan , Zisrocin, Azithromycin are Hikma pharma ,EGYpharm & AUG pharma with course cost are 108, 108 & 90 LE, respectively.

Table (8): Lower-cost alternatives for ZITHROMAX® | Pfizer :

	Company	Price	Course cost
Zithrokan	Hikma pharma	32.5	108
Zisrocin	EGYpharm	32.5	108
Azithromycin	AUG pharma	27	90

This study compared azithromycin and erythromycin's cost, side effects, and efficacy in treating PPROM. All females were randomly divided into two groups: group A, which had 135 cases and received 500 mg of azithromycin orally every 12 hours for five days; and group B, which had 134 cases and received 500 mg of erythromycin orally every eight hours for five days.

Cases' age, gestational age, body mass index, parity, and number of CS are not statistically different between the study groups.

This was supported by Musavi et al. (2022), who included 194 pregnant women with PPROM and randomly .

DISCUSSION

assigned them to group A (the azithromycin group) or group B (the placebo group) (Erythromycin group). The two groups' demographic and environmental traits (age, body mass index, gravidity, parity, abortion, live birth) were not statistically different) [11].

Mohamed et al. found similar results in 162 singleton pregnant women aged 18–40 with PPROM between 24 and 32 weeks of gestation. Patients were randomly assigned to Group A or Group B. The findings revealed that differences in gestational age, parity, and the number of prior caesarean sections between the two studied groups were not statistically significant [12].

For CRP, total leucocytic count, AFI, and foetal heart rate, non-statistically significant differences between groups were found in the current study. Additionally, this was mentioned in the study by Musavi et al (2022).

The current findings supported Gelber et al.'s that included women with PPROM at 24-34 weeks who were given azithromycin (n = 29) or erythromycin (n = 67) had no difference in AFI, CRP, or total leucocytic count [13].

This was also in line with the findings of Mohamed et al. who demonstrated that there was no statistically significant difference in the parameters of temperature, heart rate, total leucocytic count, C-reactive protein concentration, foetal heart rate, and amniotic fluid index between the erythromycin and azithromycin groups [12].

In the current study, there was no statistically significant difference between the two study groups regarding latency, mode of delivery, chorioamnionitis and maternal sepsis.

Musavi and colleagues found that erythromycin and azithromycin did not significantly differ in chorioamnionitis or delivery method.

Additionally, a meta-analysis by Seaman et al. recently found a total of 5 studies with 1289 women. Both patients receiving erythromycin and those receiving azithromycin experienced similar mean latency times in women with PPROM. Clinical chorioamnionitis was 25% (95 percent confidence interval, 12-32) in women treated with erythromycin and 14% in those treated with azithromycin (95 percent confidence interval, 9-24) [8].

Navathe et al. found no statistically significant difference in latency to delivery, as did the current study. Azithromycin 1 day group, azithromycin 5 day group, azithromycin 7 day group, and erythromycin group all had unadjusted median times from PPROM to delivery of 5.0 days, 4.4 days, 4.7 days, and

4.7 days, respectively (P = 0.98) [14].

Martingano et al. noted that there was no difference in pregnancy latency and significant differences in the rates of clinical chorioamnionitis, but not histologic chorioamnionitis, about the primary outcomes. Azithromycin had a median latency difference of 5 days, with an interquartile range (IQR) of 6–11 days, and erythromycin had 4.5 days, with an IQR of 6–10.8 days [15].

Mohamed et al. found no statistically significant difference in the latency period for erythromycin patients (1-35 days) and azithromycin patients (2-28 days). They also found no significant differences in maternal outcomes like chorioamnionitis and postpartum haemorrhage between the two study groups [12].

Additionally, the current findings supported those of Gelber et al., who found no differences in latency or maternal outcomes between PPROM-positive women at 24-34 weeks who received either azithromycin (n = 29) or erythromycin (n = 67) as a treatment [13].

This was also consistent with Pierson et al. comparison's of 93 PPROM women treated with ampicillin and a single dose of azithromycin at 24-34 weeks to 75 comparable women treated with ampicillin and erythromycin. They discovered no variation in the latency from membrane rupture to delivery. The prevalence of chorioamnionitis was comparable [16].

With PPROM at 23-33 6/7 weeks, Finneran et al. compared 78 women who received 1 g of azithromycin once orally to 84 women who received erythromycin for 7 days in 2017. The only differences in maternal and neonatal outcomes were higher incidences of caesarean delivery, which was also reflected in the median latency from PPROM to delivery [17].

Non-statistically significant differences in gestational age, birth weight, APGAR score, respiratory distress, neonatal sepsis, and death were found between the studied groups in the current study.

This was consistent with a study by Musavi et al. that found no statistically significant difference between the erythromycin and azithromycin groups for any neonatal outcomes, such as gestational age at delivery, APGAR score, birth weight, or neonatal death [11].

The current findings were consistent with those of Finneran et al., who reported that the only difference in neonatal outcomes between the erythromycin and azithromycin groups that was statistically significant was the positive neonatal blood cultures in the erythromycin group [17].

Mohamed et al. reported similar findings, finding no statistically significant difference in neonatal outcomes, including foetal outcome measures such as birth weight, Apgar score, neonatal respiratory distress syndrome, neonatal sepsis, and neonatal death [12].

Additionally, the current findings supported those of Gelber et al., who found no differences in neonatal outcomes between women with PPROM at 24-34 weeks who received either azithromycin (n = 29) or erythromycin (n = 67) as treatment [13].

Additionally, the current findings were consistent with those of Pierson et al., who contrasted 93 PPROM patients at 24-34 weeks who received ampicillin and a single dose of azithromycin with 75 patients of a similar age who received ampicillin and erythromycin. Both groups had similar neonatal complications, Apgar scores, and birthweight [16].

Although the pharmacokinetic properties of azithromycin and erythromycin are different, both antibiotics cover a similar range of microorganisms. As opposed to erythromycin, which has a half-life of about 1.6 days, azithromycin has a half-life that can be as long as more than 70 hours in the myometrium [18-20].

Additionally, it has been demonstrated that azithromycin's gastrointestinal side effect profile is better [21]. Additionally, many institutions have promoted the use of azithromycin rather than erythromycin due to widespread shortages of the latter [22].

There is a statistically significant difference in the incidence of nausea, vomiting, and diarrhea between the studied groups when it comes to the side effects of the study drugs. The Erythromycin group had a higher incidence of side effects than the Azithromycin group.

This was in line with the findings of Mohamed et al., who discovered a statistically significant difference between the two studied groups in regards to gastrointestinal side effects, specifically in the areas of nausea (19:9), vomiting (15:6), and diarrhea (9:2) in the erythromycin group compared to the azithromycin group [12].

Azithromycin was compared to erythromycin and amoxicillin for treating C. trachomatis infection in pregnant women by Pitsouni et al. The study included 587 expectant women who had C. trachomatis infections that were verified microbiologically. Regarding the success of the treatment in patients who were being evaluated clinically or with an intention to treat, there was no difference between azithromycin and erythromycin. Azithromycin also had fewer gastrointestinal adverse events, overall adverse events, withdrawals, and adherence than erythromycin. Azithromycin is just as effective but safer for treating C. trachomatis infection in pregnant women [23].

In the current study, dose of erythromycin treatment is 1*3*5 with total course is 15 tablets with 500 mg concentration and net cost is 13 LE. Dose of Azithromycin treatment is 1*2*5 with total course is 10 Caps with 500 mg concentration and net cost is 283 LE.

Similar findings were presented by Mohamed et al. (2015), who demonstrated that azithromycin is more expensive than erythromycin in terms of the cost of treatment for the two groups.

Finneran et al. estimated a 95% cost savings for azithromycin over erythromycin; however, current results were in disagreement with their findings [22]. The difference could be mostly due to different geographic locations and different companies that subsequently could affect the price of the drugs.

CONCLUSION

Premature preterm membrane rupture (PPROM) is a common problem for pregnant women. Azithromycin and erythromycin had similar effects on mothers and newborns. If erythromycin is unavailable or not recommended, azithromycin may be used instead to treat PPROM in pregnant women. Apart from its accessibility and absence of gastrointestinal side effects, azithromycin does not appear to have any additional advantages.

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Low Dose Corticosteroids in Management of Hyperemesis Gravidarum at Mansoura University Hospital

Hanan Abd Almonem Ahmed [1], MD; Maged El-shamy [1], MD; Emad Ahmed Fayala [1], MD; Mahmoud Mohamed Awad [1], MD
[1] Department of Obstetrics and Gynecology, Mansoura University Hospital, Mansoura Faculty of Medicine, Mansoura, Egypt;

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Short Running Title:
Corticosteroids in hyperemesis gravidarum.

Precis

Corticosteroids were highly effective in reducing the severity of vomiting after 48 hours from the start of the treatment using Pregnancy-Unique Quantification of Emesis (PUQE) score and improving their quality of life (QOL) score compared with the standard treatment in the control group.

Abstract

Objective: to show the effect of addition of corticosteroids to standard treatment of hyperemesis gravidarum with respect to initial response of treatment, reduction of severity of vomiting , improvement of quality of life and rate of readmission to hospital .

Design: RCT (Canadian Task Force Classification- I).

Setting: Mansoura University Hospitals

Patients: Fifty pregnant women suffering from hyperemesis gravidarum were admitted to department of obstetrics and gynecology, Mansoura University Hospitals.

Corresponding author:
Hanan Abd Almonem Ahmed
Mansoura Faculty of Medicine
Mansoura, Egypt
Tel.: 01015351915
Email:henaabdo776@gmail.com

Interventions: Patients were randomized into two groups; group 1 (Corticosteroid group; n = 25) and group 2 (non-corticosteroid group; n = 25) at 1:1 ratio.

Measurements and Main Results: The primary outcome was the severity of vomiting after 48 hours after start of the treatment using Pregnancy-Unique Quantification of Emesis (PUQE) score. The secondary outcomes were quality of life after 48 hours and 1 week from starting the treatment protocol using a rating scale with a range between zero (the worst possibly imaginable) and ten (equaled as good as she felt before the start of this pregnancy) , assessment of severity of vomiting after 1 week of start of the treatment using PUQE score , rate of readmission to the hospital within 2 weeks of treatment , extent of ketonuria , and length of hospital stay. The severity of vomiting after 48 hours of start of the treatment using PUQE score decreased significantly in the corticosteroid group, being 10.64 ± 1.62 compared to 11.88 ± 1.64 in the control group. PUQE score after 1 week of start of the treatment was comparable again between the two studied groups. The quality of life (QOL) of patients after 48 hours from starting the treatment score was statistically higher among the corticosteroid group being 5.8 ± 0.8 compared to 4.6 ±1.2 in the control group p value ≤0.05 which continued after 1 week being higher among the corticosteroid group, 8.3 ±0.9 compared to 7.6 ± 0.7 in the control group. The rate of readmission to the hospital for hyperemesis gravidarum within 2 weeks of starting the study was higher among the control group being 32% versus only 4% in the group taking corticosteroids. Also, the median length of stay was higher among the control group, 7 days ranged from 4 to 28 days compared to 4 days ranged from 3 to 12 days in the corticosteroid group.

Conclusions: Corticosteroids were highly effective in reducing the severity of vomiting in HG patients after 48 hours from the start of the treatment using PUQE score and improving their QOL score compared with the standard treatment in the control group.

Keywords: hyperemesis, Corticosteroids, PUQE score, quality of life, ketonuria.

Introduction

In the first half of pregnancy, 50% to 80% of pregnant women experience nausea and occasional vomiting (NVP) which has a significant negative influence on the health and quality of life of the mother (1, 2) .Hyperemesis gravidarum (HG) is a term that is frequently used to describe severe or prolonged vomiting. 0.2-3.6% of pregnant women experience HG, which is far less frequent than NVP. Although it occurs at a very low rate, HG is the leading cause of hospital admission (3, 4) .

As a first line of treatment for hyperemesis gravidarum, hospitalization, intravenous rehydration, and antiemetics are frequently used (5) .The most current studies on NVP treatments state that there is conflicting evidence about the efficacy of pyridoxine (vitamin B6), ginger, and antiemetic drugs (6) . In general, pyridoxine decreases nausea but not vomiting. However, pyridoxine does not have teratogenic effects when combined with antihistamines (H1-receptor blockers as doxylamine and meclizine) and considerably relieves sensations of nausea and vomiting (7). 5-hydroxytryptamine3-receptor antagonists, such as ondansetron, may be used as a second-line treatment after phenothiazines (such as Phenergan) and dopamine-antagonists (such as metoclopramide). They are all said to lessen the symptoms of nausea and vomiting but may have adverse effects on the mother, and potential teratogenic effects are less researched (5).

Although Corticosteroids (CCS) are frequently used to treat nausea and vomiting caused by chemotherapy, there is little data to support their use in the treatment of HG (8). Corticosteroids have resulted in dramatic and rapid improvement in case series of pregnant women with refractory HG.

Corticosteroids should be used after failure of treatment with intravenous fluid replacement and antiemetics .The suggested dose is intravenous hydrocortisone 100 mg twice per day , and convert to oral prednisolone 40–50 mg per day after improvement is achieved , with the dose gradually decreased until the lowest maintenance dose that controls the symptoms is reached (9) . In most cases prednisolone should be continued until the gestational age at which symptoms of HG would have disappeared and in some cases till the delivery(10) .

Materials and methods

Patient population:

A prospective, randomized, and controlled From March 2021 to March 2022 a prospective randomized study enrolled 50 pregnant women with hyperemesis gravidarum admitted at Mansoura university hospital, department of Obstetrics and Gynecology.

The study protocol was reviewed and approved by the Mansoura Faculty of Medicine Institutional Research Board (Code number # MS.21.02.1387) was obtained.

Pregnant woman less than 16 weeks with vomiting more than three times per day for the previous 72 hours not responding to first and second lines of treatment, ketonuria ++ that did not respond to dietary changes, weight loss more than 5 %, or with a second admission for hyperemesis were selected to enroll in our study. Eligible subjects were interviewed, informed about the study, and counseled for participation. They were evaluated regarding the inclusion and exclusion criteria. Women with any of the following criteria were excluded from the study: 1) molar pregnancy; 2) twin pregnancy; 3) contraindications to steroids (glaucoma, and those with cardiovascular disorders, gastrointestinal diseases, liver dysfunction, and acute pyelonephritis); 4) conditions requiring steroid use (systemic lupus erythematosus (SLE) and immunocompromised patients) ; or 5) causes of nausea and vomiting are unknown. A written informed consent was taken from each woman participating in the study.

Allocation and Randomization:

Pregnant women with hyperemesis gravidarum who met the inclusion and exclusion criteria were randomized into 2 groups. Group 1 (corticosteroid group) 25 patients. Group 2 (non-corticosteroid group) 25 patients. The randomization was determined by the patient's identification number kept within closed sealed envelopes. Women with odd identification numbers were selected for the corticosteroid group and those with even identification numbers for non- corticosteroid group.

Methods:

Afterwards the patients were admitted to the hospital, Full history including gravidity, parity, past and surgical history was taken. Clinical examination included vital signs, height, weight, body mass index (BMI), and obstetric examination. Ultrasonography was done to confirm the existence of a normal-appearing intrauterine pregnancy. Laboratory investigations included complete blood count (CBC), serum levels of sodium and potassium, serum creatinine, serum aspartate aminotransferase (AST) and alanine transaminase (ALT), and urine analysis for ketonuria. Arterial blood gas (ABG) samples were examined for any acid base disturbances.

A standard regimen for management of hyperemesis gravidarum for all the study participants were applied and consisted of administration of intravenous crystalloid solutions to correct dehydration, replacing any electrolyte disturbance, correcting acid base disturbance, administration of prophylactic anticoagulant, anti-stress ulcer drugs **(PANTOPRAZOLE 40 mg, PHARO PHARMA)** and anti-emetics (Antihistamines

(EMETREX, AMOUN), Serotonin receptor antagonist **(ZOFRAN 8 mg, SANDOZ)** according to the recent guidelines of the Royal College of Obstetricians and Gynecologists (9).

In the corticosteroid group (25 patients): Beside the standard regimen of treatment, Hydrocortisone 40 mg Intravenous (IV) **(SOLU-CORTIF 100 mg, PFIZER)** (equivalent to 10 mg prednisolone) was given every 12 hours for 2 days then prednisolone 5 mg **(SOLUPRED ORO 5 mg, SANOFI)** oral tablets were given every 12 hours for 5 days.

In the non-corticosteroid group (25 patients): Beside the standard regimen of treatment, 2 cm of IV saline 0.9% every 12 hours for 2 days followed by Folic acid tablets **(FOLIC ACID 5 mg, EPICO)** were administered as 1 tablet every 12 hours for 5 days.

Prednisolone or folic acid was administered to patients in the form of 1 tablet twice daily via standardized tablet dispensers that held a 5 day supply that had previously been packaged.

After 2 days of the start of the treatment. We used Pregnancy-Unique Quantification of Emesis (PUQE) score **figure (1)** to measure the severity of vomiting and assessment of quality of life using a rating scale with a range between zero (the worst that can be imagined) and ten (equivalent to how she felt before the start of this pregnancy) (11) .

After 4 days of the start of the treatment, patients who responded well to treatment (improvement of PUQE Score and quality of life) were sent home with their standardized pill dispensers and told to take the rest of their prescribed medication for a total of five days. Pill counts were used to confirm patient compliance with the study regimen at follow-up visits.

After 1 week of the start of the treatment, assessment of severity of emesis using Pregnancy-Unique Quantification of Emesis (PUQE) score, and length of hospital stay were recorded.

Primary outcome:

The primary outcome was the severity of vomiting after 48 hours after start of the treatment using PUQE score.

Secondary outcomes:

1. Rate of readmission to the hospital for hyperemesis gravidarum within 2 weeks of treatment
2. Quality of life after 48 hours and 1 week from starting the treatment protocol using a rating scale with a range between zero (the worst possibly imaginable) and ten (equaled as good as she felt before the start of this pregnancy).
3. Assessment of severity of vomiting after 1 week of start of the treatment using PUQE score.
4. Length of hospital stay.
5. Extent of ketonuria.

Sample size calculation and power analysis:

The primary outcome was assessment of severity of emesis after 48 hours of start of the treatment using Pregnancy-Unique Quantification of Emesis (PUQE) score. A study done by Jarvis **Sheba and Nelson-Piercy Catherine** (10) examined the effect of corticosteroids in patients with hyperemesis gravidarum, and showed decrease in severity of emesis from 50% to 80% Assuming alpha = 0.05 , beta = 0.2 (power = 80%), and using the 2-tailed Student t test with allocation ratio (1:1), 25 subjects were required in each group to detect a difference of 30% (effect size of 0.6) decrease in the severity of emesis after treatment with prednisolone which was considered to be the least clinically significant effect.

Statistical analysis:

SPSS version 20 was used to analyze the data. The histogram and Kolmogorov-Smirnov test

were used to determine whether continuous data were normal. The Student's t test was used to analyze normally distributed data, which were reported as mean standard deviation. Data that were not normally distributed were shown as median (range) values and were subjected to the Mann-Whitney U test. The chi-square test or Fisher's exact test was used to analyze categorical data, which were given as numbers (percentages). Statistical significance was defined as a P-value 0.05.

Results

As shown in the study flow diagram (Figure 2), Seventy-four patients were assessed for eligibility to participate in the study. Sixteen patients did not meet inclusion criteria and eight patients declined to participate in the study. The final number was fifty patients who were randomized, and data from them (25 patients in the corticosteroid group and 25 patients in the non-corticosteroid group) were analyzed. In the corticosteroid group 3 patients did not respond to treatment, while 22 patients responded, of them 17 patients were discharged by request and 1 patient was readmitted to hospital within 2 weeks. In the non-corticosteroid group 5 patients did not respond to treatment. However, 20 patients responded, of them 11 patients were discharged by request and 8 patients were readmitted to hospital within 2 weeks.

Patients' baseline characteristics (age, height, pre-pregnancy weight and BMI, admission weight and BMI) were similar in the 2 groups (Table 1).

There were no significant differences between the 2 groups in the obstetric history including gravidity, parity, previous abortions, living children, and gestational age (Table 2).

At admission, Pregnancy–Unique Quantification of emesis (PUQE) score was statistically non-significant between both groups. However, the severity of vomiting after 48 hours of start of the treatment using PUQE score decreased significantly in the corticosteroid group, being 10.64±1.62 compared to 11.88±1.64 in the non-corticosteroid group. PUQE score after 1 week of start of the treatment was comparable again between the two studied groups as observed in (Table 3).

As regard the quality of life (QOL) of patients, at admission there was no statistical difference between the studied groups, while after 48 hours from starting the treatment, QOL score was statistically higher among the corticosteroid group being 5.8±0.8 compared to 4.6±1.2 in the non-corticosteroid group p value ≤0.05. This improvement in QOL score continued after 1 week higher among the corticosteroid group, 8.3±0.9 compared to 7.6±0.7 in the control group (Table 4).

Ketonuria was statistically not significant among the studied groups at day 1, day2 and at discharge. Ketonuria at day1 was positive in 96% in corticosteroid group versus 92% in non-corticosteroid group. At day 2 ketonuria improved insignificantly in corticosteroid group more than non-corticosteroid group. At discharge ketonuria improved but insignificantly in both groups (Table 5).

The rate of readmission to the hospital for hyperemesis gravidarum within 2 weeks of starting the study was significantly higher among the control group being 32% versus only 4% in the group taking corticosteroids (Table 6). Also, the median length of stay was higher among the control group, 7 days ranged from 4 to 28 days compared to 4 days ranged from 3 to 12 days in the corticosteroid group (Table 6).

Table (7) shows that no statistically significant difference was observed between the studied groups regarding response to treatment, p value > 0.05. 88% and 80% in the first and second groups response to initial treatment, respectively.

Laboratory investigations on admission showing no statistically significant difference between the studied groups regarding CBC, liver enzymes, blood gases and blood electrolytes, p value > 0.05 (Table 8).

Discussion

This randomized controlled trial demonstrated that corticosteroids were highly effective in reducing the severity of vomiting in pregnant women with hyperemesis gravidarum using Pregnancy-Unique Quantification of Emesis (PUQE) score and improving their quality of life (QOL) score compared with the conventional standard treatment in the control group. Additionally, there were significant differences between groups in the rate of readmission to the hospital for hyperemesis gravidarum within two weeks of the study's beginning and the length of hospital stay.

Our study revealed that corticosteroids were effective in reducing the severity of vomiting in HG patients after 48 hours from the start of the treatment compared to the conventional standard treatment, but this finding was clinically insignificant between the two groups after 1 week from the start of the treatment. Also, patients in the corticosteroids group had higher QOL scores compared to the other control group.

The effectiveness of prednisolone in decreasing symptoms of hyperemesis was studied in two trials. Nelson-Piercy et al (12) compared between oral prednisolone (12 women) and placebo (12 women) .Compared to 5 of the 12 women who received placebo, only 1 of the 12 women who receive prednisolone enrolled in the study during the first HG hospital admission (P = 0.01). Prednisolone group had a mean gestational age of 10.6 2.1 weeks, while placebo group had a mean gestational age of 8.3 ±1.9 weeks. After 1 week of treatment, there was no differences between prednisolone and placebo in improvement of nausea (self-reported using a visual analogue scale (VAS), P=0.10), vomiting (self-reported; relative risk (RR), 1.4; 95% confidence interval (CI): 0.6-3.2) or vomiting more than 5 times per day (self-reported; RR, 2.5; 95% CI: 0.6. Additionally, there were no changes in the effects of switching to intravenous medication when oral therapy failed to produce the desired level of improvement (RR, 2.0; 95% CI: 0.6-6.2) or the need for intravenous fluids (RR, 1.0; 95% CI: 0.2-4.0). But when compared to placebo,, oral prednisolone considerably improved well-being (median VAS improvement, 6.5 vs. 3.5 points; P=0.02).

Ziaei et al (13) compared between oral prednisolone (40 women) and promethazine (40 women). In comparison to women who received promethazine, pregnant women who received prednisolone showed less improvement in their self-reported sickness after 2 days of treatment (no improvement/ becoming worse vs. any improvement) (no or mild nausea: odds ratio (OR), 0.33; 95% confidence interval (CI), 0.0.13-0.86; fewer than three vomiting attacks per day: (OR), 0.22 95% CI: 0.08-0.61; sickness improved: (OR), 0.33; 95% CI: 0.13-0.86. However prednisolone and promethazine were equally effective on all three parameters between days 3 and 10, on day 17, and also 1 week after the end of treatments. Pregnant women in Prednisolone did not experience any drowsiness, whereas promethazine did (0% vs. 15%; P=0.03).

Bondok et al (14), compared between intravenous hydrocortisone (20 women) and intravenous metoclopramide (20 women) in treatment of pregnant women with HG women in intensive care unit (ICU). After one week of treatment, pregnant women who recieved hydrocortisone experienced fewer mean vomiting attacks than those who received metoclopramide (reduction of 95.8 vs. 76.6%; P0.001) after one week of treatment.

In our study there were clinically significant differences between groups as regard length of hospital stay and readmission rates as the median length of stay was higher among the control group, 7 days ranged from 4 to 28 days compared to 4 days ranged from 3 to 12 days in the corticosteroid group and the rate of readmission to the hospital for

hyperemesis gravidarum within 2 weeks of starting the study was higher among the control group being 32% versus only 4% in the group taking corticosteroids.

The length of hospital stays in Nelson-Piercy et al did not change substantially by treatment (median, 7 days for both and placebo prednisolone), but readmission rates were lower in pregnant women who were received prednisolone than in those who were not (RR, 0.6; 95% CI: 0.3-1.4). However, In Bondok et al study (14) did not mention the hospital stay length , none of the 20 women in the hydrocortisone group were readmitted to the intensive care unit, but six of the 20 women in the metoclopramide group were (P 0.001). Yost et al (15) found that there was no difference in length of hospital stay between the methylprednisolone and placebo groups (7.618.0 vs. 4.34.3 days; P=0.18). Comparable hospital readmission rates were seen in both groups (19 of 56 vs. 19 of 54 readmissions; P=0.89).

In our study, Ketonuria was statistically not significant among the studied groups at day 1, day 2 and at discharge, p value > 0.05. Ketonuria at day 1 & day 2 was positive in 96% versus 92% while at discharge was 36% versus 44% among first and second groups, respectively. However, significant cases with Ketonuria were less among the first group.

There is inconclusive evidence that corticosteroids have lethal effect on human fetus development. Prednisolone, the biologically active component of prednisone, is metabolized mostly into the inactive form of prednisone in the placenta. Transplacental transfer of the active forms is limited. It was observed that the amount of active chemicals in fetal cord blood was 10% lower than in the mother. (16). corticosteroids has been associated with an increased risk of preterm birth and preterm premature membrane rupture, but this effect has only been noted with high dosages (17). The study by Rodriguez-Pinilla and Martinez-Frias (18) found a significant effect of first trimester steroid use and cleft lip and palate, but 3 of the 5 cases appear unlikely to be relevant. 1 of the 3 cases received only two doses of prednisolone after 8 weeks of pregnancy, when lip fusion should have already occurred. Another case was associated with several abnormalities, while a third case received replacement doses of hydrocortisone. In a bigger trial, there was no correlation between first-trimester corticosteroids use and oral facial clefts, and the frequencies of oral clefts were comparable between controls and those taking steroids (19). We did not publish neonatal outcomes in our study, however there were no side effects reported from the corticosteroids on the patients during the study.

In conclusion , Corticosteroids were highly effective in reducing the severity of vomiting after 48 hours from the start of the treatment using PUQE score and improving their QOL score compared with the conventional standard treatment in the control group. Other outcomes (severity of vomiting after 1 week of start of the treatment using (PUQE) score, rate of readmission to the hospital for hyperemesis gravidarum within 2 weeks of starting the study, and length of hospital stay) were higher in control group.

Limitations of our study are; the sample size was small as the power was 80% . the current study design neither reported adverse neonatal outcomes nor congenital anomalies after corticosteroids use, and we could not blind the patients because the color of prescribed pills (Folic acid and Prednisolone) was different.

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Figure (1) : Pregnancy-unique quantification of emesis and nausea form (11).

PUQE form

Pregnancy-Unique Quantification of Emesis and nausea

Circle the answer that suit the best your situation for the last 24 hours.

1. On average in day, for how long do you feel nauseated or sick to your stomach?

> 6 hours 5 points	4-6 hours 4 points	2-3 hours 3 points	≤ 1 hour 2 points	Not at all 1 point

2. On average in day, how many times do you vomit or throw up?

≥ 7 times 5 points	5-6 times 4 points	3-4 times 3 points	1-2 times 2 points	Not at all 1 point

3. On average in day, how many times have you had reching or dry heaves without bringing anything up?

≥ 7 times 5 points	5-6 times 4 points	3-4 times 3 points	1-2 times 2 points	Not at all 1 point

Total score (sum of replies to 1, 2, and 3): mild NVP ≤6; moderate NVP, 7-12; severe NVP ≥13.

Quality of life question:

On a scale of 0 to 10, how would you rate your well-being:_____

0 (worst possible) 10 (As good as you felt before pregnancy)

PUQE form modified from: Koren G, Boskovic R, Hard M, Maltepe C, Navioz Y, Einarson A. Motherisk-PUQE) pregnancy-unique quantification of emesis and nausea) scoring system for nausea and vomiting of pregnancy. American journal of obstetrics and gynecology. 2002;186:S228-31, with permission

Figure (2) : Study flow diagram

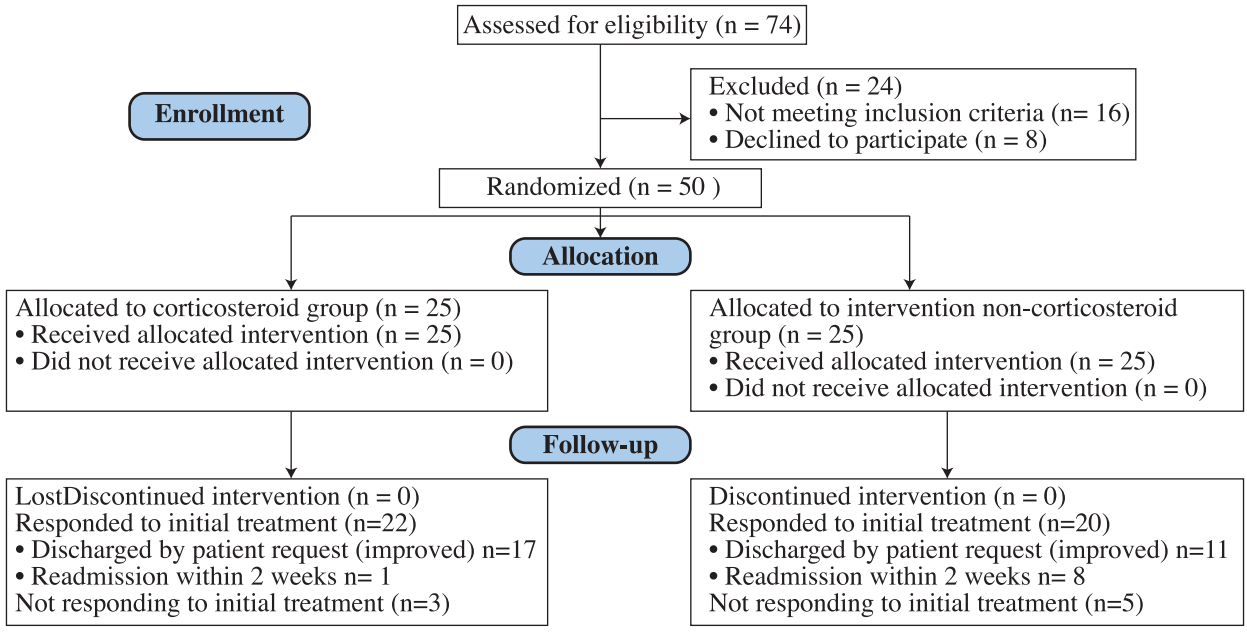

Table 1. Demographic characteristics of the study groups.

	Corticosteroid group (1) (n=25)	Non corticosteroid (2) group (n=25)	Test of significance	P value
Age (years)	26.76±4.11	26.40±5.60	t=0.259	0.797
Pre-pregnancy weight (Kg)	75.04±9.26	75.80±11.60	t=0.256	0.799
Height (Cm)	167.20±3.511	167.52±5.17	t=0.256	0.799
Pre-pregnancy BMI (Kg)	28.03±8.39	25.96±3.24	t=1.149	0.256
Admission weight (Kg)	68.56±9.81	73.48±12.57	t=1.542	0.130
Admission BMI (Kg/m^2)	24.75±2.52	25.01±3.28	t=0.307	0.760

Data are mean ± SD

Table 2. Obstetric history among the studied groups

Obstetric history	Corticosteroid group (1) (n=25)	Non corticosteroid group (2) (n=25)	P value
Gravidity Median (Min-Max) ≤3 >3	 3.00 (1.00- 5.00) 12 (48.0%) 13 (52.0%)	 3.00 (1.00- 5.00) 11 (44.0%) 14 (56.0%)	0.758
Parity Median (Min-Max) Nullipara ≤3 >3	 1.00 (0.00- 4.00) 6 (24.0%) 17 (68.0%) 2 (8.0%)	 1.00 (0.00- 4.00) 10 (40.0%) 12 (48.0%) 3 (12.0%)	0.641
Previous abortion Yes No	 6 (24.0%) 19 (76.0%)	 4 (16.0%) 21 (84.0%)	0.187
Living children No <3 ≥3	 6 (24.0%) 17 (68.0%) 2 (8.0%)	 10 (40.0%) 12 (48.0%) 3 (12.0%)	0.520
GA at admission (weeks)	10.40±2.41	10.12±2.24	0.673

Data are mean ± SD, median (range), or number (percentage).
GA, gestational age.

Table 3. Pregnancy –Unique Quantification of emesis score at different follow up periods.

	Corticosteroid group (1) (n=25)	Non corticosteroid group (2) (n=25)	Test of significance	P value
PUQE score at admission	13.84±1.21	14.40±0.70	t=1.99	0.052
After 48 hours	10.64±1.62	11.88±1.64	t=2.68	0.01*
After 1 week	5.96±1.88	6.56±1.75	t=1.16	0.250

Data are mean ± SD.

Table 4. Quality of life at different follow up among the studied groups.

Quality of life	Corticosteroid group (1) (n=25)	Non corticosteroid group (2) (n=25)	Test of significance	P value
Day 1	4.3±1.0	3.8±0.8	t=1.94	0.058
Day2	5.8±0.8	4.6±1.2	t=3.92	≤0.001*
At discharge 8.3±0.9	8.3±0.9	7.6±0.7	t=2.89	0.006*

Data are mean ± SD

Table 5. Ketonuria at different follow up among the studied groups.

Acetone	Corticosteroid group (1) (n=25)	Non corticosteroid group (2) (n=25)	P value
Day 0 +1 +2 +3	 1 (4.0%) 3 (12.0%) 5 (20.0%) 16 (64.0%)	 2 (8.0%) 1 (4.0%) 10 (40.0%) 12 (48.0%)	0.371
Day2 0 +1 +2 +3	 1 (4.0%) 8 (32.0%) 15 (60.0%) 1 (4.0%)	 0 (0%) 8 (32.0%) 10 (40.0%) 7 (28.0%)	0.08
At discharge 0 +1 +2 +3	 16 (64.0%) 8 (32.0%) 0 (0%) 1 (4.0%)	 14 (56.0%) 10 (40.0%) 1 (4.0%) 0 (0%)	0.658

Data are numbers (percentage).

Table 6. Outcome among the studied groups.

Outcome	Corticosteroid group (1) (n=25)	Non corticosteroid group (2) (n=25)	Test of significance	P value
Readmission within 2 weeks of starting treatment	1 (4.0%)	8 (32.0%)	FET	0.023*
Length of hospital stay	4.00 (3.00- 12.00)	7.00 (4.00- 28.00)	t=3.38	0.001*

Data are median (range), or number (percentage).

Table 7. Outcome among the studied groups.

Response to treatment	Corticosteroid group (1) (n=25)	Non corticosteroid group (2) (n=25)	Test of significance	P value
Not responding to initial treatment	3 (12.0%)	5 (20.0%)	FET	0.702
Responding	22 (88.0%)	20 (80.0%)		

FET: Fisher exact test

Table 8. Laboratory investigations on admission among the studied groups.

Laboratory investigations on admission	Corticosteroid group (1) (n=25)	Non corticosteroid group (2) (n=25)	Test of significance	P value
HG	11.39±1.50	11.73±0.97	t=0.958	0.343
HCT	39.10±5.19	40.37±5.20	t=0.867	0.390
WBCs	7.41±2.23	7.76±2.37	t=0.527	0.600
PLTs	229.22±73.82	259.44±66.37	t=1.52	0.135
SGPT	22 (15- 92)	30 (16- 174)	Z=1.81	0.071
SGOT	22 (16- 60)	26 (18- 80)	Z=1.73	0.083
Creatinine	0.62±0.08	0.66±0.09	t=1.71	0.094
PH	7.37±0.06	8.40±5.27	t=0.975	0.334
HCO3	17.74±3.54	17.96±2.60	t=0.241	0.811
Pco2	29.16±6.78	30.70±6.12	t=0.844	0.403
Na	139.56±25.52	149.12±17.63	t=2.99	0.074
K	3.11±0.55	2.93±0.42	t=1.31	0.194

Uterocervical angle and cervical length as predictors for preterm birth in low-risk women

Mohamed K. Etman[1], Vivian A. Youssef1, Mohamed S. Bakry[1], Sahar MY. El-Baradie[1]
[1]Obstetrics and gynecology department, Faculty of Medicine, Fayoum University
Obstetrics and Gynecology at Fayoum General Hospital

Abstract

Background: Cervical length measurement is widely used to estimate the risk of preterm birth. Another potential predictor of preterm birth is the uterocervical angle, and this additional measurement may improve the risk assessment.

Objectives: To evaluate the role of the uterocervical angle compared to the cervical length measurements in preterm birth prediction.

Study design: This prospective cohort study was carried out on 120 asymptomatic primigravida women at low risk of preterm labor attending the Gynecology and Obstetrics department at Fayoum University Hospital. Uterocervical angle and cervical length were measured by transvaginal ultrasound. Maternal history and pregnancy data were recorded. Delivery data were subsequently collected.

Results: The mean age, BMI, and gestational age at delivery were 21.79 ± 3.3, 24.6 ± 5.8, and 38.46 ± 1.98, respectively. Fifteen out of 120 women (12.5%) experienced preterm birth. The uterocervical angle was significantly larger among the preterm group than the term group (110.17 ±14.93 vs. 125.00 ±15.35, $p<0.001$). The cervical length was significantly shorter among preterm women as compared with term. An inverse linear moderate correlation existed between gestational age and the uterocervical angle ($r= -0.370$, $p<0.001$). A positive linear moderate correlation existed between gestational age and the CL-one line ($r= 0.260$, $p=0.004$). Also, a positive linear strong correlation between GA and CL-two lines ($r= 0.716$, $p<0.001$).

Conclusions: The uterocervical angle is a potential novel screening tool for predicting preterm birth better than cervical length.

Keywords: Uterocervical Angle; Cervical Length; Asymptomatic Preterm Labor.

Corresponding author:
Mohamed K. Etman, MD
Associate professor of Obstetrics and Gynecology, Fayoum University Hospital, Fayoum Faculty of Medicine.
Email: drmohamedetman@gmail.com
Tel: 01002412118
Address: Bilal, Qesm Al Fayoum, Faiyum, Faiyum Governorate.
PO Box: 63514

Introduction

Preterm birth (PTB) seriously affects about 10.6% of live births globally. It is defined as birth before the 37th week of gestation (1). It has multiple risk factors but may

occur without any possible explanation (2). Determining women at risk is challenging as the sensitivity of demographic and behavioral risk factors is low (3). Additionally, PTB is a multifactorial condition with a previous history of PTB, the powerful predictive of recurrent PTB (4). Accordingly, there is a continued need to determine tools accurate in predicting PTB in low-risk women (5). Cervical length (CL) measurement and fetal fibronectin were used to predict PTB, with conflicting results about its utility in low-risk women (6). A novel ultrasound parameter- the uterocervical angle (UCA) - was introduced as a predictor for PTB with reported improved accuracy than CL measurement (7). Both modalities are easy to perform and inexpensive; however, the UCA was not evaluated among low-risk women (8). Therefore, this study was conducted to evaluate the accuracy of UCA in predicting PTB in low-risk women.

Methods

This prospective cohort study was conducted at the obstetrics and gynecology department at Fayoum University from February 2022 to July 2022. We recruited primiparous women attending the outpatient clinic and low-risk for PTB according to specific inclusion and exclusion criteria. Inclusion criteria: a) age from 18-35 years, b) gestational age from 20-28 weeks, and c) asymptomatic women with no uterine contractions, lower abdominal pain, low back pain, pelvic pressure, vaginal bleeding, or leakage of amniotic fluid. Exclusion criteria: a) women with multiple gestations, b) cervical cerclage, c) tocolysis intake or cervical manipulation as vaginal douche, intercourse, or digital vaginal examination in the last 24 hours, and d) history of medical disorders in the current pregnancy as diabetes and hypertensive disorders with pregnancy.

Eligible women were evaluated as follows:

- Detailed history taking, including maternal age, gestational age determination at recruitment and at time of delivery, and detailed obstetric history.
- A transabdominal ultrasound was performed to determine fetal biometry, estimated fetal weight, and amniotic fluid index at delivery time.
- A transvaginal ultrasound was performed on all participants using a high-frequency endovaginal probe (3–9 MHz) on a (voluson s10 expert, EG) ultrasound system. The urinary bladder was empty, and women were laid in the lithotomy position. The vaginal probe was introduced in the anterior fornix smoothly without pressure. A sagittal view of the cervix and the anterior uterine wall was obtained. The endocervical canal was identified as the hypoechoic zone of the cervical mucosa. The external and internal cervical os was identified where the anterior and posterior lips of the cervix meet together at the vaginal canal and the lower uterine segment, respectively.
- The CL was measured using two methods: a) the single line measurement as a line extending from the internal to the external os (9), b) the two-line method where the CL was measured as the line from the internal os to the point of maximum excursion of the cervical curvature and then from this point to the external os (10).
- If a difference > 5mm was detected between the two methods, the two-line method was adopted to guarantee accurate measurements.
- The UCA was measured as the angle between a line drawn from the internal to the external cervical os and a line drawn parallel to the anterior uterine wall and passing through the internal cervical os (11).

All women were followed up according to regular antenatal care visits recommended by NICE guidelines (12). The GA at delivery was recorded, and accordingly, the cohort

was divided into two groups: Group A, who delivered before 37 weeks gestation (PTB) (3), and Group B, who delivered after 37 weeks gestation.

The primary outcome measure was the predictive role of the UCA compared to the CL in PTB. Other outcome measures included determining cutoff levels for the CL and UCA in predicting PTB.

Sample size calculation was done using the Egyptian 13 % Preterm birth rate (births <37 weeks per 100 live births), (13) a minimal total hypothesized sample size of 100 asymptomatic pregnant women was calculated at a two-sided confidence level (1-alpha) 95%, power 80%, taking into consideration 5% level of significance and 5% precision using Z- test (14). This calculation estimated a total sample size of 120 participants after adding a 20% dropout rate during follow-up.

Statistical analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 24. The qualitative data were presented as numbers and percentages, while quantitative data were presented as mean and standard deviations when their distribution was found to be parametric. The comparison between two groups with qualitative data was made using the **Chi-square test** and/or **Fisher exact test** instead of the Chi-square test when the expected count in any cell was found less than 5. The comparison between two independent groups with quantitative data and parametric distribution was made using an **independent sample t-test** and **Mann-Whitney U test** with the non-parametric distribution. We used receiver operating characteristic (ROC) curve analysis, giving a level of sensitivity and specificity, to evaluate the CL and UCA cutoff value as predictors for PTB. It was estimated as the area under the curve (AUC) with a 95% confidence interval (CI). The AUC ranged from 0.5 (no predictive ability) to 1 (predictive value). Pearson's correlation analysis was done to evaluate the linear relationship between the CL and UCA, and the GA at delivery. P value was considered significant when below 0.05.

Results

Table (1) shows the baseline characteristics of the studied women. Woman's ages ranged from 18 to 33 with an average of 21.79 ± 3.3. Their BMI ranged from 22.3 to 30.4, averaging 24.6 ±5.8 kg/ m2. The mean gestational age at delivery was 38.46 ± 1.98 weeks.

Out of the studied 120 women, fifteen (12.5%) experienced a preterm birth, while the remaining 105 (87.5%) women had term birth after 37 weeks gestation.

The UCA according to pregnancy outcome (term vs. preterm) was significantly more prominent among the preterm group as compared with the term group (110.17 ±14.93 vs. 125.00 ±15.35, p<0.001). The CL was significantly shorter among preterm women than the term (3.39 ±0.59 vs. 2.93 ±0.48, p= 0.004). We further assessed the CL by the two-line method in 22 participants, and the CL by the 2-line method was significantly shorter among preterm compared to term women (4.25 ±0.42 vs. 3.71 ±0.27, p=0.025) **(Table 2)**.

There was an inverse linear moderate correlation between GA and UCA (r= -0.370, p<0.001). Additionally, a positive linear moderate correlation existed between GA and CL-one line and CL- two lines (r= 0.260, p=0.004 and r= 0.716, p<0.001, respectively).

The area under the curve (AUC) of UCA for prediction of preterm was (AUC = 0.810, SE = 0.065, 95% CI: 0.683–0.938). UCA degrees of ≥110 could predict preterm with a sensitivity of 83.3%% and a specificity of 74.8% (Figure-). The (AUC) of the CL-one line for prediction of preterm was (AUC =

0.724, SE = 0.066, 95% CI: 0.595–0.854). A CL-one line of ≤2.9 cm could predict preterm with a sensitivity of 75.2% and a specificity of 70%. The AUC of CL-two lines for prediction of preterm was (AUC = 0.882, SE = 0.078, 95% CI: 0.729–1.000). A CL-two line of ≤3.9 cm could predict preterm with a sensitivity of 77.8% and a specificity of 99% (Table 3).

Discussion

Preterm rates were reported as 12.5%. This differed from other results (9.6%) (14), while in Egypt, higher rates were reported (28% and 26%) (16, 17). Different sample sizes and definitions used in different studies would explain these variable results.

The UCA was inversely correlated with the GA at birth. It was significantly higher among women who delivered prematurely. It also predicted PTB significantly at measurements > 110. A previous study reported a significant inverse relation between the UCA and GA at birth (18). This denotes that an increased UCA was associated with earlier gestation at delivery. Additionally, an earlier study reported a UCA measurement of 115.4◦ which was significantly higher than in women who delivered at term. A cutoff value > 105.5◦ predicted PTB significantly (19). Another one reported a cutoff value > 105 (17). However, contradictory results were reported by another researcher who stated that UCA measurement in the second trimester was a poor predictor for PTB (20).

The relation between the UCA and PTB would be explained by the exposure of the cervix to mechanical forces exerted by the pelvic organs and the uterus. An obtuse UCA commonly allows pressure transmission exerted by these forces to the cervix leading to its dilatation. However, an acute UCA would hinder force transmission to the cervix keeping its standard shape and closure (21). However, different results would be rendered to the decreased incidence of PTB in each study, different gestational ages for obtaining measurements, and different races and histories of the studied populations.

The current study reported that the CL was significantly shorter among preterm compared with term women when measured by one- and two-line methods. Also, there was a statistically significant linear positive correlation between the CL at the time of assessment (16+ 0 to 24+ 0 weeks gestation) and the GA at delivery. This agreed with previous results (22, 23), emphasizing the role of the CL in the prediction of PTB. Contradicting results reported an insignificant difference in the CL among women who delivered preterm than those who delivered at term (15, 17).

A cutoff value <2.9 cm predicted PTB with a sensitivity of 75.2% and a specificity of 70%. Another study reported a cutoff value of <2.5cm (18). The other performance of the CL was reported with a sensitivity and specificity of 27.8% and 85.8%, respectively (17). Another study reported poor performance of the CL (15). Inconsistent results would be attributed to different sample sizes, races, and patient histories among studies.

Conclusion

The CL and UCA predicted PTB with better performance reported by the UCA.

Conflict of interest

None.

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Table (1): Baseline data of the studied women; (N= 120)

		Descriptive Statistics
Age; (years)	Mean ±SD	21.79 ±3.3
	Minimum	18
	Maximum	33
BMI; (kg/ m^2)	Mean ±SD	24.6 ±5.8
	Minimum	22.3
	Maximum	30.4
GA; (weeks)	Mean ±SD	38.46 ±1.98
	Minimum	28.50
	Maximum	42.00

Table (2): comparison of Uterocervical angle between studied women according to outcome (term vs. preterm); (N= 120)

		Outcome		p-value
		Term N= 105	**Preterm N= 15**	
Uterocervical angle (degrees)	**Mean ±SD**	110.17±14.93	125.00±15.35	**<0.001***
	Minimum	67.00	76.00	
	Maximum	144.00	140.00	
		Outcome		**p-value**
		Term N= 105	**Preterm N= 15**	
Cervical Length (CL)-one line	**Mean ±SD**	3.39±0.59	2.93±0.48	**0.004***
	Minimum	2.24	2.31	
	Maximum	4.61	3.73	
		Outcome		**p-value**
		Term N= 18	**Preterm N= 4**	
Cervical Length (CL)-two lines	**Mean ±SD**	4.25±0.42	3.71±0.27	**0.025***
	Minimum	3.51	3.45	
	Maximum	4.89	3.97	

Table (3): Results of ROC curve analysis for sensitivity and specificity of UCA and CL for prediction of preterm birth

	AUC	95% CI of AUC	Cutoff	p-value	Sensitivity	Specificity
UCA	0.810	0.683 - 0.938	≥110	<0.001*	83.3%	74.8%
CL-one	0.724	0.595 - 0.854	≤2.9	0.005*	75.2%	70%
CL-two	0.882	0.729 - 1.000	≤3.9	0.019*	77.8%	99%

AUC: Area under the curve, **CI:** Asymptotic 95% Confidence Interval of AUC, **UCA:** Uterocervical angle, **CL-one:** Cervical Length-one line, **CL-two:** Cervical Length-Two lines.

A pilot study of The Efficacy of Using Chromium Salts in Reducing Hirsutism Scoring & BMI in Polycystic Ovary Syndrome Patients: Double Blinded Randomized Controlled Trial

Running title:

Chromium Salts in Reducing Hirsutism scoring

Dina Yahia Aly Mansour [1],Heba Mohammed Eid [2], Marwa Saber Snosi[1,3]

Authors affiliations:

[1]Department of Obstetrics & Gynecology, Ain Shams University ,Cairo, Egypt.

[2]Department of Obstetrics & Gynecology Sharq El Madina Hospital Alexandria,Egypt.

[3]Department of Obstetrics & Gynecology,Al Maaly Hospital ,Hafr El batin,Kingdom Of Saudia Arabia.

Corresponding author:

Marwa Saber Sayed Snosi

Lecturer of obstetrics and gynecology Faculty of medicine, Ain Shams University, Cairo, Egypt.

Consultant of obstetrics and gynecology Almaaly Hospital

Address: Hadeq El Quba, Cairo ,Egypt.

E-mail: dr.marwasnosi@yahoo.com

phone: +966546833239, +20 1115670205 .

ORCID number: 0000-0001-8917-8814

Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the most frequent endocrinopathies in women in reproductive age. the clinical image of the disease is heterogeneous. Hirsutism is one of the gynecological symptoms of PCOS with prevalence ranging from 70-80% in affected cases.

Objective: To assess the effect of chromium salts in addition to metformin on hirsutism scoring and Body Mass Index in polycystic ovary syndrome patients.

Methods: This double blinded randomized controlled trial at Ain Shams University Maternity Hospital included sixty cases of PCOS complaining of hirsutism, age between 18 and 40 years were included, while Patients known to be diabetic, hypertensive, receiving corticosteroids, psychotropic drugs, diuretics, Ovulation induction drugs, known hypersensitivity or contraindications to the used medications or those who used permanent methods of hair removal were excluded. . The patients were randomly divided into two equal groups. Group S were thirty cases who received metformin and chromium picolinate and Group C were thirty cases receiving metformin and placebo in the form of vitamin C retard.

Results: There was a highly statistically significant difference between two groups as regard weight loss, BMI, hirsutism score and menstrual regularity in favor to intervention group after 4 months follow up as p- value was <0.001. There was No significant difference between two groups as regard side effects reported about the drugs as p-value was >0.05.

Conclusion: Adding chromium picolinate to metformin in PCOS patients caused further improvement of Hirsutism score and regularity of menses, and further decrease in BMI.

Trial registration number:
PACTR202209735208004,retrospectively registered.

Keywords: Polcystic ovaries; hirsutism; chromium.

Background

Polycystic ovary syndrome (PCOS) is defined by having at least two of the following criteria: irregular or absent ovulation, elevated levels of androgenic hormones and enlarged ovaries containing at least 12 follicles each[1].

Hyperandrogenism manifests by androgenic alopecia, hirsutism, acne lesions, increased hair loss, oily skin, seborrheic lesions. Hirsutism is one of the most frequent symptoms, assessed according to the Ferriman– Gallwey score, is the presence of gruff, thick and pigment-saturated hair in women in places typical for men, e.g. upper lip, chin, chest, nape of the neck, lumbar region, abdomen, thighs and feet[2]. The increased insulin levels found in patients with PCOS appear to directly enhance LH stimulated androgen secretion from the ovary[3]. Androgens are the key factors in the growth and development of sexual hair. Androgens act on sex-specific areas of the body, converting small, straight, fair vellus hairs to larger, curlier, and darker terminal hairs[4]. Hirsutism is very common, very distressing to patients and often improves with medical management. Prompt medical attention is important because delaying treatment makes the treatment more difficult and may have long-term health consequences[4].

Metformin is one of the insulin-sensitizing medications, which reverses the majority of metabolic abnormalities of PCOS[5] by increasing its sensitivity, increasing estrogen secretion and decreasing androgen production, thus, decreasing hirsutism[6].

Another agent is chromium (III) the synthetic salt form of Cr chloride the naturally occurring trivalent variety of chromium, which is a microelement that facilitates the maintenance of normal blood glucose level by activating insulin signal transduction and sensitivity[7], thus minimizing insulin resistance, which can play a significant role in controlling PCOS[8]. Picolinic acid may serve to improve chromium absorption9 Adequate intake (AI) was set based on estimated mean intakes and amounts of 25μg/day for young women. It is indicated as a microelement facilitating in maintaining normal glycaemia[7].

The aim of the current pilot study was to assess the effects of chromium compared to placebo in addition to metformin in women with polycystic ovary syndrome patients for improving hirsutism scoring & Body Mass Index.

Materials and Methods

This is a pilot double blinded Randomized Controlled parallel arm trial has a1:1 allocation for each arm (metformin / chromium in the active arm and the placepo/metformin in the control arm) .It was conducted in Ain Shams University Maternity Hospital from November 2020 to May 2021 study included 60 patients complaining of hirsutism attending outpatient Gynaecology clinic with polycystic ovary syndrome diagnosed according to Rotterdam criteria 1 which included the presence of at least two criteria from these three: oligo or anovulation, manifestations of hyperandrogenism and polycystic ovaries in ultrasound The study was conducted after approval of Research Ethical Committee, faculty of medicine of Ain shams University Number :(FMASUMS630/2020) .Written informed consent was obtained from each patient after full explanation of the procedure before enrolment

The study was registered in The Pan-African Clinical Trial Registry PACTR202209735208004. No important changes were done to methods after trial commencement.

Patients assessed for eligibility were 60 inclusion criteria included age between 18

and 40 years who agreed to participate in the study while Patients known to be diabetic, hypertensive, receiving corticosteroids, psychotropic drugs, diuretics, Ovulation induction drugs, known hypersensitivity or contraindications to the used medications in the study or those who used permanent methods of hair removal **were excluded**.

All patient participated in this study were undergone the following procedures: Full history, history of investigations and treatment. Menstrual history: criteria, average number of menstrual cycles per year, amount. infertility history Examination: Physical examination: measurement of BMI and BP measurement was done. General examination: For hirsutism Physical examination was begun with determination of the distribution and degree of hair growth using a scoring method Ferriman-Gallwey scale (according to this score. A score of 1 to 4 was given for nine areas of the body (upper lip, chin, back, chest, nape of the neck, lumbar region, abdomen, thighs and feet). A total score less than 8 was considered normal, a score of 8 to 15 indicated mild hirsutism, and a score greater than 15 indicated moderate or severe hirsutism. A score of 0 indicated absence of terminal hair. Skin examination: was done for acne or acanthosis nigricans.

All sixty women received metformin with dose of 500mg after main meal.

The first Group (chromium group): (n=30) received chromium (chromium picolinate, Mepaco) orally in a dose of 200microgram /day for 4 months' duration.

The second Group (placepo group) :(n=30) received placebo(same shape as chromium capsules).

Follow Up: The patients were followed up for taking the drug regularly by phone calls every two weeks. Follow up was for the frequency of hair removal every month for 4 months duration with usage of temporary method of hair removal and was stopped 2 weeks before Follow up. Body mass index (BMI) was also followed up monthly (Body weight was measured using analogue scales in light clothes; height was measured barefoot using a stadiometer) and calculated as follows: weight (kg)/height2 (m) Regularity of menses: The patient was asked about her menstrual history (time between cycles, frequency, duration), and any reported side effects of chromium salts or metformin: each month throughout the period of taking the drug.

The primary outcome was chromium effect in addition to metformin on hirsutism scoring, while **secondary outcomes were** its effect on BMI ,regulation of menses and side effects of chromium salts such as mood changes irritability headache, and side effects of metformin.

Sample size calculation:

At the beginning of the study there was no available information in literature assessing effect of chromium on hirsutism all studies were assessing its effect on insulin resistance so it was considered pilot study included 60 patient (30 in each group).

And were randomly assigned using computer –generated random sequence in a ratio of 1:1 Allocation for each arm of the study .each patient was assigned a, sealed ,opaque envelope with her number (Sequentially numbered) containing either chromium tablets or placepo .doctors and patients were blinded to which group the candidate was assigned to ,only the nurse in the clinic who chose each envelop for each patient was not blinded.

Statistical analysis

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, unpaired student t-test was used to compare between two groups in quantitative data, paired Student T-test was used to compare between related samples and chi square test was used to compare between

groups in qualitative data by (IBM SPSS

Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). p value of< 0.05 was considered significant.

Results

Between November 2020 to May 2021, a total of 60 women(n=30 per group) were recruited for the study.. They were followed for 4 months duration ,Figure 1 (consort flow diagram)shows the allocation and follow-up of patients. Group 1 is the patients received chromium (chromium picolinate, Mepaco) orally in a dose of 200microgram /day .And group 2 received placebo(same shape as chromium capsules).

There were no statistically significant differences between both groups regarding age, Body Mass Index (BMI), period of infertility ,number of cycles per year (p value >0.05) as shown in table 1.

There were highly statistically significant difference between both groups as regard hirsutism scoring, weight loss and consequently BMI in favour of the intervention group after 4 months follow up (pvalue-<0.001) –as shown in table 2.

There was highly statistically significant difference between 2 groups as regards regularity of menses (pvalue <0.001) as shown in table 3

There was no statistically significant difference between two groups regard drugs reported side effects (pvalue <0.05) as shown in table 3.

Discussion

Up to 70% of patients with PCOS demonstrate overt insulin resistance and hyperinsulinism. The administration of insulinsensitizing agents has been proposed for the treatment of hirsutism as these agents have various potential advantages over traditional therapies as they correct both the metabolic and the endocrine aberrations of the disorder; thus permitting the resumption of normal endogenous ovulatory function, with little or no risk of ovarian hyperstimulation and multiple gestation, in addition to, the possible decrease in the long-term risk of type 2 DM and CVD [10].

The current study found that there were significant difference between both groups as regard hirsutism, menstrual regularity, weight loss and consequently BMI in favor of the intervention group after 4 months follow up (p-<0.001) - although hirsutism decreased, weight loss increased and menstrual regularity increased in both groups probably due to the effect of metformin given.

Teede et al [11]., suggested the use of metformin, in addition to, lifestyle modifications could be considered in adolescent females complaining of symptoms of PCOS even before the diagnosis is made, as this was shown to reduce weight and improve hirsutism [12]. Both studies agree with the results of the current study as both groups showed weight loss and improvement of hirsutism although in different degrees, which suggests that chromium augments the effects exerted by metformin.

The results of the current study agree with the results of Jamilian et al 13who found a significant reduction in hirsutism on 30 patients taking chromium 200 μg compared to the same number taking placebo in his double blinded randomized controlled trial after a follow-up period of 8 weeks, and with Ashoush et al [14]. who found a significant reduction in BMI and more menstrual regularity in 100 cases of PCO who used chromium for 6months.

However; Amr and Abdel-Rahim15 , found no significant change in BMI, acne or hirsutism; while there was an insignificant reduction in the number of cases with oligo/ amenorrhea in cases receiving chromium picolinate for 6months.

The main side-effect reported in the current study was abdominal discomfort which is

a known side-effect of metformin use and occurred equally in both groups suggesting that adding chromium did not result in additional side-effects.

The advantages of the current study is that it is the first randomized controlled study addressing the hirsutism score as the primary outcome with a follow-up period of 4 months; however, the main limitation of this study is the relatively small number of cases recruited as it is a pilot study, and the lack of comparison between different doses of chromium to decide the most effective dose to be used.

Conclusion

Adding chromium picolinate to metformin in PCOS patients augmented the effect of metformin causing further improvement of Hirsutism score, regularity of menses, and further decrease in BMI.

Larger randomized controlled trials using different doses of chromium are needed to confirm or refute these findings.

Ethics approval and consent to participate

Study approved by Research Ethical Committee, faculty of medicine ,Ain shams University, Number :(FMASUMS630/2020)

Consent :consent was taken from each patient .

Availability and data material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report there are no competing interests to declare

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Registration

The study was registered at Pan Affrican Clinical Trial Registry PACTR202209735208004.

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Authors' contributions

All authors jointly contributed to conception and design of the study.

Marwa Saber Snosi: Design of the study, helped in review of literature, revision of results and data analysis , writing the manuscript and submission to journal.

Dina yahia Aly Mansour: design of the study, revision of review of literature and revision of manuscript revision of results and data analysis.

Eid HS: registration of trial, obtaining ethical committee approval, reviewed the literature, shared in collection of Data, active participation in following up patients ensuring patients compliance by phone calls.

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Table (1): Comparison between two groups as regard descriptive data.

		Cases (N=30)	Control (N=30)	Tests	
				X^2/ t	P-value
Age (years)		27.80±5.67	27.93±5.23	0.095	0.925 NS
Marital status					
Single		30%))9	43.3%))13	1.148	0.284 NS
Married		70%))21	56.7%))17		
BMI (kg/m^2)		28.41 ± 2.19	28.72± 3.04	0.459	0.648 (NS)
Parity	0	6(28.6%)	952.9%))	2.77	0.44 NS
	1	9(42.9%)	529.4%))		
	2	5(23.8%)	17.6%))3		
	3	1(4.8%)	0.0%))0		
Parity	0	6(28.6%)	52.9%))9	2.34	0.13 NS
	>=1	15(71.4%)	47.1%))8		
Skin Examination					
Acne					
Present		30(100%))	30(100%)	0.000	1.000 NS
Acanthosis					
Present		19(63.3%)	19(63.3%)	0.000	1.000 NS
Absent		11(36.7%)	11(36.7%)		
Duration Of Infertility		1.95±1.18	1.91±0.96	0.107	0.915 NS
Average no of cycles per year		5.53±1.28	5.50±1.48	0.093	0.926

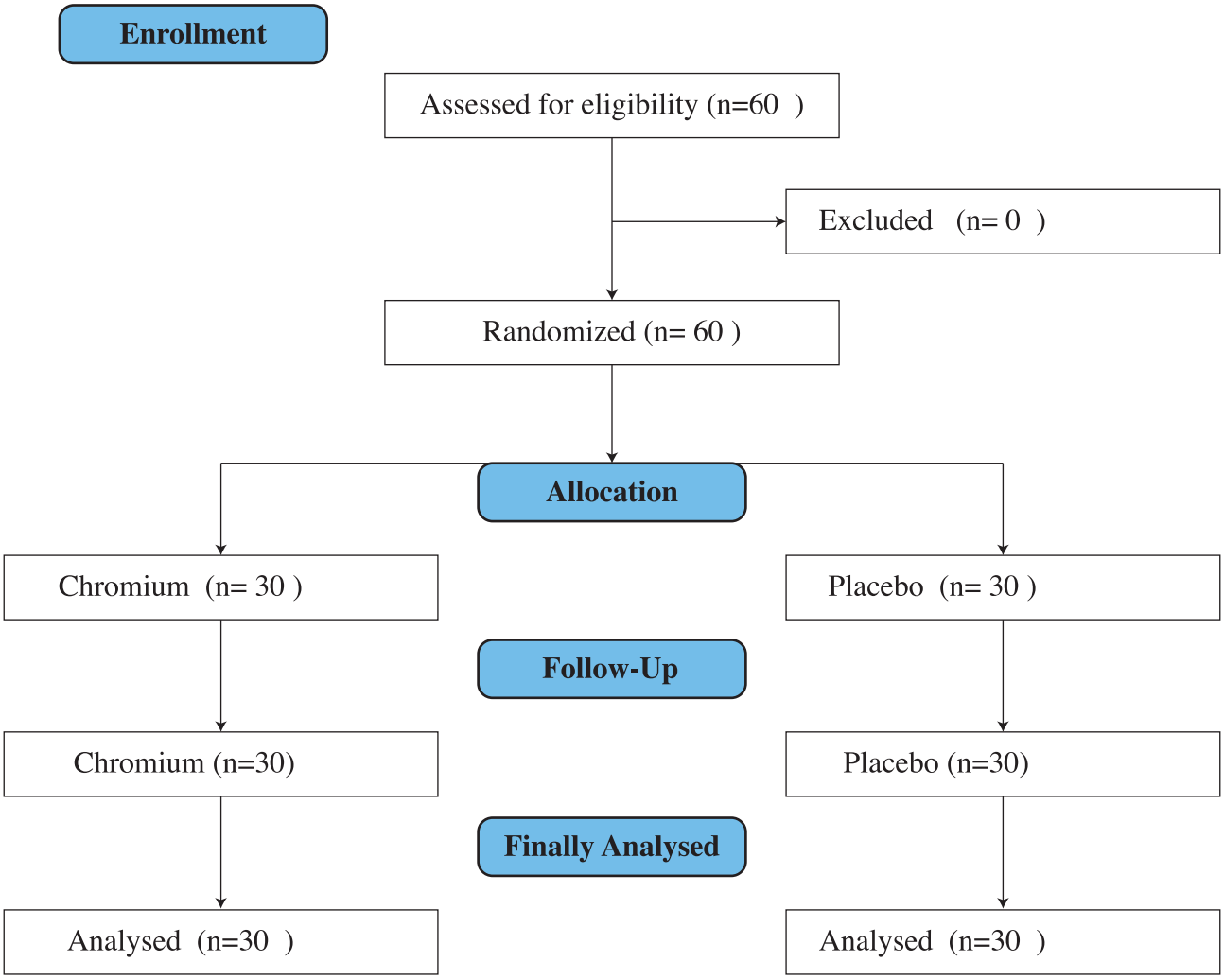
Table (2): Comparison between two groups as regard Weight loss , BMI and hirsutism scoring at different times (Baseline,1st month,2nd month, 3rd month and 4th month).

	Cases		Control		T-test	
	Mean±SD	**% of change**	**Mean±SD**	**% of change**	**t**	**P-value**
Weight						
Baseline	74.93 ± 10.83		76.40± 9.53		0.557	0.580 (NS)
At 1 mon.	68.43 ± 9.60	8.7%	75.67± 10.18	1.0%	2.831	0.006* (S)
At 2 mon.	66.60 ± 9.79	11.1%	73.93± 9.70	3.2%	2.915	0.005* (S)
At 3 mon.	63.63 ± 8.84	15.1%	72.10± 9.80	5.6%	3.514	<0.001* (HS)
At 4 mon.	60.90 ± 8.68	18.7%	70.37± 9.37	7.9%	4.060	<0.001* (HS)
BMI						
Baseline	28.41 ± 2.19		28.72± 3.04		0.459	0.648 (NS)
at 1 mon.	26.44 ± 1.97	6.9%	28.06± 3.61	2.3%	2.156	0.035* (S)
at 2 mon.	25.12 ± 1.54	11.6%	27.11± 3.46	5.6%	2.887	0.005* (S)
at 3 mon.	23.89 ± 1.38	15.9%	26.17± 3.36	8.9%	3.445	<0.001* (HS)
at 4 mon.	22.71 ± 1.23	20.1%	25.52± 3.43	11.1%	4.223	<0.001* (HS)
Hirsutism						
Baseline	15.23 ± 4.83		17.17±5.64		1.426	0.159 (NS)
at 1 mon.	13.97 ± 4.25	8.3%	16.60±5.57	3.3%	2.060	0.044*(S)
at 2 mon.	12.13 ± 3.40	20.4%	16.07±5.53	6.4%	3.317	0.002* (S)
at 3 mon.	11.00 ± 3.21	27.8%	15.43±5.73	10.1%	3.697	<0.001* (HS)
at 4 mon.	9.77 ± 3.35	35.9%	14.73±5.77	14.2%	4.077	<0.001* (HS)

Table (3): Comparison between two groups as regard Regularity of menses and side effects reported about the drugs and at different times (1st month,2nd month, 3rd month and 4th month).

Regularity of menses	Cases		Control		Total		Chi-square	
	N	%	N	%	N	%	X^2	P-value
Baseline								
present	1	3.3	1	3.3	2	3.3	0.00	1.00 (NS)
Not present	29	96.7	29	96.7	58	96.7		
At 1 mon.								
Present	10	33.3	2	6.7	12	20.0	6.667	0.010* (S)
Not Present	20	66.7	28	93.3	48	80.0		
At 2 mon.								
Present	22	73.3	16	53.3	38	63.3	2.584	0.108 (NS)
Not Present	8	26.7	14	46.7	22	36.7		
At 3 mon.								
Present	27	90.0	17	56.7	44	73.3	8.523	0.004* (S)
Not Present	3	10.0	13	43.3	16	26.7		
At 4 mon.								
Present	27	90.0	15	50.0	42	70.0	11.429	<0.001* (HS)
Not Present	3	10.0	15	50.0	18	30.0		
Side effects reported about the drugs								
at 1 mon.								
abdominal discomfort	3	10.0	3	10.0	6	10.0	0.000	1.000
No	27	90.0	27	90.0	54	90.0		
at 2 mon.								
No	30	100.0	30	100.0	60	100.0	0.000	1.000
at 3 mon.								
No	30	100.0	30	100.0	60	100.0	0.000	1.000
at 4 mon.								
No	30	100.0	30	100.0	60	100.0	0.000	1.000

Figure 1 recruitment and flow of patients



Role of Activated Natural Killer Cells (CD3, CD56, CD16) in Repeated Implantation Failure in Women Undergoing IVF/ ICSI Cycles

Noura El-Nassery[1] MD
Abdel Maguid Ramzy [1] MD
Amal Shohayeb1 MD
AyatAllah A Nassef [2] MD
Ahmed M Maged[1] MD
Nancy M Ismael [1] MD
[1] Obstetrics and Gynecology Department , Cairo University, Egypt
[2] Immunology and clinical pathology Department , Cairo University, Egypt

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- The manuscript is original work.

Abstract

Objective : to compare the level of peripheral blood natural killer (NK) cells (CD3, CD56, CD16) in cases of RIF and women with history of at least one successful ICSI trial.

Methods : A prospective cohort study conducted on 50 women underwent ICSI trial classified into 2 groups. Group I included 25 women repeated (2 or more) ICSI failures and group II included 25 women who previously achieved clinical pregnancy at least once in previous trial. Peripheral blood NKCs was assessed in all women. The primary outcome parameter was the number of NKCs in women of both groups. Other outcomes included the number and quality of retrieved oocytes, the number and quality of obtained embryos, fertilization rate, implantation rate, chemical pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate and live birth rate.

Results: No significant difference between women with previous IVF failure and those with previous IVF success regarding number and quality of retrieved oocytes, number and quality of embryos, fertilization, implantation, clinical pregnancy, ongoing pregnancy and live birth rates. However, the chemical pregnancy rate was significantly higher in women with previous IVF success.

Conclusion: NKCs (CD56. CD16.CD3.) levels did not differ significantly between recurrent implantation failure cases and recurrent successful implantation controls.

INTRODUCTION

Since the first born IVF girl “Louise Brown” in 1978, more than 8 million live birth were achieved through assisted reproduction (1).

Corresponding author:
Noura El-Nassery
Tel +201227763517
Email noura_elnassery2004@hotmail.com

IVF success is dependent on many factors, the most important one is successful implantation. Implantation success is achieved through a precise synchronization between endometrial and blastocyst maturity and development (2).

Modifications to the cellular, vascular, and immunological systems are necessary for optimal endometrial growth (3).

These modifications include the development of the stromal cells of the endometrium to decidual cells with pinopodes (apical projections) associated with growth of the endometrial glands, and the appearance of microvilli on the epithelial luminal surface of the endometrium (4).

Vascular invasion and endometrial immune cell infiltration are caused by alterations in adhesion molecules, cytokines, growth factors, and inhibitory mediators that are linked to these cellular changes (5).

Recurrent implantation failure (RIF) is a main cause of repeated IVF failure (6).

RIF has several definitions. Some consider it as failure to achieve clinical pregnancy after the transfer of at least 6 good quality embryos in fresh or frozen IVF cycles, at least 4 embryos in two egg donations, or after transfer of 10 or more embryos in multiple transfers, or the absence of a gestational sac on ultrasound at 5 weeks after embryo transfer (ET) following 3 ET with high-quality embryos (7).

Among the various reasons of RIF, uterine variables, including thin endometrium, poor endometrial receptivity, and immunological factors have drawn increased attention. Numerous factors are involved in the process of implantation, including embryo quality, endometrial receptivity, and immunological factors(6).

Natural killer (NK) cells are lymphocytes generated from bone marrow that work to eliminate foreign, infectious, and cancerous cells as well as to enhance immune response (8).

By virtue of their expression of the CD56 and CD16 cell surface antigens, they are subtyped. Most pNK cells express CD16, but have less CD56 surface antigens; as a result, they are frequently referred to as CD56dim/CD16+ cells. Cell lysis is caused by CD16 (9)

Both peripheral blood and the uterine mucosa contain NK cells. However, the NK cells at the two sites exhibit significant phenotypic and functional variations (10).

While uterine NK cells are primarily CD56 bright/CD16+ and primarily cytokine producers, peripheral blood NK cells are predominately CD56 dim/CD16+ and are cytotoxic and in direct contact with chorionic villi at inter billows space (11).

Importantly, however, it is believed that identical mechanisms govern peripheral and uterine NK cells. Therefore, determining the degree of NK cell activation in peripheral blood provides insight into the condition of uterine cells (12). In addition, some claim that pNK cells migrate into the uterus before becoming uNK cells. **(9)**.

Rai et al., reported an association between absolute count of activated NKC and reduced implantation rate in IVF cycles, which may suggest being a useful test to discriminate women who will benefit immune-modulation therapeutic intervention (10).

The aim of this study is to analyse and compare the level of peripheral blood natural killer (NK) cells (CD3, CD56, CD16) in cases of RIF and women with history of at least one successful ICSI trial.

Material and Methods

This prospective cohort study was conducted on 50 women who underwent IVF/ ICSI cycle at IVF unit of the department of obstetrics and gynecology, Cairo university between ……. and …….

An informed written consent was signed by all participating women after explanation of

the aim, procedure, risks and benefits of the trial. The study was approved by kasr Alainy ethical committee on ……. with number ……

Fifty women who underwent an ICSI trial were arranged into two groups, group I of women who experienced repeated (2 or more) ICSI failures and group II of women who previously achieved clinical pregnancy at least once in previous trial.

Inclusion criteria included age between 20 and 35 years. Women in group I inclusion criteria were unexplained infertility, mild male factor, tubal factors (not including hydrosalpinx), anovulation (not PCOS nor POI) with normal anatomical, hormonal and gynecological profile and had at least two failed attempts of ICSI (fresh or frozen) with total embryo transfer of six embryo of good quality. Women in group II had a minimum of one successful (with confirmed clinical pregnancy) ICSI trial with no history of repeated miscarriage not due to abnormal hormonal, gynecological nor anatomical causes (unexplained).

Exclusion criteria included women with immunological diseases such as anti-phospholipids proven by (normal anticardiolipin IgG & IgM and lupusanticoaglant antibodies) previously done by the patient already, chronic diseases such as DM, uterine anomalies (fibroid, polyp, septum), documented chromosomal rearrangement in either parent, hydrosalpinx, endometriosis, endocrinological and metabolic disease, gynecological intervention: endometrial polypectomy, myomectomy) and severe male factor (severe oligo-atheno-terato zoospermia).

All participants were evaluated through full history (especially the details of previous IVF cycles) and examinations (general, abdominal and pelvic) Laboratory and ultrasonographic evaluation were done to ensure stickiness to inclusion and exclusion criteria.

During the preparatory follow up visit peripheral blood NKC assessment using 3 mL of blood using flow cytometry (13).

At the Flow- Cytometry Laboratory, the flow-cytometric analysis was completed. Peripheral blood samples weighing three millilitres were drawn into heparinized tubes. Before staining, anticoagulated blood can be kept at room temperature (20°C–25°C) for up to 6 hours. 10 ml of each of the following monoclonal antibodies were pipetted into 100 l of each specimen before being tagged with the patient's name. Anti-CD3 antibody FITC conjugated; clone SK7, is composed of mouse IgG1 heavy chains and kappa light chains, , Anti-CD56 antibody R phycoerythrin-cyanin 5 (PC5) conjugated; clone MY31, is composed of mouse IgG1 heavy chains and kappa light chains and, Anti-CD16 antibody R phycoerythrin-cyanin 5 (PC5) conjugated; clone B73, is composed of mouse IgG1 heavy chains and kappa light chains, (all supplied by BD Biosciences, Becton, Dickinson and Company, USA). Reagents were provided in 1 mL of buffered saline with gelatin and 0.1% sodium azide.

Incubation for 10 to 12 minutes at room temperature (20°C–25°C) in the dark. Immediately after incubation, tubes were centrifuged at 300g for 5 minutes at room temperature (20°C–25°C) and the supernatant was discarded. The cell pellet in the residual fluid was Re-suspended, and then 2 mL of PBS was added with 0.1% sodium azide to each tube. Flow cytometric analysis was performed on the Becton Deckinson FACS calibre flow cytometer using. Cells negatively stained for CD3, positively for CD56 were selected and CD16 expression was analyzed.

Ovarian stimulation was performed using GnRH antagonist protocol. On day 2 of the therapy cycle, rFSH (Gonal-f; Merck Serono) subcutaneous injections were initiated daily. The daily subcutaneous administration of cetrorelix (Cetrotide; Merck Serono) at 0.25 mg was started as soon as one or more of the following conditions—one or more follicles reaching a diameter of 14 mm, the blood level of estradiol reaching 2203 pmol/L, and the serum

level of LH reaching 10 IU/L—were met. The GnRH antagonist and rFSH were given every day up until the triggering day. Ovum collection was carried out under transvaginal ultrasound supervision 34–36 hours following hCG triggering. Under ultrasound guidance, a Day 3 embryo transfer was carried out utilising a Labotect semirigid catheter (Labotect, Göttingen, Germany). From the day of ovum collection until serum -hCG testing, luteal phase support is provided by daily intramuscular injections of 100 mg progesterone (Prontogest; Amsa, Rome, Italy) (14).

The primary outcome parameter was the number of NKCs in women of both groups. Other outcomes included the number and quality of retrieved oocytes, the number and quality of obtained embryos, fertilization, implantation, chemical pregnancy, clinical pregnancy, ongoing pregnancy and live birth rates.

Sample size calculation was done using the comparison of CD56/16 between Cases with recurrent implantation failure and those with no implantation failure in couples doing IVF-ET, as it was the primary outcome of our study. As reported in previous publication (15), the mean ±SD of CD56/16 level in recurrent implantation failure group was approximately 13.62 ± 4.6, while in control group it was approximately 4.51 ± 0.98. Accordingly, we calculated that the minimum proper sample size was at least 21 women in each group to be able to detect a real difference of 9.1 with 99% power at $\alpha = 0.05$ level using Student's t test for independent samples. Sample size calculation was done using Stats Direct statistical software version 2.7.2 for MS Windows, Stats Direct Ltd., Cheshire, UK.

Data analysis was done using IBM SPSS statistics (V. 26.0, IBM Corp., USA, 2019). For quantitative non-parametric measures, data were expressed as median and percentiles, and for categorised data, both number and percentage were used. For non-parametric data, the Wilcoxon Rank Sum test was used to compare two independent variables, and the Kruskall Wallis test was used to compare more than two patients. Chi-square test to investigate the relationship between each of the two variables or to compare the two independent variables in relation to the categorised data. At 0.05, the likelihood of mistake was regarded as significant, whereas at 0.01 and 0.001, it is highly significant.

Results

We assessed 721women for eligibility, 615 of them did not fulfill our inclusion criteria and 56 declined to sign the informed consent. So, 50 women were included in our study (25 in each group). All of them underwent the treatment and followed up till completion of the study and none were excluded from analysis.

No significant difference between women with previous IVF failure and those with previous IVF success regarding age, BMI, AMH or the number of NKCs. However, the duration of infertility was significantly longer in women with previous IVF failure (table 1).

No significant difference between women with previous IVF failure and those with previous IVF success regarding number and quality of retrieved oocytes, number and quality of embryos, fertilization, implantation, clinical pregnancy, ongoing pregnancy and live birth rates. However, the chemical pregnancy rate was significantly higher in women with previous IVF success (table 2).

When comparing women who achieved pregnancy in the current cycle and other women, we found no significant difference between them regarding age, BMI, duration of infertility, AMH, the number of NKCs (table 3), number and quality of retrieved oocytes (table 4) but women with successful current pregnancy showed a significantly higher number of embryos especially of good quality (A and B)(table 4).

When comparing women who underwent fresh ET to those who underwent frozen ET, there was no significant difference between them regarding age, BMI, duration of infertility while AMH and the number of NKCs were significantly higher in women with frozen ET (table 5).

All outcome parameters named the number and quality of embryos, fertilization, implantation, chemical pregnancy, clinical pregnancy, ongoing pregnancy and live birth rates were not statistically different between women with fresh and frozen ET (table 6).

Discussion

Our study found no statistical significant difference between recurrent implantation failure (RIF) history group and patients with history of successful ICSI trial as regard natural killer cells population, this agrees with Zhang et al., (16), Kolanska et al., (17) and Tohma et al., (18) in the same time Mardanian et al., (15), Wafa et al., (19) and Azargoon et al., (20) found that the level of NKC is higher in the RIF, while Prado-Drayer et al., (21) and Ghafourian et al., (22) reported remarkably low proportions of NK cells in healthy controls. Moreover Sacs et al., (23) found a decreased proportion of NK cells in an infertile population compared with the control group.

These conflicted results may be explained by the heterogeneity in population recruited characteristics between studies especially as regard recruited population type, age, NKC subpopulation measured and method of analysis.

As regard number and quality of oocyte retrieved, no statistical significant difference was found between both groups this is supported by the findings of Ocal et al., (24) and Roy Choudhury et al., (25). This may be due to the age convergence between both groups (age min is 22 and age max is 35 with average 31.6 and 31 for group with history of successful ICSI and RIF group respectively), as the female age is known to be an important determinant of the ovarian reserve which is an independent factor for the no. and quality of oocyte retrieved as well established by de Bruin et al., (26), Amanvermez and Tosun (27) and Shahrokh Tehraniejad et al., (28).

Our current study showed a statistically significant difference between recurrent implantation failure (RIF) history group and patients with history of successful ICSI trial as regard duration of infertility which is consistent with findings observed by Ocal et al., (24), meanwhile, a conflict appear with the later study regarding the absence of statistical significant difference for age, BMI and AMH (as a marker for ovarian reserve). This is goes with the observations of Roy Choudhury et al., (25). This can be explained by the wider range of patients demographic data in Ocal et al.,(24) and the similarity of patients characteristics between our study and that of Roy Choudhury et al.,(25) and reflects the homogeneity of patients distribution rather than a true contradiction.

Multiple influential factors are involved in such situation among which the immunological factors (29), which have been proven to be a prominent cause for recurrent implantation failure through testing the efficacy of multiple immune-modulator interventions like; intravenous immunoglobulin (IVIG) (30), intralipid (31). Granulocyte colony-stimulating factor (G-CSF) intrauterine administration (29) and endometrial scratch (32).

Natural killer cells role in human fertility in general and recurrent implantation failure in specific has been discussed extensively (32) to make use of the gained knowledge to improve the assisted reproduction outcomes especially the implantation rate which has been described as the barrier in assisted reproduction to be overcome (33).

Also there was no statistical significant difference between the two groups as regard number and quality of embryo resulted and

transferred which goes in a line with Ocal et al., (24) and Roy Choudhury et al., (25) results. Oocyte quality affects the quality of embryos as suggested by Khalili et al., (34); Balaban and Urman (35) and since the two groups were comparable to each other as regard the oocyte quality, so it was logic to be again comparable to each other as regard embryo quality.

Multiple interrelated reasons may determine the number of resultant embryos such as the preference of physicians to transfer more than one embryo, that become a policy in many centers now wide world to gain higher implantation and pregnancy rate (33), and make every effort to produce more oocytes and subsequently more embryos to be transferred using for such purpose protocols and stimulation drug doses able to achieve this to maximize the chances of pregnancy (24).

Single embryo transfer is becoming more and less popular nowadays and only 15% of doctors are still choosing single embryo transfer in their practice especially in the presence of signs of good prognosis (36).

In the same contest, our study showed no statistical difference between RIF group and successful ICSI history group regarding fertilization and implantation rates. Oocyte quality plays a major role in fertilization process and embryo development in ART program as suggested by Khalili et al., (34) and in the same time it also determines the implantation potential of the derived embryo as reported by Balaban and Urman (35). So having no statistical difference regarding oocyte and embryo quality between the two groups resulted in having such a similar indifference regarding implantation and fertilization rates.

As regard achieving pregnancy, a statistical significant difference was found between the two groups regarding chemical pregnancy (which may represent the overall pregnancy rate) in the favor of the successful ICSI group, while when it comes to achieving clinical pregnancy and detection of a pulsating G.S. in ultrasound no statistical significant difference was detected this is agrees with Sacs et al., (23) and partially disagrees with Ocal et al., (24).

Our study also showed no statistical significance between the two groups as regard continuation of pregnancy past 1st trimester, miscarriage rate and live birth rate. This came in contrast to Sacs et al., (23) who recorded that RIF women had a higher miscarriage rate and lower live birth rate while Chin et al., (37) found that RIF patients' obstetric and perinatal results are comparable to those of control IVF-ET patients, indicating that they do not experience overtly negative effects when compared to controls.

Our results may be attributed to underlying parental risk factors that are more prevalent in patients suffering from infertility (38), taking into consideration the homogenous presence of the factors that influence miscarriage and live birth rates such as age (39).

We tried to look into our data from a different prospective, so we rearranged our cases into two new groups; the group of women who fail to achieve pregnancy in the current cycle and the one who got pregnant and we found that:

No statistically significant differences between both groups as regard demographic data (duration of infertility, age, BMI and AMH) which agrees with the findings of Roy Choudhury et al., (25) while Mardanian et al., (15) disagree with our results regarding the age.

Also no statistical significant difference was found regarding natural killer cells (CD56. CD16.C3.), this goes in line with Fukui et al., (40), Thum et al., (41) and Baczkowski et al., (42) while Mardanian et al., (15) found significant rise of The level of CD56dim CD16+ cells in women with IVF failure compared to successful IVF women but fail to record the same result for CD56bright CD16- cells levels.

This might be explained by differences in sample size, inclusion criteria or method of analysis between our study and the compatible studies from one side and Mardanian et al.,(15) on the other side.

A statistical significant difference was found between the two newly created groups as regard number and good quality embryos (A&B) resulted despite absence of any statistical difference has been found regarding number and quality of retrieved oocyte, however, the number of retrieved oocyte from the successful ICSI trial cases, although not statistically significant, were higher than those from failed ones which agree with Labarta et al., (43) and Vermey et al., (44) who confirmed a strong positive association between oocytes retrieved and top/good-quality embryos and that the group with the most oocytes aspirated had the most top/good-quality embryos

The previous result may spot some light on factors that lead to the success of the ICSI trial in these women.

The quality of transferred embryo, as well as the number of good quality embryos, is one of the most important determinants of for the success of ICSI and the chances of achieving clinical pregnancy (45).

Moreover, some studies in the literature stated that if an aged woman is producing a good number of good quality embryos, the pregnancy rate is almost same as that of young women (46).

A statistical significant increase in the NKC (CD56.CD16.CD3.) in the frozen embryos group compared to the fresh embryos patients; this finding seem to be strange and unrelated at the first instant . This provoked us to search for the possible relation between NKC and causes lead to freezing embryos.

By analyzing the causes of freezing embryos in our study, we found that in 11 patients out of 20, freezing the embryo was a necessity. Eight patients are for the fear of OHSS and three patients are for anticipation of poor endometrium receptivity. OHSS is then comprised around 70% of the inevitable causes of embryo freezing and 40% of all causes.

Digging deeper, cytokines (particularly IL2) are suggested to be the link between OHSS and NKC.

Exposure of hyper stimulated ovaries to hCG leads to the production of pro-inflammatory mediators. This includes vascular endothelial growth factor (VEGF) and varieties of cytokines among which is IL2.cytokines are likely to be involved in the pathogenesis and clinical features of OHSS (47).

In the same time, IL2, which is secreted predominantly from T lymphocytes, found to have a regulatory role in NKC function and number (48).

From the above it may be suggested that cytokine especially IL2 may be a common factor, a result or even a cause for increased NKC in the frozen embryo transfer group. However, more researches and studies are needed to confirm this result, figure out its impact on the success of IVF/ICSI and exclude the possibility of being a statistical coincidence rather than a true association.

The main strength of our study is its prospective nature, proper selection of participants and long term follow up duration while its main limitation was the relative small sample size

Our study concluded that NKCs (CD56. CD16.CD3.) levels did not differ significantly between recurrent implantation failure cases and recurrent successful implantation controls. This result is questioning their role in RIF and does not support the routine use of such marker in RIF outside the research work; accordingly, the use of immunomodulaors may be not justified based on this marker alone. Larger prospective studies are needed to confirm and extend our results. Further studies are needed to evaluate the correlation

between peripheral and endometrial NKC as the later role in cases of RIF is more evident in the literature.

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Effect of Luteal Estrogen Priming in Women with Poor Ovarian Response Ungergoing IVF Using Antagonist Protocol

Noura El-Nassery[1] MD
Amal Shohayeb[1] MD
Mostafa Gamea[1] MD
Ahmed M Maged[1] MD
Mahmoud Soliman [1] MD
[1] Obstetrics and Gynecology Department, Cairo University, Cairo, Egypt

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Keywords: Estrogen priming; Poor ovarian responders; clinical pregnancy rate; IVF

Abstract

Objective : to explore the benefits of the E2 priming during the luteal phase in women with poor ovarian response (POR) undergoing IVF cycles using the GnRH antagonist protocol.

Methods : This randomized controlled study conducted on 100 POR underwent ICSI trial using GnRH antagonist protocol assigned to 1 group of 2. Group I included 50 women received oral E2 valerate 4 mg/day started on luteal day 21 and continued up to the 1st day of the cycle group II included 50 control women who did not receive estrogen priming. The main outcome parameter was the number of MII oocytes. Other outcomes included implantation, chemical pregnancy and clinical pregnancy rates.

Results: No statistically significant difference between women in the estrogen priming group and other women regarding the retrieved oocytes number and quality, implantation, clinical pregnancy, or chemical pregnancy rates.

Conclusion: estrogen priming did not increase the number of MII retrieved ova in POR.

INTRODUCTION

Infertility affects 25% of couples in developing countries (more than 186 million women) (1). In vitro fertilization accounts for 2-3% in low-income countries (2).

Controlled ovarian hyperstimulation (COH) is the first phase in IVF. Poor ovarian response (POR) is not uncommon event during COH as it represents about 5 to 35 % of women with infertility (3).

POR is diagnosed by cancellation of one or more IVF cycles or failure of the long agonist protocol (4).

Corresponding author:
Noura El-Nassery
Tel +201227763517
noura_elnassery2004@hotmail.com

POR women produce inadequate number of oocytes that results in cycle cancellation (76%) (5) or insufficient numbers of embryos for transfer with the resultant lower probability of pregnancy (3.2-14%) (6).

Several approaches were tried to optimize the outcome of IVF in POR women. These include the use of different protocols as antagonist (7), agonist stop (8), short flare up or microdose flare up protocols (9), Pretreatment with different drugs as letrozole, hCG, or AndroGel (10), adjuvant therapy with aromatase inhibitors (10), clomiphene citrate or luteinizing hormone (LH) (11), maximize the starting Gn dose (12) or luteal phase support with FSH (13).

A suggested mechanism for POR is the short follicular phase with the resultant lowered ability of adequate oocyte cohorts or the increased sensitivity to corpus luteum suppressive agents (14).

Adding estrogen to GnRH antagonist during the mid luteal phase of the IVF preceding cycle was suggested to improve IVF outcomes and lower cancellation in POR (15).

E2 pretreatment enhances the negative feedback of natural estrogen on the hypothalamus–pituitary–ovary axis and results in prevention of intercycle increases in FSH and improving oocytes synchronization (14).

However, the studies evaluating the E2 pretreatment studies were not designed to detect IVF outcomes and used the same participants in their previous failed cycle as a control. Moreover, these studies failed to define the appropriate time of Gn induction after luteal E2 or the time of stop of E2 therapy.

The aim of the present study is to explore the benefits of the luteal phase E2 priming in POR undergoing ICSI cycles using the GnRH antagonist protocol.

Material and Methods

This open label randomized controlled trial included 100 women candidates for IVF/ ICSI in IVF unit, Obstetrics & Gynecology Department, Cairo University between December 2019 and May 2020. The study protocol was approved by Kasr Alainy ethical committee. Signing of informed consents was done by all participants after full explanation of POR condition and possible beneficial and hazardous effects of E2; we also mentioned the lack of such studies on poor responders.

All the participants were diagnosed as POR according to Bologna criteria. The criteria require the presence of 2 or more of the following criteria: female age ≥40 years or other risk factors for poor ovarian reserve, poor ovarian response to COH with a conventional stimulation protocol (produced 3 or less oocytes) and low ovarian reserve test (AFC 5-7 or AMH 0.5 – 1.1 ng/ml) (3).

Exclusion criteria included severe male factor, uterine abnormalities (as fibroid, polyps, congenital anomalies or intrauterine synechia (evaluated by hysteroscopy), ovarian cysts, ovarian or pelvic endometriosis, hydrosalpinx, endocrinological disorders as thyroid, adrenal or hyperprolactinemia), uncontrolled metabolic or medical disorders.

All included participants were subjected to complete history, general, abdominal and pelvic examinations and ultrasonographic evaluation.

The participants were randomized using computer generated random numbers, each patient chosen a sealed envelope containing the randomized assignment to either the study or the control group. They were assigned equally to one of 2 groups. E2 priming group who received oral E2 valerate 4 mg/day (Cycloprogynova white tablets, Bayer Pharma™) started on luteal day 21 and continued up to the 1st day of the cycle (14). Control group did not receive such E2 therapy.

All participants were subjected to stimulation using GnRH antagonist conventional protocol. Starting from day 2 of the cycle, each woman received 150 U urinary Gn

(Menogon; Ferring, Switzerland) in addition to 300 U recombinant FSH

(Gonal-f; Merck Serono, Germany) then the dose was adjusted according to monitored ovarian response. Monitoring was done through transvaginal ultrasound done on alternate days using (Mindray trans-vaginal 4-7.5 MHz V6) for follicular count, size, endometrial thickness and pattern. Subcutaneous daily injection of 0.25 mg Cetrolix (Cetrotide, MerckSerono, Germany) was started when the leading follicle reached 12 mm till the day of triggering. When at least 3 follicles reached more than 14 mm, triggering was done using 10,000 IU hCG intramuscularly (Choriomon, IBSA™) (4).

Under general anesthesia transvaginal ultrasound guided oocytes retrieval was performed 34-35 hours after the hCG dose. ICSI procedure was performed in all

cases. Assessment of fertilization was done 16 - 18 hours after oocyte injection then evaluation of embryos was done 48 – 72 hours after ICSI,

Collection of Oocytes and culturing of embryos were done in ISM1 culture medium (Origiomedicult media, Denmark).

Day 2-5 embryo transfer was done using Labotect semirigid catheter; labotect GmbH, Germany).

The main outcome was the number of MII oocytes. Other outcomes included clinical pregnancy rate (one or more gestational sacs detected by transvaginal ultrasound with possible fetal pulsations), implantation rate (the number of gestational sacs divided by the number of embryos transferred) and the number of ET.

Sample size calculation was calculated by comparing the number of metaphase II (MII) oocytes in POR ICSI cycles treated with E2 priming throughout the luteal phase and those un treated matched women. As reported in previous publication (14), the mean ± standard deviation (SD) of number of retrieved oocytes in E2 primed group was approximately 4.5±2.9 oocytes, while in un-treated group it was approximately 3.2±1.9 oocytes. As a result, we determined that using the Student's t test for independent samples, 35 participants in each group were the bare minimum necessary to reject the null hypothesis with 80% power at the 0.05 level. Utilizing Stats Direct statistical software for MS Windows, version 2.7.2, Stats Direct Ltd., Cheshire, UK, calculated the sample size.

Data were statistically described using the mean, standard deviation (SD), median, and range, or, when appropriate, frequencies (number of occurrences), and percentages. Using a Student t test for independent samples, the study groups' numerical variables were compared. An analysis using the Chi-square (2) test was done to compare categorical data. When the anticipated frequency is less than 5, an exact test was used in its place. It was deemed statistically significant when the two-sided p value was less than 0.05. IBM SPSS (Statistical Package for the Social Science; IBM Corp., Armonk, NY, USA) release 22 for Microsoft Windows was used to perform all statistical calculations.

Results

There were no significant differences between E2 priming group women and controls regarding female age, BMI, number of previous IVF failures, AFC or AMH (table 1).

There were no significant differences between women in the 2 study groups regarding all outcome parameters named Gn dose, number and quality of oocytes, number and day of ET, chemical pregnancy, clinical pregnancy or implantation rates (table 2).

There was no correlation between estrogen priming and baseline characteristics or outcome parameters (table 3).

Discussion

Our study found that estrogen priming did not increase the number of MII follicles in women with POR.

The theory of estrogen therapy of PORs is to minimize the levels of FSH during the late luteal and early follicular phases to allow better recruitment of higher number of follicles for growth and maturation (16).

Addition of E2 to GnRH antagonist started during the mid luteal phase of the IVF preceding cycle was suggested to lower cancellation and improve IVF outcomes in POR as it suppresses early follicular recruitment that occurs in the perimenopausal women during the late luteal phase and enhances synchronized follicular development (15).

Although both combined estrogen and progesterone in combined oral contraceptive pills and GnRH agonist can be used for the same purpose, they can adversely affect ovarian responsiveness (17).

The results of the present study are consistent with Di Luigi and his co-workers (18). Using a micro dose leuprolide acetate (LPA) regimen or a GnRH antagonist treatment that included a luteal phase E2 patch and GnRH antagonist in the prior menstrual cycle, they conducted a randomized experiment to examine the IVF outcomes in 54 poor responder patients. In comparison to the control group, the results showed that E2 priming in GnRH antagonist cycles had no appreciable impact on the success rates of IVF in terms of oocyte retrieval, clinical pregnancy rates, and ongoing pregnancy rates.

The luteal phase synchronization of follicular growth was another method Elassar and his team (19) proposed for increasing ovarian responsiveness in underperformers. In low responders, they contrasted luteal E2 alone (n=57) with luteal E2 in addition to antagonist (n=256). When poor responders are used for IVF, the addition of GnRH antagonist to luteal E2 for luteal suppression prior to ovarian stimulation does not increase the success of the procedure. showed that E2 priming during a GnRH antagonist cycle had no discernible effect on the success of IVF in patients who had poor responses.

Stands with our findings, the McGill Reproductive Center's application of the Ata study on 75 women undergoing IVF after stimulation with luteal E2 patch (LPA)-GnRH antagonist and micro dose (MD) flare-up protocols in anticipated poor responders. Despite the fact that the clinical pregnancy rate of 38.9% and the embryo implantation rate of 16.7% in the LPA group were both nearly 50% higher than the corresponding rates of 10.3% and 22.2% in the MD group, respectively, the differences were not statistically significant (p values > 0.05 for all comparisons). This might be because patients in this trial were matched for age and markers of ovarian reserve, whereas in our investigation, age was used as an inclusion criterion with no upper limit.

Although the results do not support our findings that the E2 priming effect is not related to oocyte yield or follicular synchronization with the LPA protocol, Ata suggested that the observed trend towards higher embryo implantation and clinical pregnancy rates needs further study.

The study by Fanchin and his colleagues who studied prospectively 90 IVF-ET candidates (16), showed that by utilizing the hypothalamic-pituitary E2 priming's natural negative feedback, it was possible to successfully suppress the rise in FSH during intervals between cycles. This method may be helpful in synchronizing follicular growth during controlled ovarian hyperstimulation. Although this study's results indicate that luteal E2 administration reduces the size and enhances the homogeneity of early antral follicles on day 3, its correlation to finally bring about better growth of mature follicles with final net effect on IVF results on poor

responders is earlier to be concluded and need some more studies especially because of the variable factors implement this process.

A similar study was carried out by Ghasemzadeh and her associates (21), involving two groups of patients who were receiving gonadotropin and a GnRH antagonist for inadequate response. There were 53 patients in each group, and the oocyte producing results varied slightly. The average number of large follicles (2.9 against 2.3), M2 oocytes (3.6 against 2.8), and type II and type III embryo quality (1.3 against 0.9 and 0.7 against 0.3) were all significantly higher in the intervention group compared to the control group (P values were 0.05, 0.05, 0.05, and 0.01) on average. However, the success rate of pregnancies was 8.3%: 6.7%. which supported our findings of pregnancy rate (16: 18%) but was not statistically significant (P = 0.50), Endocrine differences due to racial disparities across different populations could be the explanation.

Yucel and his colleagues (22), compared E2 to progesterone priming in POR in micro dose flare-up and GnRH antagonist combined letrozole protocols during ICSI. They concluded that these protocols did not significantly improved the outcomes.

Our finding that E2 priming has no significant effect on the picked ovum count agrees with study by Elassar et al., (19). They compared E2 priming to non-priming in antagonist cycles and found a significantly lower total dose of Gn and E2 levels in E2 primed women with similar number and quality of retrieved oocytes, cancellation and pregnancy rates.

Contrary to our findings, the study by Frattarelli (23) used 60 patients who had a poor response rate and 120 IVF cycles, with the main outcome measure(s) being the number of embryos with 7 cells on day 3 of development. The patients themselves were used as controls, and the previous failed cycles belonging to these patients were used as the control. The number of ovum and embryos produced by the usual IVF treatment would dramatically rise as compared to the method without E2 priming, according to the results. This study did not assess and monitor the therapeutic efficacy of E2 therapies, which may account for the discrepancy between our findings despite its minimal effect on pregnancy rate.

In the retrospective investigation carried out by Hwajeong Lee and colleagues (24), 64 more poor responders were enrolled as the control group and completed standard protocols without pretreatment, while 65 poor responders underwent the E2 priming regimen. Two groups' clinical outcomes were contrasted. However, in this study, fertilized oocytes had significance of (2.251.74 vs. 1.321.26; P=0.001), good embryos (1.620.91 vs. 1.140.90, P=0.021) and number of retrieved oocytes (3.582.24 vs. 1.701.45; P=0.000) compared to mature oocytes (1.651.23; P=0.000). Additionally, the clinical pregnancy rate was significantly higher in the E2 priming group than in the control group (26.2% vs. 12.5%; P=0.048), but the variation in racial factors suggests that more multi-centric studies be conducted.

Reynolds and his coworkers (25), in a meta-analysis research, looked at the impact of E2 priming during the luteal phase. It was discovered that this approach enhanced the rate of clinical pregnancy while decreasing the risk of cycle cancellation. The original search turned up 2249 publications in all, while the bibliographies, abstracts, and other sources produced 11 more. Duplications were eliminated, leaving 1227 research, of which 8 eventually matched the requirements for inclusion. Women exposed to LE priming (n = 468) had a lower risk of cycle cancellation compared to women undergoing non-E2 primed protocols (n = 621) and an improved chance of becoming clinically pregnant (RR: 1.33, 95% CI: 1.02-1.72), but the curious thing was that there was no discernible improvement in the quantity of mature oocytes or zygotes obtained per cycle.

Finally, they reported despite its limitations,

this meta-analysis supports the use of E2 priming prior to COH in poor responders as a promising hope, this hope despite non-significant differences is the principle and the motive that promotes more trials with more priming or adjuvant agents.

The main strength of our study is its randomized nature with proper sample size calculation while its main limitation was the absence of long term follow up to calculate the live birth rate (the most important outcome in IVF cycles).

Based on a quantitative and qualitative analysis of responses, it can be concluded that no significant relation between the luteal E2 priming in the antagonist protocol in poor responders and a better ICSI outcome, neither in the number and quality of oocytes retrieved nor the pregnancy rate as well as the embryo implantation rate. Further studies are needed on a larger scale with bigger multi-centric samples to obtain more data about the effect of the luteal E2 priming on poor responders ICSI outcome. While this study limits the generalizability of the results, this approach provides new insight into other protocols and medications to be introduced as a pretreatment for poor responders.

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Single versus Double Frozen Embryo Transfer in PCOS Patients

Walid M Elnagar MD, Khaled F Helal MD
Department of Obstetrics & Gynecology, Faculty of Medicine, Zagazig University

Objectives : The study targets to evaluate the outcomes of polycystic ovarian syndrome (PCOS) infertile women undergoing intracytoplasmic sperm injection (ICSI) using single (SBT) versus double blastocyst transfer (DBT) strategy.

Patients & Methods : 271 infertile PCOS women diagnosed according to the Rotterdam criteria, aged 20-35 years, had body mass index (BMI) of <35 kg/m2 and mild hyperandrogenemia were randomly categorized into SBT and DBT groups and underwent elective frozen-thawed autologous day-5 good-quality (5A) BT. Study outcomes included the live birth rate (LBR), and the incidence of pregnancy-related maternal, fetal and neonatal complication.

Results: Chemical and clinical pregnancy rates were non-significantly higher with DBT. However, the incidence of early pregnancy loss and multiple pregnancy was significantly higher, while the incidence of gestational hypertension and diabetes mellitus, ectopic pregnancy, premature preterm rupture of membrane were non-significantly higher with DBT. On contrary, the incidence of preterm labor (PTL) and the need for cesarean section were significantly higher among women of DBT group. Neonatal outcomes were better with SBT with significantly lower incidence of low birth weight (BW), need for NICU admission and hospital neonatal mortality with significantly higher LBR.

Conclusion: DBT even of good quality worsens the outcomes of ICSI for PCOS women. DBT is significantly associated with small BW, high incidence of PTL, need for operative delivery and NICU admission with subsequent reduction of LBRs. Also, DBT non-significantly increased the incidence of maternal complications than SBT.

Keywords: PCOS, ICSI, Blastocyst transfer, Single blastocyst transfer, double blastocyst transfer, Live birth rate

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is condition characterized by being complex, familial and polygenetic metabolic condition [(1)] with heterogeneous involvement of several genes in the hypothalamic-pituitary-gonadal axis [(2)]. PCOS is the most incident endocrine-

Corresponding author:
Walid M Elnager,
Whitewhale1977@gmail.com,
01224252626

metabolic syndrome that is characterized by hyperandrogenemia, menstrual irregularities, and/or small cysts in one or both ovaries [3] and could be considered as the main cause of female infertility [2].

Assisted reproductive technologies (ART) were defined by the American Center for Disease Control as any fertility-related treatments where eggs or embryos are manipulated [4]. In vitro fertilization involves the transfer of fresh embryos, however, the freeze-all strategy that entails cryopreservation of all embryos to be transferred subsequently in un-stimulated cycle to guard against the negative effects of controlled ovarian stimulation on the endometrium and to minimize the risk of ovarian hyper-stimulation syndrome [5]. Intracytroplasmatic sperm injection (ICSI) is a common procedure used to improve reproductive results, even among couples without male factor infertility [6].

However, there are several points of controversy in the fields of ART traditionally, multiple embryo transfer strategy was used to increase the chance for getting a baby [7], however, multiple pregnancy is a common complication for this strategy with subsequent adverse fetal, maternal and neonatal outcomes [8]. Also, the developmental stage of embryos at time of transfer; cleavage versus blastocyst was a matter of debit, but giving the chance for in-vitro maturation to the stage of blastocyst increased the possibility of getting good quality embryo to be transferred [9]. However, the exaggeration of prolongation of the in-vitro duration appeared as a trend, but recent studies found no advantages for transfer of D7 or D6 blastocyst over D5 blastocyst transfer (BT) [10].

Objectives

The study tried to evaluate the outcomes of PCOS infertile women assigned for ICSI using single (SBT) versus double blastocyst transfer (DBT) strategy.

Design

Prospective randomized comparative study.

Setting

Gynecology & Obstetrics Department, Faculty of Medicine, Zagazig University and multiple private centers

Participants

Infertile PCOS women who attended the Infertility clinic from June 2021 till Aug 2022 were evaluated for inclusion criteria

Inclusion criteria

Infertile women fulfilling at least two of the Rotterdam criteria for diagnosis of PCOS (11, 12), aged 20-35 years, had body mass index (BMI) of less than 35 kg/m2 (13, 14), mild hirsutism on Ferriman-Gallwey (FG) visual scoring system (15, 16), their hormonal profile of serum AMH >4.5, FSH <5.85 LH>5.39 and had no previous attempts of ICSI were included in the study.

Exclusion criteria

Patients who were out of the predetermined age range and BMI, had uterine abnormalities, hydrosaplnix, recurrent miscarriage, medical diseases interfering with or being complicated by occurrence of pregnancy, and cases requiring ICSI with TESE samples, had obesity-inducing endocrinopathy, severe symptomatizing hypovitaminosis or maintained on hormonal contraception within the last 6 months before inclusion in the study, were excluded from the study.

Clinical evaluation

The enrolled patients were clinically evaluated for determination of demographic data, fertility status and obstetric history. Then, patients underwent clinical and ultrasonographic examinations, and gave fasting blood samples for routine

investigations, estimation of serum AMH, FSH, LH, testosterone and estradiol.

Ethical Consideration

The study protocol was discussed with each couple after being preliminary acceptance by the University Ethical Committee and couples accepted to participate in the study according to the randomization sequence were enrolled in the study after signing the written consent. At the end of the study, the final approval was obtained at 27-11-2022; approval number: ZU-IRB#10066/27-11-2022. The study design, protocol and the probable outcomes were registered at ClinicalTrials.gov. by the number: NCT05854810

Randomization and grouping

Patients were randomly divided into two groups (SBT & DBT) using the random block sizes of 2 and 4 by 1:1 allocation computer randomization method (Excel 2007, Microsoft, Redmond, WA, USA) to generate the sequence of patients between both groups.

Study rational

All women were assigned to receive frozen embryo that was frozen at D-5 blastocyst stage using the vitrification ultra-rapid cryopreservation as previously described (17). The blastocysts were staged according to the ASEBIR classification system that included evaluation of the internal cell mass (A-C grades), trophectoderm (A-C grades) and assessment of the degree of blastocele expansion on 2-6 scale; good quality blastocysts must be graded as AA6 on day-5 (18). Only good quality (AA6) blastocysts were selected for transfer.

Patients' preparation and BT

At 1-week before the expected date of menses, gonadotropin-releasing hormone (GnRH)-agonist therapy was started as subcutaneous injection of triptoreline (Decapeptyl, Ferring Pharmaceuticals Ltd., Wittland, Germany) in a dose of 0.1 mg. On the 2nd day of the menstrual cycle, estradiol valerate (Progynova, 2 mg, Bayer Schering Pharma, UK) was given 6-mg daily for 4 days and then dose was adjusted according to the endometrial thickness. Endometrial thickness was judged by TVU (Sonoline Prima 7.5 MHz, Siemens) in the midsagittal plane as the distance between the outer edges of the endometrial/myometrial interface on days 10 to 12. When the endometrial thickness was 8 mm, progesterone therapy 400 mg twice daily as progesterone vaginal supp (Cyclogest; Actavis Co., USA) for 5 days and BT was commenced after rapid thawing on the 6th day of start of progesterone therapy. Progesterone therapy was continued after BT for 14 days at time of chemical diagnosis of pregnancy that was assured clinically depending on detection of viable embryo with pulsating heart by US examination.

Study outcomes

1. The primary outcome is the live birth rate (LBR) after SBT and DBT.
2. The secondary outcomes include

- The chemical (CHPR) and clinical pregnancy rates (CPR) and the incidence of early pregnancy loss (EPL).
- The incidence of pregnancy-related maternal complications especially gestational diabetes mellitus (GDM) and gestation hypertension (GH), and need for cesarean section (CS).
- The incidence of adverse pregnancy outcomes including preterm and very preterm labor, premature preterm rupture of membranes, low and very low-birth weights
- The incidence of adverse neonatal outcomes as low APGAR score, need for NICU admission, duration of NICU stay and neonatal mortality rate.

Statistical analysis

Results were analyzed using paired t-test and Chi-square test (X2 test) using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) with P-value cutoff point for significance is <0.05.

Results

During the study duration 312 PCOS infertile women were evaluated; 22 women were excluded and 290 women had fulfilled the inclusion criteria and were randomly divided into two groups according to number of BT; single (SBT group) and DBT (DBT group). Unfortunately, 19 women were missed after BT and were excluded from the statistical analysis as shown in figure 1. The enrollment data of the studied women are shown in table 1.

Table 1: Enrollment data of the studied women categorized according to number of the transferred blastocysts

Data		SBT (n=136)	DBT (n=135)	P-value
Age (years)	<25	5 (3.7%)	12 (8.9%)	**0.209**
	25-30	85 (62.5%)	80 (59.3%)	
	>30	46 (33.8%)	43 (31.8%)	
	Average (±SD)	29.5 (2.9)	29 (3)	**0.163**
BMI (kg/m²)	Overweight (<30)	23 (16.9%)	36 (26.6%)	**0.052**
	Obese (30-34.9)	113 (83.1%)	99 (73.4%)	
	Average (±SD)	31.5 (1.8)	31.4 (1.9)	**0.634**
Infertility	Primary	80 (58.8%)	86 (63.7%)	**0.409**
	Secondary	56 (41.2%)	49 (36.3%)	
Lab findings	AMH (ng/ml)	4.66 (1.65)	4.52 (1.9)	**0.513**
	FSH (mIU/ml)	5.2 (1.3)	5.15 (1.4)	**0.758**
	LH (IU/ml)	5.5 (1.6)	5.8 (1.7)	**0.133**
	Testosterone (ng/ml)	1.035 (0.15)	1.03 (0.14)	**0.782**

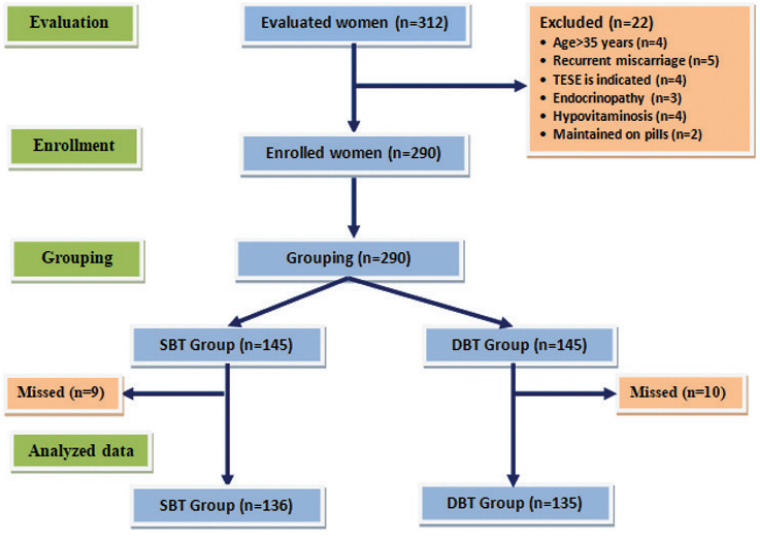

Figure 1: Study Flow Chart

Table 2: Pregnancy data of the studied women categorized according to number of the transferred blastocysts

Data	Group	SBT (n=136)		DBT (n=135)		P-value
		No.	%	No.	%	
Chemical pregnancy (CHP)	Positive	78	57.4	88	65.2	**0.232**
Clinical pregnancy (CP) among	Total women	52	38.2	63	46.7	**0.160**
	Women had +ve CHP		66.7		71.6	**0.422**
Ectopic pregnancy		0	0	1	0.74	**0.315**
Early pregnancy loss among	Total women	14	10.3	27	20	**0.026**
	Women had +ve CP		26.9		42.9	**0.021**
Women completed follow-up	Total women	38	27.9	36	26.7	**0.814**
	Women had +ve CP		73.1		57.1	**0.076**

Chemical pregnancy (CHP) test was positive in 166 women for a chemical pregnancy rate of 61.3% with non-significantly (P=0.232) higher CHP rate for women of DBT (n=88; 65.2%) than for women of SBT (n=78; 57.4%). US examination detected viable embryo in 115 pregnant women for clinical pregnancy (CP) rate of 42.4% with non-significantly (P=0.160) higher CP rate among women of DBT group (n=63; 46.7%) in comparison to women of SBT group (n=52; 38.2%). The CP rate among women had positive CHP rate (n=166) was 69.3% and was non-significantly (P=0.422) higher rate among women of DBT group (71.6%) compared to the rate detected for SBT group (66.7%). Only one woman in DBT group had ectopic pregnancy for an incidence of 0.74% and 0.37% among the total studied women. Unfortunately, 41 women had early pregnancy loss (EPL) for an incidence rate of 35.7% among women had CP, 24.7% among women had CHP and 15.1% among the total enrolled women. Women of DBT group had significantly higher EPL rate (n=27) 42.9% among women had CP (P= 0.026) and 20% among total DBT women (P=0.021) in comparison to EPL rates for women of SBT; 26.9% and 10.3%, respectively. Seventy-four women continued their pregnancy for pregnancy rate of 44.6%, 64.3% and 27.3% among women had CHP, CP and total enrolled women, respectively with non-significantly (P=0.076) high rate among women had SBT (n=38) than women had DBT (n=36) as regards rate among women had CP (73.1% vs. 57.1%) and among total studied women (27.9% vs. 26.7%) as shown in table 2

Forty-three women; 38 had SBT and 5 had DBT developed single gestational sac containing singleton fetus, while 31 women had DBT developed two gestational sacs for a multiple pregnancy rate of 23% among total women of DBT group and 86.1% among women who completed their pregnancy. The incidence of singleton fetus was significantly (P<0.001) higher in women had SBT as shown in figure 2.

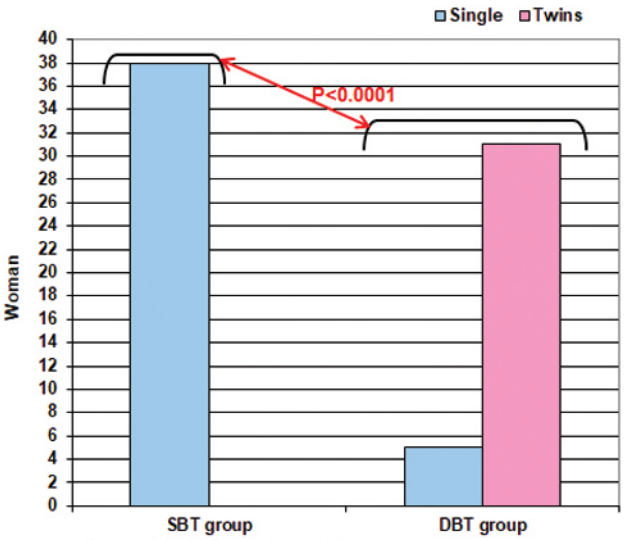

Fig. (2): Incidence of multiple pregnancy among women of both groups

Among women continued their pregnancy 22 women (29.7%) developed hypertensive manifestation; 5 had early preeclampsia (6.7%), 8 women developed late preeclampsia (10.8%) and 9 women (12.2%) had gestational hypertension without proteinuria with non-significantly (P=0.093) higher hypertensive complication rates among women had DBT than women had SBT. Further, 10 pregnant women (13.5%) developed gestational diabetes mellitus with non-significantly (P=0.440) higher incidence of gestational diabetes mellitus among women had DBT. Seven women (9.5%) had preterm premature rupture of membranes with non-significantly (P=0.205) higher incidence among DBT women. Fifteen women (20.3%) had preterm labor with significantly (P=0.0065) higher incidence among women of DBT (33.3%) than women of SBT group (7.9%). Twenty-six women (35.1%) required cesarean section with significantly (P=0.034) higher cesarean section rate among women of DBT group (47.2%) than among women of SBT (23.7%) group (Table 3, Fig. 3).

Table 3: Maternal complications of the studied women categorized according to number of the transferred blastocysts

Outcomes			SBT (n=38)	DBT (n=36)	P-value
Gestational hypertension (GH)	Preeclampsia	Early-onset	2 (5.3%)	3 (8.3%)	**0.093**
		Late-onset	3 (7.9%)	5 (13.9%)	
	GH		3 (7.9%)	6 (16.7%)	
	No hypertension		30 (78.9%)	22 (61.1%)	
Gestational diabetes mellitus		Yes	4 (10.5%)	6 (16.7%)	**0.440**
		No	34 (89.5%)	30 (83.3%)	
Preterm premature rupture of membrane		Yes	2 (5.3%)	5 (13.9%)	**0.205**
		No	36 (94.7%)	31 (86.1%)	
Preterm labor	<37 gestational weeks		3 (7.9%)	12 (33.3%)	**0.0065**
	≥37 gestational weeks		35 (92.1%)	24 (66.7%)	
Cesarean section	Yes		9 (23.7%)	17 (47.2%)	**0.034**
	No		29 (76.3%)	19 (52.8%)	

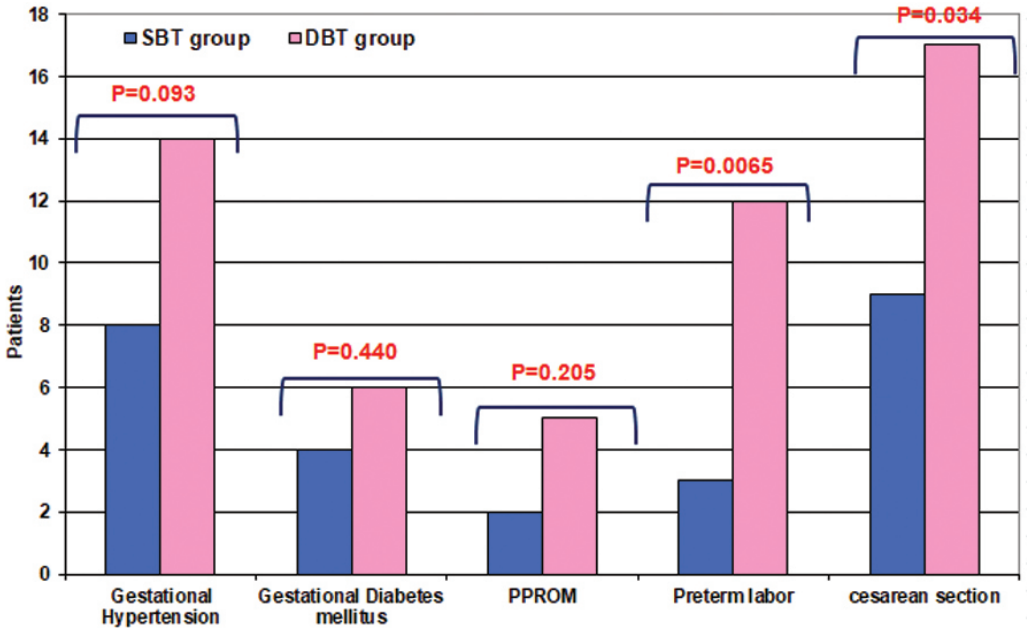

Fig. (3): Patients' distribution according to maternal complications

The applied study protocol resulted in 105 neonates; 64 neonates (61%) had normal average birth weight and 41 neonates (39%) had birth weight of <2.5 kg with significantly (P=0.031) higher incidence of low birth weight among neonates of DBT women (53.7%) than SBT women (73.7%). Further, 49 neonates (46.7%) required admission to NICU; 11 neonates of women of SBT group (28.9%) and 38 neonates of women of DBT (56.7%) with significantly (P=0.006) higher incidence of NICU admission among neonates of DBT group. Thirty-five neonates (33.3%) died; 8 neonates of SBT and 27 neonates of DBT (21.1% vs. 40.3%) groups with significantly (P=0.044) higher mortality rate among neonates of DBT group (Table 4, Fig. 4).

Table 4: Neonatal outcomes

Outcomes		SBT (n=38)	DBT (n=67)	P-value
Birth weight (kg)	<2.5	10 (26.3%)	31 (46.3%)	**0.031**
	Normal weight	28(73.7%)	36(53.7%)	
NICU admission	Yes	11 (28.9%)	38 (56.7%)	**0.006**
	No	27 (71.1%)	29 (43.3%)	
Live births	Yes	30 (78.9%)	40 (59.7%)	**0.044**
	No	8 (21.1%)	27 (40.3%)	

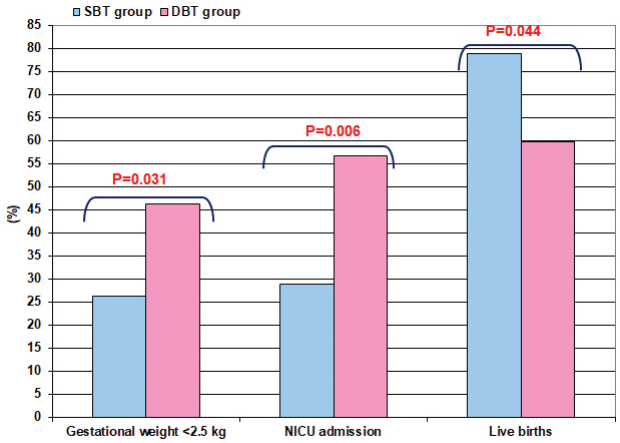

Fig. (4): Neonatal outcomes of pregnant women of both groups

Discussion

Considering the live birth rate (LBR) not the pregnancy rate as the target for any ART trial, the current study reported significantly higher LBR after single blastocyst transfer (SBT) than after double BT (DBT) despite of the significantly higher rate of multiple pregnancy after DBT. Additionally, the frequency of preterm premature rupture of membrane and preterm labor was higher women had DBT, this deleteriously affected neonatal outcome as evidenced by higher percentages of neonates with birth weight (BW) of <2.5 kg, required NICU admission and hospital neonatal mortality rate. Further, DBT was associated with higher incidence of gestational hypertensive disorders especially early-onset preeclampsia.

These data are in line with previous studies evaluating a similar topic, wherein, Peng et al. (19) in a meta-analysis detected a significant increase in cumulative LBR, higher gestational age at birth and BW after two consecutive cycles of single embryo transfer (SET) than one cycle of double ET (DET) with a lower risk of CS, antepartum hemorrhage, preterm birth, low BW, and NICU admission, and concluded that the 2 SETs strategy is beneficial especially for women younger than 35 years and BT provides more favorable outcomes than cleavage stage ET. Huang et al. (20) found an elective single cleavage ET can maintain the relatively high LBR with an acceptable low twin birth rate than double cleavage ET.

Further, Williams et al. (21) found DET significantly increased the multiple pregnancy LBR, with 43% twins and 0.9% triplets and BT had higher LBR than cleavage stage embryos (52.5% vs. 39.5%). Chen et al. (22) investigated the risk factors for twin pregnancy in IVF and found transfer of one good-quality embryo (GQE) was associated

with significantly reduced rate of twin pregnancy, low BW and preterm birth than transfer of two GQE.

Recently, Ozmen et al. (23) detected higher incidence of multiple pregnancy, CS and NICU admission and duration of NICU stay per neonate after DBT versus SBT. Also, Rodriguez-Wallberg et al. (24) revised the 10-y outcomes after SET versus DET or BT in the Sweden registry and detected higher risk of neonatal deaths in singletons that were born after DET than SET, and in case of frozen embryo, DET was associated with higher risk of low BW, and in case of BT, DBT was associated with higher risk of very preterm births and low BW and concluded that DET or DBT is associated with higher risk of adverse outcomes even if it resulted in singleton fetus in comparison to singletons that were born after SET or SBT.

Fortunately, one woman (0.74%) in DBT group, but none in SBT group developed ectopic pregnancy (EP). This low rate of EP among the studied population (0.37%) could be attributed to the selection of good blastocyst to be transferred, irrespective of being SBT or DBT. In line with this attribution, Anzhel et al. (25) retrospectively reported significantly lower EP rate after top-quality than poor-quality ET and significantly higher risk of EP after DET than SET. Further, Xue et al. (26) retrospectively investigated the impact of previous EP on outcomes of subsequent IVF and found the odds of EP were lower by 82.2% lower after BT than after cleavage embryo and were 6-times higher after DET than SET.

The current study tried to avoid bias of the reported LBR due to multiple effectors, so the study looked-like selective study including only PCOS as an underlying pathogenesis for infertility and all women were free of other etiological factors for infertility, women were in age range of 20-35 years and had BMI of <35 with average BMI of about 31.4 kg/m2 and mild PCOS-induced hormonal disturbances. Also, to equalize the chance for all women to get a live born baby, the study rational was to transfer frozen not fresh, blastocyst not cleaved embryo and of good quality of 5A grade

In line with the selective rational of the current study, Williams et al. (21) reported progressive reduction in the LBR with increasing the recipient age (OR, 0.8 for 40-44, 0.77 for age of 45-49 and 0.65 for age >49 years), a steady decline in the LBR with increases in recipient BMI above normal and with cleavage stage embryos than BT. Also, Yang et al. (27) tried to determine the outcomes of SET versus DET according to the recipient's age and concluded that for women younger than 35 years SET is the appropriate, while for older women individualized choices are required because the outcomes for women aged 35-39 depends on the number of oocytes retrieved and blastocyst quality, while for women older than 39 years the outcomes were low in both single and double ET with non-significant differences

Unfortunately, review of literature could not provide an explanation the reported low LBR, irrespective of the number of the transferred blastocyst and despite of the strict inclusion criteria. Some attributions were provided depending on the effect of uterine receptivity (28) intrauterine inflammatory milieu (29), intrauterine and systemic oxidative stress (30). Also, endometrial dysbiosis was found to affect embryo implantation through inflammation-related endometrial changes (31).

The obtained results after adjustment of inclusion criteria and the lack of explanation for the high pregnancy loss rate indicated the necessity for optimization between the benefits and risks before taking the decision for the elective frozen-thawed autologous blastocyst transfer as single or double BT for PCOS women undergoing ICSI.

Conclusion

Double BT even of good quality worsens the outcomes of ICSI for PCOS women. DBT is significantly associated with small BW, high incidence of preterm labor, need for operative delivery and NICU admission with subsequent reduction of LBRs. Also, DBT non-significantly increased the incidence of maternal complications than SBT.

Recommendation

Single good quality BT is the appropriate policy for PCOS women fulfilling the inclusion criteria of the current study and no need to take the risk of DBT with preservation of the second blastocyst for another session of ICSI to increase the chance for getting more offspring.

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