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The Impact of Cervical Mucus, Blood, and Bacterial Contamination on the IVF Outcome

Mohamed Shehata[1]*, Eman A. Hassan[2]

[1]*Department of Obstetrics and Gynecology, International Islamic Center for Population Studies, and Research, Assisted Reproduction Unit, Al-Azhar University, Egypt*

[2]*Embryology Department, International Islamic Center for Population Studies, and Research, Assisted Reproduction Unit, Al-Azhar University, Egypt*

Corresponding Author:** ***Mohamed Shehata, *Department of Obstetrics and Gynecology, International Islamic Center for Population Studies and Research, Assisted Reproduction Unit, Al-Azhar University, Egypt. Email: drmshehata0@gmail.com*

Abstract

Background: *There is insufficient evidence about the effects of different antibiotic prophylaxis regimens on ARTs outcome. Authors suggest that the cervical mucus may interfere with the correct placement of the embryos into the uterine cavity, and the cervical mucus aspiration increased the clinical pregnancy rate. This study designed to evaluate the impact of cervical mucus, blood, and bacterial contamination on the IVF outcome.*

Materials and Method: *Eighty-one women scheduled for controlled ovarian hyper stimulation (COH), and intra-cytoplasmic-Embryo transfer (ICSI-ET) cycles included in this study. Studied women received long pituitary down regulation protocol, and Controlled Ovarian Hyperstimulation (COH). COH, followed by ovulation trigger, oocytes retrieval, and Day 2 or 3 embryo transfers. The cervical mucus aspirated, and the embryo transfer catheters examined after the ETs for the presence of mucus and/or blood, followed by bacteriological examination, and culture of the cervical mucus, and embryo transfer catheters.*

Results: *The clinical pregnancy rate was 58.1% (18/31) in positive culture versus 52% (26/50) in negative culture group (no significant difference; P=0.77). The clinical pregnancy rate was 56% (14/25) in presence of blood versus 53.6% (30/56) in absence of blood in embryo transfer catheters, and it was 60.9% (14/23) in presence of mucus versus 53.6% (30/56) in absence of mucus in embryo transfer catheters with (no significant difference; P=1 and 0.68; respectively).*

Conclusion: *Bacterial contamination of the cervical mucus, and/or embryo transfer catheters did not affect the clinical pregnancy rates after ICSI. In addition; the cervical mucus aspiration before embryo transfers is time consuming, and may stimulate the unwanted uterine contractions.*

Keywords: *Cervical, Mucus, Blood, Bacterial, IVF*

1. INTRODUCTION

Assisted reproductive techniques (ARTs), such as trans-vaginal oocyte pick-up, and catheter insertion for intrauterine insemination (IUI) or embryo transfers (ETs), considered relatively safe procedures.[1] Acute pelvic infection following ARTs is uncommon despite the invasive nature of the procedures. [2-3]

Risk of infection arises from the transfer of microorganisms such as normal vaginal flora to the uterine cavity, pelvic peritoneum, and the infection may interfere with the implantation of the transferred embryos. [4]

Clinical studies showed that bacterial contamination of the embryo transfer catheters has a significant negative effect on the clinical pregnancy rates. [5-8]

In addition, the routine uses of the antiseptics to clean the uterine cervix before embryo transfers have negative impact on the quality of the embryos transferred. [9]

Moreover, there is insufficient evidence about the effects of different antibiotic prophylaxis regimens on ARTs outcome. [8,10-13]

In addition; many authors suggest that the cervical mucus may interfere with the correct

placement of the embryos into the uterine cavity, and the cervical mucus aspiration increased the clinical pregnancy rate. [14-16]

2. Materials And Methods

Eighty-one women scheduled for controlled ovarian hyper stimulation (COH), and intra-cytoplasmic-Embryo transfers (ICSI-ETs) cycles included in this prospective comparative study after informed written consent at the ART unit of C PLAS - Hospital in Sana'a Yemen from July 2012 to March 2013. Studied women evaluated by; thorough history, hormonal profile (Follicle stimulating hormone (FSH), Luteinizing hormone (LH), prolactin, Thyroid stimulating hormone (TSH), and Anti-Mullerian hormone (AMH)), trans-vaginal sonography (TVS) to detect the antral follicle count (AFC), and hysteroscopy for the uterine abnormalities.

Inclusion criteria includes; infertile women for ≥2 years due to male or tubal factors, polycystic ovary syndrome, unexplained infertility with normal uterine cavity, 20-40 years old, ≥5 oocytes retrieved, endometrial thickness ≥8 mm, and basal FSH <10 mIU/ml.

Exclusion criteria includes; women refused to participate, women with endocrine disturbance (thyroid and/or hyperprolactinaemia), > 40 years, <5 oocytes retrieved, endometrial thickness < 8 mm, and basal FSH more than 10 mIU/ml.

Studied women received long pituitary down regulation protocol using 0.1 mg GnRH agonist triptorelin (Decapeptyl®, Ferring, Kiel, Germany) subcutaneously daily started from day 21 of the cycle, and continued till the day of hCG administration. Day 3 Serum FSH, Estradiol (E2), and TVS done to confirm proper down regulation.

Pituitary down regulation followed by COH with hMG (75 IU FSH and 75 LH; Menogon®, Ferring, Kiel, Germany) intramuscularly from day 2 of the cycle until ≥3 follicles 18-22 mm diameter detected during the folliculometry.

The calculated hMG dose given to studied women according to their; age, body mass index (BMI), AFC, hormonal status (AMH, FSH). Folliculometry started on Day 7 using Mindray DP 8800 ultrasound machine (KeeboMed Inc., Rand Road, USA) with 7.5 MHz trans-vaginal probe. When ≥3 follicles 18-20 mm in diameter detected during folliculometry, the ovulation triggered by hCG. Oocytes retrieval 34-36 hours after hCG trigger under general anesthesia.

The retrieved oocytes incubated for 1 hour in global fertilization media, and denudation of the retrieved oocytes carried out chemically and mechanically before insemination. Post-insemination the oocytes incubated overnight in global total media, triple gas incubator and observed after 16-18 hours post insemination for fertilization. The fertilized oocytes then transferred into cleavage medium, and incubated, followed by day 2 or 3 embryo transfers (4-8 cell stages).

Cervical mucus aspirated using a sterile insulin syringe before embryo transfer. The aspirated cervical mucus placed onto a 5% sheep blood agar plate. The vagina, and cervix cleaned with normal saline or culture media (no antiseptic solution used), followed by embryo transfer.

All embryo transfers done using a non-touch sterile replacement technique (sterile drapes, speculum, and disposable non-latex gloves), and contact between the embryo transfer catheters, the vaginal walls and ecto-cervix avoided.

After embryo transfers, the embryologist examined the transfer catheters under microscopy to confirm or exclude the presence of mucus or blood in the catchers and to confirm that all loaded embryos transferred followed by cut off the distal 2 cm of the catheter tip using sterile scissors. Each catheter tip rolled onto a 5% sheep blood agar plate, using sterile forceps. The cervical mucus, and catheter tip samples incubated aerobically, and under 5% CO2 at 37°C for 72h, followed by microbiological assessment of the plates. Bacteria identified by standard laboratory techniques [13] and quantified using a semi-quantitative four point grading system for gram-positive and gram negative organisms: the absence of growth after 48h (no growth (NG)), <10 bacterial colonies (+), >10 bacterial colonies (++) and semi-confluent or confluent growth (+++) on the blood agar plate. Studied women received luteal phase support by micronized progesterone (Cyclogest; Actavis; Barnstaple, UK) started at the day of embryo transfers, followed by quantitative β-hCG 14 days after the embryo transfers for diagnosis of pregnancy. Women proved to be pregnant by quantitative β –hCG, scheduled for TVS 20 days after the quantitative β –hCG for detection of gestational sac or sacs.

The studied women categorized according to presence of cervical mucus, blood in the embryo transfers catheters, and bacterial contamination in the cervical mucus and/or embryo transfer catchers into; positive group (positive for cervical mucus or blood in the catheters or bacterial contamination of cervical mucus and/or catheters), and negative group (negative for cervical mucus or blood in the catheters or bacterial contamination of cervical mucus and/or catheters).

The clinical pregnancies considered for the studied women following detection of gestational sac or sacs or histological detection of the products of conception if miscarriage occurred.

Statistical Methods

The collected data statistically analyzed using the Statistical Package for Social Science (Chicago, IL, USA). Categorical variables presented as number and percentage. Chi-square test ($\chi 2$) used for statistical analysis and *P*-value <0.05 considered statistically significant.

3. RESULTS

Eighty-one women scheduled for COH and ET cycles included in this prospective comparative study. The mean number of collected oocytes was 12.3 ± 6.2, and the mean number of transferred embryos was 2.4 ± 0.7. Positive bacterial contamination of the cervical mucus and/or embryo transfer catheters samples detected in 38.35 (31/81) of the studied women, and the microorganisms isolated from the cervical mucus and/or embryo transfer catheters after culture were Escherichia coli in 58.1% (18/31), streptococcus species in 32.3% (10/31), and Kleibsiella Spp. in 9.6% (3/31).

The clinical pregnancy rate was 58.1% (18/31) in positive culture group versus 52% (26/50) in negative culture group with no significant difference between the two studied group (*P*=0.77). Table 1

This explains why the antibiotic prophylaxis are not recommended by any of the ARTs societies during the ARTs procedures.

Table1. *The clinical pregnancy rate in relation to positive or negative cultures, and the age of the studied women*

Age	Pregnancy Rate (%)			*P* value
	Negative bacteria in cervical mucus and/ or transfer catheter	Positive bacteria in cervical mucus and/or transfer catheter	Total	
20-25 Years	4/9 (44.4%)	9/14 (64.3%)	13/23 (56.5%)	0.61*
26-30 Years	8/12 (66.7%)	10/24 (41.7%)	18/38 (50%)	0.42*
31-38 Years	6/10 (60%)	7/12 (58.3%)	13/23 (59.1%)	1.0*
Total	18/31 (58.1%)	26/50 (52%)	44/81 (54.3%)	0.77*

** Non-significant difference*

Chi-square (X2) test used for statistical analysis

Data presented as number and percentage (%)

In this study; the presence of blood in the embryo transfer catheters during ETs, and did not affect the clinical pregnancy rates. The clinical pregnancy rate was 56% (14/25) in presence of blood in embryo transfer catheters versus 53.6% (30/56) in absence of blood in embryo transfer catheters with no significant difference (P=1). Table 2

Table2. *The clinical pregnancy rate in relation to the blood in the ET catheters, and the age of the studied women*

Age	Pregnancy Rate (%)			P value
	Positive blood in embryo transfer catheters	Negative blood in embryo transfer catheters	Total	
20-25 Years	3/5 (60%)	10/18 (55.5%)	13/23 (56.5%)	1.0*
26-30 Years	7/10 (70%)	11/26 (42.3%)	18/38 (50%)	0.41*
31-38 Years	4/10 (16%)	9/12 (75%)	13/23 (59.1%)	0.39*
Total	14/25 (56%)	30/56 (53.6%)	44/81 (54.3%)	1.0*

* Non-significant difference

Chi-square (X2) test used for statistical analysis

Data presented as number and percentage (%)

ET: Embryo transfers

In this study; the cervical mucus aspiration before ETs did not affect the clinical pregnancy rates. The clinical pregnancy rate was 60.9% (14/23) in presence of mucus in embryo transfer catheters versus 53.6% (30/56) in absence of mucus in embryo transfer catheters with no significant difference (*P*=0.68). Table 3

Table3. *The clinical pregnancy rate in relation to the mucus in the ET catheters, and the age of the studied women*

Age	Pregnancy Rate (%)			P value
	Positive mucus in embryo transfer catheters	Negative mucus in embryo transfer catheters	Total	
20-25 Years	5/7 (71.4%)	8/16 (50%)	13/23 (56.5%)	0.62*
26-30 Years	8/12 (66.7%)	10/24 (41.7%)	18/38 (50%)	0.42*
31-38 Years	1/4 (25%)	12/18 (66.7%)	13/23 (59.1%)	0.39*
Total	14/23 (60.9%)	30/56 (53.6%)	44/81 (54.3%)	0.68*

* Non-significant difference
Chi-square (X^2) test used for statistical analysis
Data presented as number and percentage (%)
ET: Embryo transfers

4. DISCUSSION

The female genital tract contains a variety of bacterial flora including aerobic, and anaerobic spices. [17-18]

Moreover, the diversity of the organisms that comprise the vaginal microbial community varies among women. [19]

Since, the lower genital tract is naturally inhabited with vaginal flora, and pathogenic organisms, operative procedures through the genital tract may lead to moderate to high risk of infection. Therefore, the antibiotic prophylaxis recommended in many of the genital tract procedures as vaginal hysterectomy, and abdominal hysterectomies. In spite of the fact that all ARTs procedures such as ovum retrieval, intrauterine inseminations, and embryo transfers are done through the vagina, the antibiotics prophylaxis is not recommended in any of the ARTs societies.

This study designed to detect whether the presence of mucus, blood or bacterial contamination of the cervical mucus or embryo transfer catheters affecting the ARTs outcome or not?

In this study; the bacterial contamination detected in the cervical mucus and/or embryo transfer catheters samples in 38.35% (31/81) of the studied women, and the clinical pregnancy rate was 58.1% in positive culture group versus 52% in negative culture group with no significant difference between the two studied group (*P*=0.77). Our findings did not support the routine use of antibiotics, because the presence of bacterial contamination of the cervical mucus and/or embryo transfer catheters did not affect the clinical pregnancy rates after the ARTs, and explains why the antibiotic prophylaxis are not recommended by any of the ARTs societies during the ARTs procedures till now.

In addition; *Brook et al*, evaluated the effect of co-amoxiclav on the rates of bacterial contamination of transfer catheters, and the clinical pregnancy. They found that the antibiotics therapy given during the embryo transfers significantly reduced catheter contamination rates (49.4 versus 62.3%; *P* = 0.03), with no significant difference detected in the clinical pregnancy rates between the two studied groups. [13]

Brook et al, concluded that the Co-amoxiclav reduces catheter contamination, but this is not translated into better clinically relevant outcomes such as clinical pregnancy rates. [13]

In this study; the presence of blood in the embryo transfer catheters during ETs, and did not affect the clinical pregnancy rates. The clinical pregnancy rate was 56% (14/25) in presence of blood in embryo transfer catheters versus 53.6% (30/56) in absence of blood in embryo transfer catheters with no significant difference.

In addition; the cervical mucus aspiration before ETs did not affect the clinical pregnancy rates. The clinical pregnancy rate was 60.9% (14/23) in presence of mucus in embryo transfer catheters versus 53.6% (30/56) in absence of mucus in embryo transfer catheters with no significant difference.

Eskandar et al, and *Moini et al*, suggested that the cervical mucus during embryo transfer may interfere with the correct placement of the embryos into the uterine cavity, and the cervical mucus aspiration increased the clinical pregnancy rate. [14,15]

Although, *Shrimali et al*, suggested that the cervical mucus may trap the embryos, and facilitate their expulsion during catheter withdrawal. [16]

Visschers et al, concluded that it is unlikely that removal of cervical mucus prior to embryo transfer has a significant effect on live birth rates [20], and this study also concluded that the presence of blood and/or mucus in the embryo transfer catheters did not affect the clinical pregnancy rates.

In addition the cervical mucus removal or aspiration before embryo transfers is time consuming procedure, and may stimulate the unwanted uterine contractions during the transfer procedures.

This study concluded that the bacterial contamination of the cervical mucus and/or embryo transfer catheters during ARTs procedures did not affect the clinical pregnancy rates, and this explains why the antibiotics prophylaxis are not recommended by any of the ARTs societies during the ARTs till now. Although, many authors suggest that the cervical mucus may interfere with the correct placement of the embryos into the uterine cavity, and the cervical mucus aspiration increased the clinical pregnancy rate. This study concluded that the presence of cervical mucus and/or blood in the catheters during embryo transfer did not affect the clinical pregnancy rates, so, large controlled studies needed to confirm the effect of cervical mucus aspiration on the pregnancy rates after ARTs.

Women refused to participate in this study was the only limitation faced during conduction of this study. This study explains why the antibiotic prophylaxis are not recommended by any of the ARTs societies during the ARTs procedures till now, and concluded that the cervical mucus aspiration before ETs is time consuming, and did not increase the clinical pregnancy rate.

5. CONCLUSION

Bacterial contamination of the cervical mucus, and/or embryo transfer catheters did not affect the clinical pregnancy rates after ICSI. In addition; the cervical mucus aspiration before embryo transfers is time consuming, and may stimulate the unwanted uterine contractions.

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