PROSTAGLANDIN LEVELS AND SEMEN QUALITY IN MALE PARTNERS OF INFERTILE COUPLES IN ILE-IFE, NIGERIA.

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ABSTRACT:

***Objective**:* To provide data on semen prostaglandins in Nigerian men and relate this to fertility potential as provided by semen analysis results.

***Design**:* Prospective study

***Setting**:* Infertility Clinic of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria

***Subjects**:* All male partners of infertile couples who reported for male factor test at the clinic and agreed to be part of the study by completing informed consent forms.

***Results**:* The study revealed a high percentage of men with sub-normal semen .Range of PGF2α in the subjects was 0.15-11.05µg/ml with a mean of 2.77± 0.23 µg/ml while that of PGE was 21.8- 652.0 µg/ml with a mean of 248.79± 13.88 µg/ml. Among men with normal semen profile, mean PGF2α and PGE levels are 2.1± 0.32 µg/ml and 325.1± 28.3 µg/ml respectively while that in semen of men with sub-normal semen are 3.0± 0.28 µg/ml and 225.1 ± 15.1µg/ml respectively. Despite the wide range of PG values in all the groups, significant differences($P<0.05$) were found to exist between the PG values of men in the normal and sub normal semen groups. Significant differences were also found when they were grouped according to sperm count alone. However, differences observed when in the grouping according to other individual semen characteristics are not significant.

***Conclusion**:* The wide range of PG values obtained in all the groups make it difficult to make far reaching conclusions as to the relationship between PG levels and semen quality. Further research is desirable in establishing the role of PGs in sperm function.

INTRODUCTION

Prostaglandins (PGs) are lipid soluble fatty acids derived from arachidonic acid and similar precursors. They were first identified in seminal fluid and this remains the richest mammalian source. Prostaglandins are now known to have a wide distribution in mammalian tissues and much research has been carried out on their physiological role (1). In the female reproductive

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tract, they are known to contribute significantly to uterine contraction during parturition, dysmenorrhea and menorrhagia. Their role in the ovary have also been extensively described (2) .From the 19th century, seminal prostaglandins have been thought to be important in ejaculation and perhaps the facilitation of sperm transport within the female reproductive tract through a local effect on the uterine muscle(3,4)

The possible contribution, association and quantification of the various PGs in human semen and their exact roles in male reproductive tract are poorly understood .In addition, even though the levels of different semen prostaglandins have been determined in many parts of the world and various attempts made to study the relationship between prostaglandin levels and other parameters of semen quality (5,6). studies on human semen prostaglandins are scarce in Africa where high incidence of male factor infertility resulting from oligospermia, azoospermia and urinary tract infection are reported (7). This study aims at obtaining quantitative data on prostaglandin levels in male partners of infertile couples in Ile-Ife, a Nigerian community to provide baseline data on which further research could be based. Prostaglandin levels were also related with the socio economic status of the respondents to examine possible relationships.

MATERIALS AND METHODS

Subjects were made up of men who reported for routine male factor tests after their wives had presented for infertility in the Obstetrics and Gynaecology clinics of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife Nigeria in the course of the study. The men agreed to participate in the study by completing informed consent forms after the procedures and objectives of the study were explained to them. Structured Questionnaires were administered to obtain demographic data and determine the socio economic status of these men. They were asked to produce semen after 3 days of sexual abstinence by masturbation (only samples received within one hour of collection were included in the study). Complete semen analysis was carried out on the samples according to WHO procedures (8). After the determination of spermatozoal characteristics, seminal fluid was obtained from the remaining fraction by centrifugation at 3,000 G for 30 minutes using a refrigerated centrifuge. The plasma was then collected into a 1.5 ml tubes and stored in liquid nitrogen for prostaglandin assay.

PGF2α was assayed using ELISA kits. The label used is a conjugate of PGF2α with biotin. Samples were diluted 1:5,000. All standards and samples were run in duplicates while PG values were determined by reference to standard curves generated with Assay zap analysis software (Bio soft)

Total PGE was measured by a Spectroscopic assay which was a modification of the method described by Andersen (9) .This method measures the PGB isomer formed by dehydration of PGE in the presence of Alkali. Standard solution containing 1mM (350µg/ml) PGE2 was used for calibration.

Subjects were placed in 2 groups, depending on whether they had primary or secondary infertility. They were also grouped on the basis of semen analysis results as having normal or sub-normal semen .Those in the former group consisted of men whose semen met all WHO criteria as follows

1. Sperm count ≥15,000,000 spermatozoa/ml
2. Total (Progressive+non-progressive) Sperm motility ≥40% active spermatozoa
3. Sperm morphology ≥40% normal forms
4. Sperm viability ≥58% live spermatozoa.
5. Semen volume ≥1.5ml
6. pH 7.2-8.0

Those who fell short of one or more of these criteria were placed in the sub-normal semen group. Grouping was also done according to single semen characteristics-

sperm count, motility, morphology and viability. Socio economic group was determined using a scoring system based on education and occupation as described by Olusanya (10).

SPSS version 11.0 was used for statistical analysis.

RESULTS

Age range of subjects was 26-62 years with a mean of 38.8±7.3 years. Primary infertility was seen in 41.2% while 58.8% claimed to have impregnated a woman or fathered a child at one time or another. Semen analysis results show that

23.7% of the subjects had "normal" semen, going by all WHO criteria put together, while others (76.3%) were deficient in one or more criteria and grouped as having "subnormal" semen. Sperm count results show that 44.7% are normospermic, 43.9% are oligospermic while 11.4% are azoospermic.

Table I describes Prostaglandin profile of all 114 subjects involved in the study. Mean and median values obtained for PGF2α was 2.77± 0.23 and 1.98 respectively while that for PGE was 248.79± 13.88 and 250.35 respectively.

Table II gives the Prostaglandin profile obtained when the respondents were grouped by six WHO criteria put together (as described in the methodology) , by sperm count alone and then by socio economic status. Significant differences were observed in the first two groupings for both PGE and PGF2α while the socio economic groups did not differ significantly in their PG values.

Table III shows the relationship of other semen characteristics i.e. morphology, motility, viability and infection on the concentration of the PG values. Differences observed in PG values of the groups are not significant.

Table IV describes the differences in prostaglandin levels among subjects with primary infertility and those with secondary infertility.

Tables V & VI show there are no significant correlation between PGE and PGF2α as well as age and other semen quality parameters.

DISCUSSION

Our results show that a high proportion of men do not meet the WHO criteria for normal semen. This decline in semen quality is consistent with other studies carried out in this region (11,12) .The figure obtained here of men with subnormal semen (76.3%) is a reflection of worsening semen profiles showing poor reproductive health of men in this environment.

In this study PGE and PGF2 are measured using UV spectroscopy and ELISA methods respectively. Earlier studies have employed Radioimmunoassay (13), Gas chromatography (14) and Bioassay(6) among others.. PGE values are slightly higher while PGF2α is comparable with what previous authors have obtained for men in infertile marriages (15,16) Differences in methods of analysis as well as environmental factors may have accounted for the differences seen. It will be interesting to compare this with the PG profile of men with recently documented evidence of fertility in this environment. This could be a subject of future research.

In this study, PGE and PGF2α levels differ significantly in the sperm count groups (normospermic, oligospermic and azoospermic) as well as the "normal" versus "subnormal" semen groups. However, we have to interpret this with caution because of the wide range of values obtained in each group (Table II). A previous study (17) reported a wide range of PG levels even in men with evidence of fertility. However, some studies have linked high PGE levels with increased sperm quality. It is believed that PGEs enhance the acrosome reaction through the influx of extracellular calcium into the cytoplasm of human spermatozoa and they may be responsible for delay in spermatozoa transport from the epididymis, giving enough time for spermatozoa maturation (18,19). Low levels of PGF2α have also been linked to good testicular function (14,20) All this put together indicates a need to further investigate the role played by these compounds in male fertility.

This is the first time that PG levels will be related to socio economic class. However, significant differences were not observed in the groups. Previous workers have linked micronutrient and omega 3 fatty acid deficiencies with impaired fertility and abnormal spermatogenesis in men (21).

This study provides PG levels in group of infertile men and demonstrates the need to carry out further research in finding the link between male infertility and prostaglandin metabolism. Such studies may provide new treatment options for infertile men in this environment.

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CONFLICT OF INTEREST: Authors report no conflicts of interest.

ETHICS

Ethical Clearance was obtained from the research and ethical committee of the Obafemi Awolowo University Teaching Hospitals Complex for the study. Written informed consent was obtained from all participants and they were assured of confidentiality of all information obtained from the study.

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Table III: Mean Prostaglandin Levels by individual semen characteristics

Status	% of Population	PGF2 α ± SEM (µg/ml)	Total PGE ± SEM (µg/ml)
Motility= 50%	65.8	2.81±0.28	249.34±16.56
Motility< 50%	10.5	3.61±0.91	259.8±37.3
		NS	NS
Morphology = 30%	71.0	2.72±0.27	260.52±16.0
Morphology< 30%	8.6	3.13±0.95	260.52±16.0
		NS	NS
Viability= 75%	32.4	2.55±0.32	267.11±22.1
Viability<75%	44.7	2.94±0.31	242.62±21.1
		NS	NS

Table IV: Mean prostaglandin levels by primary vs secondary infertility.

	% of Population	PGF2α	PGE
Primary infertility	42.2	2.96±0.28	234.62±20.68
Secondary infertility	57.8	2.63±0.39	259.09±18.7
		NS	NS

Table V: Correlation of PGF2α levels with PGE, age and semen quality parameters

	Total PGE	Age	Morphology	Motility	pH	Semen Vol
Pearson correlation(r)	-.147	.009	.016	-.016	.016	.102
Sig (2-tailed)	.119	.921	.869	.867	.866	.280