Assessment of cervical cytomorphological changes in infertile women undergoing IVF/ICSI

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Abstract

The aim of this study is to assess cervical cytomormophological changes in cervical smears taken from infertile women undergoing IVF/ICSI compared with normal fertile women. A case-control study of 100 cases were included, 50 infertile group and 50 normal fertile control group both in age range between (20-44), Pap smear taken prior to ova pickup in case group and in preovulatory day in control group. The mean age for case group was 28.9 ±5.6 year; and the mean age of control group was 29.7 ±5.5 years with no significant difference between them t=1.99, P=0.5, the most frequent cause of infertility is polycystic ovaries or with polycystic ovarian syndrome which diagnosed in (36%) of patients then (28%) of patients diagnosed with unexplained infertility. Case group has (22%) diagnosed with atypical squamous cells with undetermined significance (ASC-US) while control group has just 1 case (2%) with ASC-US and this affects pregnancy test result since (18%) out of (22%) of patient with (ASC-US) pap smear report has negative pregnancy test. Statistical analysis shows significant correlation between positive cervicitis and negative pregnancy test (P value = 0.04) because (52%) out of (66%) with positive cervicitis has negative pregnancy test. Beside a significant relation between positive infection in pap smear report and negative pregnancy test because (30%) out of (34%) with positive infection in pap smear has negative pregnancy test. In Conclusions, infertile women eligible for IVF are more probable to have ASC-US when compared with normal fertile women, ASC-US worsen pregnancy test results in women undergoing IVF/ICSI because (18%) out of (22%) of patient with (ASC-US) have negative pregnancy test.

Keywords: IVF/ICSI; Cervical cytomorphological; Cervical cancer

Introduction

Knowledge regarding the role of the cervix in fertility has expanded considerably over the past 20 years [1].

It is estimated that abnormalities of cervical secretions may account for ~3% to 8% of cases of infertility in

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women [2]. The facts that women with infertility are exposed to sexual transmitted diseases (STDs) and usually treated with exogenous hormones supported the theory that women with infertility are at high-risk of developing cervical intraepithelial lesions (CIN) or even carcinoma [3]. Papanicolaou test (pap) and pelvic exam are best simple methods in detecting cervical cancer, other problems with the cervix, or active sexually transmitted diseases (STDs) , Any of these can interfere with fertility in women [4]. The techniques used in assisted reproductive technologies (ART) have advanced considerably since the first in vitro fertilization (IVF) birth in 1978. However, despite these advances, pregnancy rates are still relatively low and have not increased significantly in the last decade [5, 6].

Based on the facts that embryo implantation is considered as the last barrier in ART and that inadequate uterine endometrial receptivity is responsible for approximately two-thirds of implantation failures, intensive research work has been performed to understand the physiology, regulation, and the clinical assessments of the uterine receptivity to improve the success rate of In-Vitro-Fertilisation/ Embryo Transfer and the cervix is considered as the 1st indirect site to assess uterine receptivity [7]. Cervicitis is an inflammation of the cervix (the neck and outlet of the uterus). It can be an acute or chronic condition.& if untreated it may lead to endometritis and infertility [8]. Pelvic inflammatory disease (PID) and infections of female reproductive system that is often caused

by bacteria resulting from a common sexually transmitted infection (STI), such as gonorrhea or Chlamydia also may cause infertility [9]. Chronic infections in the cervix can also reduce the amount or quality of cervical mucus, the sticky or slippery substance that collects on the cervix and in the vagina. Reduced amount or quality of cervical mucus can make it difficult for women to get pregnant [10]. The aim of the study is to assess cervical cytomormophological changes in cervical smears taken from fertile and infertile women undergoing IVF/ICSI. And to determine whether a relationship exists between in-vitro fertilization and embryo transfer (IVF-ET) outcome and cervical cytomorph-ological changes.

Methods

The study was prospectively designed. A total 100 samples were included in the study. Case group include: 50 infertile women undergoing IVF/ICSI aged 20-45 year , 35 cases had consulted High Institute of Infertility Diagnosis are and Assisted Reproductive Techniques/Al Nahrain University and 15 cases had consulted Kemal Al-Samerai'i Hospital for Infertility Treatment and IVF in the period from October 2015 to June 2016. Clinical data were collected from case group (women undergoing IVF/ICSI). Included; gravid, parity, history of abortion, history of dysfunctional uterine bleeding, and history of vaginal discharge.
The cause of sub-fertility categorized into 5 classes:

- Poly Cystic Ovaries (PCO)
- Poor Ovarian Reserve (POR)
- Hypogonadotropic Hypogonadism
- Tubal and uterine factor of infertility
-Unexplained infertility

Also we look for the number of ova pickup, number of transferred embryos, and history of coasting during IVF cycle program.

Cervical smears (Pap smear) were taken prior to ova pickup, and stained with conventional papanicolaou stain and screened by a pathologist according to the Bethesda system (TBS) 2001 to classify the epithelial cells abnormalities [83]. Operative ova pick numbers and embryo transfer notes and then pregnancy test result were documented. Control group composed of 50 fertile women (their last baby delivered in not more than 2 year) who consulted department of obstetrics and gynaecology at Al-Imamein Al Kadhimein Medical City in the same period , their age range between 20-41. Clinical data included; gravida, parity, history of abortion, history of dysfunctional uterine bleeding.

History of vaginal discharge
The Pap smear applied in preovulatory day according to transvaginal ultrasound scan, and stained with conventional papanicolaou stain and screened by pathologist according to TBS 2001.

Results

Age range for case group patients included in this study was (20-44 years), their Mean age was 28.9 ±5.6 year; and age range for control group patients was (20-44 yr) the mean age of control group was 29.7 ±5.5 years with no significant difference between them t=1.99, p=0.5, df=98 as shown in Figure 4.

Case group include 22 patients (44%) in age group (20-29) while 21 patients (42%) in (30-39), and 7 patients (14%) in (> or = 40).

Control group include 24 woman (48%) in age group (20-29) and 21 patients (42%) in (30-39) and just 5 woman (10%) in age group equal or more than 40.
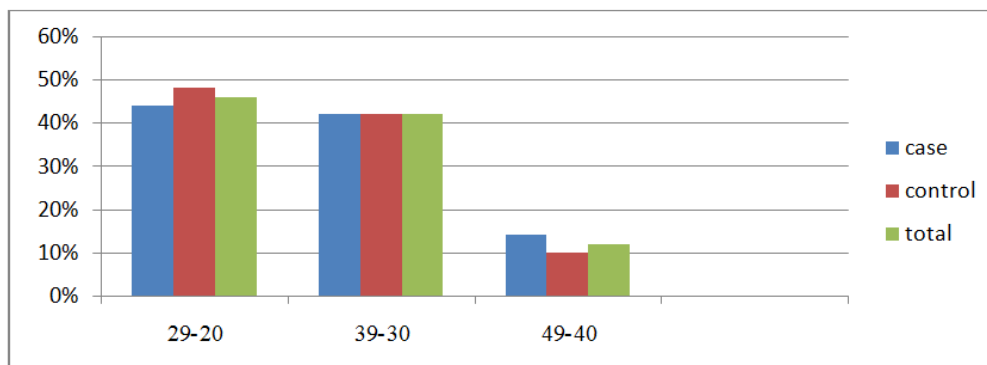

Figure 1.
Distribution of case and control groups according to 10 years age interval

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Cytomorphologic examination
Pap smear

Cytological cervical smear results of (100 cases) were categorized according to the Bethesda System (TBS) 2001: (10). Forty eight cases (96%) out of 50 cases in the case group were satisfactory for evaluation (presence of endocervical/transformation zone components with adequate squamous cellularity), and forty nine (98%) out of 50 cases in the control group were satisfactory for evaluation, as shown in table 1 and in figure 2. Two cases (4%) of case group were unsatisfactory for evaluation (absence of endocervical/transformation zone components, autolysis, obscuring blood, obscuring inflammation and small amount of material) and one case (2%) was unsatisfactory for evaluation in control group. As shown in table 1 and in Figure 2. Eighty four cases (84%) from the total 100 cases had negative cervical smears for intraepithelial lesions or malignancy (NILM). as shown in table 1 and in figure 2. Case group has 37 case (74%) cases negative cervical smear for intraepithelial lesion or malignancy (NILM) while control group has 47 cases (94 %) with (NILM) as shown in table 1and in figure 2. Case group has 11 cases (22%) with atypical squamous cells with undetermined significance (ASC-US) while control group has just 1 case (2%) with ASC-US as shown in table 1 and in figure 2. One case (2%) in the control group was found with low significance squamous intraepithelial lesion (LSIL) in a normal multiparous woman prior to insertion of intrauterine contraceptive device. as shown in table 1and in figure 2. The relation between pap smear in both case and control groups appears be significant difference $\chi2=13.06$, df=3; P=0.005 as shown in table 1.

Table 1.
Pap smear result in case and control groups

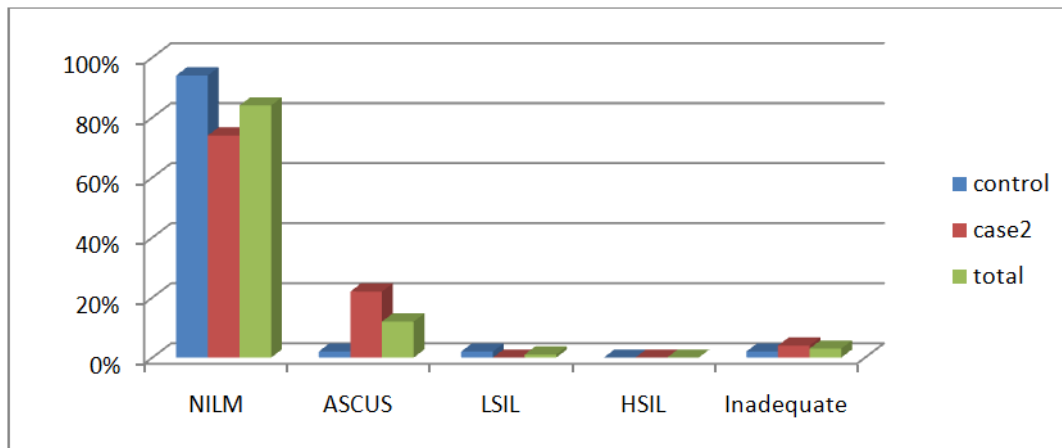| Pap smear analysis								
		NILM	ASC-US	LSIL	HSIL	inadequate	Total	P value
Case	Number	37	11	0	0	2	50	
	percent	74%	22%	0%	0%	4%	100%	
Control	Number	47	1	1	0	1	50	0.005
	percent	94%	2%	2%	0%	2%	100%	
Total	Number	84	12	1	0	3	100	
	percent	84%	12%	1%	0%	3%	100%	

$\chi2=13.06$, df=3; P=0.005

Figure 2.
Distribution of pap smear result in 50 cases and 50 controls.

IVF and embryo transfer outcome in case group

Relationship between Pap smear result and IVF outcome

Statistical analysis shows non-significant relation between pap smear result and negative pregnancy test (P=0.3) because 35 patients (70%) out of 50 patient (100 %) has negative pregnancy 2 patients (4%) of them were inadequate for evaluation test and gives negative pregnancy test and 24 patients (48%) out of 37 cases (74%) with (NILM) has negative pregnancy test and 9 patients (18%) out of 11(22%) cases of (ASC-US) has negative pregnancy test, as shown in table 2. In spite of that statistical analysis a new relation appear between abnormal pap smear and negative pregnancy test where 9 cases (18%) out of 11(22%) of

patient with (ASC-US) reported pap smear has negative pregnancy test and this appear to be significant as shown in table 2 and one of their pap smear shown in Figure 3 which shows Cervical smear from 26 year old infertile women(case group)showing atypical squamous cells with enlarged nucleus more than 3 times without hyperchromasia (ASC-US) and she has 1st IVF trial ends with negative pregnancy test . While Figure 4shows cervical smear taken from 27 year old infertile women (case group) with 5 year duration of unexplained infertility showing normal squamous epithelial cells with absence of atypical changes or inflammation she has positive pregnancy test and successful IVF outcome; single healthy baby delivered by cesarean section.
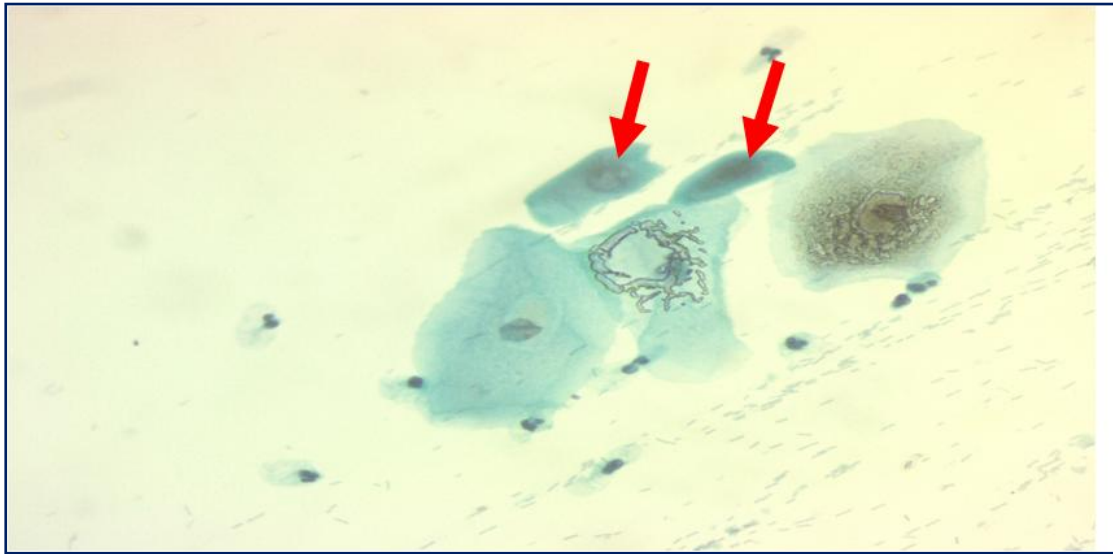
Figure 3.
Cervical smear from 26 year old infertile women(case group)showing atypical squamous cells with enlarged nucleus more than 3 times without hyperchromasia (ASC-US) (arrows) and she has 1st IVF trial ends with negative pregnancy test .Pap stain 40X.
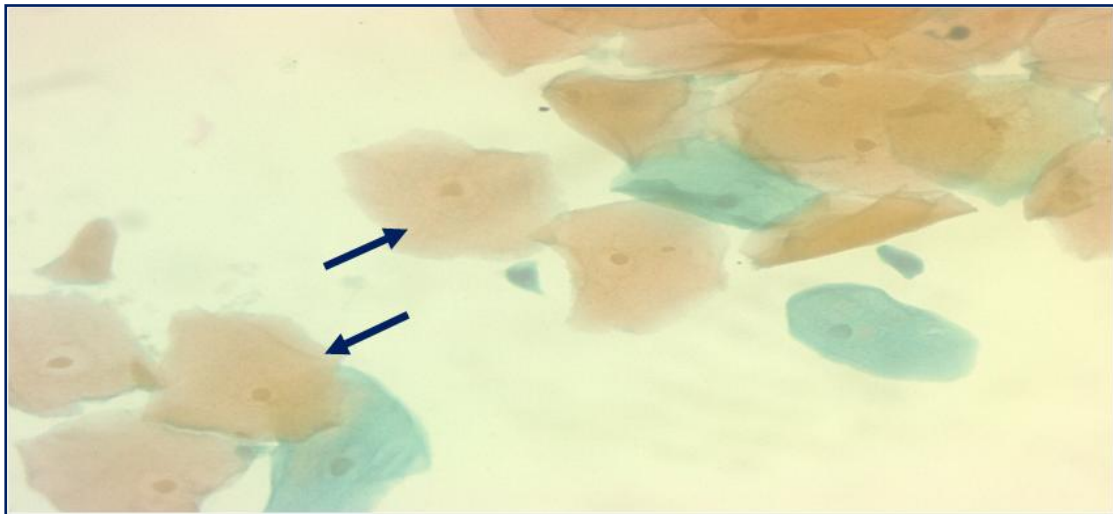

Figure 4.
Cervical smear taken from 27 year old infertile women (case group) with 5 year duration of unexplained infertility showing normal squamous epithelial cells (arrows) with absence of atypical changes or inflammation she has positive pregnancy test and successful IVF outcome; single healthy baby delivered by cesarean section. Pap stain, 40X.

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Discussion

The present study was conducted on 100 women 50 of them considered case group from patients eligible for IVF/ICSI and 50 of them considered control group from normal fertile women. This study is comparable with the literature and other studies done by (Almobarak *et al*., 2013(11); Al-Refaee *et al*., 2012(12); Hamont *et al*., 2006 [13]. Age range of patients included in the study was (20-44 years), the Mean age of the case group was 28.9 ±5.6 year and the mean age of control group was 29.7 ±5.5 years with no significant difference between them. Majority of case group 22 cases (44%) belonged to age group (20-29) and majority of control group taken in the same age group were 24 cases (48%) from (20-29). There are approximately similar to (Almobarak, *et al* 2013) [11] where the majority of cases (41%) belonged to age group (20-29) years but the mean age was 34 years because the age range were (20-60) years. In contrast to the study, Lundqvist *et al*, 2002 [14] did not apply age limits the case group were aged 20-40 years from women eligible for IVF and ICSI, whereas the control group were aged 25–59 years all fertile women including postmenopausal women. So the results could not be compared to our results because statistical analyses are not performed. In this study, epithelial cell abnormalities (ASC-US) were observed significantly more often in women with infertility (22%) as compared with fertile women (2%) These results are consistent with study conducted by other study [11] they observed (15.4%)

of infertile women against (4.2 %) of fertile and by Hamont, *et al* [13]. They observed abnormal pap smears in (6.1%) of cases compared with (3.9%) in the controls. Another study conducted in Saudi Arabia in 2010, reported abnormal cervical cytology in (29.5%) in sub-fertile women [15]; however, the study lacked the control group of fertile women. Other researchers suggested abnormal cervical smear cytology in (2.3%) of case group and (4.1%) of the controls [14].

However their study have some limitations because of the difference in the sample size of the controls in comparison with case and in the criteria which used in the selection of the patients, and the age was not matched well between case and control groups. In this study one smear (2%) reported as LSIL in control group in patient consult gynaecology clinic prior to insertion of IUCD while no case (0%) reported as LSIL in case group this is lower than what was found by al Mobarak, *et al* [11] where the cases and controls showed similar results (46.2%) of infertile women and (45.8%) of control respectively showed the low-grade squamous intraepithelial lesion (LSIL) and this difference may be due to the inclusion of larger age range (20-60 yr).

One Limitation in our study are the difficulty between cytomorphologic exam correlation and patients follow-up, we depend on patient compliance and phone number to call them back, but the address were absent as some of subjects of the study (women) were coming from remote areas.

In conclusions, infertile women eligible for IVF are more probable to have ASC-US when compared with normal fertile women. ASC-US worsen pregnancy test results in women undergoing IVF/ICSI because (18%) out of (22%) of patient with (ASC-US) have negative pregnancy test.

Competing interests

Author declares that they have no competing interests.

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