

The role of oxidative stress on oocyte yield in women undergoing *in vitro* fertilization at a tertiary centre in Benin City

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Abstract

Over the years, infertility and its management have been a global challenge of public health concern. Approximately 5-8% of couples globally experience an inability to achieve pregnancy within a year of regular sexual intercourse. Infertility affects couples' harmonious existence adversely in all spheres, economically, socially, emotionally as well as mentally. *In vitro* Fertilization (IVF) technology is one of the advances in the management of infertility. This technology has been able to help couples achieve their fertility desires. Despite the advancement in IVF, the success rate is still low. This study aimed to determine the effect of oxidative stress on oocyte yield among women undergoing IVF. This prospective cross-sectional study involved women undergoing oocyte retrieval for IVF procedures. An assay of antioxidant enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and catalase was carried out on follicular fluid samples collected during oocyte retrieval. Relevant data were obtained from the study participants. They were grouped into good, poor, and no oocyte yield; the levels of oxidative stress markers were analyzed. There was a statistically significant higher level of antioxidant enzymes in women with good oocyte yield compared to those with poor and no oocyte yield (SOD 0.56 ± 0.06 vs 0.47 ± 0.10 , vs 0.40 ± 0.14 , $p < 0.0001$; catalase 0.30 ± 0.07 vs 0.20 ± 0.06 vs 0.12 ± 0.06 , $p < 0.0001$, GPX 1.17 ± 0.16 vs 0.86 ± 0.20 vs 0.66 ± 0.18 , $p < 0.0001$). There was a positive correlation between oocyte quality, oocyte maturation and levels of antioxidant enzymes ($r = 0.42$ SOD, 0.69 catalase, 0.68 GPX). Younger participants (< 30 years) and those with normal Body Mass Index (BMI) had good oocyte yield compared to older participants and those who were overweight/obese (68.8% vs 38.5% , $p < 0.001$, and 64.8% vs 37.0% $p = 0.01$), respectively. This study suggests that oocyte yield, quality and maturation are affected by oxidative stress within the follicular fluid, as individuals with good oocyte yield, maturity and quality had higher mean levels of antioxidant enzymes in their follicular fluid.

Introduction

Infertility is a global public health challenge. Its management has continued to be challenging globally. Infertility affects 5-8% of couples worldwide.^{1,2} The prevalence of infertility is higher in low and middle-income countries, with a rate as high as 30% in countries within the Sub-Saharan region.^{1,3,4}

One of the most common reasons for seeking gynaecological care has been reported to be infertility.^{1,5} The burden of infertility is not limited to the couples' lives only but also extends to involve the healthcare services and the social environment. With thorough evaluation and application of current treatments, 50-60% of infertility couples will conceive.^{4,6,7}

Assisted Reproductive Technologies (ART) have been instrumental in proffering solutions to this burden of infertility, which has multifactorial causes. These technologies have evolved tremendously and have recently become the most popular *In Vitro* Fertilization (IVF).^{4,8}

Some couples have been able to fulfil their fertility desires through these technologies, especially in Sub-Saharan Africa, where a premium is still placed on childbirth.⁹ These facilities are still limited in access and services in low- and middle-income countries of the world, as it has been reported that less than 1.5% of the African population can access ART.¹⁰

Despite the advancement in IVF and other ART procedures, success rates are reported in only about 1/3 of cases with over 60% failure rates.^{4,11} Unsuccessful IVF procedure has been attributed to many factors, ranging from age, Body Mass Index (BMI), genetic factors, serum levels of hormones, and semen and oocyte characteristics as well as IVF protocols.^{12,13} The contributions of these factors to unsuccessful IVF differ between individuals.

Consequently, in a bid to improve the outcome of IVF, many studies have been done, especially on female infertility, with the ovary playing a central role.¹¹⁻¹⁴

The ovarian follicular fluid provides a micro-environment which is of significant influence on the growth, maturation as well and quality of oocytes, and this is a substantial determinant of the success of IVF.¹¹⁻¹⁵

The ovarian follicular fluid has been found to contain leucocytes, macrophages and cytokines.¹⁴ The activities of these substances have been shown to produce reactive oxygen species, which affects oocyte growth and maturation.^{14,15} Reactive oxygen species also play a beneficial role in folliculogenesis, signalling the proteomic activities leading to oocyte maturation.¹⁶ For effective oocyte development and maturation, there has to be a balance between reactive oxygen species and antioxidants within the ovarian follicular fluid microenvironment.

The cause of female infertility with the aim of improving the success of IVF procedures has received attention from researchers.¹⁶⁻²¹ Despite these studies, there seems to be little or no improvement in the success rate of IVF.^{20,21} Emerging evidence has shown that oxidative stress could harm the oocyte microenvironment due to the imbalance between reactive oxygen species and inherent antioxidants, resulting in poor oocyte quality, fertilization capacity and embryo quality.^{24,25} However, the few studies done in this regard were in developed countries, with a paucity of data in our setting despite geographical and individual differences that may affect such studies.^{19,24}

This study aims to evaluate the role of oxidative stress on oocyte yield in women undergoing IVF by assaying the levels of enzymatic oxidative stress markers such as superoxide dismutase, catalase and glutathione peroxidase in the follicular fluids. The emanating data may show the need to introduce antioxidants in the protocol for the management of female infertility.

Materials and Methods

A prospective cross-sectional study among women planned for IVF was carried out over 12 months. The participants were all consenting women undergoing oocyte retrieval for IVF procedures, including oocyte donors. Women with medical disorders such as hypertension, diabetes, chronic renal or liver diseases, and those who do not give consent were excluded.

A non-probability sampling technique was used to recruit study participants until the required sample size of 90 was obtained. All par-

ticipants had their menses synchronized with combined oral contraceptive pills, as the IVF procedure was done in batches. Agonist or antagonist protocol was used for ovarian stimulation. A transvaginal ultrasound was done for the antral follicular count on day three, as well as the anti-Mullerian hormone before the treatment cycle, as this was used as the basis for treatment decisions. Follicular fluid devoid of macroscopic blood contaminants at oocyte retrieval was collected in a sterile universal sample bottle. Superoxide dismutase level was determined according to the methods of Misra and Fridovich. Catalase was determined using the method described by Cohen *et al.* Glutathione peroxidase was determined by Nyman's method. Participants were grouped into good responders (>10 oocytes), poor responders (<10 oocytes) and non-responders (no oocytes). Oocyte assessment was based on oocyte maturation (oocytes at metaphase II) and oocyte quality. Good quality oocytes refer to oocytes without shape anomalies, perivitelline space granularity and zona pellucida defects.¹⁷ Analysis of obtained data was done using IBM Statistical Package for Social Sciences (SPSS) version 25. The information provided was encoded and appropriately entered. The association between categorical exposure variables such as age, education level,

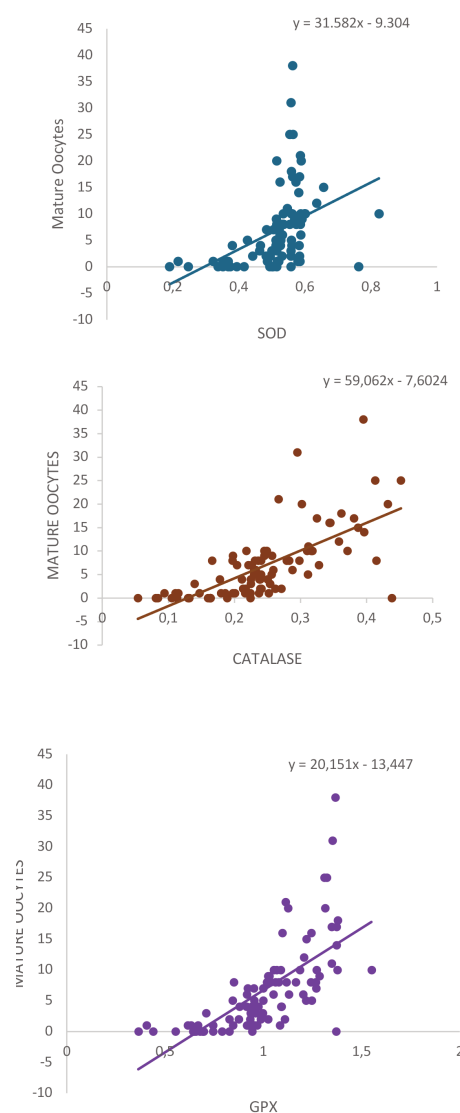


Figure 1. Correlation matrix graph of oxidative stress markers and oocyte maturity.

and oxidative stress was analyzed using Chi-square. Association between continuous variables such as obesity, duration of infertility, identifiable cause of infertility and the outcome variable (oxidative stress) was measured using the Student t-test. Bivariate analysis was done to show the relationship/association between oxidative stress and outcome measures. Correlation was done to show the relationship between antioxidant enzymes and oocyte maturation and quality. Statistical test of significance was done at $p < 0.05$ while the confidence interval was set at a 95% confidence limit. The results of the analysis were presented as frequency distribution tables and figures. A data extraction sheet was used to document participants' sociodemographic and clinical characteristics. Ethical approval was obtained from the Ethical and Research Committee of the University of Benin Teaching Hospital.

Results

A total of 213 follicular fluids were retrieved and subjected to analysis. Over 53.3% of the study participants were below 30 years of age, with a mean age distribution of 29.3 ± 7.2 . Most had normal BMI, as 60% of the study participants fall within this group with a mean BMI of 24.9 ± 3.4 . Over 70% of the participants had a tertiary level of education, with the most common indication for ART being male factor infertility (38.6%) (Table 1). Most participants

(51.1%) had an excellent ovarian response, with ten or more oocytes retrieved. Poor ovarian response was noted in 43.3%, while 5.6% had no oocyte retrieved from their follicular fluids (Table 2). A statistically significantly higher number of oocytes was retrieved from participants younger than 30 years and those within normal BMI (Table 3). The individuals with good ovarian response were found to have statistically significant higher levels of these antioxidant enzymes (Table 4). A positive correlation was noted between oxidative stress markers and the maturity of oocytes (M11). The mean levels of these oxidative stress markers were significantly higher in mature oocytes' follicular fluids than in immature oocytes (Table 5). The correlation matrix graph shows a positive correlation between oxidative stress markers and oocyte maturity (Figure 1). Good-quality oocytes had higher mean levels of these antioxidant enzymes (Table 6). Correlation matrix graphs show a positive correlation between these antioxidant enzymes and oocyte quality (Figure 2).

Table 1. Sociodemographic and clinical characteristics of study participants.

Variables	Frequency	Percentages
Age group		
<30	48	53.3
30-35	13	14.4
35-40	25	27.8
>40	4	4.4
Mean age	29.3 ± 7.2	
BMI group (kg/m ²)		
Normal weight	54	60.0
Overweight	27	30.0
Obese	9	10.0
Mean BMI (kg/m ²)	24.9 ± 3.4	
Education		
Secondary	17	18.9
Tertiary	73	81.1
Tribe		
Bini	30	33.3
Igbo	16	17.8
Yoruba	9	10.0
Others+	35	38.9
Indication for ART		
Male factor	17	38.6
Tubal factor	8	18.2
Anovulation	4	9.1
Endometriosis	7	15.9
Unexplained	8	18.2
Duration of infertility (Group)		
1-4	11	25.6
5-9	26	60.5
≥ 10	6	14.0

+Others: Agbor, Esan, Etsako, Ijaw, Isoko, Ogoni, Owan, Tsekiri, Urhobo. BMI, Body Mass Index; ART, Assisted Reproductive Technologies

Table 2. Ovarian response.

Variables	Frequency	Percentages
Good responses (>10 oocytes)	46	51.1
Poor responses (<10 oocytes)	39	43.3
No responses (No oocytes)	5	5.6

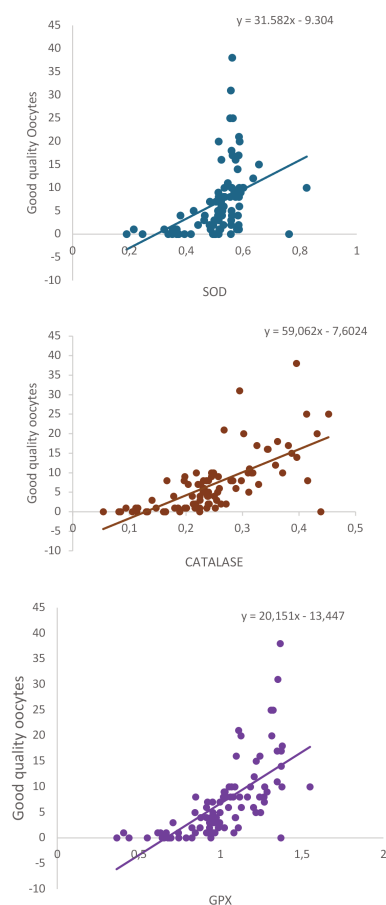


Figure 2. Correlation matrix graph of antioxidant enzymes and oocyte quality.

Table 3. Chi-square test of association of variables and oocyte yield.

Variable	No response	Poor response	Good response	Chi-square (χ^2)	p-value
Age group					
<30	0 (0.0)	15 (31.3)	33 (68.8)	22.150	<0.001*
30-35	0 (0.0)	8 (61.5)	5 (38.5)		
35-40	4 (16.0)	13 (52.0)	8 (32.0)		
>40	1 (25.0)	3 (75.0)	0 (0.0)		
BMI group (kg/m ²)					
Normal weight	1 (1.9)	18 (33.3)	35 (64.8)	13.344	0.01*
Overweight	3 (11.1)	14 (51.9)	10 (37.0)		
Obese	1 (11.1)	7 (77.8)	1 (11.1)		
Indication for ART					
Male factor	0 (0.0)	8 (47.9)	9 (52.1)	15.010	0.059
Tubal factor	1 (12.5)	5 (52.5)	2 (25.0)		
Anovulation	0 (0.0)	4 (100.0)	0 (0.0)		
Endometriosis	3 (42.9)	3 (42.9)	1 (14.3)		
Unexplained	5 (12.5)	25 (62.5)	14 (25.0)		
Duration of infertility (Group)					
1-4	0 (0.0)	5 (45.5)	6 (54.5)	8.200	0.085
5-9	3 (11.5)	16 (61.5)	7 (26.9)		
≥10	2 (33.3)	4 (66.7)	0 (0.0)		

* Statistically significant. BMI, Body Mass Index; ART, Assisted Reproductive Technologies.

Table 4. Comparing oocyte yield with mean values of oxidative stress markers.

Variable	Good response (N=46)	Poor response (N=39) Mean±SD	No response (N=5)	F-statistics	p-value
SOD	0.559±0.056	0.474±0.103	0.402±0.1416	15.179	<0.0001*
Catalase	0.2968±0.070	0.201±0.065	0.124±0.056	29.894	<0.0001*
GPX	1.172±0.1615	0.864±0.1998	0.663±0.182	40.610	<0.0001*

*Statistically significant. SOD, Superoxide Dismutase; GPX, Glutathione Peroxidase.

Table 5. Correlation matrix of oxidative stress markers with oocyte maturity.

Variable		Mature oocytes	Immature oocytes	SOD	Catalase	GPX
Mature oocytes	r	1				
	p					
Immature oocytes	r	0.641	1			
	p	<0.0001*				
SOD	r	0.417	0.276	1		
	p	<0.0001*	0.008*			
Catalase	r	0.693	0.489	0.634	1	
	p	<0.0001*	<0.0001*	<0.0001*		
GPX	r	0.676	0.470	0.809	0.860	1
	p	<0.0001*	<0.0001*	<0.0001*	<0.0001*	

*Statistically significant at the 0.05 level. r, Pearson correlation; SOD, Superoxide Dismutase; GPX, Glutathione Peroxidase.

Table 6. Correlation matrix of oxidative stress markers with oocyte quality.

Variable		Good quality oocytes	SOD	Catalase	GPX
Good quality oocytes	r	1			
	p				
SOD	r	0.426	1		
	p	<0.0001*			
Catalase	r	0.683	0.634	1	
	p	<0.0001*	<0.0001*		
GPX	r	0.665	0.82	0.85	1
	p	<0.0001*	<0.0001*	<0.0001*	

*Statistically significant at the 0.05 level. r, Pearson correlation; SOD, Superoxide Dismutase; GPX, Glutathione Peroxidase.

Discussion

Assisted reproductive technologies have impacted positively in reducing the burden of infertility globally. Success rates of these procedures continue to require improvement, a goal shared by medical professionals and patients.

Oocyte maturation and quality are crucial to the success and effectiveness of assisted reproductive technologies as they influence fertilization rate, embryo quality and pregnancy rate.²⁴⁻³¹ The development of oocytes occurs in the follicular fluid micro-environment, as this micro-environment has been found to determine oocyte growth, maturation and quality.³²⁻⁵⁶

The key findings of this study were statistically significant higher mean levels of oxidative stress markers SOD, GPX and catalase in the follicular fluids of women with good oocyte yield compared to those with poor oocyte yield. Also, there was a positive correlation between these oxidative stress markers with oocyte maturity and quality.

In this study, mean levels of antioxidants SOD, GPX and catalase were found in the follicular fluid of women undergoing oocyte retrieval for ART procedures, with GPX having the highest concentration. Similarly, Carbone *et al.* noted the presence of these antioxidant enzymes within the follicular fluid.⁵⁷ Oyawoye demonstrated the effects of these antioxidants by determining the total antioxidant capacity within the follicular fluid.²²

Superoxide dismutase activity was shown in this study to be associated with oocyte yield, maturation and quality. A higher level of superoxide dismutase enzyme was found in the follicular fluids of women with good oocyte yield than those with poor oocyte yield. Glutathione peroxidase and catalase antioxidant enzyme levels were equally higher in women with good oocyte yield. These findings align with the study by Nunez Calonge *et al.*⁴⁷ The study further noted that high ovarian response patients had higher follicular fluid antioxidant profiles when compared with those with low ovarian response, confirming that reduced antioxidant capacity in follicular fluid correlates positively with reproductive failure.^{42-47,50-54} Thaker *et al.* also noted high mean levels of antioxidant enzymes in the follicular fluids of women with good oocyte yield compared to those with poor ovarian response.⁵⁸ Babuska *et al.*, in a similar study comparing oxidative stress markers for infertile women and fertile oocyte donors, noted higher levels of mean values of follicular fluid antioxidants in fertilized oocytes as against oocytes with fertilization failure.⁵⁹⁻⁶¹

In contrast, Appasamy and colleagues find no significant association between levels of antioxidants and oocyte yield.^{18,55-57} Pasqualotto *et al.* noted no correlation between the total antioxidant levels and the patient's age, oocyte maturity, fertilization rate or quality of the embryos. However, it was observed that the Total Antioxidant Capacity (TAC) in the follicular fluids of the patients who got pregnant was significantly higher than that of the non-pregnant group.⁴⁵

Like the studies by Babuska *et al.*⁵⁹ and Nunez Colonge *et al.*,⁴⁷ a positive correlation between mean levels of oxidative stress markers and oocyte maturity was noted in this study. Participants with M11 oocytes were found to have higher levels of oxidative stress markers, SOD, GPX and catalase in their follicular fluids. Also, individuals with low ovarian response had low mean levels of antioxidants (Glutathione peroxidase, glutathione reductase and glutathione transferase). On the contrary, Oyawoye *et al.* found no relationship between TAC and oocyte maturity or quality.²² This difference might be due to estimating the effect of single antioxi-

dant enzymes against the TAC.

The quality of oocytes is crucial in determining the success of IVF. Oxidative stress has been shown to affect oocyte quality and fertilization rates. A positive correlation was found in this study between oocyte quality and mean levels of antioxidants. Das *et al.* corroborated this, noting a significant negative correlation between ROS levels in follicular fluid and embryo quality.³⁷

Conclusions

Oxidative stress has been identified as one of the factors that could influence IVF outcomes as it affects oocyte maturation and quality as well as fertilization rates and embryo quality. The routine evaluation of oxidative stress could serve as an adjunct in the management of infertile women.

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