Babandi *et al.*
Middle East Fertility Society Journal (2023) 28:8
https://doi.org/10.1186/s43043-023-00132-y

Middle East Fertility Society Journal

RESEARCH

Open Access

Does seminal fluid bacterial isolate(s) affect in vitro fertilization — embryo transfer outcome?

Rukayya Muhammad Babandi[1*], Rais Shuaibu Ibraheem[1], Rayyan Muhammad Garba[2], Idris Muhammed Liman[1], Amina Ismail-Are[1] and Yakubu Samuel[3]

Abstract

Background The chance of achieving a successful pregnancy through in vitro fertilization and embryo transfer (IVF-ET) is limited despite recent scientific advances in this field due to several factors that are known to affect the outcome. There are studies linking the presence of bacteria in the male genital tract to poor semen parameters and IVF-ET outcomes. Results are, however, contradictory. The finding of confirmed genital tract infection warrants treatment, especially when dealing with infertile couples, but treating asymptomatic bacteriospermia is controversial. This study assessed the prevalence and effects of seminal fluid bacterial isolates on semen quality and rates of fertilization and biochemical and clinical pregnancies in IVF-ET.

Methodology This is an analytical cross-sectional study conducted at the IVF Center of National Hospital Abuja, Nigeria. Due to the low turnout of clients, we enrolled all consecutive consenting eligible male partners of women undergoing the procedure during the study period to obtain a *sample size of 242*. Participants observed sterile techniques to prevent contamination of the seminal fluid during collection. Growth of bacteria > 10,000 colony-forming units (CFU)/ml was considered significant and tested for sensitivity to a panel of antibiotics. We determined the influence of positive bacterial isolates on fertilization, biochemical pregnancy, clinical pregnancy (primary outcome), and multiple pregnancies. Data were analyzed using SPSS version 22. Student's *t*-test, chi-square test, and Fisher's exact tests were employed as appropriate. p-value < 0.05 at a 95% confidence interval was regarded as statistically significant.

Results Seminal fluid culture was positive in 57 patients (47.11%). *Staphylococcus aureus* was the predominant organism cultured (43.90%), followed by *Streptococci* spp. (21.05%), *Escherichia coli* (17.54%), *Klebsiella* spp. (8.77%), *Pseudomonas aeruginosa* (5.26%), *Staphylococcus saprophyticus* (1.75%), mixed *Staphylococcus aureus*, and *Streptococcus* spp. organisms (1.75%). The fertilization rate was 95.4%, the biochemical pregnancy rate was 42.2%, the clinical pregnancy rate was 38%, and the multiple pregnancy rate was 16.53%. Significant factors found to be associated with positive clinical pregnancy were primary infertility (p-value = 0.001) and negative seminal fluid culture result (p-value = 0.033).

Conclusion The prevalence of bacteriospermia was relatively high, and the presence of bacterial isolates adversely affected fertilization and clinical pregnancy rates among couples undergoing the IVF-ET program.

*Correspondence:
Rukayya Muhammad Babandi
rmbabandi@yahoo.com
Full list of author information is available at the end of the article

Springer Open

© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Keywords Seminal fluid, Bacteriospermia, Infertility, Semen contamination, In vitro fertilization (IVF), Assisted reproductive technology (ART)

Background

Semen is a mixture of spermatozoa and fluidsderived from the epididymis, the bulbourethra, the urethra, and the prostate glands. Each area that contributes to a semen sample is considered a sterile area, yet the culturing of semen for bacteria is usually positive [1]. Microbes contained in semen can result in the overt contamination of embryo culture media. The demise of valuable embryos due to contamination will, at least, result in a patient with a wasted cycle and zero chance of pregnancy [2].

Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process [3], causing qualitative and quantitative sperm alterations. Recent studies have shown that the simple presence of bacteria in semen samples may compromise sperm and embryo quality [4]. The bacteria responsible for semen contaminations generally originate from the urinary tract of patients or can be transmitted by the partner via sexual intercourse. The presence of microorganisms within the upper genital tract and those that contaminate the IVF culture system may result in poorquality embryos (possibly due to oocyte DNA fragmentation), early pregnancy loss, or preterm birth [5].

Generally, bacteriospermia affects the normal fertility process by a deterioration of spermatogenesis, decreased sperm motility, altered acrosome reaction, altered morphology, formation of reactive oxygen species leading to increased DNA fragmentation index, formation of antisperm antibodies due to breach in the blood-testes barrier, and genital tract obstruction due to inflammation and fibrosis [6].

The direct association between the presence of infectious factors in semen and sperm-fertilizing potential has been intensely discussed and constitutes a significant problem in andrology. Studies linking semen urogenital infection/inflammation with a higher number of spermatozoa with DNA fragmentation are contradictory [7]. Published studies also reported conflicting results on the harmful impact of leukocytospermia on sperm DNA integrity as measured by DNA fragmentation assays [8]. Difficulties arise when determining which bacteria are significant and which merely represent skin contamination.

Infertility is one of the most trying challenges of the married state; it is considered a national health problem in many countries, including Nigeria. In vitro fertilization (IVF) has recently become very attractive in Nigeria, representing the definite solution to barrenness and stigmatization because, unlike many of the more traditional ways of treating infertility, it is both medically accepted and socially tolerated. Despite these advantages, centers providing the services are few, with attending high costs and technical challenges. Data on the influence of seminal fluid bacterial isolates on IVF-ET outcome is scanty and almost nonexistent in our environment.

National Hospital Abuja is one of the few public centers providing IVF-ET service in the country; with an ever-increasing patient population who can afford the high cost of the service, the improved outcome is still desirable. This study is aimed at determining the prevalence of bacterial isolates in seminal fluid and its effect on semen quality, fertilization rate, and biochemical and clinical pregnancy rates in the IVF-ET program.

Methods

This is an analytical, cross-sectional study carried out at the IVF Center of the National Hospital, Abuja, from June 2017 to May 2018. National Hospital Abuja is a multidisciplinary tertiary hospital located in the central business district sub-locality, Abuja, the Federal Capital City of Nigeria. IVF started in National Hospital, Abuja, in August 2006, and the birth of the first IVF baby was recorded in February 2007.

The IVF-ET approach involves pituitary downregulation, followed by ovulation induction with gonadotropins using the long or short protocols, during which a transvaginal scan is used to monitor the follicular growth. At the National Hospital Abuja IVF-ET Center, treatment is usually done in batches of 80–150 couples. The women receive 10,000 IU human chorionic gonadotropin (hCG) for ovulation trigger when two or more follicles reach a diameter of $\geq$ 18 mm, and oocytes are retrieved by the transvaginal route 34–36 h after hCG administration.

Intravenous amoxicillin/clavulanic acid 1.2 g or ceftriaxone 1 g is given immediately to all patients after egg retrieval. The oocytes are assessed by the embryologist and fertilized with the previously potentiated sperm. The resulting embryo is transferred on the third day transcervically using a standard embryo transfer catheter (HG Wallace Ltd., UK). Embryo transfer is usually done by three fertility experts (the gynecologist, embryologist, and a trained nurse), aided by abdominal ultrasound.

Study population and sample size

Due to the low turnover of clients, all consecutive consenting male partners of women undergoing IVF-ET from June 2017 to May 2018 were included. Exclusion criteria included female partners above 40 years of age, congenital genital tract abnormality, clinical evidence of epididymitis and/or orchitis, and male partners on antibiotics at the time of sample collection. *A sample size of 217 was obtained using Cochran's formula* ($n = z^2pq/d^2$), with a proportion of clinical pregnancy rate per transfer in a culture-negative and culture-positive group of 17% obtained from a previous study [9]. We approximated this to 242 to account for possible nonresponse as shown below:

Where:

n = minimum sample size.

Z_α= the standard normal deviate corresponding to 95% confidence interval. The value obtained from the normal distribution table is 1.96.

Z_β = the standard normal deviate corresponding to the power of the test to detect differences, set at 95%. The value obtained from the normal distribution table is 1.64.

p = proportion of clinical pregnancy from previous studies[9] = 17% = 0.17.

q = complimentary probability = 1-p = 1-0.17 = 0.83.

d = degree of precision = 0.05.

Data collection

Data was collected over 12 months. IVF specialists and the researcher took consent at the routine counseling session before the IVF procedure. All the men were instructed to abstain from sexual intercourse for 3–5 days before collection, and *detailed instructions were given concerning sterile techniques to prevent contamination of the semen from normal flora in that body region. These instructions included thoroughly cleaning the head of the penis with a moist sterile towelette, semen collection by masturbation with clean, dry hands using no creams or lubricants, directing the entire ejaculate into the container, and avoiding contact with the interior of the sterile wall of the container.*

Seminal fluids collected on the day of oocyte retrieval were immediately taken to the IVF center laboratory. Aliquots were taken out of the collecting bottle using a sterile pipette and used for the study. Semen samples were transported to the National Hospital laboratory within 60 min of collection by the researcher or the research assistant. Certified microbiologists immediately carried out the laboratory procedures with the full involvement of the researcher.

The remaining samples were quickly analyzed at the IVF center, prepared, and used for the procedure.

Microscopy

Smears of the seminal fluid were made on a sterile grease-free glass slide, covered with a cover slip (wet preparation), and viewed with ×10 and ×40 objective lenses to take note of pus cells and other microscopic abnormalities in the semen.

Culture

Semen samples were plated on 5% blood agar, chocolate agar, MacConkey agar, Thayer Martins, and Columbia agar (Becton Dickinson BBL Microbiology Systems, Franklin Lakes, NJ, USA) using sterile wire loops. These were then placed in the incubator (Memmert GmbH + Co. KG, Schwabach, Germany) aerobically at 37 °C for 18–24 h. Any growth of bacteria > 10,000 colony-forming units (CFU)/ml on the 5% blood agar was considered significant and subsequently identified and tested for sensitivity to a panel of antibiotics.

Gram stain

We placed a drop of normal saline on the center of a grease-free slide. With the aid of a sterile wire loop, a small colony of the suspected bacteria was picked and emulsified on a glass slide and then heat fixed. The smear was then flooded with crystal violet (primary stain) and allowed to stand for 60 s before rinsing with water. Lugol's iodine was next added to the slide and allowed to act for another 30–60 s and rinsed with water. Acetone was added drop by drop for 5–10 s and immediately rinsed. Safranin (a counterstain) was added and washed with water after 2 min. After drying, slides were examined with oil emersion objective ×100.

Biochemical tests

Media plates with significant bacteria growth (positive plates) were further subjected to various biochemical tests, including Simon Citrate, Kligler Iron Agar (KIA), Urea Agar Base, and hydrogen peroxide. This is to identify and classify the bacteria isolate(s) as shown in the two tables below.

Data management and analysis

The generated data were entered into two separate Excel Sheets by two clerical staff and checked for (in) consistencies. Analysis was performed using SPSS version 22; for categorical variables, proportions and frequencies were calculated and compared using Pearson's chi-squared test (χ^2) or Fisher's exact test, as the case may be. Continuous variables, on the other hand, were summarized as means and standard deviation

and compared using a *t*-test. In addition, minimum, maximum, and missing values were reported for all variables.

Those factors significantly associated with the primary outcome were entered into a binary logistic regression model to control for confounding. Any *p*-value < 0.05 at a 95% confidence interval was regarded as statistically significant.

Results

The age range of the patients was 24 to 40 years, with a mean of 36.67 ± 3.809 (years). The majority of the patients had secondary infertility (71.1%). Female factor accounted for 42.2% (tubal — 17.4%, uterine —16.5%, ovarian — 8.3%), male factor infertility was 23.1%, combined male and female factors were 21%, while 13.2% had unexplained infertility. When the patients were grouped into two based on the seminal fluid culture results (positive or negative), there was no significant difference between the groups concerning the sociodemographic and clinical characteristics except for the type of infertility. Up to 42 out of 70 patients with primary infertility were pregnant (60% success rate), while only 50 out of 172 patients with secondary infertility conceived (29.1% success rate). Most patients (89%) had three or more embryos transferred during the cycle. Five patients had their cycles canceled due to failure of fertilization.

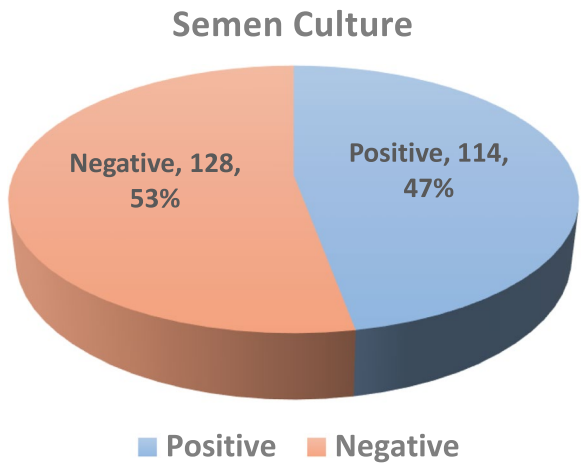

Fig. 1 Semen culture

Of the 242 patients, 114 (47%) had positive seminal fluid culture results, while more than half of the patients (53%) had negative culture results (Fig. 1).

In Table 1, when the patients were grouped into two based on the seminal fluid culture results (positive or negative), there was no significant difference between the groups concerning the clinical characteristics except for the type of infertility. More clients with primary infertility had positive cultures (60%) compared to those with secondary infertility (29.1%).

In Fig. 2, *Staphylococcus aureus* was the predominant organism cultured, followed distantly by streptococci spp. Mixed organisms were seen in two patients. A rarer species, *Staphylococcus saprophyticus*, was also seen in two patients.

As seen in Table 2 below, there was no statistically significant difference in sperm count between culture positive and culture-negative patients (A). Overall, fertilization was positive in 95.10% of the patients, and more than 50% of the oocytes were fertilized in the majority (78.50%) of the patients. There was a statistically significant difference in fertilization rate between

Table 1 The clinical characteristics of the study participants

Variables	Culture		Test of significance
	Positive	Negative	
Mean age in years ± SD (male partner)	43.52 ± 7.561	42.33 ± 5.691	$t = -0.978; p = 0.330$
Mean age in years ± SD (female partner)	36.02 ± 4.171	37.07 ± 3.539	$t = 1.472$; p = 0.144
The mean number of previous IVF per patient ± SD	0.5 ± 0.837	0.56 ± 0.874	$t = 0.373; p = 0.710$
The mean number of embryos transferred per patient ± SD	3.24 ± 0.794	2.84 ± 1.231	$t = 1.962; p = 0.052$
Type of infertility			$X^2 = 10.099; p = 0.001$*
Primary	42 (60%)	28 (40%)	
Secondary	50 (29.1%)	122 (70.9%)	
Causes of infertility			$X^2 = 16.297; p = 0.06$
Combined	8 (15.4%)	44 (84.6%)	
Male factor	32 (57.1%)	24 (42.9%)	
Ovarian factor	4 (20%)	16 (80%)	
Tubal factor	24 (57.1%)	18 (42.9%)	
Uterine factor	10 (25%)	30 (75%)	
Unexplained	14 (43.8%)	18 (56.3%)	

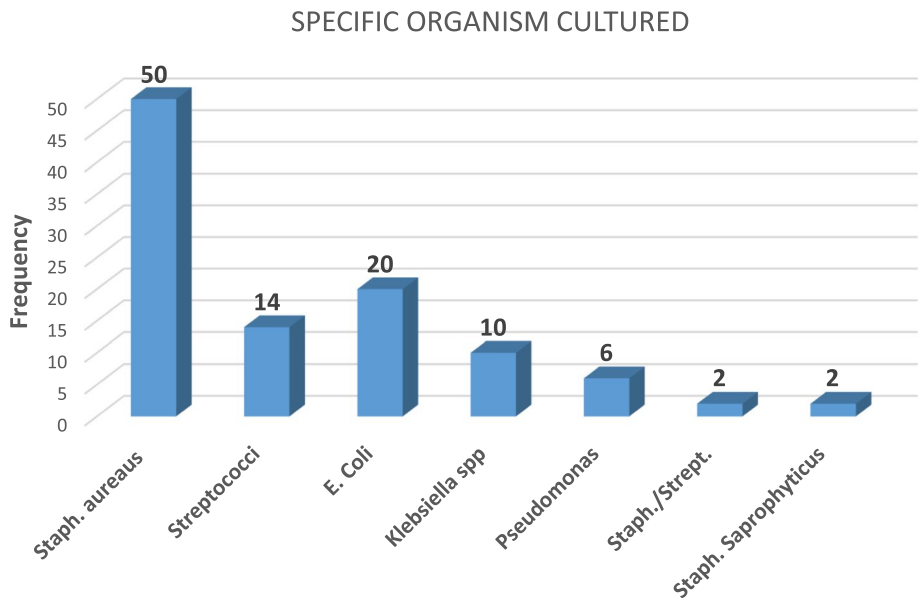

Fig. 2 Specific organisms cultured

Table 2 Relationship between semen culture and pregnancy outcomes

A. Semen culture	***Sperm count***		***Total***	χ^2	***p-value***
	≥ 15 M/mL	***< 15 M/Ml***			
Negative n (%)	98 (76.60)	30 (23.40)	128 (100)	1.461	0.227
Positive n (%)	76 (66.70)	46 (33.30)	114 (100)		
Total	174 (71.9)	68 (28.10)	242 (100)		
B. Semen culture	***Fertilization rate***		***Total***	χ^2	***p-value***
	Good	***Poor***			
Negative n (%)	112 (87.50)	16 (12.50)	128 (100)	6.505	0.011*
Positive n (%)	78 (68.40)	36 (31.6)	114 (100)		
Total	190 (78.50)	52 (21.50)	242 (100)		
C. Semen culture	***Biochemical pregnancy***		***Total***	χ^2	***p-value***
	Yes	***No***			
Negative n (%)	64 (50.0)	64 (50.0)	128 (100)	3.435	0.064*
Positive n (%)	38 (33.3)	76 (66.7)	114 (100)		
Total	102 (42.1)	140 (57.9)	242 (100)		
D. Semen culture	***Clinical pregnancy***		***Total***	χ^2	***p-value***
	Positive	**Negative**			
Negative n (%)	60 (46.9)	64 (53.1)	128 (100)	4.524	0.033*
Positive n (%)	32 (28.1)	82 (71.9)	114 (100)		
Total	92 (38.0)	150 (62.0)	242 (100)		

culture-positive and culture-negative patients (B). Patients with negative semen cultures were more likely to have good fertilization ($\chi^2 = 3.435$, p-value $= 0.064$). Biochemical pregnancy (serum b-hCG) test was positive in up to 42.10% of the patients (C). There was no statistically significant difference in biochemical pregnancy between culture-positive and culture-negative patients ($\chi^2 = 6.505$, p-value $= 0.011$). Clinical pregnancy, defined as the presence of a gestational sac by transvaginal ultrasound scan (done at 6-week gestation), was positive in 38% of all the patients (D).

Out of the 92 positive clinical pregnancies, 10 (4.1%) were ectopic gestations, while 40 (16.5%) 284 had multiple pregnancies (Table 3).

Table 3 Relationship between semen culture result and pregnancy outcome

Semen culture	*Pregnancy outcome*				*Total*
	No pregnancy	*Singleton*	*Multiple*	*Ectopic*	
Negative n (%)	68 (53.10)	26 (20.30)	24 (18.80)	10 (7.80)	128 (100)
Positive n (%)	82 (71.90)	16 (14.00)	16 (14.00)	0 (0.00)	114 (100)
Total	150 (62.00)	42 (17.40)	40 (16.50)	10 (4.10)	242 (100)

* This has not been found to be significant ($F = 10.135$; $p = 0.165$)

As seen in Table 4 below, factors found to be significantly associated with clinical pregnancy rate at bivariate analysis (semen culture result and type of infertility) as well as a priori confounders (age, previous IVF, number of embryos transferred, and specific cause of infertility) were entered into a multiple logistic regression model to adjust for possible confounding effects as shown in Table 5 below. Only semen culture and type of infertility remained significant, which means the two factors are independent (intrinsic) determinants of clinical pregnancy in the study population.

Table 4 Relationship of variables with clinical pregnancy

Variables	*Clinical pregnancy*		*Total*	*p-values*
	Yes	*No*		
Age group				
21–30 yrs.	16 (66.7)	8 (33.3)	24 (100)	0.031
31–40 yrs.	76 (34.9)	142 (65.1)	218 (100)	
Semen culture				
Negative	60 (46.9)	68 (53.1)	128 (100)	0.033
Positive	32 (28.1)	82 (71.9)	114 (100)	
Previous IVF				
No	62 (39.7)	94 (60.3)	156 (100)	0.598
Yes	30 (34.9)	56 (65.1)	86 (100)	
Type of infertility				
Primary	42 (60.0)	28 (40.0)	70 (100)	0.001
Secondary	50 (29.1)	122 (70.9)	172 (100)	
Specific cause				
Combined	8 (15.4)	44 (84.6)	52 (100)	0.006
Male	32 (57.1)	24 (42.9)	56 (100)	
Ovarian	4 (20.0)	16 (80.0)	20 (100)	
Tubal	24 (57.1)	18 (42.9)	42 (100)	
Unexplained	14 (43.8)	18 (56.2)	32 (100)	
Uterine	10 (25.0)	30 (75.0)	40 (100)	
Sperm count				
Normospermia	64 (36.8)	110 (63.2)	174 (100)	0.654
Oligospermia	28 (41.2)	40 (58.8)	68 (100)	
Organism cultured				
Negative culture result	58 (45.3)	70 (54.7)	128 (100)	0.125
Escherichia coli	2 (10.0)	18 (90.0)	20 (100)	
Staph. saprophyticus	0 (0.0)	2 (100)	2 (100)	
Klebsiella spp.	2 (20.0)	8 (80.0)	10 (100)	
Pseudomonas aeruginosa	2 (33.3)	4 (66.7)	6 (100)	
Staph./Strep.	2 (100)	0 (0.0)	2 (100)	
Staphylococcus spp.	22 (44.0)	28 (56.0)	50 (100)	
Streptococci	4 (16.7)	20 (83.3)	24 (100)	
Fertilization rate				
Good	80 (42.1)	110 (57.9)	190 (100)	0.077
Poor	12 (23.1)	40 (76.9)	52 (100)	
Number of embryo transferred				
1–2	16 (27.6)	42 (72.4)	58 (100)	0.185
3 and above	76 (41.3)	108 (58.7)	184 (100)	

Discussion

The current study found that prevalence of primary infertility was 28.9%, while 71.1% had secondary infertility. This agrees with global distribution, whereby the prevalence of secondary infertility is higher than primary infertility [10–14]. Regarding the specific etiologies, female factors accounted for 42.2%, male factors contributed 23.1%, combined male and female factors were 21%, while 13.2% had unexplained infertility. This result agrees with findings in a similar population [12]. The age of both male and female partners, duration of infertility, number of previous IVF attempts, and number of embryos transferred did not differ between culture-positive and negative results.

We isolated bacteria in 114 of the 242 patients (47.11%); this is similar to a value of 46.3% obtained by Ricci et al. in Italy [13]. Higher and lower bacteriospermia prevalence rate of 65.7–85% [14–16] and 22.3–35.3% [17–19] were reported by other researchers, respectively. The commonest organisms cultured were *Staphylococcus aureus* (43.9%), followed by *Streptococci* (21.05%) and *Escherichia coli* (17. 54%). This finding is similar to that obtained in other studies where *Staphylococcus aureus*was the commonest isolated organism (with a prevalence of 28.3–62.5%) [12, 20]. Other researchers found less common organisms like *Enterococcus faecalis* [21], *Escherichia coli* [17, 22], and *Peptostreptococcus* [23] as the principal isolates. Variations in prevalence and microbial compositions reported by different studies can be attributable to differences in center protocols, isolation techniques, and study population. The high prevalence of Staphylococcus aureus in our study raises suspicion of contamination. Microorganisms isolated from seminal fluid may originate from the surrounding penile skin, hands, and genital tract [22], sexual intercourse, or hematogenous spread resulting in either contamination, colonization,

Table 5 Logistic regression model

Covariates	*B*	*p-values*	*Adjusted OR*	*95% CI for AOR*	
				Lower	*Upper*
Age (years)					
21–30 yrs.	−0.832	0.285	0.435	0.095	2.003
31–40 yrs.	Reference				
Semen culture					
Negative	−0.437	0.339	0.646	0.263	1.583
Positive	Reference				
Type of infertility					
Primary	−1.310	0.012	0.270	0.097	0.750
Secondary	Reference				
Specific cause					
Combined	0.768	0.339	2.155	0.446	10.401
Male	−1.043	0.154	0.352	0.084	1.478
Ovarian	0.249	0.803	1.283	0.181	9.090
Tubal	−1.442	0.045	0.237	0.058	0.969
Unexplained	−0.686	0.371	0.504	0.112	2.263
Uterine	Reference				
Sperm count					
Normospermia	0.152	0.775	1.164	0.412	3.289
Oligospermia	Reference				
Fertilization rate					
Good	−0.821	0.190	0.440	0.129	1.503
Poor	Reference				
Number of embryo transferred					
1–2	−0.204	0.718	0.815	0.269	2.472
3 and above	Reference				
Constant	2.308	0.015	10.057		

or infection [23]. Although we ensured semen collection by masturbation and the use of aseptic collection techniques to minimize the possibility of contamination, resident skin flora on the skin may be difficult to remove even with hours of scrubbing. However, contamination and infection are detrimental. Various reports signified a decrease in sperm motility and agglutination of sperms when spermatozoa were incubated with *Staphylococcus aureus* [24], and this explains the negative impact of *S. aureus* on semen parameters and, consequently, IVF success rate.

Our study did not find a statistically significant association between sperm count and seminal fluid culture result ($p = 0.227$); this is similar to the findings by Vlaisavljevi et al. and Jue et al. [8–20, 22–28], whereby the presence of asymptomatic bacteriospermia did not correlate with abnormal semen parameters. Other researchers found a strong association between the presence of bacteria in sperm samples with abnormal semen parameters [15, 16, 18, 19, 29, 30]. Our finding could be explained by the fact that we screen and treat oligospermic patients for infection during initial screening in the clinic before IVF-ET. In addition, the quality of semen is a function of several other parameters aside from the absolute count.

The fertilization rate was reasonable in the study population, with positive fertilization in up to 95.1% of the patients. This is perhaps due to strict inclusion criteria and standard IVF techniques employed in the study center. There is a statistically significant difference in fertilization rate between culture-positive and culture-negative patients $\{\chi^2\ (1, N = 121) = 6.505, p < 0.05\}$. It is also noteworthy that most couples with unfertilized oocytes had a positive culture result. This agrees with an earlier similar study which noted that the presence of organisms like *Staphylococcus aureus*in seminal fluid correlated with a low pregnancy rate [20]. Also, in vitro studies on gram-positive organisms have demonstrated some effect of bacteria on sperm morphology, possibly mediated by its virulence factor hemolysin [3]; hence, contamination of the culture system with seminal microbes may result in suboptimal fertilization rates and impaired embryonic development [31].

Although the fertilization rate was high and embryo transfer was done for 95.1% of the patients, the pregnancy test was positive in only 42.1%. This suggests significant implantation failure, possibly due to poor endometrial receptivity and low embryo quality. Even though more patients with negative seminal fluid culture results had a positive (biochemical) pregnancy test (50% versus 33.3%), the difference was not found to be statistically significant ($p = 0.064$). Novel studies are ongoing to evaluate the effect of the endometrial microbiota on endometrial receptivity and infertility. It is now evident that sites in the body that are historically sterile, such as the uterine cavity and the placenta, are colonized with their unique microbiome [32] and perhaps low embryo quality.

In addition, a transvaginal ultrasound scan done at 6-week gestation demonstrated clinical pregnancy (demonstrable gestational sac) in 38% of the total patients. The lower clinical pregnancy rate compared to biochemical pregnancy is due to early pregnancy failure, which is also seen in natural conception. However, the presence of bacteria in the seminal fluid was noted to associate with less clinical pregnancy rate $\{\chi^2\ (1, N = 121) = 4.524, p < 0.05\}$; when specific isolated organisms were analyzed, the difference was, however, not found to be statistically significant (p-value = 0.165). In the study by Guillet-Rosso et al.,[9] pregnancy rate per cycle was significantly reduced when the semen culture contained organisms compared with axenic semen ($p < 0.05$). This was independent of the cleavage rate of oocytes and the number of embryos transferred, as observed in this study.

We also noted that out of the 92 positive clinical pregnancies, 10 (4.13%) were ectopic gestations, while 40 (16.53%) had multiple pregnancies. The multiple-pregnancy rate following assisted conception is reported at 4.9% (Sweden), 19.8% (UK), and 20.5% (Canada) to up to 46% in the USA. The low value in Sweden is due to the adoption of elective single embryo transfer (eSET) [33]. This study found no statistically significant difference between culture result and type of pregnancy (Fisher's exact test = 7.09, p-value = 0.062). Not unexpected, as it is known that younger women are significantly more likely to achieve pregnancy following infertility treatment [11], in this study, two-thirds of those less than 31 years became pregnant, while only about one-third of those between 36 and 40 conceived. The difference was, however, not statically significant ($\chi^2 = 4.798$; p-value = 0.91).

A multiple logistic regression model was used to adjust for possible confounders like age, type of infertility, previous IVF, and the number of embryos transferred; we found that only semen culture and type of infertility remained significant, which means the two factors are an independent (intrinsic) determinant of clinical pregnancy in the study population.

Limitations in this study include the fact that culture was limited to aerobic organisms only, our inability to culture some fastidious organisms like mycoplasmas and chlamydia, and contamination not objectively ruled out by skin swab culture before and after cleaning (prior to masturbation).

Conclusion

This study determined that the prevalence of bacteriospermia was high among the study population, and the presence of these bacterial isolates negatively affected fertilization and clinical pregnancy rates. The onus is to prevent semen and culture contaminations, and confirmed infections should be treated before IVF-ET treatments to maximize chances of success.

Acknowledgements

Our sincere appreciation to the Microbiology and IVF Center staff members of the National Hospital Abuja, Nigeria, for their support during this study. Also, special thanks to all the patients that participated in the study.

Authors' contributions

RMB and IRS, conception, design, and IVF-ET laboratory procedures; MRG, study design and data interpretation. IA, manuscript writing and revision. YS, microbiological laboratory procedures and result interpretation. All authors read and approved the final manuscript.

Funding

This research received no specific grant.

Availability of data and materials

The datasets generated and analyzed during the study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

We presented a copy of the research proposal to the Research and Ethics Committee of the National Hospital, Abuja. An approval letter (NHA/EC/088/2016) was obtained before data collection. We also ensured that informed written consent from every participating couple at the regular counseling session is performed by the IVF specialists and the researchers before the IVF procedure.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]In Vitro Fertilization Unit, Department of Obstetrics and Gynaecology, National Hospital Abuja, Abuja, Nigeria. [2]Department of Community Medicine, Aminu Kano Teaching Hospital, Kano, Nigeria. [3]Department of Microbiology, National Hospital Abuja, Abuja, Nigeria.

Received: 15 April 2022 Accepted: 17 March 2023
Published online: 04 April 2023

References

1. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM et al (2010) World Health Organization reference values for human semen characteristics. Hum Reprod Update 16(3):231–45. https://doi.org/10.1093/humupd/dmp048
2. Kastrop PM, de Graaf-Miltenburg LA, Gutknecht DR, Weima SM (2007) Microbial contamination of embryo cultures in an ART laboratory: sources and management. Hum Reprod 22(8):2243–8. https://doi.org/10.1093/humrep/dem165
3. Moretti E, Capitani S, Figura N, Pammolli A, Federico MG, Giannerini V, Collodel G (2009) The presence of bacteria species in semen and semen quality. J Assist Reprod Genet 26:47–56
4. Štšepetova J, Baranova J, Simm J et al (2020) The complex microbiome from native semen to embryo culture environment in human in vitro fertilization procedure. Reprod Biol Endocrinol 18:3. https://doi.org/10.1186/s12958-019-0562-z
5. Keck C, Gerber-Schäfer C, Clad A, Wilhelm C (1998) Breckwoldt M (1998) Seminal tract infections: impact on male fertility and treatment options. Hum Reprod Update 4(6):891–903
6. Fujita Y, Mihara T, Okazaki T, Shitanaka M, Kushino R, Ikeda C et al (2011) Toll-like receptors (TLR) 2 and 4 on human sperm recognize bacterial endotoxins and mediate apoptosis. Hum Reprod 26(10):2799–806
7. La Vignera S, Condorelli R, D'Agata R, Vicari E, Calogero AE (2012) Semen alterations and flow-cytometry evaluation in patients with male accessory gland infections. J Endocrinol Invest 35(2):219–23
8. Fraczek M, Wiland E, Piasecka M, Boksa M, Gaczarzewicz D, Szumala-Kakol A et al (2014) Fertilizing potential of ejaculated human spermatozoa during in vitro semen bacterial infection. Fertil Steril 102(3):711–12
9. Guillet-Rosso F, Fari A, Taylor S, Forman R, Belaisch-Allart J, Testart J et al (1978) Systematic semen culture and its influence on IVF management. Br J Obstet Gynaecol 94(6):543–7. https://doi.org/10.1111/j.1471-0528.1987.tb03148.x. (PMID: 3620403)
10. Sun H, Gong T. T, Jiang Y. T, Zhang S, Zhao Y. H, Wu Q. J (2019) Global, regional, and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990-2017: results from a global burden of disease study. Aging 11(23):10952–10991. https://doi.org/10.18632/aging.102497
11. Menuba IE, Ugwu EO, Obi SN, Lawani LO, Onwuka CI (2014) Clinical management and therapeutic outcome of infertile couples in southeast Nigeria. Ther Clin Risk Manag 10:763–8. https://doi.org/10.2147/TCRM.S68726
12. Panti A, Sununu Y (2014) The profile of infertility in a teaching hospital in North West Nigeria. Sahel Med J 17(1):7. https://doi.org/10.4103/1118-8561.129145

13. Ricci S, De Giorgi S, Lazzeri E, Luddi A, Rossi S, Piomboni P, De Leo V, Pozzi G (2018) Impact of asymptomatic genital tract infections on in vitro fertilization (IVF) outcome. PloS one 13(11):e0207684
14. Isaiah IN, Nche BT, Nwagu IG, Nnanna II (2011) Current studies on bacterospermia, the leading cause of male infertility: a protégé and potential threat towards man's extinction. N Am J Med Sci 3(12):562–4
15. Audu BM, Massa AA, Bukar M, Melah GS, Kudi A (2010) Effect of bacterial isolates on the seminal indices of men. Niger J of Clinical Pract 13:16–9
16. Mamdooh A. Al_ Nasrawi, Loqman J Tawfiq (2020) Bacterial infection and seminal fluid parameters in Iraqi sub fertile men. Indian J Forensic Med Toxicol 14(3):1405–1410. https://doi.org/10.37506/ijfmt.v14i3.10604
17. Agyepong E, Bedu-addo K (2019) Semen parameters and the incidence and effects of bacteriospermia in male partners of infertile couples attending a fertility clinic in the Kumasi Metropolis, Ghana. Int J Reprod Contracept Obstet Gynecol 8(1):99–107
18. Ahmad S, Wasim S, Tiwari N, Verma V, Gupta N, Mishra N (2016) Evaluation of bacteriospermia as etiology for oligospermia: an analysis. Int J Sci Study 4(2):194–7. https://doi.org/10.17354/ijss/2016/283
19. Omosigho OP, Emumwen EGlH (2015) Prevalence of bacteriospermia among male partners of infertile couples in Bida. Niger State. Niger Biomed Sci 2:211
20. Cottel E, Lennon B, McMorrow J, Barry-Kinsella CHR (1997) Processing of semen in an antibiotic-rich culture medium to minimize microbial presence during in vitro fertilization. Fertil Steril 67:98–103
21. Owolabi AT, Fasubaa OB, Ogunniyi SO (2013) Semen quality of male partners of infertile couples in Ile-Ife. Nigeria. Niger J Clin Pract 16(1):37–40
22. Zuhair, A., Hussein, G., & Al-bassam, A. N. (2019). Bacterial contents of seminal fluid of Iraqi infertile men. Al-Nisour J Med Sci, 1(1). http://lms.nuc.edu.iq/journals/web/articles/1-5.pdf.
23. Vlaisavljevi V, Relji M (2002) Does the in vitro fertilization procedure require bacteriological investigation of normozoospermic ejaculate ? Gynaecol Perinatol 11(2):69–72
24. Shalika S, Dugan K, Smith RD, Padilla SL (1996) The effect of positive semen bacterial and Ureaplasma cultures on in-vitro fertilization success. Hum Reprod 11(12):2789–92
25. Wang H, Chow SC (2014). Sample size calculation for comparing proportions. https://doi.org/10.1002/9781118445112.stat07091
26. Król J, Żarski D, Bernáth G, Palińska-Żarska K, Krejszeff S, Długoński A, Horváth Á (2018) Effect of urine contamination on semen quality variables in Eurasian perch Percafluviatilis L. Anim Reprod Sci 197:240–246. https://doi.org/10.1016/j.anireprosci.2018.08.034
27. Colonization v. infection - Louisiana Department of Health. https://ldh.la.gov/assets/oph/Center-PHCH/Center-CH/infectious-epi/HAI/MDRO2017/handouts/ColonizationvInfection.pdf.
28. Kaur, S., Prabha, V., & Shukla, G. (2010). Interference of human spermatozoal motility by live Staphylococcus aureus. Am J Biomed Sci, 91-97.
29. Jue JS, Ramasamy R (2017) Significance of positive semen culture in relation to male infertility and the assisted reproductive technology process. Transl Androl Urol 6(5):916–922
30. Shakeel W, Isaac A, Sonia R, Rabia S, Moh'd N et al. (2017) Bacteriospermia among asymptomatic infertile males, a trigger for morphological and physiological deterioration of spermatozoa. Glob J Intellect Dev Disabil 2(2). https://doi.org/10.19080/GJIDD.2017.02.555584.
31. Shu JH, Zhang B, Feng GX, Gan XY, Zhou H, Zhou L et al (2010) Influence of sperm morphology on the outcomes and neonatal status in IVF-ET. Zhonghua Nan Ke Xue 16(10):897–900
32. Franasiak JM, Scott RT Jr (2015) Reproductive tract microbiome in assisted reproductive technologies. Fertil Steril 104(6):1364–71. https://doi.org/10.1016/j.fertnstert.2015.10.012
33. Multiple pregnancies following assisted conception: Scientific Impact Paper No. 22. (2018). BJOG 125(5), e12–e18. https://doi.org/10.1111/1471-0528.14974

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:

- ► Convenient online submission
- ► Rigorous peer review
- ► Open access: articles freely available online
- ► High visibility within the field
- ► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com