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Comparison of serum reproductive hormones, antioxidants, PGE2 and PGF2- α between primary and secondary infertile women in Calabar

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ABSTRACT

Objective: To compare the prolactin, progesterone, luteinizing hormone, follicle stimulating hormone, estradiol, antisperm antibodies, tumor necrosis factor α , total antioxidant capacity, prostaglandin E₂ and F_{2 α} between primary and secondary infertile women in Calabar. **Methods:** One hundred and two volunteers infertile women (test group), aged 20-45 years attending the infertility clinic in the University of Calabar Teaching Hospital (UCTH) were recruited. Fourteen of the women had primary infertility while 88 of them had secondary infertility. Sixty apparently healthy, age matched women served as the control group. Five millilitres of blood was collected, allowed to clot and serum was obtained from the subjects and Enzyme Linked Immunosorbent Assay method was used for prolactin, progesterone, estradiol, follicle stimulating hormone (FSH) and luteinizing hormone (LH), prostaglandin E₂ and F_{2 α} , human TNF- α , *Chlamydia trachomatis* IgG, antisperm antibodies assay, while serum total antioxidant capacity was assessed spectrophotometrically. **Results:** The result shows on comparison that primary infertile women has a significantly higher level of progesterone than those with secondary infertility ($P>0.05$). There was a significant difference in the levels of prolactin and total antioxidant capacity in the primary and secondary infertility when compare to the control group at $P>0.05$. Their mean ages were (31.10 \pm 5.37) years and (33.10 \pm 4.91) years respectively. There was a positive correlation between TAC and FSH, TNF and anti-sperm anti-bodies in the test group of $r=0.207$ and $r=0.632$; $P>0.05$ respectively. **Conclusions:** These findings suggest no alterations in levels of prostaglandin F_{2 α} , TNF and anti-oxidant between primary and secondary infertile females.

1. Introduction

Childlessness is generally regarded a tragedy to the married woman in Africa and can be a cause of marital upset as well as of personal unhappiness and ill health. Such couples usually feel ostracized and depressed; this feeling is more pronounced in the females than in males. Infertility is defined as the inability of a couple to achieve conception despite frequent unprotected, well

timed sexual intercourse for one year duration. It also includes the inability of a woman to carry a pregnancy to the delivery of a live baby[1]. Infertility occurs in one out of five couple of reproductive age and in 10%-20% of these cases, there seems to be no definitive

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cause, therefore these are classified as unexplained infertility[2,3]. Infertility can be broadly classified as primary or secondary infertility. The term primary infertility is used to describe a couple who have never been able to conceive a pregnancy after at least 1 year of unprotected intercourse. While secondary infertility describes couples who have previously been pregnant at least once but have not been able to achieve another pregnancy[2,4]. Causes of infertility include a wide range of factors, with 30%-40% of infertility due to a male factor such as retrograde ejaculation, impotence, hormone deficiency, scarring from sexually transmitted disease or decreased sperm count[5]. The male factor is associated with more cases of primary infertility than secondary infertility[6]. While female factor causes are associated more with secondary infertility with 25%-40% prevalence; these female factors include ovulatory problems, cervical factors, pelvic factors, tubal factors, and uterine factors. Oftentimes, combinations of these problems exist[3,7]. Adetoro and Ebomoyi[8] reported infertility prevalence of 30.3% among women living in Nigeria. Studies by Sule *et al.*[9] done on the prevalence of infertility in woman in South Western Nigerian and 22.5% had the prevalence of primary infertility, while 77.5% had secondary infertility. A retrospective study done by Ekwere *et al.*[10], on patients coming to the Obstetrics and Gynaecology clinic in Calabar, the causes of infertility was 58% female, 30% male while 12% were both causes. Primary infertility was found in 69.7% of the males and 34.5% of the female, while secondary infertility was 30.3% in males and 65.5% in females. Infection is the strongest predisposing factor. In addressing infertility, it should be recalled that social and environmental factors, as well as physiological and genetic ones, contribute to the condition[11]. The aim of this study therefore was to estimate the comparison of prolactin, progesterone, luteinizing hormone, follicle stimulating hormone, estradiol, antisperm antibodies, tumor necrosis factor α , total antioxidant capacity, prostaglandin E_2 and $F_{2\alpha}$ between primary and secondary infertile women.

2. Materials and methods

A group of women attending the infertility clinic in the Department of Obstetrics and Gynecology and coming for infertility test in the Department of Chemical Pathology of the University of Calabar Teaching Hospital (UCTH) were selected for the study. The selection of patients was done with the help of a Gynaecologist in the Obstetrics and Gynaecology Department of the hospital. One hundred and two volunteers infertile women (test group), aged 20-45 years were recruited and further grouped as primary and secondary infertility as appropriate. Fourteen of the women had primary infertility while 88 of them had secondary infertility. Sixty

apparently healthy, age matched women who had given birth to at least one child within the last three years, were selected to serve as the control group. Approval was given from the Health Research Ethical Committee (HREC) of the Hospital and the subjects all gave informed consent to participate in the study. Their confidentiality was maintained. Inclusion criteria used was that apparently healthy women with infertility problems and healthy women who had given birth to at least one child within the last three years while women who were pregnant or older than 45 years of age and those with high blood pressure and diabetes were excluded.

Five milliliters of venous blood was obtained from these test subjects in the luteal phase from day 21 to day 23 of their menstrual cycle into clean plain bottles (containing no anticoagulants). The blood was allowed to clot and was centrifuged at 3 000 r/min for 5 min. The serum was then separated by the use of pasteur pipettes into serum containers with tight screw caps and was stored at -20°C in aliquots of 1 mL until ready for use. The sample was used for prolactin, progesterone, estradiol, follicle stimulating hormone (FSH) using DRG Enzyme immunosorbent assay while luteinizing hormone (LH), prostaglandin E_2 and $F_{2\alpha}$, Human TNF- α , Chlamydia trachomatis IgG, antisperm antibodies assays using ELISA method and total antioxidant capacity using the total antioxidant status assay kit ([RL0017 (product code)]; Rel Assay Diagnostics, turkey).

The data was analysed using Microsoft Excel and PASW (Predictive Analysis Software) version 18 packages from SPSS Inc. USA.

3. Results

Prolactin, progesterone, LH, FSH, estradiol (E_2), Chlamydia trachomatis IgG, TNF- α , anti-sperm anti-bodies, PGE_2 and $F_{2\alpha}$ and total anti-oxidant capacity levels were estimated in a total of 102 infertile female. Sixty women who have had at least one child within the last three years were used as controls (fertile women). Their mean ages were (31.10 ± 5.37) years and (33.10 ± 4.91) years, respectively. Fourteen of the women had primary infertility while 88 of them had secondary infertility. Table 1 shows the comparison of prolactin, progesterone, luteinizing hormone, follicle stimulating hormone, estradiol, anti-sperm anti-bodies, tumor necrosis factor α , total anti-oxidant capacity, prostaglandin E_2 and $F_{2\alpha}$ between primary infertile women was significantly higher than those with secondary infertility ($P < 0.05$). While there was no significant difference among prolactin groups of LH, FSH, E_2 , ASA, TNF, $PGE_2/PGF_{2\alpha}$, TAS, PGE_2 and $F_{2\alpha}$ ($P < 0.05$).

Table 1

Comparison of prolactin, progesterone, luteinizing hormone, follicle stimulating hormone, estradiol, antisperm antibodies, tumor necrosis factor α , total antioxidant capacity, prostaglandin E₂ and F_{2 α} between primary and secondary infertile women.

Group parameter	Primary infertility	Secondary infertility	Calc t	Crit t
Prolactin (ng/mL)	47.60±12.50	45.20±3.80	0.185	2.1
Progesterone (ng/mL)	17.70±0.48*	11.60±0.69	7.200	2.1
LH (μ U/L)	7.90±1.72	10.20±1.51	0.992	2.1
FSH (μ U/L)	8.30±2.60	5.20±0.90	1.110	2.1
E ₂ (pg/mL)	93.30±8.80	87.20±4.30	0.625	2.1
Antisperm (U/mL)	90.60±4.00	91.20±4.00	0.105	2.1
TNF- α (pg/mL)	49.30±17.60	30.10±6.80	1.020	2.1
TAC (mmol TROLOX EQIV/L)	1.27±0.13	1.25±0.03	0.166	2.1
PGE ₂ (pg/mL)	340.70±43.10	305.00±21.40	0.740	2.1
PGF _{2α} (pg/mL)	1800.00±116.70	3408.10±761.00	2.090	2.1
PGE ₂ /PGF _{2α}	0.20±0.03	0.20±0.02	0.090	2.1
N	14.00	88.00	-	-

Values are expressed as mean±SEM; $P>0.05$; * $P<0.05$.

Table 2 shows the analysis of variance in prolactin, ASA, TNF, PGE₂, PGF_{2 α} and TNF among the primary, secondary infertile and control. There was a significant difference in the levels of prolactin and total anti-oxidant capacity ($P>0.05$), while there was no significant variation in the levels of ASA, TNF α , PGE/PGF ratio, prostaglandin E₂ and F_{2 α} ($P<0.05$).

Table 2

Comparison of prolactin, antisperm antibodies, tumor necrosis factor- α , prostaglandin E₂ and F_{2 α} , and total antioxidant capacity level in controls, primary and secondary infertile.

Group parameters	Primary infertility	Secondary infertility	Controls	Calc f	Crit f
Prolactin(ng/mL)	47.60±12.50*	45.20±3.80*	9.20±1.60	27.17	3.05
Antisperm(U/mL)	90.60±4.00	91.20±4.00	88.80±3.50	0.10	3.05
TNF- α (pg/mL)	49.30±17.60*	30.20±6.80	15.30±3.00	2.90	3.05
TAC(mmol TROLOXEQIV/L)	1.28±0.13*	1.25±0.03*	1.10±0.03	5.76	3.05
PGE ₂ (pg/mL)	340.70±43.10	305.10±21.40	264.60±27.20	1.14	3.05
PGF _{2α} (pg/mL)	1800.00±116.70	3408.20±761.20*	1371.70±105.80	2.80	3.05
PGE ₂ /PGF _{2α}	0.20±0.03	0.20±0.02	0.20±0.03	0.03	3.05
N	14.00	88.00	60.00	-	-

Values are expressed as mean±SEM; *significantly higher than controls; $P<0.05$.

There was a negative correlation ($r=-0.330$; $P>0.05$) between of PGF_{2 α} and anti-sperm anti-bodies in the control group in Figure 1. The correlation graph of TAC against TNF in the control group was positive ($r=0.335$; $P>0.05$) in Figure 2. While Figure 3 shows the correlation graph of TAC against FSH in the test group. It was a positive correlation ($r=0.207$; $P>0.05$). Figure 4 shows the correlation graph of TNF against antisperm antibodies in the test group. There was a positive correlation ($r=0.632$; $P>0.05$). Figure 5 shows the correlation graph of TAC against PGF_{2 α} in the control group. There was a positive correlation ($r=0.296$; $P>0.05$).

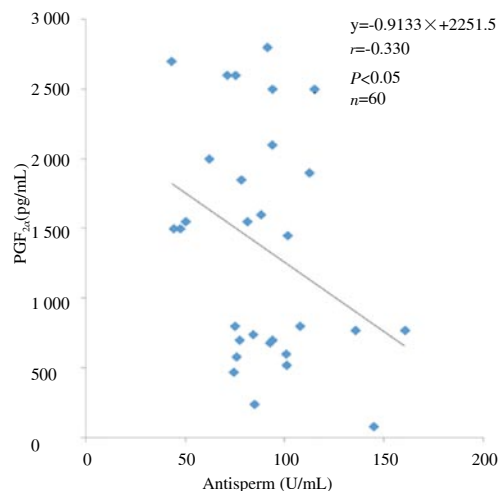


Figure 1. Correlation graph of PGF_{2 α} against antisperm antibodies in the control group. There was a negative correlation.

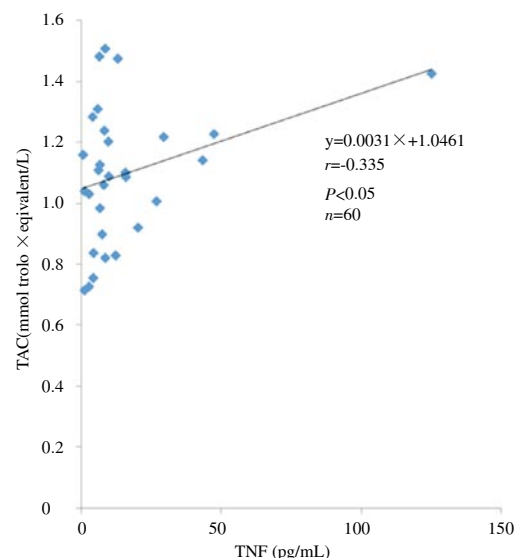


Figure 2. Correlation graph of TAC against TNF in the control group. There was a positive correlation.

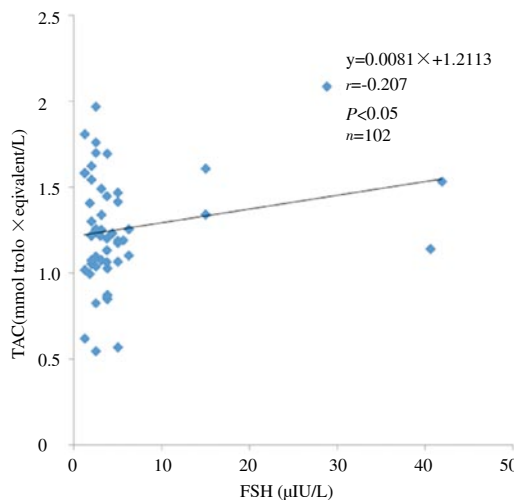


Figure 3. Correlation graph of TAC against FSH in the infertile group. There was a positive correlation.

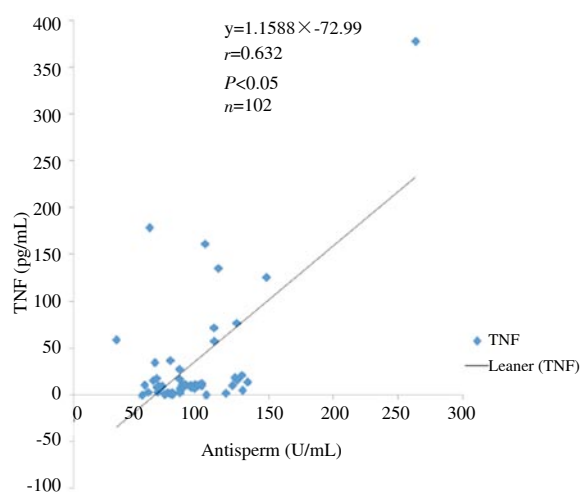


Figure 4. Correlation graph of TNF against antisperm antibodies in the infertile group.

There was a positive correlation.

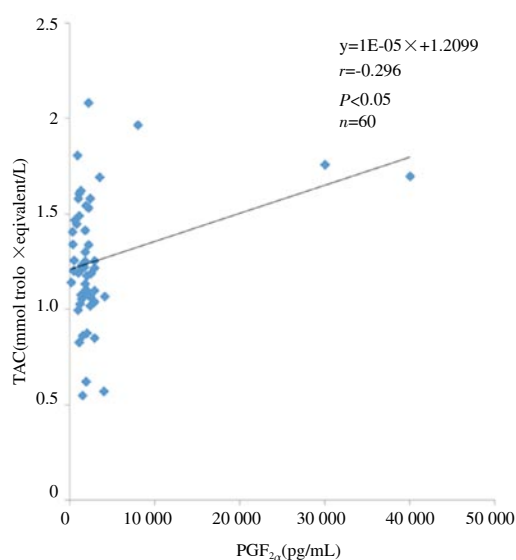


Figure 5. Correlation graph of TAC against $PGF_{2\alpha}$ in the control group. There was a positive correlation.

4. Discussion

This study was conducted to determine and compare serum levels of prolactin, progesterone, LH, FSH, estradiol, ASA, TAC and Chlamydia trachomatis IgG in fertile and infertile (primary and secondary) women. There was a positive correlation between tumor necrosis factor and total antioxidant capacity in control subjects. $TNF-\alpha$ and $IFN-\gamma$ generates immune response which leads to enhanced proinflammatory cytokines and free radical production[12]. The positive correlation may be because glutathione, which is an antioxidant, is known to modulate cellular production of cytokine T helper cells responses, TH_1 or TH_2 [13]. Patients with a history of recurrent abortions were associated with elevated levels of the TH_1 response and glutathione, and depletion of this antioxidant inhibits TH_1 type cytokines. Rainer *et al.*[14] also found another antioxidant; mitochondrial manganese superoxide dismutase (MnSOD)

readily inducible by various cytokines such as tumor necrosis factor alpha ($TNF-\alpha$), interleukin-1 (IL-1), and $IFN-\gamma$. This up-regulation compensates for increased oxygen consumption in situations of oxidative stress. Manganese superoxide dismutase is highly inducible by various cytokines, and the SOD_2 transgene contains the promoter and intron II elements known to confer inducibility by cytokines.

There was a strong positive correlation between tumor necrosis factor and anti-sperm antibody (ASA) in the infertile women. The positive correlation may be due to the fact that patients with immunological infertility usually have high levels of ASA and such patients usually have higher concentrations of $TNF-\alpha$ in their follicular fluid compared to the control group. Under physiological conditions, TNF is involved in immune surveillance and defence, in cellular homeostasis, protection against certain neurological insults as well as in the control of cell survival, proliferation, migration and differentiation. Owing to its strong pro-inflammatory and immune stimulatory activities, TNF is associated with a number of pathological events. The cytokine is involved in the progression of many autoimmune diseases.

There was a negative correlation between prostaglandin $F_{2\alpha}$ and antisperm antibody. The mechanism of interaction is poorly understood, but studies by Kim *et al.*[15] found that prostaglandin inhibited antibody dependent cytotoxicity by binding effector and target cells. This effect was dose dependent. The concentration of $PGF_{2\alpha}$ in semen samples correlated negatively with motility in normal men and was always higher in men with disturbed fertility[16].

In this study, the levels of prostaglandin E_2 did not differ significantly between the infertile group and control nor did it vary when compared among the infertile groups ($P > 0.05$). Our work differs from those by Khan *et al.*[17], Badamy *et al.*[18], and Ryantova *et al.*[19] who observed higher levels of PGE_2 in patients with repeated unexplained miscarriages and endometriosis. Our findings however were similar to work done by Holme *et al.*[19] who did not have E_2 significantly higher in endometrial infertile women when compared to controls. Slater *et al.*[20] proposed that PGE_2 in women reduces the release of factors associated with cervical ripening and may act to down regulate some of the processes that contribute to the onset of human labour and may be beneficial in helping to maintain pregnancy towards term. Despite several investigations, a precise role for PGs other than $PGF_{2\alpha}$ in regulation of corpus luteum function is still obscure, especially their intercellular receptors and their intracellular signalling mechanisms[21]. Our recent work reveal prolactin and E_2 values were significantly ($P < 0.05$) higher in the high group compared to the normal ovarian profile and moderate group while progesterone levels were significantly higher in the normal group compared to the moderate and high groups. There was no significant difference in the values of LH and FSH among the groups ($P > 0.05$)[22].

There was a positive correlation between total antioxidant capacity and prostaglandin $F_{2\alpha}$ in controls. The reason for this is not clear but it is proposed that ovarian senescence is caused by increased oxidative stress in the follicular fluid; and $PGF_{2\alpha}$ is important for follicular rupture and it is reported as being capable of inducing ovulation[19]. The positive correlation is suggestive of increase in

the anti-oxidants to overcome the oxidative damage induced by ROS[23] reported recently that mean prolactin and total antioxidant capacity of the infertile group was significantly higher than that of the fertile group ($P<0.05$) while there was a negative correlation between TAC and prolactin in infertile women ($r=-0.196$; $P<0.05$). No significant difference ($P>0.05$) was found in the total antioxidant capacity and antisperm antibodies between the infertile women with normal hormonal profile and the controls while prolactin was high significantly ($P<0.05$) in the infertile women with normal ovarian profile than the controls.

There was a positive correlation between total antioxidant capacity and follicle stimulating hormone in the infertile women. The observed relationship may be due to the fact that the corpus luteum (CL) has a high concentration of antioxidants, particularly beta-carotene, which gives the CL its bright yellow colour[24]. Other carotenoids and vitamins C and E are also present in relatively high concentrations in the CL where they may play an important role in scavenging reactive oxidative species (ROS)[25,26].

Progesterone was significantly higher in subject grouped under primary infertility than those with secondary infertility. There is also an association between TAC and FSH, TNF and antisperm in the infertile women. Our findings that apart from progesterone, there were no significant differences in other reproductive hormones, antioxidants and prostaglandins between primary and secondary infertile women.

Conflict of interest statement

We declared that we have no conflict of interest.

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