

# Microbiology of Follicular Fluid Aspirated in Women with Polycystic Ovarian Syndrome (PCOS) Attending Fertility Clinic on Southern Part of Nigeria

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Received: 30 Aug 2023; Accepted: 28 Sep 2023; Published: 04 Oct 2023

**Citation:** Ibadin Ngozi Ogbenyalu, Okojie Rachael O, Ibadin Kennedy Osegua. Microbiology of Follicular Fluid Aspirated in Women with Polycystic Ovarian Syndrome (PCOS) Attending Fertility Clinic on Southern Part of Nigeria. *Gynecol Reprod Health*. 2023; 7(5): 1-6.

## ABSTRACT

Polycystic ovarian syndrome (PCOS) is a heterogenous endocrine disorder frequently diagnosed in women attending fertility clinics in Nigeria. It is characterized by anovulation, hyper androgenism and polycystic ovary, several factors have been associated with the pathophysiology of this disorder including microorganisms. Microorganisms frequently and transiently colonize the female genital tract.

A prospective cross-sectional study was conducted in four selected fertility clinics in south-south and south south-east part of Nigeria. The purposive sampling technique was used to recruit participants. A total of 206 women were recruited in this study, 101 (49.1%) women fulfilled the Rotterdam criteria for the diagnosis of PCOS, while 105 women (50.9%) without PCOS were included as control. Paired follicular fluid samples and high vaginal swabs were collected from each woman at the time of oocyte retrieval and sent to the laboratory for microbiological analysis.

Microbial biochemical identification was done using the analytical profile index (API). Statistical analysis was performed using SPSS version 20.0.  $p$ -value of  $<0.05$  was considered significant.

A total of 206 F.F samples were cultured, colonizing bacteria were isolated in 109 (52.9%) samples while 97 (47.1%) samples had isolates graded as contaminants. *Lactobacillus* spp. was the most frequent isolate found in colonized Follicular fluid with 67 (62.5%) On the other hand, *Escherichia coli* was the least common isolate in colonized Follicular Fluid (F.F) 4 (3.7%), there was no statistical difference between bacterial colonization in women with PCOS and the control group ( $p>0.05$ ). The present study shows that the follicular fluid is not sterile, oocytes are exposed to colonizing microbes even before fertilization and subsequent embryo culture. The main aim of the study or research was to investigate the microbiological characteristic of the follicular fluid in women with PCOS.

## Keywords

Microbiology, Infertility, PCOS.

## Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder prevalent in women attending fertility clinics around the world, this disorder was first described in 1935 [1]. PCOS is often diagnosed clinically by symptoms mainly seen as menstrual

disturbance including, oligomenorrhea, menorrhagia, anovulation, all these can results in infertility [2].

The reported prevalence of PCOS ranges from 2.2% to 26% in various countries, depending on the recruitment process of the study population, the criteria used for its definition, and the method used to define each criterion [3]. A systematic review reveals that globally, the prevalence of PCOS using the Rotterdam applicably

doubles that under the NIH 1990; however, the prevalence of PCOS using the AES 2006 appears to lay in between [4]. A recent study in Benin City, Nigeria has estimated the local sub Saharan African prevalence of PCOS using the Rotterdam, NIH and AES criteria as 27.6%, 16.9 and 20.7% respectively [5]. Common signs and symptoms of PCOS include oligomenorrhea, amenorrhea, hypergonadism, polycystic ovaries, bursitis, infertility, etc.

Concerning the pathophysiology of PCOS, this syndrome has been found to be heterogeneous, characterized mainly by menstrual dysfunction, polycystic ovarian morphology and hyperandrogenism.

The microbiological invasion and colonization of the follicular fluid and its possible effect in the development of PCOS or infertility can not be over emphasized it may be possible that follicular fluid and other genital secretions such as semen and cervical secretions inherently contain microbes, which can be transferable to IVF medium contain the retrieved oocyte or embryos and these can result in adverse effects on the developing embryos [6]. Previous studies have shown that opportunistic microorganisms present in the female lower genital tract can result in poor pregnancy outcomes such as repeated miscarriages, eclampsia, placenta previa and birth deformities both in natural and medically assisted conception. Pelvic inflammatory disease and bacterial vaginosis have been previously linked to low fertilization rate, reduced pregnancy rate, increased first trimester miscarriages and increased premature birth rates in women that received IVF treatment [7]. Microbial colonization of the follicular fluid may be a thing of concern in fertility laboratories were these colonizing microorganisms from both the follicular fluid or lower genital tract can contaminate cell culture media containing oocytes or embryos. The colonizing bacteria may be resistant to common antimicrobial agents used in the IVF laboratories, for instance *Candida* species can contaminate IVF media from oocystic retrieval process through the vaginal wall. Bacteria such as *Lactobacillus spp*, which is a normal vaginal flora can also affect the development of granulosa cell growth this has been establish *in vitro* in different species. Bacterial endotoxins dramatically disrupt granulosa cells growth and function *in vitro* in different species [8]. Contamination of IVF cell culture media is caused mainly by bacterial strains, which have become resistant to the common antibiotics used in such culture media or by *Candida* species colonizing the vaginal environment [9]. In recent years the use of assisted reproductive technologies in the treatment of infertility has become increasingly popular as a preferred method of infertility treatment, IVF treatment involves the retrieval of oocytes from the ovarian follicles using a special needle which is passed through the vaginal wall and after fertilization *in vitro*, a special catheter is used in transferring the developing embryo through the cervix in to the uterus, these invasive manipulations can increase the possibility of bacterial introduction into uninfected areas of the genital tract. There is research evidence showing that uterine bacterial contamination as a result of bacterial introduction during the process of oocyte retrieval or embryo transfer can adversely affect the blastocyst development rate and pregnancy outcome

[10]. Although assisted reproductive technology including IVF have become the part preferred treatment of choice for most infertile couples, studies aimed at establishing the effects of microbial colonization of the upper female genital tract and their effect on IVF treatment outcomes remain elusive. This study will provide clues about follicular fluid bacterial colonization and its effect on IVF treatment outcome.

### Study Area

This study was carried out in selected fertility clinics across Southern part of Nigeria, and where independent Private and Public Hospitals are equipped with needed facilities and expertise for *in-vitro* fertilization treatment and PCOS diagnosis.

Ethical clearance was obtained from the University of Benin Teaching Hospital Ethics and Research committee.

Consent was obtained from each study participant before collection of samples. Participant were informed that participation is voluntary and the confidentiality and privacy of all the participant was respected They were assured that there was no penalty for refusal or withdrawal from participation. Information on medications, menstrual and clinical history were collected by administering a structured questionnaire. Cross sectional study design and purposive sampling techniques was utilized in this study. This involved identifying and selecting study participants who fit into the inclusion criteria for this research and recruiting them based on their availability and willingness to participate in the study.

### Study Population

The study group was taken from patients receiving *in-vitro* fertilization treatment in the selected hospital spread across the geographical zone of Nigeria.

### Inclusive/ Exclusion Criteria

The inclusive criteria as based on the definition of PCOS adopted at the Joint consensus meeting of the American Society for Reproductive Medicine and the European society of Human Reproduction and Embryology (ASRM/ESHRE),

1. Oligo-and /or anovulation
2. Hyperandrogenism (clinical and /or biochemical) and
3. Polycystic ovaries with the exclusion of other aetiologies. (Rotterdam ESHRE/ASRM, 2004).

### Exclusion Criteria

This study excluded women who has any of the following condition:

1. History of chronic hypertension
2. Known autoimmune disorder
3. Women who did not give constant
4. Diabetes mellitus or treatment with oral glucocorticoids
5. Congenital adrenal hyperplasia.

The sample size was calculated using the Cochran formula for sample size determination.

## Sample Collection

Higher vaginal swab were collected from each participant before trans-vaginal oocyte retrieval to obtain the follicular fluid (f.f)

## Culture and Identification of Bacterial Isolates

The principle of Monica cheesbrough on Medical Laboratory manual of 2000/Analytical providence Index (API) were applied

Gram positive bacilli and Gram positive catalase negative cocci were identified using the Analytical profile index (API) 50 CH strips/API 50CHL and Rapid ID 32A test kit (API system; bioMerieux, Marcy l'Etoile,) respectively. Gram-negative bacilli colonies were identified using API120E (bioMerieux, Inc., Mary l'Etoile, France).

## Principle of the API Identification System

A suspension is made in the medium with the microorganism to be tested and each tube of the API strip is then inoculated with the suspension. During inoculation, carbohydrates are fermented to acids, which produce a decrease in the PH, detected by the change in the colour of the indicator. The result make up the biochemical profile, which is use by the identification software to identify the strain. To identify *Lactobaccillus spp.* using the API system, a standardized system, consisting of API 50 CH is used in conjunction with API 50 CHL medium. It is a ready-to-use medium, which allows the fermentation of the 49 carbohydrate on the API 50 CH strip to be studied. The API 20E/Nacl medium was used to identify Gram-negative rods belonging to the *Enterobacteriaceae* families. Also *streptococcus* and *peptostreptococcus* genera were identified using the Rapid ID32 A kit.

## Results

A total of 206 women attending fertility clinics in 4 facilities across the study area were recruited for this study and the study subjects were divided into two group according to the Rotterdam

diagnostics criteria for PCOS group 101 (49%) women who met the Rotterdam criteria for diagnosis of PCOS were grouped as the PCOS group while, 106 (51%) apparently healthy women who had male factor infertility were recruited and grouped as the control group. The baseline demographic characteristic of the study population are shown in table 1. The study group age ranged from 26 to 53 years and there was no significant difference between the means ages of the PCOS group vs. 30.44±3.1 for the control group. The mean body mass index (BMI) in the PCOS (25.37±5.9) group were significantly higher than mean BMI of the control group (24.29±3.8) on the other hand, the mean number of menstrual cycle/yr.in the PCOS group (5±4.63) were significantly lower than that of the control group (5±4.63 and 3.04).

The frequency tabulation of follicular fluid bacterial colonization of the study subject is show in table 2. Bacterial isolate were detected in 100% of the high vaginal swab (HVS) culture but HVS culture were used in the study as a quality control system, it was considered that that if bacterial isolates present in the high vaginal swab culture was also isolated in the follicular fluid culture, the isolate was considered a contaminant but if the isolate were present only in the follicular fluid culture it was considered as true colonizing bacteria. out of 206 follicular fluid samples cultured, bacterial colonization were confirmed in 109 (52.9%) while 97 (47.1%) were found to be contaminants, a comparison between the PCOS were colonizing bacterial while 55.95 were no bacterial colonization.

To measure the association between PCOS and follicular fluid bacteria colonization, a logistic regression analysis of sample were conducted, Table 3 below shows the odds of PCOS, similarly after adjusting for the control group, there was no significant association found between follicular fluid bacteria colonization and either the PCOS or control group.

**Table 1:** Demographic characteristic of the study population.

Demographic Characteristics	PCOS (Mean ± SD)	Control (Mean ± SD)	P value
Means age (years)	29.46 ± 4.3	30.44 ± 3.1	0.124
Number of menstrual cycle/year	5 ± 4.63	9 ± 3.04	0.001*
Body Mass index (kg/m <sup>2</sup> )	25.37 ± 5.9	24.29 ± 3.8	0.021*

Values are means ± standard deviation (n=206) p<0.5=significant.

**Table 2:** Frequency of follicular fluid bacterial colonization in study subjects.

Bacterial Colonization	Count	PCOS	Control	Total
No	Count	53	44	97
	%	53.6%	46.4%	100%
Yes	Count	48	61	109
	%	44.1%	55.9%	100%
Total	Count	101	105	206
	%	45.1%	54.9%	100%

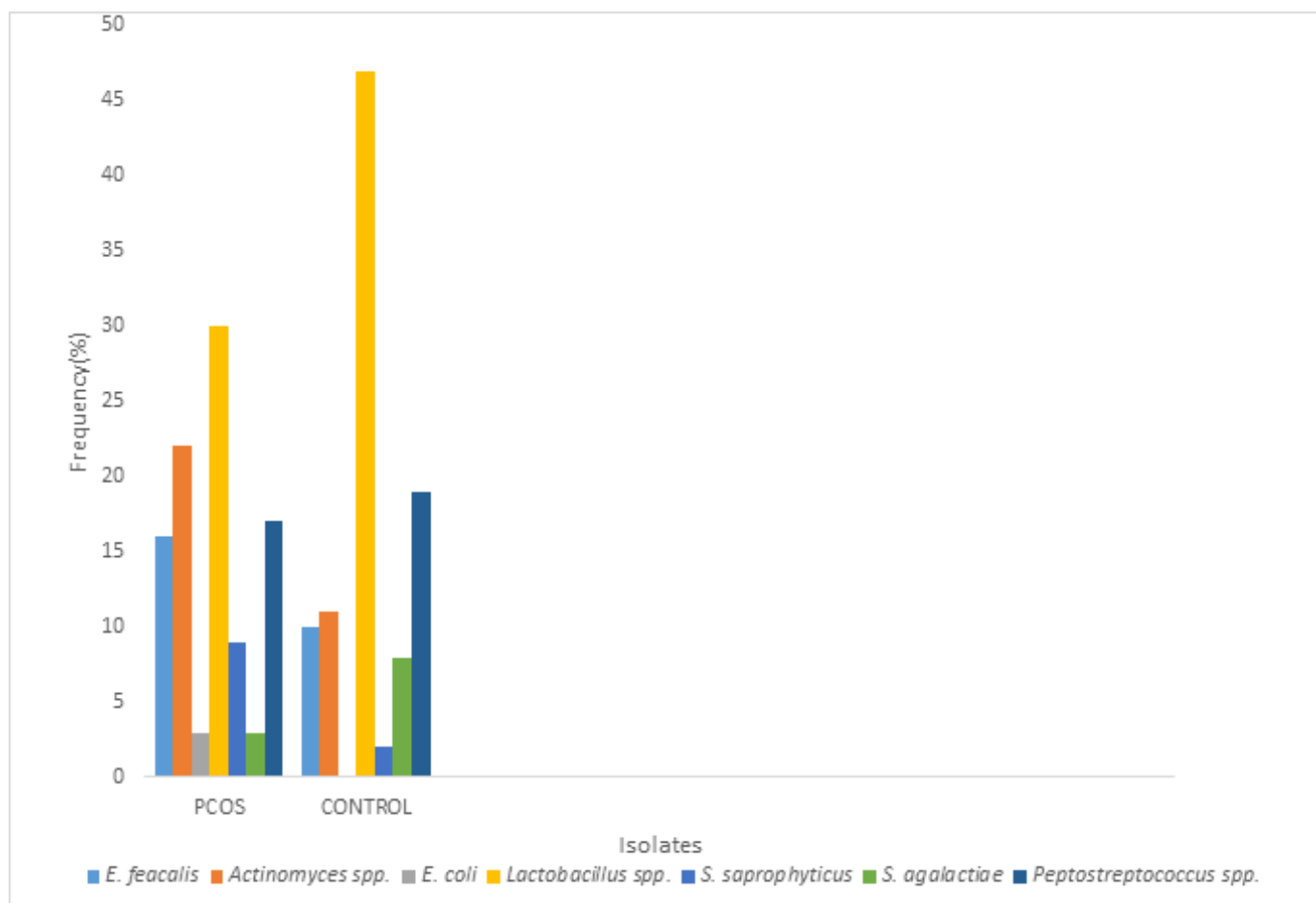
**Table 3:** Logistic regression analysis for association between PCOS and bacterial colonization of follicular fluid.

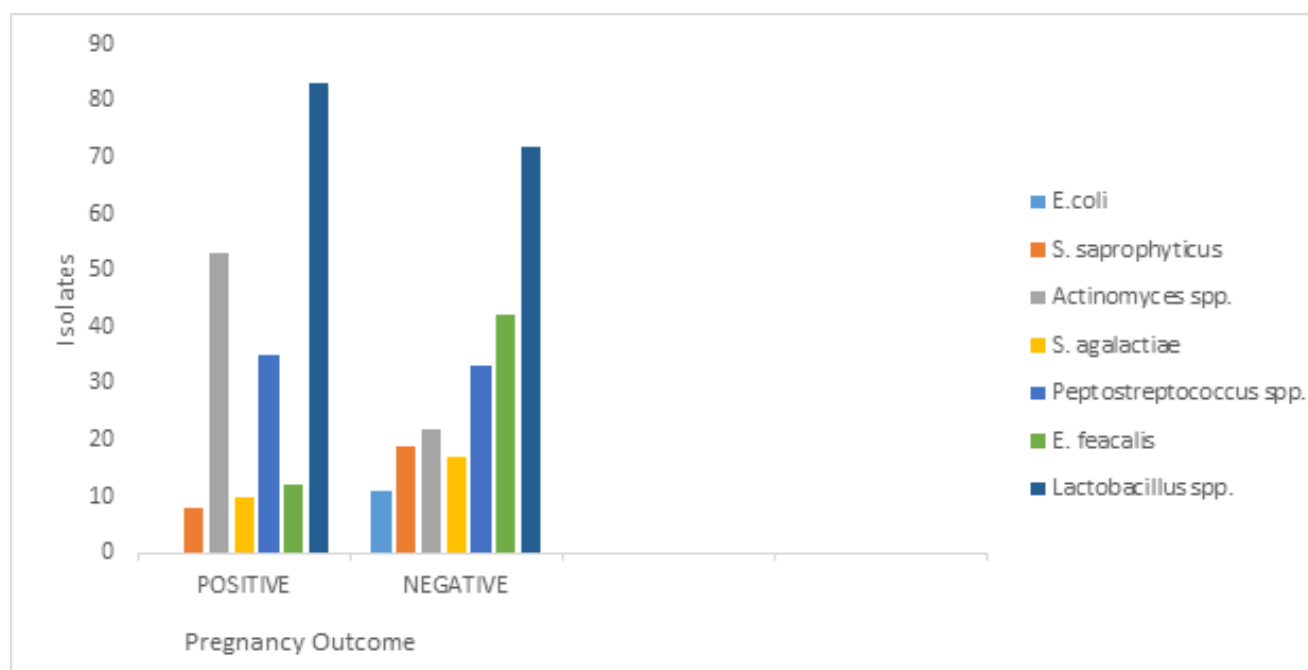
F.F Bacterial Colonization		Odds ratio	95%CI	P Value	F.F Bacterial Colonization		Odds ratio	95%CI	P Value
PCOS	Positive	0.78	0.25-2.41	0.316	PCOS	Positive	0.83	0.39-8.11	0.424
	Negative	0.64	0.47-3.18	0.633		Negative	0.53	0.61-5.27	0.320
	Positive	1.26	0.32-4.78	0.291		Control Positive	0.77	0.05-2.84	0.637
	Negative	0.53	0.21-2.09	0.564		Negative	1.36	0.41-8.25	0.226

\*Adjusted for follicular fluid colonization of PCOS and control.

**Table 4:** Frequency of follicular fluid bacterial colonization and post-IVF pregnancy outcome.

Bacterial Colonization			Count	PCOS	Total
			Yes	No	
Colonized	Yes	Count	49	60	109
		%	44.9%	55.1%	100%
	No	Count	36	61	97
		%	37.1%	62.9%	100%
	Total	Count	85	121	206
		%	41.3%	58.7%	100%





## Discussion

This study was undertaken to investigate the role of microbial agents and bacterial colonization of follicular fluid in the pathophysiology of PCOS in a cross section of women attending infertility clinic in a southern Nigeria. Many study in the field of Reproductive immunology are serum based and investigate mainly the outcomes of fertility treatment in women with PCOS or the metabolic characteristic of PCOS and its pathological consequences however few studies have focused on follicular fluid of microbiology of PCOS. Since the follicular fluid is central in the physiological communication between cells of female reproductive system, it seems its study may hold great potentials in revealing the pathophysiology of infertility in PCOS and improves its treatment outcome.

The study have demonstrated the presences of the bacterial (either as colonizer or contaminants) within follicular collected from women undergoing ART cycles, this study also identified bacteria colonizing the human follicular fluid. The presence of microorganism within ovarian follicles may be as a result of prior ascending bacterial infection from the fallopian tube to the ovaries, or as a result of bacteria being introduced into the ovary at the time of oocyte retrieval. Alternative, bacterial from other site such as the oral cavity or the respiration tract could access the ovary after haematogenous dissemination and subsequently colonize the ovary. Microbial colonization or contamination of the follicular fluid, compared bacterial colonization of the follicula in women with PCOS and finally evaluated the effect of bacterial colonization of the follicular fluid in pregnancy outcome following IVF treatment.

This study have shown that the presence of some colonizing bacterial within follicular may be beneficial for successful

pregnancy outcome following IVF, while some may adversely affect pregnancy outcome. This observation were made from women included in the study, and in particular for women within infertility due to PCOS (when compared with women from the control group who had colonized follicular fluid specimens), this could be as a result of damage to the oocyte *in vivo* after exposure to the disadvantageous bacterial metabolic products. This study observed *Lactobacillus spp.*, as the prevalent colonizing bacteria. Present in the follicular fluid if women in this study this was followed by *peptostreptococcus spp.*, these two predominant bacterial isolates were associated with higher pregnancy whom studied the effect of endogenous steroid hormones on lactobacilli in IVF patient they also reported that during IVF treatment with increasing estrone levels, three major vaginal Lactobacilli were found predominant in the IVF media.

This study did not isolate *Bifidobacterium spp* in the follicular fluid of women investigated. Both genera can metabolise carbohydrate from glycogen degradation in response to elevated estradiol levels, making the female genital tract a niche environment, enhancing the ability of the lactobacilli to persist and protect the genital tract and follicular epithelium from opportunistic infection. In the study *E. Coil* was the least isolated bacteria colonizing follicular fluid, this bacteria was also associated with negative pregnancy outcome in women in this study, *E. Coil* is a hyaluronidase producing bacterial, the hyaluronidase virulence factor of this species could lead to poor quality cumulus cells via enzymatic hyaluronic degradation decreased steroid hormone synthesis and ultimately, detrimental defects of the follicle resulting in poor quality oocytes.

## Conclusion

This study identified colonizing bacteria present in the follicular fluid and include that *Lactobacillus spp.*, is the prevalent colonizer

of the human follicular fluid and that follicular fluid colonizing bacteria can either have beneficial or adverse effect on pregnancy rate following medically assisted reproduction treatment or even natural conception. These findings are a valuable addition to the huge knowledge gap on the pathophysiology of PCOS and also the effect of bacteria on the post-IVF outcomes. Further characterization of microorganism present in the follicular fluid and their metabolite will increase our understanding of their effects on pregnancy rate and early pregnancy events.

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