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BIOCHEMICAL AND HORMONAL ASSESSMENT OF CERVICAL MUCUS SCORE IN IRAQI WOMEN WITH PRIMARY VERSUS SECONDARY INFERTILITY

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ABSTRACT

Background: WHO reports indicate that one in four women in developing countries suffers from infertility. The total fertility rate for Iraqi women is about 4.5 children per woman; other studies have indicated that the maternal fertility rate has been declining in Iraq since 2003 and that war conditions have contributed to this decline. **Objectives:** To study the relationship between primary and secondary infertility and mid-cycle cervical mucus variables. **Materials and Methods**: 50 female patients were included in this study; they were recruited from private infertility clinic patients. The patients divided in to two groups: first group[25] female with Primary infertility. The second group[25] female with Secondary infertility. The serum level of estradiol (E2), lutinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin were measured at the day 2 of cycle (pretreatment). At the same time the cervical mucus (CM) score was done. **Results:** The results showed no significant differences in demographic data and hormone levels between the primary and secondary infertility groups. There was a significant difference in the mid-cycle estrogen level ($p < 0.001$) with higher levels in the primary infertility group. There were also significant differences in cervical mucus volume ($p = 0.029$), consistency ($p = 0.046$), spinocarcinoma ($p < 0.001$), cellularity ($p = 0.002$) and total cervical mucus score ($p < 0.001$) with higher levels in the secondary infertility group, while there was no significant difference in the ferning and pH change of cervical mucus between the two groups. **Conclusions:** Most infertility cases were found in women from normal weight, FSH, LH, Prolactin, but E2 and cervical mucus effect and indicate the problem of infertility in our study group.

KEYWORDS: primary infertility, secondary infertility, cervical mucus score, E2, Prolactin.

INTRODUCTION

Cervical mucus is important for spontaneous conception. Fertile cervical mucus fluid is required to assist sperm survive and swim from the cervix into the uterus and inevitably the fallopian tubes.[2] The cervical mucus is a heterogeneous secretion regulated by steroid hormones that are regularly created by the ovary Estrogen and Progesterone) that changes in concert with the hormonal alteration observed during normal menstrual cycle. In general, estrogen stimulates the secretion of large amount of watery mucus.[3] Cervical mucus is measured over that standard level of dampness. It increments in water substance as a lady approaches ovulation. For the most part, the higher the water substance, the more prolific the cervical liquid. After ovulation, the water substance will diminish. Note: all cervical bodily fluid is possibly rich.[4] At the second phase day 8-21 OF cycle the CM increase into 10 folds, clear, watery mucus sufficient quantity, distinct fern, mucus highly elastic, long thread (spinbarkitt) when ejected into slide, bubbles like soap may appear when E2 level peak just prior to ovulation, the cervical os is open from 1 to 3 mm in diameter to allow for better sperm passage.At this stage the female is fertile and intercourse at this stage will increase the chance of pregnancy. it's the most fertile period.[3]

Trans Vaginal Ultrasound A strategy utilized to look at the vagina, uterus, fallopian tubes, ovaries, and bladder (It provides a direct visualization of the follicular growth and maturation process, and endometrial thickness in the mid cycle when the when the dominant follicle approach 18 mm diameter allow a more meaningful interpretation of E2 concentration.[5]

The cervix is the lower round and hollow parcel of the uterus the cervix contrasts histologically from the rest of the uterus. The endocervical mucosa could be a basic columnar epithelium on a thick lamina propria with numerous bigger, branched, mucus-secreting cervical organs the cervical locale around the outside os ventures somewhat into the upper vagina and is secured by the exocervical mucosa with nonketinized stratified sequamous epithelium ceaseless with that of the vagina. The intersection between this sequamous epithelium and the bodily fluid –secreting columnar epithelium of the endocervix occursin the change zone.[6]

The cervix has a lesser muscular component compared with the myometrium and these muscles are dispersed in ground substance.[7] This substance contains glycosamine glycans and collagen fibrills and the fibrillar structure of cervical mucin allows the cervix to participate actively in sperm transport.[15]

The cervix is the opening between the vagina and the uterus and it is lined with various little organs (cervical organs) which create bodily fluid. Once sperm are ejaculated, they swim through this bodily fluid from the vagina through the cervix and into the uterus. The bodily fluid too gives food for the sperm. On the off chance that the cervical bodily fluid is lacking, the sperm cannot reach and treat the egg.[21]

Hormonal dysfunctions, primarily characterized by insufficient estrogen generation and/or untimely progesterone rise, may render cervical bodily fluid lacking for sperm infiltration, and may result in infertility.[6] Drugs that lower estrogen levels, such as Clomid, can contrarily influence the cervical mucus fluid.[11]

The CM is a complex substance (AnnaWorms,2020), composed of 92% - 98% water and around 1% inorganic salts of which sodium chloride is the most constituent. Examination of biochemical and biophysical structure of cervical bodily fluid remains a challenge due to complex basic proteins, tall substance of oligosaccharides (such as sialic acid) and cyclic variability of its structure.[12] The total content and proportion of sialic acid is of great importance during the ovulatory period and can be increased by the use of E2.[13]

Positive sperm-cervical mucus interaction has been known as a major cause of infertility which cannot be managed easily. At present, IUI is considered as the treatment of choice for this condition, though its success is debatable. The female genital tract has a potential space which is filled by fluid. The fluid in diverse parts of the female genital tract (eg. cervical canal, uterine depression and fallopian tube) differs in composition which changes with the phases of the menstrual cycle. Currently available infertility investigation techniques can only assess the sperm-cervical mucus interaction to find out the hostility of the cervical mucus.[14] From a normal of 200 to 300 million sperm kept within the vagina, as it were some hundred accomplish vicinity to the oocyte. Given this anticipated tall spermatozoon misfortune, a slight alteration in cervical mucus may quickly change the cervix into a "threatening" environment, which, along side changes in vaginal environment and cervix structure, may anticipate common conception and be a cause of infertility.[15]

Within the early minutes after ejaculation, the sperms stay generally stable conceivably since of the formation of viscous semen coagulum by clotting prostatic enzyme. This coagulum holds the semen within the more profound locale of the vagina where the uterine cervix lies. The coagulum at that point breaks down amid the following 15 - 30 minutes since of lysis by prostatic enzyme also. As the coagulum dissolve, the sperm becomes highly motile.[16]

CM may act as a filter which selectively allow penetration and migration of the motile best functioning spermatozoa and to serve as a barrier for non-motile sperms.[17] Sperm motility has a significant influence on the success of sperm transport and the penetration of sperm to CM is considered to be related to sperm motility.[18] Some spermatozoa ascend into the uterine cavity, while others remain in the cervical crypts from where they are subsequently released into the upper portions of the upper genital tract.[19] At this stage, there is a reduction in quantity and improvement in quality because in the cervical crypts the motility of the sperm decreases. The cervical crypts represent a reservoir of sperm, which save energy and protect from phagocytic activity and antibodies.[20] The sperm can survive in the cervical cavity for 3 - 4 days as compared to 20 minutes in the vagina and 20 minutes in the seminal plasma, but the very fertile period of sperm is only 24 hr within the female genital tract.[21]

Spermatozoa require a certain period of home within the female genital tract to gotten to be practically competent cells. As spermatozoa navigate through the female genital tract, they experience different biochemical and physiological changes collectively alluded to as capacitation. As it were capacitated spermatozoa associated with the extracellular egg coat, the zona pellucida.[22]

CM exhibits a number of rheological properties as.
Consistency: consistency is influenced by the molecular arrangement and by the protein and ionic concentration of CM.

Spinnbarkeit: is the term used to describe thread ability or elasticity characteristic of CM, the glycoproteins in CM supports the property of spinnbarkeit.

Ferning: refers to the degree and pattern of sodium chloride crystallization observed when CM is dried on glass slide.[23]

CM physical characteristic is a direct expression of the hormonal activity in female[24] because E2 and P secreted by the ovary cause cyclic changes in the cervix.[25] E2 make the CM thinner and more alkaline (changes that promote survival and transport of sperm). P make CM thick, tenacious, and cellular.

Study objective: To study the relationship between primary and secondary infertility and mid-cycle cervical mucus variables.

ART Assisted Reproductive Technique
BMI Body Mass Index
AFC Antaral follicle count
CM Cervical Mucus
COS Controlled Ovarian stimulation

2-MATERIALS AND METHODS

2.1: Samples
3.1. Patients.
50 female patients were included in this study, they were recruited from private infertility clinic patients. The patients divided in to two groups: first group (25 female patient Primary infertility. The second group (25 female patient treated Secondary infertility. The study exteneded from November 2024 to January 2025.

Criteria for selection of patients to be included in the study depend on.
1- Regularity of the menstrual cycle (28 - 30 days).
2- Absence of congenital anomalies of the reproductive system.
3- Absence of acquired abnormalities of the cervix, such as cervical cauterization and biopsy.
4- Absence of any ovarian cyst detected by Ultra sound at the 2 days of cycle.
5- Patent fallopian tubes as shown by hysterosalpinography or hycosy.
6- Normal base line reproductive hormonal level (FSH, LH, Prolactin (PRL), E2, Serum Testosterone, TSH).
7- Concerning the seminal fluid analysis of the husband, the wives of males with normal semen analysis were included in the study.

Table 3.1: Patient clinical data.

parameters	Data
Number of patients	50
Age	20- 40 year
Duration of infertility	2 - 15 year

3.2. Patient protocol

The experimental design include.

3.2.1. Cervical mucus score
The parameter includes: amount of mucus (volume), consistency (viscosity), spinnbarkeit, ferning, and cellularity (39).
The pH of the mucus is not included in the total CM score, but should be measured as an important factor of sperm-mucus interaction.[33]

The current study extended from November 2024 to January 2025. Fifty patients were included in this study, recruited from patients of a private infertility clinic. The patients were divided into two groups according to the type of infertility

Group I, 25 patients with primary infertility.
Group II (25 patients with secondary infertility. On the second day of the menstrual cycle, follicle growth was monitored serially (in both cycles) by vaginal ultrasound from the 10th to the 14th day of the cycle until the dominant follicle reached a size of 17 - 20 mm, at which time serum E2 measurement and cervical mucus score were performed. Cervical mucus was evaluated according to the system developed by Mogisi.[15],[33]

The evaluation is as follows.
1. Volume: The volume of cervical mucus collected is measured in the collection syringe and scored as following 0 = 0 ml,1 = 0.1 ml, 2 = 0.2 ml and 3 = 0.3 ml or more.
2. Consistency: The consistency is scored as the following: 0 = thick, highly viscous premenstrual mucus, 1 = moderate viscosity, 2 = thin or mildly viscous and 3 = watery or minimally viscous.
3. Spinnbarkeit: A drop of cervical mucus put into slide and lifted gently with pipette and the length of C thread is measured in centimeters before it break and scored as follows: 0 = < 1 cm,1 = 1 – 4 cm,2 = 5 – 8 cm and 3 = ≥ 9 cm.
4. Cellularity: An estimate of the number of leukocyte and other cells in five high power field (× 40).The cellularity is scored as follows: 0 = ≥ 11 cells / HPF,1 = 6 – 10 cells / HPF, 2 = 1− 5 cells / HPF and 3 = 0 cells / HPF.
5. Ferning: Ferning is examined by spreading cervical mucus on the glass slide and allowed for air dryness. Examination under low power (× 10) objective lens in contrast phase microscope. The ferning is scored as follows: 0 = no crystallization,1 = atypical fern formation (lineal), 2 = primary and secondary stems and 3 = tertiary and quaternary stems.
6. pH: The pH of CM from the endocervical canal was measured using pH indicator strips immediately following collection.

THE RESULTS

The demographic features and hormonal assay of the studied groups were presented in table 1 and the results demonstrated no significant differences regarding the age (p= 0.202), BMI (p= 0.688), the duration of infertility (p= 0.816), FSH level (p =0.468), LH level (p

=0.432), prolactin level (p =0.069) and E2 level (p =0.088). There was also significant difference in mid-cycle E2 level ($p < 0.001$).

Table 1: Demographic data and hormonal levels of the study group.

Hormonal assay	primary infertility group	secondary infertility group	*p* value
	Mean±SD	Mean±SD	
Age (years)	25.32 ± 4.51	27.24 ± 5.89	0.202
BMI (Kg/m²)	26.69 ± 4.34	27.12 ± 3.17	0.688
Duration of infertility	4.68 ± 2.89	4.88 ± 3.13	0.816
FSH (mIU/ml)	7.56 ± 2.18	7.93 ± 1.36	0.468
LH (mIU/ml)	6.74 ± 2.79	7.47 ± 3.71	0.432
Prolactin (ng/ml)	15.07 ± 8.38	11.34 ± 5.54	0.069
E2 (pg/ ml)	36.04 ± 15.29	47.16 ± 28.08	0.088
Mid-cycle E2 (pg/ ml)	304.65 ± 182.19	153.59 ± 79.02	< 0.001*

SD: Standard deviation; BMI: Body mass index; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; *: p value < 0.05 (significant).

The comparison of cervical mucus score parameters between the two study groups demonstrated a significant differences in the cervical mucus volume (p=0.029), the consistency (p=0.046), spinnbarkeit ($p < 0.001$),the cellularity (p=0.002) and total cervical mucous (CM) score ($p < 0.001$) with higher parameters levels in secondary infertility group.There was also no significant difference between the two groups regarding the ferning (p=0.626) and PH (p=1.0), as demonstrated in table 2.

Table 2: Comparison of Cervical Mucous score parameters between the study groups.

parameters	primary infertility group	secondary infertility group	*p* value
	Mean±SD	Mean±SD	
Volume	2.16 ± 0.94	2.64 ± 0.49	0.029*
Consistency	1.56 ± 1.23	2.12 ± 0.6	0.046*
Spinnbarkeit	0.8 ± 1.00	1.80 ± 0.65	< 0.001*
Cellularity	0.84 ± 1.18	1.76 ± 0.72	0.002*
Ferning	2.24 ± 0.52	2.32 ± 0.63	0.626
Total CM score	7.56 ± 1.19	10.64 ± 1.18	< 0.001*
PH	6.08 ± 0.28	6.08 ± 0.28	1.00

SD: Standard deviation; CM: Cervical mucus; *: p value < 0.05 (significant)

Comparison of cervical mucus grade parameters between the two study groups showed statistically significant differences in cervical mucus volume (p=0.029), consistency (p=0.046), spina bifida (p<0.001), cellularity (p=0.002) and total cervical mucus grade (p<0.001) with higher levels of the criteria in the secondary infertility group. There was also no significant difference between the two groups regarding ferning (p=0.626) and pH (p=1.0), as shown in Table 2.

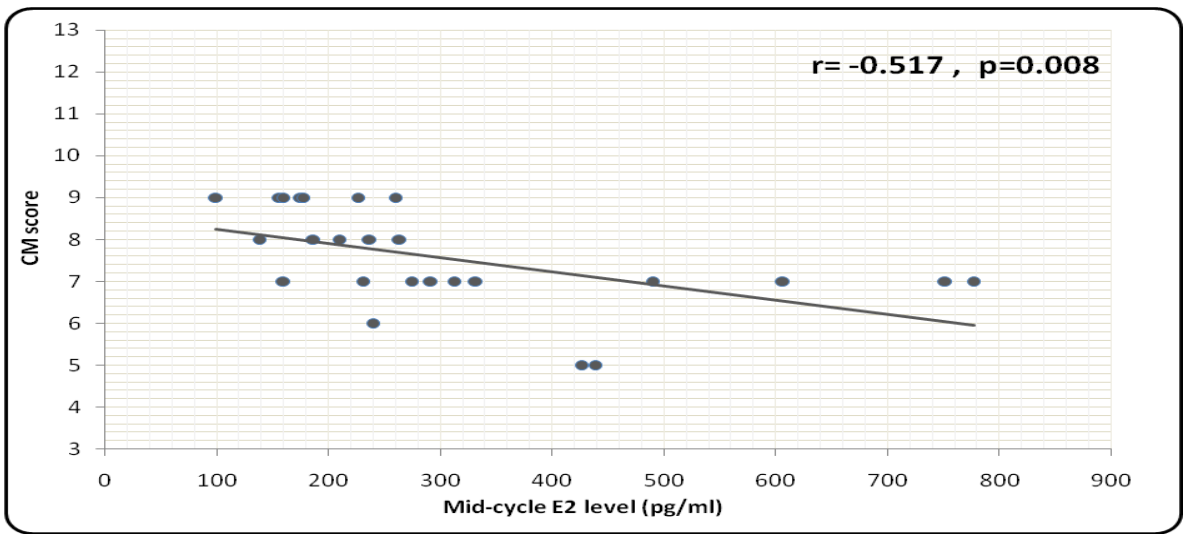

Figure 1: Correlation between mid-cycle E2 and total CM score in primary infertility group.

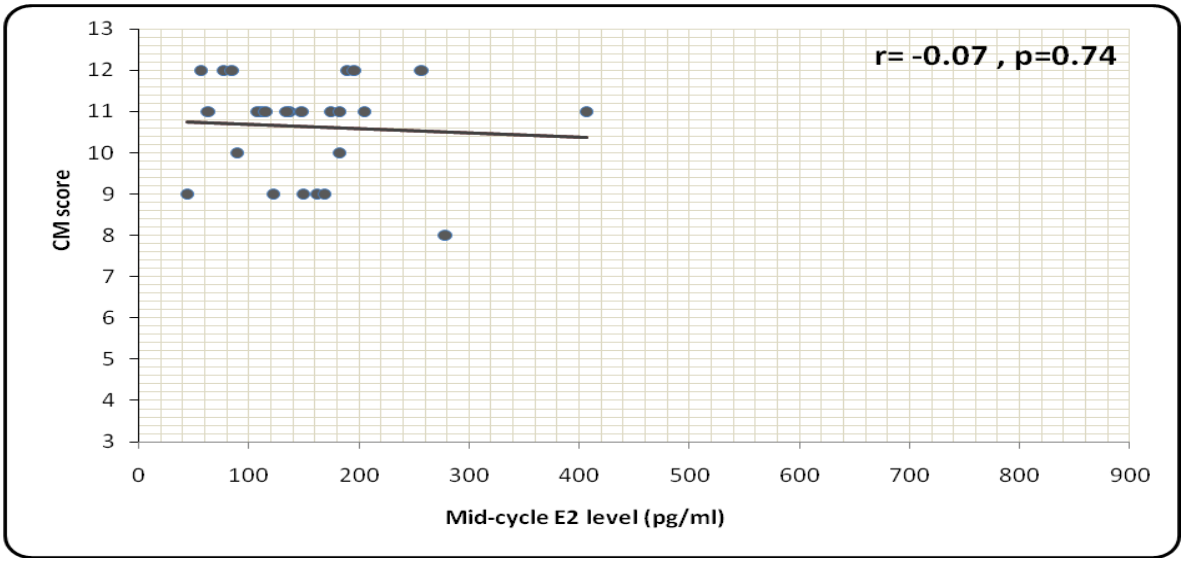

Figure 2: Correlation between mid-cycle E2 and total CM score in secondary infertility group.

DISCUSSION

Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in a woman under 35 years of age) or 6 months (in a woman over 35 years of age) despite regular and adequate (3-4 times per week) of unprotected sexual intercourse[1] Primary infertility in which the couples have never been able to conceive; while in secondary infertility difficulty in conceiving after having conceived (either carried a pregnancy to term or miscarriage).

Main factors associated with infertility are weight, smoking and age, and other factors are nutrition, physical activity, psychological stress, caffeine and alcohol all affect the reproductive ability. Women between 20 and 24 years of age (have the highest reproductive ability and chance of pregnancy). On average, 85-90% of young and healthy couples achieve pregnancy within one year of unprotected intercourse.[26]

There was no significant difference between the age of females in the sample, and that may reflect the trend in Iraqi society. Unexplained infertility among couples' percentage was found 14.7% of the studied couples in the sample and this result is close to that in Lagos, Nigeria study (11.1%) and Africa (10.4%).[27]

The relationship between demographic factors and fertility should be further explored to reduce infertility-related diseases. Table 1 shows the association between infertility type at menarche in patients, BMI, Duration of infertility and age of infertility patients. BMI and Duration of infertility and age did not reveal any significant positive association with infertility type ($p > 0.05$). These associations found that the most common cases of primary and secondary infertility were among women with normal weight. This is consistent and does not contribute to a 2016 study in northern China that reported that the most common cases of female infertility were among underweight women.[36]

The reported prevalence of infertility due to abnormal transport of spermatozoa in cervical mucus (cervical factor) ranges from 10% to 30%1,2 This diagnosis is made by microscopic examination of postcoital cervical mucus obtained just prior to ovulation in the midcycle. Scot, et.al. study investigateed and treated a group of patients referred for "idiopathic" infertility in whom no apparent cause for infertility, apart from inadequate cervical mucus.[28]

It is generally believed that another potentially important feature of human cervical mucus is its ability to restrict migration of abnormal spermatozoa, thus acting as a “filter” that eliminates deficient sperm. It has been shown that abnormal sperm have a poorer hydrodynamic profile compared with morphologically normal motile sperm. Moreover, sperm movement is probably influenced by the interaction between the mucus and the surface properties of the sperm head; for instance, sperm antibodies on the sperm head may inhibit sperm movement through the mucus.[29]

Like the vagina, the cervix can assemble immune responses. Studies have shown that vaginal insemination stimulates the migration of leukocytes, particularly neutro phils and macrophages, into the cervix as well as into the vagina. This leukocytic invasion protects against microbes that are often seen in the semen, but it does not represent a barrier to normal sperm under physiological conditions. On the other hand, it has been demonstrated that neutrophils bind to and ingest human sperm if the female serum contains both serological complement and complement-fixing antisperm antibodies.[30]

As already mentioned, immunoglobulins, mainly IgG and IgA, have been detected in human cervical mucus. Secretory IgA is produced locally by plasma cells in subepithelial connective tissue. Although immunoglobulins provide protection from microorganisms, immunological infertility can occur

when antibodies present in the cervical mucus recognize sperm-bound antigens. Since comp lement proteins are present in the cervical mucus, antibody-mediated sperm destruction as well as leukocytic sperm capture may occur. Despite the fact that not all antisperm antibodies are complement-activated, they can still interfere with sperm progression by attaching the sperm head and avoiding spermatozoa to enter the microarchitecture of the cervical mucus network. Furthermore, the presence of ASA in the male can also result in infertility since such antibodies have been shown to affect sperm function.[31]

Our results about E2 can explain by facts that cervical mucus is a heterogeneous mixture of secretions whose rate of production depends on several factors. These factors include the number of mucus-secretory units in the cervical canal, the percentage of mucus-secreting cells per unit and the secretory activity of the cells in response to circulating hormones. There are several types of mucus Type E is thin and watery (with approximately98% of water), which is characteristic of estrogen dominance. Type G is thick and sticky, and reflects the stimulation of progestogenic hormones. Under the influence of progester one, the water content decreases to approximately 90% and the mucus becomes more viscous. Therefore, type E is predominant at the time of ovulation in a proportion of Medical Express.[32]

The very significant decrease in total cervical mucus score seen in primary infertility cases may be due to decrease in cervical mucus volume, spindle cell score, consistency, and cellularity which implies that infertility is related to the quality and quantity of cervical mucus. This finding is consistent with the study by Fauzia HaqNawaz et al., Volume, consistency, and spindle cellity can be considered as different sides of the same coin related to cervical mucus hydration. Cervical mucus hydration can lead to changes in cellularity through a dilution effect.[38] The result of the present study suggests that the cellularity score of the menstrual cycles of primary infertile women was significantly decreased (i.e., increased cell count) when compared to that of spontaneous menstrual cycles of secondary infertile women. This may be due to the effect of decreased cervical mucus volume causing an increase in cell concentration. Ferning occurs due to the presence of sodium chloride in the mucus under the influence of estrogen. When high levels of estrogen are present, just before ovulation, cervical mucus forms fern-like patterns due to the crystallization of sodium chloride on the mucus fibers.[37] Sodium chloride is the main salt component of cervical mucus and the determining factor for its osmolar pressure. Prins et al. found that the concentration of sodium chloride (NaCl) in cervical mucus is constant during the mid-cycle (estrogenic state) and luteal phase (gestational state) to maintain isotonicity of cervical mucus.[35] This leads us to propose that the concentration of sodium chloride is constant as estrogen levels change. Therefore, when thick or thin cervical mucus dries, the concentration of sodium chloride will remain the same and the crystallization will not change.[34]

Nakano et al. suggested that estrogen makes cervical mucus more alkaline. In the present study, the large increase in serum estrogens resulting from primary infertility had no significant effect on cervical mucus pH.[15] Cervical mucus volume increases near ovulation.[15] In the present study, there was an increase in cervical mucus volume in women in the secondary infertility group. Jamie L. Bigelow et al. showed that: “Changes in mucus quality across the fertility window predict the observed pattern in day-specific pregnancy probability[20] and the watery, elastic mucus required for optimal sperm permeability.[15]„

5. CONCLUSION

Most infertility cases were found in women from normal weight, FSH, LH, Prolactin, but E2 and cervical mucus effect and indicate the problem of infertility in our study group.

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