IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 5 Ver. 9 (May. 2018), PP 80-87
www.iosrjournals.org

Microbial colonization of human follicular fluid and adverse outcome on in vitro fertilization cases in Kamal al-Samarrai's Hospital for fertility and In vitro fertilization treatment in Baghdad, Iraq 2016

*lnas Taha A. Hamad, ** Arkan T. Ahmed, ***Salema Mohamed Sadeq and ****Baydaa Ismaeel.

**Consultant OBG , Elwyia Maternity Teaching Hospital Baghdad ,MBChB. FICOG Baghdad, Iraq.*
***Consultant anesthesiologist, Rusafa Directorate Baghdad, FICMS anesthesia. Baghdad, Iraq.*
****Specialist OBG . Elwyia Maternity Teaching Hospital Baghdad, MBChB . DGO, Baghdad, Iraq.*
*****Specialist OBG. Kamal Alsamarai Hospital Baghdad , MBChB. CABOG, Baghdad, Iraq.*
*Corresponding author: * lnas Taha A. Hamad*

Abstract

Background: *There are various assisted reproductive technologies such as in vitro fertilization for treatment of infertility, So IVF culture system is not a sterile system. Not only is the environment we work in full of bacteria, fungi and viruses, but our patient's bodies (follicular aspirates, semen and the vaginal and cervical regions for egg retrieval and embryo transfer) also contain microbes, this can lead to adverse pregnancy outcome after IVF.*

Objectives: *To demonstrated differences in women with colonized and contaminated follicular fluid and estimate the prevalence of the possible risk factors for developing microbial colonization of the follicular fluid.*

Methodology: *A cross sectional study was conducted during the period from 1st of June 2015 to 30th of March 2016 in Kamal al-Samarrai's Hospital for fertility and IVF treatment in the Baghdad city. A purposive sampling technique was used to collect data, there were 106 women underwent in vitro fertilization that were consented and interviewed directly, using a questionnaire form prepared for the study as well as reviewing their medical records. From each woman who underwent in vitro fertilization and had a pregnancy test positive, two samples (High vaginal swab and Follicular fluid sample)were collected at the time of oocyte retrieval and sent to the hospital laboratory for culture, then the patients had been followed by phone or when they come to outpatient clinic at the end of their first trimester, for 1st trimester outcome to identify if the female is still pregnant or had abortion. A descriptive statistics (frequency and percentages tables, pie and bar charts) and inferential statistics (A chi square or Fisher Exact test and T test) were used for data analysis.*

Results: *A total of 106 studied pregnant women underwent in vitro fertilization, 57(54%) women got abortion at their first trimester; women who had colonized follicular fluid 95.2% got abortion at their 1st trimester. There was a significant association between follicular fluid colonization and residence, secondary infertility. First trimester abortion, tend to occur more in women who live in rural areas, who had history of secondary infertility, women who underwent ovulation induction. The most common cause of infertility was endometriosis 34.9%followed by PCOS 27.3%, genital tract infection 25.4%, male factor 7.5%, idiopathic 4.7%. Culture analyses revealed that cultivable bacterial species were present in 21(45.7%)of follicular fluids tested, Lactobacillus and staphylococcus comprised 8(38.1%) respectively; other bacteria comprised 44.4%, 4.8% which include streptococcus, Actinomyces respectively. No single species tend to be associated more with first trimester abortion.*

Conclusions : *The women who had colonized follicular fluid tend to get abortion at their first trimester more than women whose follicular fluid categorized as contaminated. No single species of bacteria tend to associated more with first trimester abortion. Women may benefit from microbial screening of vaginal swabs prior to IVF cycle, to detect microorganisms or by culturing the follicular fluid collected at the time of trans-vaginal oocyte retrieval for the presence of microorganisms. Treatment with antimicrobials may increase IVF treatment success.*

Keys words*: Fertility, Follicular Fluid, In Vitro Fertilization, Microbial Colonization, Outcome*

Date of Submission: 07-05-2018 Date of acceptance: 22-05-2018

I. Introduction

Infertility is a complex disorder with significant medical, psychosocial, and economic problems [1]. Infertility is not always a woman's problem. Both women and men can have problems that cause infertility. About one-third of infertility cases are caused by women's problems. Another one third of fertility problems are due to the man. The other cases are caused by a mixture of male and female problems or by unknown problems [2]. Of infertility cases, one-third remains unexplained, while the others are attributed to infection or to anatomical and physiological problems. Some studies investigating unexplained infertility have shown the presence of lactobacilli in human follicular fluid. These bacteria, known for their beneficial effects on health and their usage in food, have been recently associated with embryo maturation and transfer. This fascinating study also reported a correlation between cultivable microbes and the cause of infertility and in vitro fertilization (IVF) outcomes [3,4]. STDs are a leading cause of infertility. They are often asymptomatic but may display few symptoms, with the risk of failing to seek proper treatment in time to prevent decreased fertility. Some of the identified STDs (such as syphilis, trichomoniasis, chancroid, Chlamydia, gonorrhea, herpes simplex virus, human papilloma virus, HIV, lympho granuloma venerum) are treatable while many are not, with HIV virus being the most serious sexually transmitted infection as it eventually leads to death. STDs can also be transmitted vertically from mothers to children during pregnancy and childbirth [5].

For treatment of infertility there are exist various assisted reproductive technologies such as in vitro fertilization, which is become a common and increasingly used treatment [6, 7]. In vitro fertilization treatment (IVF) involves the placement of embryos into the uterine cavity using a catheter that passes through the cervix; the possibility of bacterial contamination during the embryo transfer procedure clearly exists, but has been poorly explored. In fact, there is growing evidence that bacterial contamination of the uterine cavity following trans cervical embryo transfer can negatively affect the implantation rates and the pregnancy outcome. Such contamination can occur during embryo replacement through the tip of the embryo transfer catheter from various vaginal–cervical microorganisms[8]. Besides the well-established prognostic factors for success, such as the age of the women, the hyper stimulation protocol used, the number and the quality of transferred embryos other factors such as contamination and transmission of infection can also impinge on the success rates of an IVF programme[9]. In spite of advances in the field of assisted reproductive techniques including in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI), the pregnancy rate remained low. One of the major events in fertility is implantation process. Researchers believe that introduction of cervical bacteria into uterus during embryo transfer might have an inhibitory effect on implantation and thereby pregnancy rate [10]. More recent studies had suggested that IVF pregnancies may be at risk for preterm birth, low birth weight, congenital anomalies, perinatal mortality and several other pregnancy-related complications compared with unassisted pregnancies [11].

The biochemical characteristics of the Follicular fluid (FF) surrounding the oocyte may play a critical role in determining oocyte quality and the subsequent potential to achieve fertilization and embryo development [12-13]. The IVF culture system is not a sterile system. Not only is the environment we work in full of bacteria, fungi and viruses, but our patient's bodies (follicular aspirates, semen and the vaginal and cervical regions for egg retrieval and embryo transfer) also contain microbes[14,15]. The presence of opportunistic pathogens in the lower female reproductive tract has been associated with adverse pregnancy outcomes after both natural and IVF conceptions [3, 16]. Bacterial vaginosis has been associated with PID and, in ART, may decrease implantation rates, increase early miscarriages, and may also increase the risk of preterm labor[17]. Microbial contamination of long-term cell culture is a current concern for assisted reproductive techniques (ARTs) laboratories. Although trans vaginal oocyte retrieval for IVF is considered to be relatively safe, occasionally microorganisms colonize culture dishes of oocyte and embryos. These infections are mainly caused by bacterial strains insensitive to the antibiotics used or due to yeast colonization by *Candida* species, which frequently reside in the vagina or even by other normal vaginal flora, like lactobacillus as bacterial endotoxins dramatically disrupt granulosa cells growth and function *in vitro* in different species[18]. Infections in IVF culture dishes are mainly caused by bacterial strains insensitive to the antibiotics used or due to yeast colonization by Candida species which frequently reside in the vagina [19].

Rationale for implement this study: The studies about microbial colonization of the follicular fluid and its effects on in vitro fertilization were negligible. Nowadays with excess proportion of infertility, IVF have been used very commonly for conception. This study will provide clues about follicular fluid colonization effect on the fate of IVF.

So the ***objectives were included:*** To demonstrated differences in women with colonized and contaminated follicular fluid and estimate the prevalence of the possible risk factors for developing microbial colonization of the follicular fluid.

II. Methodology

A cross sectional study was conducted during the period from 1st of June 2015 to 30th of March 2016 in Kamal al-Samarrai's Hospital for fertility and IVF treatment in the Baghdad city. A purposive sampling technique was used to collect data, there were 106 women underwent in vitro fertilization that were consented and interviewed directly, using a questionnaire form prepared for the study as well as reviewing their medical records. From each woman who underwent in vitro fertilization and had a pregnancy test positive, two samples (High vaginal swab and Follicular fluid sample)were collected at the time of oocyte retrieval and sent to the hospital laboratory for culture, then the patients had been followed by phone or when they come to outpatient clinic at the end of their first trimester, for 1st trimester outcome to identify if the female is still pregnant or had abortion. A descriptive statistics (frequency and percentages tables, pie and bar charts) and inferential statistics (A chi square or Fisher Exact test and T test) were used for data analysis.

Ethical considerations

- A written informed consent from each participant was obtained after explaining the study objectives and prior to the start of data collection.
- Minimal risks and more beneficence for the participants must be considered.
- Respecting for human dignity.
- Data were collected anonymously and confidentiality of data throughout the study was assured.
- All collected data were used for research purpose and held in a password-protected computer. Access to data was limited to research team members only.

III. Result

Demographic features: A total of 106 women underwent In vitro fertilization, the mean women age was 31.32± 3.53years ranged from 25-37 years. There was 80(75.5%) women resident in urban areas while 18(17%) women resident in rural areas, 26(24.5%)women. 28(26.4%) women were governmental employee; free worker constituted 12(11.3) women and 66(62.3%) women were house keeper table (1).

Table 1. Frequency distribution of demographic features (n 106).

Variable		Frequency	Percent (%)
Residence	Urban	88	83
	Rural	18	17
Occupation	Governmental	28	26.4
	Free work	12	11.3
	House keeper	66	62.3

Clinical features of the women: Of the total sample 72(67.9%) women had a history of primary infertility and 34(32.1%) women had a history of secondary infertility. 89 (84%) women participated in previous IVF procedure. There was 89(84%) women had a history of contraception, ovulation had been induced in 40(37.7%) women. 35(33%) women had a history of HSG, table (2).

Table 2. Distribution of the sample according to the clinical features (n 106).

Variable		Frequency	Percent (%)
Infertility type	Primary	72	67.9
	Secondary	34	32.1
Participation in previous IVF procedure	Yes	89	84
	No	17	16
Ovulation	Spontaneous	66	62.3
	Induction	40	37.7
History of HSG	Yes	35	33
	No	71	67

The mean infertility duration was 4.7± 1.3 years.

The women was followed to the end of first trimester, some of them get abortion before completing the first trimester, mean gestational age the women reached was 8.1± 1.9 weeks. Serum HCG level was 282±300 m IU/ ml table (3).

Table 3. Distribution of the of the sample according to clinical features (continuous variables).

Variable	Mean	±SD	Minimum	Maximum
Gestational age at abortion (Weeks)	8.1	1.9	5	12
Infertility duration (Years)	4.7	1.3	2	8
S. HCG level (mlU/ml)	282	300	46	1100

Causes of infertility: The infertility causes percentages of the sample were as following, fig 1: Endometriosis 34.9%, PCOS 27.3%, Genital tract infection 25.4%, Male factor 7.5% and Idiopathic 4.7%

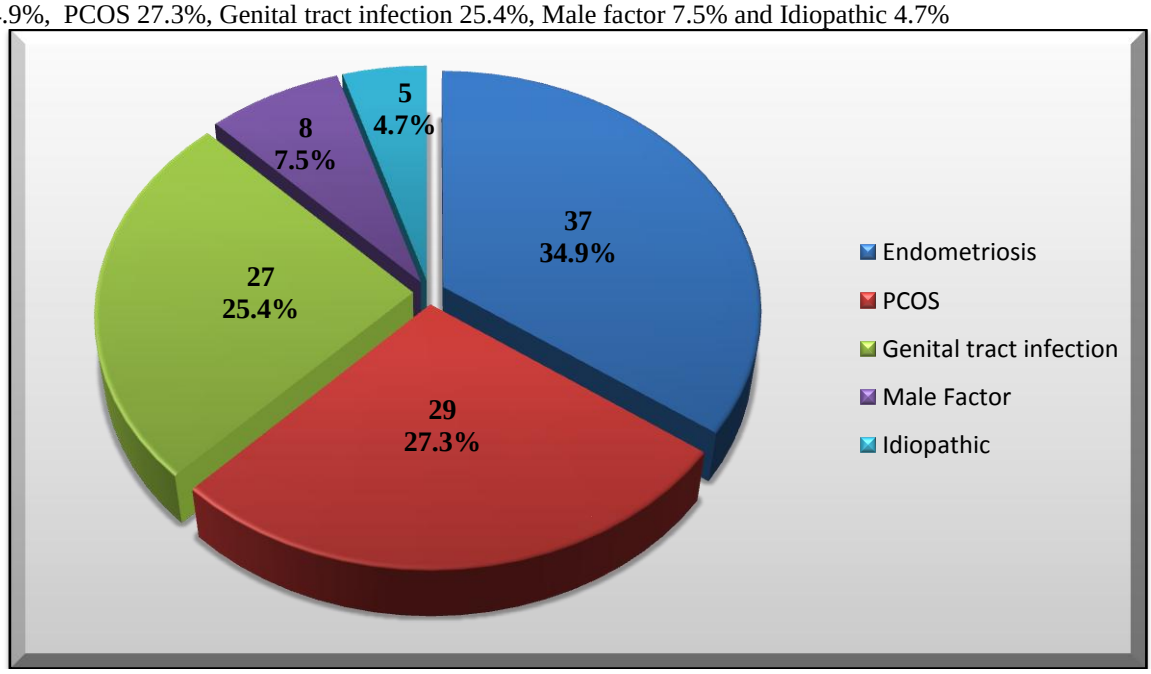

Fig.1: distribution of the sample according to the Causes of infertility.

Abortion in the first trimester:
Of the total sample 57(54%) women get abortion through the first trimester of pregnancy fig 2.

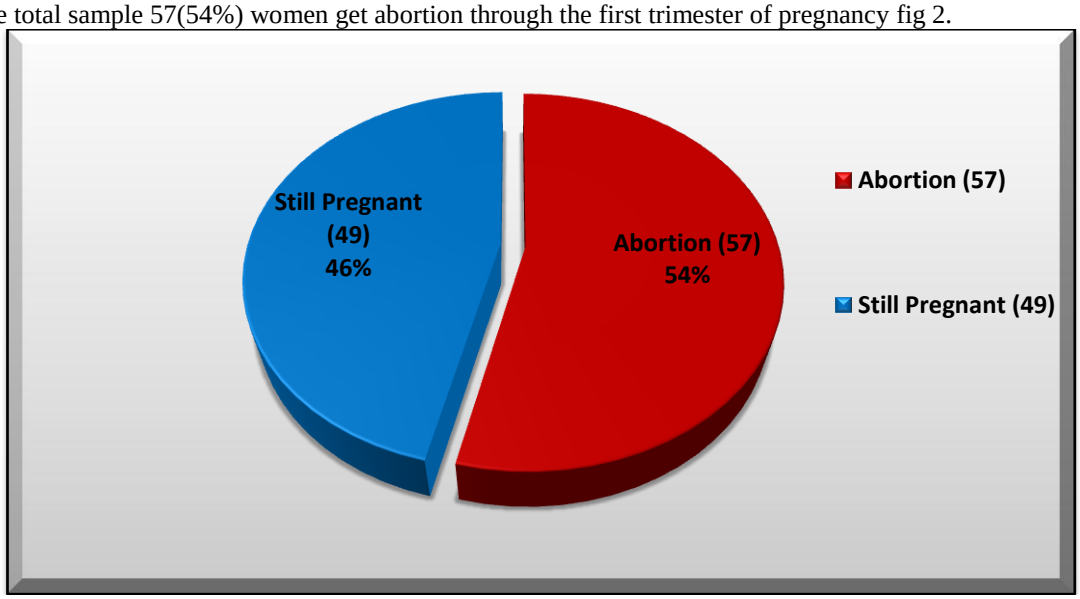

Figure 2: Frequency distribution of the sample according to the first trimester outcome.

Follicular fluid colonization: The result of vaginal swab culture was positive in 45(42.5%) women and negative in 61(57.5%) women. The result of FF. culture was positive in 46(43.4%) and negative in 60(56.6%) women. It was considered that if microorganisms present in the follicular fluid culture and vaginal swab culture is classified as contaminated FF, while if they present in FF culture without being in vaginal swab culture is classified as colonized FF. there was 25(55.6%) women had contaminated FF. and 21(34.4%) women had colonized FF. fig.3

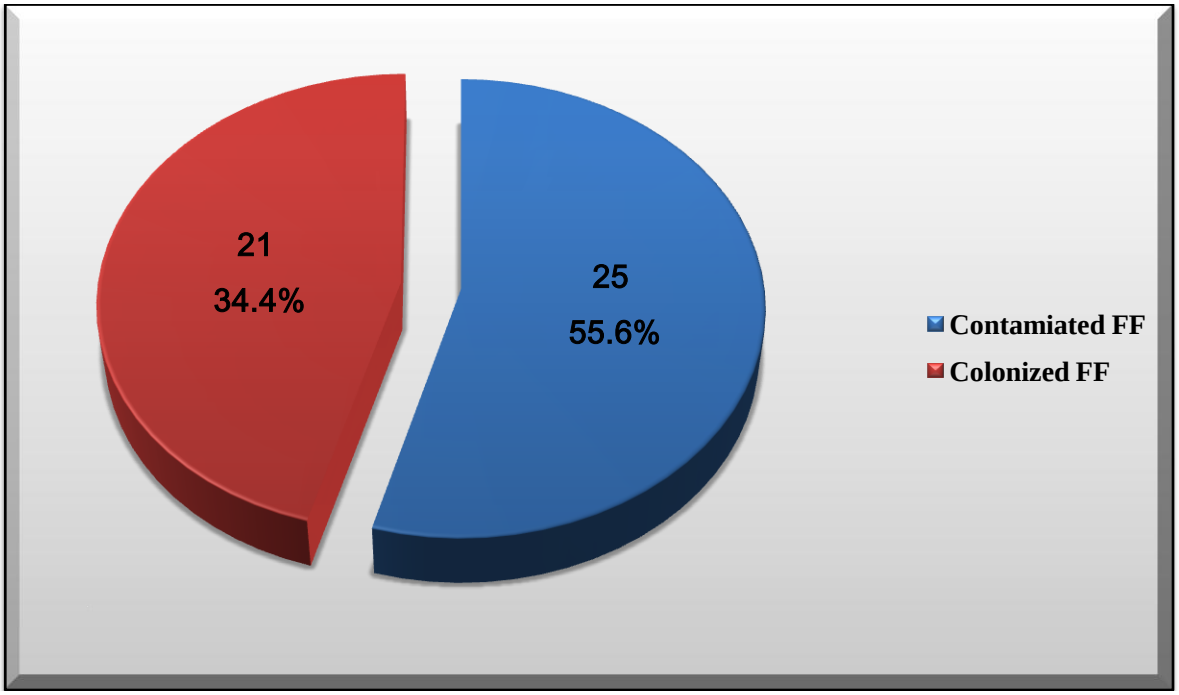

Fig.3: Frequency distribution of the contaminated VS colonized FF (n=46).

The culture analyses revealed that cultivable bacterial species were present in 21(45.7%)of follicular fluids tested(colonized). The most frequent bacterial species was *Lactobacillus* and *staphylococcus* comprised 8(38.1%) and 8(38.1%) respectively; other bacteria which include *Streptococcus*, *Actinomyces* fig.4.

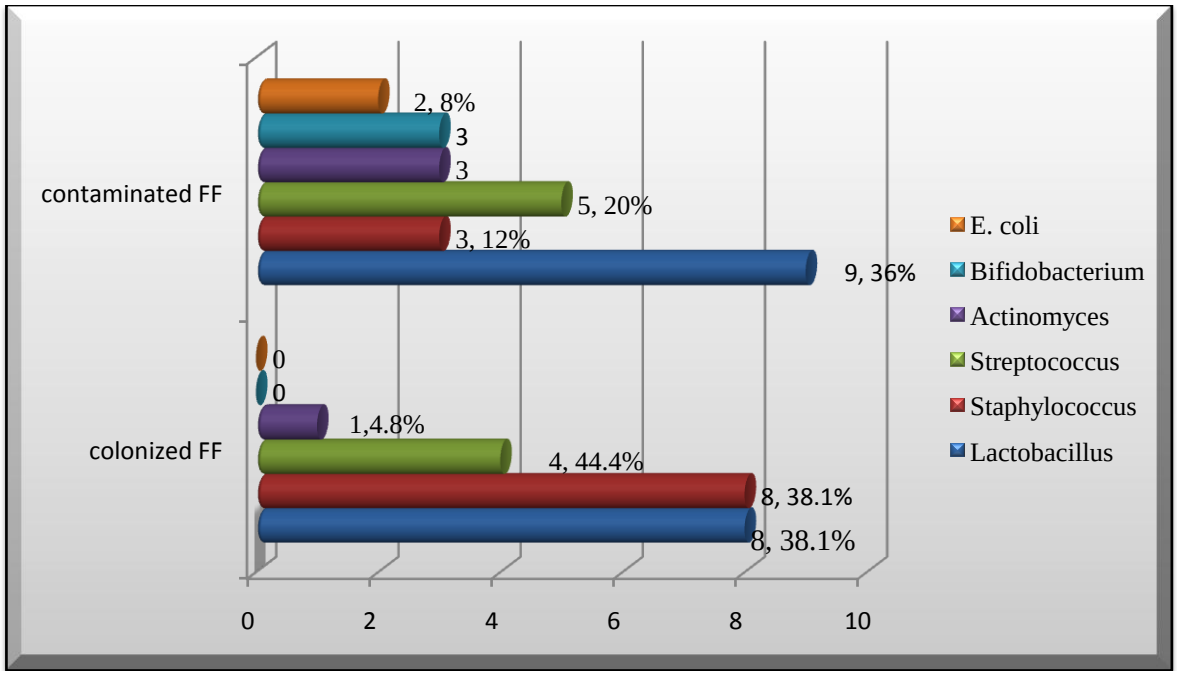

Fig.4: Distribution of the sample according to bacterial species isolated from FF culture.

Relationship between FF. culture and first trimester outcome: Table 4 represents the relationship between FF colonization and pregnancy outcome during the 1st trimester and it shows that the majority (95.2%) of the women whom follicular fluid revealed colonization got abortion within first trimester of their pregnancy and this relation was statistically significant.

Table 4. Relationship between the FF colonization and pregnancy outcome (1st trimester). (n46).					
	1st trimester outcome				P value
	Still pregnant		Abortion		
Follicular Fluid (**FF**)	**Freq.**	**%**	**Freq.**	**%**	
Colonized	1	4.8	20	95.2	*0.003*
Contaminated	11	44	14	56.0	
(***P*** statistically significant when alpha level less than 0.05)					

Relationship between FF culture and women features: The table 5 shows Pearson Chi square distribution to measure the association and there were significant associations of the follicular fluid colonization with residence (highest occurrence in rural, 66.7%), whereas there were no significant associations with occupation.

IV. Discussion

First trimester outcome following IVF: Of the women who had colonized follicular fluid 95.2% got abortion at their 1st trimester, While 56 % of women with contaminated follicular fluid got abortion at their 1st trimester. Significant association was found between colonization of follicular fluid and first trimester abortion. Women who had colonized follicular fluid tend to get abortion more than those had contaminated follicular fluid.

The high statistically significant association (less than P 0.001) [20] between follicular fluid culture and pregnancy outcome during the first trimester , such an association revealed that most cases (73.9%) with colonized FF had abortion during the first trimester, whereas just 38.3 % of the cases with sterile FF had abortion within the same time, this study agree with current study. Decreased pregnancy rates were also observed for women with microbial colonization of follicular fluid, p =0.008 compared to women with contaminated follicular fluid. This study found that human follicular fluid contains microorganisms. The presence of some microorganisms (Propionibacterium spp., Streptococcus spp. Actinomyces spp., Staphylococcus spp. and Bifidobacterium spp.) correlated with adverse IVF outcomes[21] and this resemble current study which reveal Lactobacillus and staphylococcus (comprised 16% and 10.3% respectively) and other bacteria comprised 17% which include *streptococcus*, *Actinomyces*, *Bifidobacterium* and E coli. However, the presence of bacteria other than *Lactobacillus* spp. (including Escherichia coli, Streptococcus spp., other *Enterobacteriaceae*, *Staphylococcus* spp., *Haemophilus* spp. and mixed cultures), reported as cervico vaginal flora, were associated with a reduced number of successful IVF pregnancies and an increase in early miscarriage rates.

causes of infertility: The current study show that Endometriosis consist most of infertility causes 34.9% followed by PCOS 27.3% and Genital tract infection 25.4%, Male factor and idiopathic consist 7.5% and 4.7% respectively. First trimester abortion and follicular fluid colonization were more in women with endometriosis and PCOS. A study done at 2013 found that poor IVF outcomes affecting embryo quality, pregnancy rates and ultimately the live birth rate for women with endometriosis with colonized follicular fluid (63%, p,0.0001) but not for women with contaminated follicular fluid (34%) and colonization of follicular fluid and endometriosis were found to be associated (p,0.05). There were no other statistically significant results (polycystic ovary syndrome, genital tract infection, male factor and idiopathic etiologies) [2]. This related to the trend towards increased numbers of follicular fluid colonizers in women with endometriosis may be due to the underlying disease pathology, which requires the development of an extensive vasculature to support explanted endometrial tissue [22]. Abortion during the 1st trimester found to be more associated with female sex.

This result is inconsistent with studies conducted in 2005 and 2002[22, 23] which concluded that the main infertility diagnosis had no effect on the early abortion. Colonizing microorganisms in follicular fluid were associated with: decreased fertilization rates for fertile women (P ¼ 0.005), women with endometriosis (P ¼ 0.0002) or PCOS (P ¼ 0.002) compared with women whose follicular fluid was contaminated at the time of oocyte retrieval and with decreased pregnancy rates for couples with idiopathic infertility [3].

Factors affecting follicular fluid colonization: There were factors that associated with and may had effect on follicular fluid colonization include: Age , There were highest occurrence of the follicular fluid colonization in women who resident in rural areas, Follicular fluid colonization found more in women with secondary infertility, Women who underwent ovulation induction had follicular fluid colonization, Follicular fluid colonization found more in women underwent HSG.

There were factors that not associated with and may had no effect on follicular fluid colonization include : Participation in previous IVF procedure, occupation, there were no differences with Gestational age at abortion by weeks, infertility cause(male or female cause), years of infertility duration, HCG level (mlU/ml)

Factors affecting first trimester outcome after IVF: There were factors that associated with and may had effect on first trimester outcome after IVF

- Maternal age: There was a significant higher mean age within the abortion; increasing maternal age is a risk factor for almost all pregnancy and perinatal complications. The average age at which women attempt to conceive continues to rise and consequently IVF is increasingly used by older women who are already predisposed to pregnancy complications [24,25].

- higher mean of infertility duration found in abortion,
- Highest prevalence of abortion during the 1st trimester with residence in rural areas.
- abortion during the 1st trimester more in secondary infertility
- Ovulation

There were factors that not associated with and may had no effect on first trimester outcome after IVF

- Occupation.
- Participation in previous IVF procedure.
- History of HSG.
- HCG level (mlU/ml), it was concluded that a discriminatory HCG value on day 12 after IVF and embryo transfer cycles may be useful in predicting pregnancy outcome and may guide clinicians in identifying those pregnancies at risk for adverse outcomes and instituting more intensive surveillance in this population [26].

Carmona et al. (2003) concluded that A single, early (days 12–13 after embryo transfer) HCG quantitative serum measurement in IVF cycles not only is diagnostic but also has good predictive value for pregnancy outcome [27].

V. Conclusions

A total of 106 studied pregnant women underwent in vitro fertilization, 57(54%) got abortion at their first trimester, The women who had colonized follicular fluid tend to get abortion at their first trimester more than women whose follicular fluid categorized as contaminated. Follicular fluid colonization tend to occur more frequently in women who resident in rural areas, in women who had secondary infertility and in women who had history of HSG. First trimester abortion tend to occur more in women who live in rural areas, women who had history of secondary infertility, female cause infertility and in women who underwent ovulation induction. Colonized FF. revealed more in women with endometriosis and PCOS. First trimester abortion that tend to be occur more in women with endometriosis and PCOS. The culture analyses revealed that cultivable bacterial species were present in 21(45.7%) of follicular fluids tested (colonized). Women who got pregnant with IVF procedure, ninety percent ended with abortion at their first trimester. The women who had colonized follicular fluid tend to get abortion at their first trimester more than women whose follicular fluid categorized as contaminated. No single species of bacteria tend to associated more with first trimester abortion.

VI. Recommendations

- Women with failed IVF cycles may benefit from microbial screening of vaginal swabs prior to entry into an IVF cycle to detect microorganisms, or by culturing the follicular fluid collected at the time of trans-vaginal oocyte retrieval for the presence of microorganisms. Treatment with antimicrobials may increase IVF treatment success.
- Prognostic features to identify high risk patients needing to receive more laboratory investigations and closer monitoring of the patients.
- ➥ Identification of bacteria colonizing the follicular fluid in couples experiencing a prolonged failure to conceive may present the clinician with an opportunity to initiate antimicrobial treatment prior to the next attempt at conception.
- ➥ For further research, large population based studies with analytic design are recommended in order to determine the scope of this problem nationwide and for implementation of public health program.

References

[1]. Kuohung W., Hornstein M.D., Barbieri R.L., Barss V.A., **Evaluation of female Infertility**, version 17.3. **2009**.
[2]. Eisenberg E , Shriver E.K, Brumbaugh K, Brown-Bryant R, Warner L, **Infertility**, 2009, U.S. Department of Health and Human Services, Office on Women's Health, 1-800-994-9662.
[3]. Pelzer E S., Allan J A,Waterhouse M. A, Ross T, Beagley K.W, Knox Ch.L, **Microorganisms within Human Follicular Fluid: Effects on IVF**, 2013 , 8 (Issue 3), e59062.
[4]. Reid G, Brigidi P., Burton J.P., Contractor N., Duncan S., Fargier E., et al, **Microbes Central to Human Reproduction**, 2015, doi:10.1111/aji.12319, 73: 1–11.
[5]. Eniola O.W, Adetola A.A, Abayomi B.T, A review of Female Infertility; important etiological factors and management, 2012, **J. Microbiol. Biotech. Res**., 2 (3):379-385.
[6]. Hjelmstedt A., in vitro fertilization emotional reactions to treatment, pregnancy and parenthood, 2003, **ISBN** 91-7349-715-0.
[7]. Scott M. Nelson, Debbie A. Lawlor, In vitro fertilisation (IVF) is now widely used for the treatment of infertility. Predicting Live Birth, Preterm Delivery, and Low Birth Weight in Infants Born from In Vitro Fertilisation: **A Prospective Study of 144,018 Treatment Cycles**, 2011 , 8, (Issue 1), e1000386, PLoS Medicine.
[8]. Moore DE, Soules MR, Klein NA, Fujimoto VY, Agnew KJ, Eschenobach DA. Bacteria in the transfer catheter tip influence the live-birth rate after in vitro fertilization. **FertilSteril** 2000;74:1118–24.

[9]. S.R.Steyaert, G.G. Leroux-Roels, M.Dhont, Infection in IVF: **review and guidelines**, 2000, Human Reproductive Update,6,5: 432-441.

[10]. Nasr-Esfahani M.H., Fazeli S. A., Kianpoor F., Tabibian S. A., Ahmadi S. M., Kalantari S. A., **The effect of bacterial flora of cervical canal on fertility outcome in patients referring to Isfahan fertility and infertility center**,2003, 4 (16,Issue 4) / October-December: 280-7.

[11]. Kalra S.K., MolinaroTh.A, the association of in vitro fertilization and perinatal morbidity, 2008, **seminReprod Med**, 26(5):423-435.

[12]. Basuino L, Silveira CF Jr, Human follicular fluid and effects on reproduction, 2016, **J Bras Reprod Assist**., 1;20(1):38-40. doi: 10.5935/1518-0557.20160009.

[13]. Elder K., Dale B**., In vitro fertilization**, 2000, 2nd edition, Cambridge university press.

[14]. Pomeroy K. O., Contamination of Human IVF Cultures by Microorganisms: A Review, **The Journal of Clinical Embryology**, 13(Issue 4), 2011

[15]. SowerbyE., Parsons J., Review IVF: how can we reduce the risks of infection, 2006, **The Obstetrician &Gynecologist**, 8:159–163.

[16]. Gautam A., Complications of IVF, 2010, **J ObstetGynaecol India**, 60(4): 297-8.

[17]. Eckert LO, Moore DE, Patton DL, Agnew KJ, Eschenbach DA. Relationship of vaginal bacteria and inflammation with conception and early pregnancy loss following in-vitro fertilization. **Infect Dis Obstet.Gynaecol** , 2003;11(1):11–17.

[18]. C. O. Campos, M. P. Bernuci, A. A. Vireque, J. R. Campos, M. F. Silva-de-S´ a, M. C. Jamur et al. ,Preventing Microbial Contamination during Long-Term In Vitro Culture of Human Granulosa-Lutein Cells: An Ultra structural Analysis, International Scholarly Research Network, ISRN **Obstetrics and Gynecology**, 2012, Article ID 152781, 6 pages, doi:10.5402/2012/152781.

[19]. Kastrop P.M., de Graaf-Miltenburg L.A., Gutknecht D. R., Weima S.M., Microbial contamination of embryo cultures in an ART laboratory: sources and management, 2007, **Human Reproduction**: 2243–8, doi:10.1093/humrep/dem16.

[20]. Eckert L.O., Moore D.E., Patton D.L., Agnew K.J. and Eschenbach D.A., Relationship of vaginal bacteria and inflammation with conception and early pregnancy loss following in-vitrofertilization, **Infect Dis Obstet Gynecol** 2003;11:11–17

[21]. Vignali B.M., Endometrioma excision and ovarian reserve: a dangerous relation, **J Minim Invasive Gyneco**l, 2009, 16: 142–8.

[22]. Lintsen AM, Pasker-de Jong PC, de Boer EJ, Burger CW, Jansen CA, Braat DD, Effects of subfertility cause, smoking and body weight on the success rate of IVF, **Hum Reprod,** 2005, 20(7): 1867–1875.

[23]. Wang JX, Davies MJ & Norman RJ, Obesity increases the risk of spontaneous abortion during infertility treatment, **Obes Res.** 2002, 10(6): 551–554.

[24]. Reddy UM, Wapner RJ, Rebar RW, Tasca RJ. Infertility, assisted reproductive technology, and adverse pregnancy outcomes: executive summary of a National Institute of Child Health and Human Development workshop **Obstet Gynecol**, 2007,109:967–77.

[25]. Schieve LA, Tatham L, Peterson HB, Toner J & Jeng G, Spontaneous abortion among pregnancies conceived using assisted reproductive technology in the United States, **Obstet Gynecol** ,2003, 101(5 Pt 1): 959–967.

[26]. Bjercke, Tanbo T., Olav Dale P., MørkridL.andÅbyholm T., Human chorionic gonadotrophin concentrations in early pregnancy after in-vitro fertilization Sverre, **Human Reproduction, 1999,** 14 (6): 1642–6.

[27]. Carmona F., Balasch J., Creus M., F´abregues F., Casamitjana R., C´ıvico S., Early Hormonal Markers of Pregnancy Outcome After In Vitro Fertilization and Embryo Transfer, **Journal of Assisted Reproduction and Genetics**, 2003, 20(12).

* lnas Taha A. Hamad "Microbial colonization of human follicular fluid and adverse outcome on in vitro fertilization cases in Kamal al-Samarrai's Hospital for fertility and In vitro fertilization treatment in Baghdad, Iraq 2016. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 5, 2018, pp 80-87.