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Cytological Assessment of Vaginal Epithelial Cells in Undergraduate Females of Reproductive Age in Ogun State, South-West Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author APO designed the study and wrote the protocol. Author SAA performed the statistical analysis and wrote the first draft of the manuscript. Authors AI and NW managed the analyses of the study. Author AVF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study assessed the vaginal epithelial cells and its relationship with the reproductive hormones as a determinant for fertility. 100 students participated in this cross-sectional study. The established approach to the evaluation of ovarian function and endocrine disorders in the woman is based on serial biochemical analyses of hormones, such as estrogen, progesterone, luteinizing hormones and their metabolites. This study analyzed the ratio of parabasal, intermediate and superficial cells in comparison with the presenting hormones. The biochemical analyses can be effectively supplemented by the old-fashioned endometrial biopsies, or studies of endocervical mucus. In addition, the vaginal smear may sometimes provide useful information and has the advantage of being easy to obtain, rapidly evaluated, and inexpensive. Cytology is the study of cells collected by

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various means through microscopic examination. In the case of humans, the cytologic approach is particularly valuable if laboratories specializing in endocrine analysis are not readily available. The principle of the cytologic hormonal analysis is simple. The degree of maturation of the squamous epithelium of the female genital tract depends on steroid hormones, mainly estrogen. Various factors contribute to the fertility status of a woman. In the case of this study, age, abortion and use of contraceptives are the significant contributing factors ($p < 0.05$, $t > 1.96$).

Keywords: Vaginal maturation index (VMI); infertility; vaginal smear; cytology.

1. INTRODUCTION

Infertility is experienced by an estimated 48.5 million couples worldwide Mascarenhas et al. [1] and around 1 in 7 couples in the UK NICE, [2]. However, prevalence estimates of lifetime infertility vary widely, in part because there is no agreed or consistent definition of infertility Bell, [3]. There are various factors that can predispose a woman to infertility which includes smoking, alcohol abuse, being underweight, being overweight Hassan, [4]. Although this does not imply that people who live perfectly healthy life cannot be infertile. Infertility is majorly treated by treating the underlying cause Heard, [5], Homan et al. [6], Kristner [7], Olatunji and Sule-Odu, [8].

Cytology is the study of cells collected by various means through microscopic examination. In the case of humans, cytology can be divided into gynaecological and non-gynaecological. In cytodiagnosis, specimens are analysed to determine presence of malignant, premalignant or non-malignant cells which through certain procedures may be further classified as beningn, malignant, inflammatory or degenerative.

George Papanicolaou started his study in gynaecological cytology in the year 1917. He published the results of his research in the year 1943 which helped to shed light in the diagnosis of cervical cancer. This staining technique, Pap stain, has helped in the revolution of cytodiagnosis and is the gold standard as at today.

The Papanicolaou staining technique can also be used in diagnosing non-gynaecological samples such as sputum or urine which contain squamous epithelial cells similar to that in gynaecologic samples and they demonstrate excellent results Bryan, [9].

The vaginal maturation index (VMI) is a clinical measure that uses exfoliated vaginal cells to evaluate hormonal status and thickness of the vaginal epithelium which is determined using the vaginal smear Parakkai and Gregoire [10]. A vaginal smear is usually taken from the vaginal mucosa for cytological diagnosis. A scraping of the mid-third layer lateral area of the vaginal wall mucosa, where the degree of maturation is hormone dependent, produces the best specimen Pritts, [11], Sankpai et al. [12]. The vaginal epithelium is responsive to sex steroids, particularly estrogen, and it also undergoes predictable changes in blood concentrations of ovarian hormones. Increased levels of estrogen will cause the vagina to become cornified. i.e the surface cells become large and flattened with small or absent nuclei Schwartz and Mayaux, [13].

The changes in the morphology of desquamated vaginal epithelial cells provide a convenient means of assaying changes in estrogen levels. There are many factors that can influence the accuracy of the Maturation Index including endocervical cell contamination, presence of microorganisms or large numbers of inflammatory cells Gurunath et al. [14]. Patient history, especially menstrual status, and information about medications that the patient is taking at the time of the Pap smear, is very important in providing an accurate hormonal evaluation therefore maturation Index should only be performed on specimens collected from the vaginal wall not showing confounding inflammation, atypical cells, or endocervical contamination.

Maturation index helps in the evaluation of therapies designed to treat vaginal hormonal symptoms. The VMI also gives insight into the estrogen status of the related genital structures including the vulva, urethra and the bladder Zegers-Hochschild, [15]. In contrast to serum measure of estrogen, the VMI indicates the net effect of biologically active sex hormones (circulating levels of estrogens, androgens and progestrogens) on the vaginal epithelium and provides an integrated measure of hormonal bioactivity over time rather than blood levels at a single time point McEndree [16]. The fertility status of a woman is dependent on the hormonal

status gotten from the vaginal maturation index. Therefore, the vaginal maturation index is used to determine if a woman is fertile or infertile.

The VMI quantifies the ratio of parabasal, intermediate and superficial cells P/I/S. It is based on the assumption that the sex hormones bring about maturation in squamous cells which can be detected by cytological examination. Parabasal cells are not affected by estrogen and progesterone, being immature cells Lis et al. [17], Ljubin-Sternak et al. [18]. Intermediate cells are somewhat mature having been affected by estrogen and progesterone and superficial cells are the most mature having been affected by estrogen. If there are lots of parabasal cells, it indicates a lack of estrogen hitting the tissues but if the percentage of superficial cells is higher, it means that there is a lot of estrogenic stimulation while intermediate cells have no value here. In essence, vaginal cytology is a type of endocrine assay Macaluso et al. [19].

Even though there are other methods used to determine fertility in females, most are either expensive or inconvenient for the patient. Maturation index on the other hand is a cheaper method and does not cause significant pain to the patients. It can also provide additional diagnostically important information including if fertility is being disturbed due to presence of abnormal cells or high variation in the normal number of cells Maheshwari, [20]. This study is to ascertain the accuracy of maturation index as a method for determining fertility.

Infertility is one of the medical conditions experienced by millions of people across the world including Nigeria Makar and Toth, [21] and Munne et al. [22]. Being a condition that does not necessarily show symptoms, it is quite important that an easy and cheap method of diagnosis be developed. The study aimed to use vaginal maturation index to access fertility status; it also estimated the maturation index of stained vaginal smears.

2. MATERIALS AND METHODS

This study was carried out in Babcock University, Ilishan-Remo, Ogun state. Babcock University is a Seventh Day Adventist institution with a student population of about 6000 students located in the South-Western region of Nigeria. Undergraduate female students of reproductive age within Babcock University who showed no signs or symptoms of any sickness were the target for this study. Random sampling was used to select females for this study. Adult females that have delivered at least one baby were recruited into the control group.

A total of 193 vaginal smears were collected randomly from selected female students of different departments and study level. Using formula $n= Z^2 \times P(1-P)/d^2$ described by Naing et al. 2006. Where: n = sample size, d=desired level of significance, Z= confidence interval, P= prevalence rate of infertility in Ogun state (14.8% for both female and male infertility). Therefore n=193. To be included in the study group, participants met the following criteria: Female of reproductive age with No signs or symptoms of any major ailment. Those who are presently showing symptoms of a major ailment and those who are treating bacterial vaginosis were excluded from the research study.

Smears were taken from the upper two-thirds of the lateral vaginal wall of the subjects using a sterile swab stick. A smear was made from this on clean grease free, non-aluminized glass slide. The slide was immersed in 95% methanol while wet for fixation and then stained using the Papanicolaou staining method NCCP, [23], Okonofua, [24]. Nuclear staining is done by the basic dye, Haematoxylin which stains the acidic part of the cell which is the nucleus. Cytoplasmic staining is done by the acidic dye, the counterstain which stains the basic part of the cell, the cytoplasm. Clearing in xylene results in cellular transparency and precedes mounting.

Briefly, immediately after the smear has been made on the glass slide, it is fixed by immersing in 95% ethanol for two minutes. Afterwards it is briefly rinsed in distilled water. The smear is then covered with Harris haematoxylin for two minutes and then rinsed with tap water for 5 minutes. It is then transferred to 95% ethanol for 15 seconds. Thereafter, the smear is covered with EA 50 for five minutes and then rinsed in two changes of absolute ethanol for thirty seconds each. It is then cleared in two changes of xylene for two minutes each.

Statistical analyses were performed with the aid of Statistical Package for Social Science (SPSS).

3. RESULTS

Table 1 shows that complete results were available for a total of 100 females of reproductive age at the study site. It also shows the frequency and percent of respondents that fall under the various parameters to be analyzed and compared with the results gotten.

Table 1. Complete results were available for a total of 100 females of reproductive age at the study site

Parameters	Frequency (%)
Age	
17-24 Years	92
25-32 Years	5
33-40 Years	3
Parturition	
Test	93
Control	7
Sexual Intercourse	
No	59
Yes	41
Menarche Age	
10-12 YEARS	63
13-15 YEARS	37
Regular Menstrual Cycle	
Not sure	12
No	14
Yes	74
Use of contraceptive	
No	73
Yes	27
Alcohol consumers	
No	76
Yes	24
Smoking	
No	93
Yes	7
Abortion	
No	98
Yes	2
Estrogenic effect (m.i)	
Non estrogenic	3
Estrogenic	97

Table 2. Variations in the various parameters

Variable	N	Mean ± Std. deviation	SEM	t- value	p- value
Age	T	20.57 ± 1.74	0.181	-13.172	0.000
	C	31.29 ± 4.89	1.848		
Sexual Intercourse	T	0.39 ± 0.49	0.051	-1.705	0.091
	C	0.71 ± 0.49	0.184		
Respondent's menarche age	T	1.39 ± 0.49	0.051	1.289	0.201
	C	1.14 ± 0.38	0,143		
Regular menstrual cycle	T	1.61 ± 0.69	0.072	-0.372	0.711
	C	1.71 ± 0.76	0.286		
Use of contraceptives	T	0.24 ± 0.43	0.044	-2.827	0.006
	C	0.71 ± 0.49	0.184		
Alcohol consumption	T	0.25 ± 0.43	0.045	0.619	0.537
	C	0.14 ± 0.38	0.143		
Smoking	T	0.08 ± 0.27	0.027	0.747	0.457
	C	0.00 ± 0.00	0.000		
Abortion	T	0.00 ± 0.00	0.000	-6.038	0.000
	C	0.29 ± 0.49	0.185		

Key: T = Test samples; C = Control samples

Table 2 shows the variation in the various parameters. It shows the significant and less significant parameter as relating to the study. The statistically significant parameters are age, use of contraceptives and abortion that is p-value is <0.05 and t-value is >1.96. The other parameters are not statistically significant, that p-value is >0.05 and t-value is <1.96.

Vaginal maturation index also shows the various menstrual cycle phases based on the result gotten. When VMI = 0%:40%:60%, it indicates that the person is in a proliferative phase. When the VMI = 0%:70%:30% this indicates that the person is in the secretory phase, 0%:90%:0% or 0%:100%:0% VMI indicates pregnancy, 50%:50%:0% indicates menopause or atrophy and 100%:0%:0% indicates late menopausal phase.

The photomicrographs below also showed abundant intermediate cells (Fig. 1) and superficial cells (Fig. 2).

4. DISCUSSION

This study assessed the cytology of vaginal epithelial cells in females of reproductive age of Babcock University, Ogun state. Among these 7% had given birth before while 93% had not.

Vaginal maturation index (VMI) assesses the vaginal epithelial cells. It is a cytological method of assessing the hormones present. The predominance of a particular type of cell gives an insight of the hormone that is predominant.

The results gotten from VMI can be grouped based on the predominant cells and their indication. When there is a shift to the right it shows as predominance in superficial cells, shift to the middle shows as predominance of intermediate cells which can be categorized as marked estrogenic effect, moderate estrogenic effect or slight estrogenic effect. A shift to the left (predominance of parabasal cells) which can either be categorized as marked estrogenic deficiency, moderate estrogenic effect or slight estrogenic deficiency.

It was shown statistically in this study that the most significant variable that affects the vaginal epithelial cells are age, use of contraceptives and abortion. In literature, long term heavy alcohol drinking is associated with endocrinological abnormalities, loss of sexual are perhaps clinically the most prominent in women Turner, [25]. This is contrary to the finding in this study which showed no significance with alcohol.

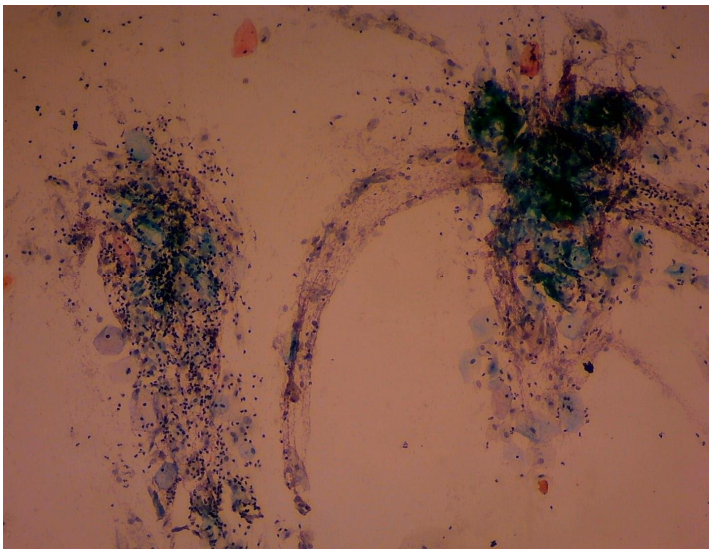

Fig. 1. Photomicrograph showing abundant intermediate cells in vaginal smear stained with Papanicolaou staining technique. The polygonal-shaped intermediate squamous cell size ranges 1,256-1,618 µm. The cell is found in the stratum spongiosum (midzone) layer of the squamous epithelium. The intermediate cell's cytoplasm is thin, transparent, and typically stains basophilic. The centrally placed nucleus is 35 µm. The nucleus is vesicular with fine evenly dispersed granular chromatin. (X100)

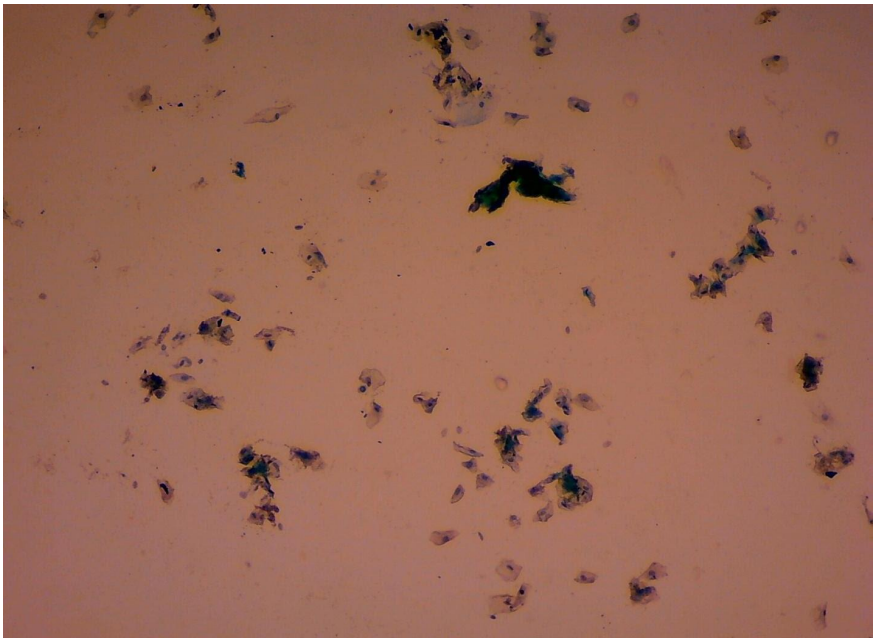

Fig. 2. Photomicrograph showing abundant superficial cells in a vaginal smear stained with Papanicolaou staining technique. The superficial squamous cell comprises the outermost layer of the non-keratinizing epithelium. The 1,604 µm eosinophillic polygonal shaped cell houses a 25 µm centrally placed pyknotic nucleus. No nuclear detail can be seen due to nuclear degeneration. (X100)

In this study, use of contraceptives was also a statistically significant parameter. Respondents who were positive to the use of contraception had lesser estrogenic effect when compared to those who had never used contraceptives. Although this was not applicable in every case as some respondents who had used contraceptives still had marked estrogenic effect. This conforms to the study carried out by Habenicht and Aitken [26] which reported that alterations to the production and or actions of estrogen can disrupt these processes leading to infertility.

5. CONCLUSION

This study suggests that maturation index can be used as a method of hormonal assay to determine fertility status. The study also shows the significant factors that may be responsible for infertility. It is a more accessible and cheaper method of hormonal assay and does not cause any harm. In addition, predominance of parabasal cells which may be marked, moderate or slight estrogenic deficiency does not indicate infertility at all times. It may sometimes be as a result of atrophy.

In the past years females have been advised to get their pap test done but there has been a barrier as ladies who have not started having sexual intercourse or not yet in the parturition stage cannot get this test done. The VMI can be used as an alternative. Females should abstain as much as they can from harmful forms of contraceptives and abortion. As age increases, fertility rate may at some point may be reduced because women approach menopause at different ages.

CONSENT AND ETHICAL APPROVAL

Written informed consent was obtained from the participants prior to the study which was approved by the Research and Ethical committee of the University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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