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Using the Lateral Vaginal Swabs for Determination of the Ovulation Status

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Abstract: ***Aims of the study: The objective of the present work was to study the effect of ovarian sex steroid hormones, estrogen and progesterone on the lateral vaginal squamous epithelia cells to determine the day of ovulation and compare it with the hormonal levels and transvaginal ultrasonography (TVUS). Methodology: The study was conducted on 40 infertile women whose ages ranged from 16 to 43 years and duration of infertility from 1 to 17 year. The reproductive history of all patients was available through filling a questionnaire form prepared for this purpose. The method depends on taking lateral vaginal smear on cycle day 11-18 (ovulatory period) and on cycle day 21-23 (midluteal phase) using Papanicolaou stain (Pap) method and compare it with the hormonal profile which were taken from all patients, serum FSH, LH and PRL on cycle day two, E2 on cycle day 11-18, P on cycle day 21-23 and TVUS at ovulatory period to determine the reliability of vaginal smears as a method for determination of ovulation especially in rural areas of Iraq where no facilities for hormonal profile and ultrasound are available. Infertile women were subdivided into three groups according to their vaginal smear results: group I compatible with the hormonal profiles and TVUS (n=29), group II incompatible showing an opposite picture with hormonal profiles and TVUS (n=6), group III incompatible because of the inflammation of the genital system with polymorphonuclear infiltration (PMNI) masking the picture of the squamous epithelial cells (n=5). Correlation study of the vaginal smears results were performed with age of the patients, duration of infertility, hormonal levels, ovulation induction, ovulation (if present or absent) and genital infection. Results: A significant correlation was observed between vaginal smears results with LH level on cycle day 21-23, ovulation as evidenced by TVUS. The control group consisted of 10 fertile parous women with regular menstrual cycle, from which vaginal smears were taken on cycle day 21-23, where 4 of them showed ovulatory cycles, and the other 6 women showed anovulatory menstrual cycle, while the vaginal smears of 3 of the 6 women showed moderate to marked inflammation with PMNI. Conclusions: It is concluded from the results of this study that 72.5% of the vaginal smears results were compatible with the hormonal levels and TVUS. Thus, the lateral vaginal smears may be more informative method for determination of ovulation, and examination of smears can be utilized as a tool for hormonal profile changes in the menstrual cycle in rural areas of Iraq.***

Keywords: Vaginal smear, Ovulation, Reproductive hormones

1. Introduction

The female reproductive system is composed of 3 main organs: the ovaries, fallopian tubes and uterus. The ovaries, also known as the female gonad, are located on both sides of the pelvic body cavity adjacent to the superior portion of the uterus [1, 2].

The center of fertility in women is ovarian function; the various causes of ovarian dysfunctions may lead to infertility. Common causes of infertility in women are ovulatory problems (such as PCOS), tubal blockages, pelvic inflammatory disease (PID), advanced maternal age, previous tubal ligation and endometriosis. Some additional factors that may contribute to female infertility are behavioural factors such as diet, exercise, smoking, alcohol and drug use [3].

Ovulatory disorders account for 30% of women's infertility and are one of the most common reasons women are unable to conceive. The causes of failed ovulation can be divided into five main categories: genetic, hormonal, ovarian scarring, premature menopause and improper folliculogenesis.

Hormonal infertility is the most common, as it relates to the delicate and complex balance of hormones and their interaction with one another. Approximately 50% of cases of anovulation are caused by failure of the ovary to produce normal follicles in which an egg can mature. The most common cause of this is polycystic ovary syndrome (PCOS) [4].

Malfunctioning of the hypothalamus or the pituitary gland also can cause hormonal infertility. The hypothalamus is responsible for sending signals to the pituitary which then stimulates the ovaries with FSH and LH to initiate egg maturation through a feedback loop known as the HPG axis. If the hypothalamus fails to trigger and control this process, eggs do not progress and improper ovulation results [4].

The hormonal control of ovarian function by gonadotrophins: follicle stimulating hormone (FSH) and luteinizing hormone (LH) play a key role in the physiological process of follicular growth and differentiation [5] and consequently the evaluation of the hypothalamic-pituitary-ovarian axis (HPOA) through assay of the following hormones: FSH, LH, estradiol (E2), prolactin (PRL), and progesterone (P) is very essential to the diagnosis of any abnormality of HPOA [6,7].

In the present study we, tried to detect the possibility of testing ovarian function and ovulation through studying cytological changes in vaginal smears in response to sex steroid hormones.

Such method if goes through successfully it will provide the physicians with cheap and good facility in ovulation detection especially in rural areas and poor patients where availability of sophisticated equipment of diagnosis, such as ultrasound and hormonal assays are not available or expensive.

2. Subjects and Methods

Vaginal smears were obtained from (40) infertile women whose ages ranged between 16-43 years, and attended the Institute of Embryo Research and Infertility Treatment, Al-Nahrain University.

The control group consisted of 10 multiparous fertile women whose ages ranged between 25–40 years. The correlation was performed with other parameters e.g. serum hormonal levels (estradiol E2 on day 14 and progesterone P on day 21 of 28 day menstrual cycle) and vaginal sonography for determination of ovulation through prepared smears on day 8, D14 and D21 of 28 day of menstrual cycle.

Two slides were prepared for each patient labeled with name, date and last menstrual period (LMP).

Cotton-tipped applicator swabs were used to scrap the lateral wall of the proximal third of the vaginal wall, and smeared in the same previous mentioned method. A number of indices were used for cytological assessments of the hormonal condition of the vaginal smear.

The smears were kept for at least 15-20 minutes in the fixative before staining with Papanicolaou (Pap) stain.

The steps used in Pap stain included: rehydration of the slides after removal from the fixative and successively transferring them into Harris haematoxylin for 1 minute.

Slides were then dipped in alcohol of gradual increases in concentration as follow to ensure dehydration. Then they were stained with orange G stain for 1 minute to stain the cytoplasm.

The slides were then carefully mounted with Canada Balsam, covered with cover slides, allowed to dry for 1-2 hours and examined.

The following reproductive hormones were assayed in the sera of the studied women using Minividas, Biomerieux, France: Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin (PRL), Estradiol (E2) and Progesterone (P).

Analysis of the 1st three hormones was performed on day 2 of the cycle, while determination of E2 was performed on cycle day 11 to 18.

The transvaginal sonography (TVUS) examination was done and the results were verified by specialist in the clinic of the Institute. Ovarian and uterine changes were visualized using a transvaginal probe. Baseline examination was done on cycle day 2 or 3 to detect any ovarian cyst that may be later confused with a growing follicle. Ovulation was then detected by serial scans scheduled daily starting from cycle day 8 onwards.

Statistical Analysis

The statistical program (SPSS 11) was used for analysis of results, and significance of difference in mean of a continuous response variable was assessed by ANOVA test and independent samples t-test. The chi-square test was used to detect degree of significance of the relation between two variables. P-value < 0.05 was considered as statistically significant.

3. Results

According to the cytological changes in the examined vaginal smears in response to the ovarian sex steroid hormones estrogen and progesterone on day 11-18 and day 21-23 of the m.c., the patients were divided into three groups:-

Group I: The vaginal smears results are compatible with the hormonal levels, estradiol (E2) on cycle day 11-18 (ovulatory period) and progesterone (P) on cycle day 21-23 (in the midluteal phase) together with TVUS on day 11-18 whether the menstrual cycles are ovulatory or anovulatory. Their total numbers are 29 (72.5%) 18 of them had ovulatory menstrual cycle and 11 of them had anovulatory menstrual cycle.

Group II: The vaginal smears results are incompatible giving a picture opposite to the finding of hormonal levels and TVUS. Their total number is 6 (15%), 3 of them had ovulatory cycles and the other three are anovulatory cycles.

Group III: The vaginal smears results are incompatible because of inflammation and polymorphonuclear infiltration (PMNI) changing the morphology of the intermediate cells and masking the picture of the vaginal squamous cells. Their total number are 5 (12.5%), 2 of them are ovulatory and the other 3 are anovulatory.

Correlation study of the 3 groups of the vaginal smears showed the following:-

1) **Age of the patient:** The correlation is not significant (P>0.05) as shown in table (1).
2) **Duration of the infertility:** The correlation is not significant (P>0.05) as shown in table (1).
3) **Hormonal levels (FSH-LH-PRL-E2-P):** The correlation is significant with LH only (P<0.05) as shown in table (1).
4) **Ovulation induction:** The correlation is not significant (P>0.05) whether ovulation is natural or induced as shown in table (2).
5) **Ovulation:** The correlation is not significant (P>0.05) whether mature follicle is present or absent as evidenced by ultrasound as shown in table (3).
6) **Genital infection:** The correlation is not significant (P>0.05) whether genital infection is present or absent as shown in table (4).

Table 1: Correlation study of vaginal smears of infertile women with age, duration of infertility, hormonal levels FSH, LH, and PRL on cycle day 2, E2 on cycle days 11-18, and P on cycle days 21-23

		Sum of Squares	df	Mean Square	F	Sig.
Age	Between Groups Within Groups Total	61.108 1341.292 1402.400	2 37 39	36.251 30.554	0.843	0.439
Duration	Between Groups of Within Groups Inf. Total	730.407 28.368 758.775	2 37 39	14.184 19.741	0.719	0.494
FSH	Between Groups Within Groups	1.394 178.578	2 37	0.697 4.826	0.144	0.866

	Total	179.972	39			
LH	Between Groups Within Groups Total	24.348 99.950 124.299	2 37 39	12.174 2.701	4.507	0.05*
PRL	Between Groups Within Groups Total	206.117 9434.408 9640.525	2 21 23	103.059 449.258	0.229	0.797
E2	Between Groups Within Groups Total	28939.366 953974.61 982913.97	2 20 22	14469.683 47698.730	0.303	0.742
p	Between Groups Within Groups Total	140.897 1913.196 2054.093	2 37 39	70.449 51.708	1.362	0.269

* Significant correlation (P<0.05)

Table 2: Correlation study of three groups of vaginal smears of infertile women with ovulation induction

Groups		Cycle		Cycle
		Natural	Natural	
1	Count % within vaginal smear % within cycle	19 65.5% 70.4%	10 34.5% 76.9%	29 100.0% 72.5%
2	Count % within vaginal smear % within cycle	6 100.0% 22.2%	0 0% 0%	6 100.0% 15.0%
3	Count % within vaginal smear % within cycle	2 40.0% 7.4%	3 60.0% 23.1%	5 100.0% 12.5%
Total	Count % within vaginal smear % within cycle	27 67.5% 100.0%	13 32.5% 100.0%	40 100.0% 100.0%

	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	4.665	2	0.097

Table 3: Correlation study of three groups of vaginal smears of infertile women with ovulation

Groups		Ovulation		Ovulation
		Present	Absent	
1	Count % within vaginal smear % within ovulation	18 62.1% 78.3%	11 37.9% 64.7%	29 100.0% 72.5%
2	Count % within vaginal smear % within ovulation	3 50.0% 13.0%	3 50.0% 17.6%	6 100.0% 15.0%
3	Count % within vaginal smear % within ovulation	2 40.0% 8.7%	3 60.0% 17.6%	5 100.0% 12.5%
Total	Count % within vaginal smear % within ovulation	23 57.5% 100.0%	17 42.5% 100.0%	40 100.0% 100.0%

	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	1.012	2	0.603

	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	3.218	2	0.200

Table 4: Correlation study of three groups of vaginal smears of infertile women with genital infection

Groups		Ovulation		Ovulation
		Present	Absent	
1	Count % within vaginal smear % within Infection	3 10.3% 75.0%	26 89.7% 72.2%	29 100.0% 72.5%
2	Count % within vaginal smear % within Infection	1 16.7% 25.0%	5 83.3% 13.9%	6 100.0% 15.0%
3	Count % within vaginal smear % within Infection	0 0% 0%	5 100.0% 13.9%	5 100.0% 12.5%
Total	Count % within vaginal smear % within Infection	4 10.0% 100.0%	36 90.0% 100.0%	40 100.0% 100.0%

	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	0.856	2	0.652

4. Discussion

In the present study, regarding the cytological changes in the examined vaginal smears in response to ovarian sex steroid hormones, estrogen and progesterone, (72.5% of infertile women) were in (Group I), this confirm that the epithelium of the vaginal vault is much more sensitive to estrogen and progesterone in quality, quantity and rapidity of response more than any other epithelial tissues [8]. Thus, rendering the possibility of using the lateral vaginal smear as a method for determination of ovulation.

The remaining 12.5% patients were in (Group III) under the effect of local genital inflammation which cause increase in the maturation of the squamous epithelial cells due to inflammatory irritation, and there is a corresponding increase in the exfoliation of superficial cells more than intermediate cells [9].

A large number of inflammatory cells can mask the epithelial cells or produce severe cellular degenerative changes like vacuolization, perinuclear halo, hypertrophy and nuclear pyknosis, also they produced eosinophilia of the squamous epithelial cells ([10]. Therefore, women in Groups II and III needed a repeated hormonal cytological evaluation after taking antibiotics and anti-inflammatory treatment because they are not diagnosed at time of genital examination, and were diagnosed after microscopic examination of vaginal smears, so their results may be changed toward group I after taking treatment.

The high concentration of estradiol secreted by dominant Graafian follicle, triggering the pre ovulatory LH surge and causing the maturation of vaginal squamous epithelia to superficial cells which have pyknotic nuclei and eosinophilic cytoplasm (11). Since the epithelial cells of the vagina like other tissues of the female reproductive tract, respond to cyclic changing levels of ovarian sex steroids, they are much more sensitive to estrogen than to any other substance, they

are the most accessible end organ reflecting hormonal activity [12] and study of vaginal cytology after Pap stain helps to form a general impression of hormonal activity of infertile women.

It is also necessary for the maintenance of luteal function [13], but high prolactin secretion may interfere with the function of corpus luteum as demonstrated by short luteal phase [14] and decrease progesterone secretion [15].

Also high prolactin decreases specific binding of LH to FSH primed granulosa cells which lead to decrease androgen production and subsequently estrogen synthesis and these will be reflected on vaginal cytology [16].

Ovarian dysfunction (agenesis, neoplasm) will produce a partial vaginal mucosal atrophy, because of the normal activity and hormonal production of the adrenal cortex which produces a proliferation of the intermediate layer in the squamous vaginal epithelium. In congenital abnormalities of the uterus (atresia or absence of the uterus), the vaginal mucosa has a normal cyclic ovulatory changes [17].

In the study done by [9], it was observed that polymorophonuclear leukocytes (neutrophils, eosinophils, and basophils) in small amounts may be found in a normal vaginal smear but in acute, subacute or chronic inflammatory disease they are abundant.

Certain infections particularly Trichomonas Vaginalis cause irritation and hyperchromasia of the squamous epithelia which in turn, enhance maturation leading to erroneous results [10].

Cocci and other bacteria cause cytolysis and can hamper the correct evaluation. Therefore the smear should be re-evaluated after anti-inflammatory treatment [9].

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