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Effect of sex hormones level on the external genital tract infection in pregnant and non-pregnant women in Dhi Qar governorate, Iraq

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Abstract:

The current study was conducted in Dhi Qar governorate, Iraq to determine the main types of microorganisms that cause female genital tract infection and the extent of the effect of the level of sex hormones (prolactin, progesterone, estrogen) on the presence of infection **,** 245 samples were collected for both vaginal swabs and blood (where take a vaginal swab and blood from every woman) , where 150 women were pregnant and 95 women non-pregnant women , aged 16-45 years **.**

Among non-pregnant women, most were women who had a reproductive tract infection within the leuteal phases , where our study included 58 women at leuteal phase , 20 women were in follicular phase , 17 women within ovulation phase of the menstrual cycle .

The study showed that *Candida albicans , Staphylococcus aureus , Escherichia coli , Gardnerella vaginalis , Klebsiella pneumonia , Staphylococcus epidermids , Enterobacter cloaceae* were isolated from women in follicular phase , the diversity of infections was lower in women in the ovulation stage, where they were isolated only *Candida albicans , Staphylococcus aureus , Escherichia coli , Gardnerella vaginalis* ,and from women with leuteal phase was obtained *Candida albicans , Staphylococcus aureus , Escherichia col ,Gardnerella vaginalis , Klebsiella pneumonia , Staphylococcus epidermids , Proteus mirabilis, Pseudomonas aeruginosa* , the precence of all type of pathogens in our study was associated with elevated estrogen and progesterone levels except the growth of *Staphylococcus epidermids , Proteus mirabilis, Pseudomonas aeruginosa , Enterobacter cloaceae* was associated with normal range of each hormones,as for the prolactin hormone did not have a clear effect as the levels ranged between natural and high for all women in the three phases.

Pregnancy was accompanied by high levels of estrogen, progesterone and prolactin hormones, this is accompanied with existence *Candida albicans , Staphylococcus aureus , Escherichia coli , Gardnerella vaginalis , Klebsiella pneumonia , Streptococcus agalactiae , Trichomonas vaginalis.*

Key words: Sex hormones , microorganisms , vaginitis.

1-Introduction:

Genital tract infection is one of the most common diseases in women during childbirth(Vazques and sobel,2002).Genital tract infection can produce from a defect the vaginal environment because excessive intake of antibiotic (Robert, 1990), pregnancy, diabetes, Acquired immunodeficiency syndrome (AIDs), anatomical changes,vaginal doching, hormonal imbalance (Ness *et al*.,2002;Ramzan *et al*., 2004; Pitt *et al* .,2005;Lan *et al* .,2007).An inaccurate diagnosis of the condition of the woman with vaginitis and rushing to take treatment without a sensitivity test (Weber *et al*.,2002). Female genital tract (uterus, cervix, and vagina) mucosal tissues are regulated by several factors commonly produced in the mucosa, as well as by sex hormones especially estrogen and progesterone (Witkin et al., 2007). Innate and adaptive mucosal immunity in the FGT are regulated by the female sex hormones estrogen and progesterone (Bouman *et al*.,2005; Butts and Sternberg,2008).This hormones regulate the transport of Igs, the levels of

cytokines,the expression of TLR genes(Aflatoonian *et al*.,2007; Aflatoonian and Fazeli,2008) and the distribution of immune cells and antigen presentation in the genital tissues during the reproductive cycle(Wira *et al*.,2005).Immune protection in the FRT varies with the phases of the menstrual cycle(Wira and Kaushic,1996).The hormonal changes play a role in change the natural flora in the vagina Leading to inflammation , where the normal level of estrogen necessary to maintain vaginal balance on the basis that this hormone stimulates and activates the growth and integration of the vaginal epithelial membrane (Reid *et al*.,2004) ,in normal women,estrogen supports vaginal epithelium in glycogen accumulation which in turn helps in maintenance of vaginal pH(Lehman,2009).As that the reproduction of lactobacilli depends on sex hormones oestrogen levels the numbers of these bacteria may change during the menstrual cycle depending on change of estrogen level . As acidification by vaginal secretion stimulates the growth of lacltobacilli .If the level of this hormone is changed, the number of lactobacilli will be reduced and vaginal environment changes to become an appropriate place for pathogens(Galask, 1988;Miller *et al*.,2000).

2-Materials and methods :

2.1.Collection of specimens:

The vaginal swab samples are taking and then placed in the carrier media (stuart transport media) until arrival to the lab and work on them .Blood samples collected by the conventional way of taking blood from the vein (serum tubes are placed in a small refrigerator so that they do not damage until they reach the laboratory).

2.2.Vaginal samples procedure:

2.2.1 Direct examination:

The vaginal swabs are directly examined to check the presence of each of the clue cell , yeast cell , WBCs , *Trichomonase vaginalis* and epithelial cell by working stuck to the sample by using an aqueous salt solution (normal saline) where the drop of stuck is placed on glass slide and covered by the cover slide and examined under the large lens of the microscope(Atlas,1995).

2.2.2 Specimens Culture:

The vaginal swabs are cultivation on blood agar, macconky agar,chocolate agar and sabouraud dextrose agar to diagnose all kinds of microbes could exist within it , then incubated at 37 ° C for 24-18 hours, and incubated dishes that did not appear to grow within 24 hours for another 24 hours before counting negative result.

2.3.Blood Sample Procedure:

2.3.1 Measurement of progesterone, estrogen and prolactin hormones:

The concentration of the three hormones (progesterone, estrogen and prolactin) in the blood serum by using the " ichroma" device with kit ready-analysis of each hormone by following the instructions supplied with the kit for each hormone.

2.4. Statistical Analysis:

The statistical analysis proceeded in groups of study, descriptive statistics analyzed by using one-way analysis of variance (ANOVA) were performed using means and standard deviations (SDs) with LSD test for continuous variables ($p< 0.05$) was considered to be significant, and X^2 distribution (P-value 0.05) was considered to be significant.All analyses were performed with the Statistical Package for the Social Sciences SPSS for Windows (version 17.0, SPSS Inc, Chicago, III).

3.Results:

3.1Type and number of isolates taken during the study:

A total of 245 vaginal swabs were collected from 150 pregnant women and 95 non-pregnant women in age avarge16 - 45 years .The pathogens isolated in this study are shown in table (4-1) . They included *Candida albicans* 75 which were predominant in pregnant women 55(73.3%) than non pregnant women 20(26.7%), as obtained 35 isolate of *Streptococcus agalactiae* were isolated from pregnant women only ,other are 27 isolate of *Staphylococcus aureus* and 25 isolate of *Escherichia coli* which were isolated from 20 (74.15%) and 15(60%) pregnant women and 7(25.9%) , 10(40%) from not pregnant women respectively. As isolated *Staphylococcus epidermidis* from 21 non pregnant women ,*Gardnerella vaginalis*

which also has a higher rate of isolates from pregnant women 12(63.2%) than not pregnant women 7(36.8%) .while *Klebsiella pneumoniae* was isolated in nearly equal proportions from the pregnant and not pregnant women with 7(46.7%) , 8(53.3%) respectively .*Proteus mirabilis* and *Pseudomonas aeruginosa* were isolate only from non pregnant women in 11 and 9 respectively. finally , *Trichomonas vaginalis* , *Enterobacter cloacae* was isolate from 6 , 0 of pregnant women and 0 , 2 from not pregnant women respectively , there was a significant difference at level (P<0.05) between the rate of infection in pregnant women and not pregnant women where significant increase was observed in pregnant women.

Table 1: microorganisms isolated from the vaginal swabs samples

Pathogens	case		
	Pregnant %	Non pregnant %	Total %
Candida albicans	55(73.3%)	20(26.7%)	75(100.0%)
Streptococcus agalactia	35 (100 %)	0(0.0%)	35 (100 %)
Staphylococcus aureus	20(74.15)	7(25.9%)	27(100.0%)
Escherichia coli	15(60.0%)	10(40.0%)	25(100.0%)
Staphylococcus epidermids	0(0.0%)	21(100.0%)	21(100.0%)
Gardnerella vaginalis	12(63.2%)	7(36.8%)	19(100.0%)
Klebsiella pneumonia	7(46.7%)	8(53.3%)	15(100.0%)
Proteus mirabilis	0(0.0%)	11(100.0%)	11(100.0%)
pseudomonas aurgenosa	0(0.0 %)	9(100.0 %)	9(100.0%)
Trichomonas vaginalis	6(100.0%)	0(0.0%)	6(100.0%)
Enterobacter cloacae	0(0.0%)	2(100.0%)	2(100.0%)
Total	150(61.2%)	95(38.8 %)	245(100.0%)
X^2 (Cal.)	101.756		

Tab. X2 : (df:10 ; P_value: 0.05) = 18.307

3.2The relationship between hormones and infections:

About 245 blood samples were collected from women, 150 women were pregnant, 58 women at leuteal phase , 20 women were in follicular phase and 17 women within ovulation phase of the menstrual cycle .*Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Staphylococcus epidermids* and *Enterobacter cloaceae* were isolated from women in follicular phase as shows in table (2) . The study recorded increase in the level of progesterone and estrogen hormones in women infected with *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* and *Klebsiella pneumonia* , there was also significant differences (P<0.05) between the level of progesterone hormone in infected women with *Candida albicans* and women infected with *Escherichia coli* , also precence significant differences (P<0.05) between the level of progesterone hormone in infected women with *Candida albicans* and women infected with *Gardnerella vaginalis*,as recorded Significant differences (P<0.05) between the level of progesterone hormone in women infected with *Candida albicans* , *Staphylococcus aureus* and each of women infected with *Staphylococcus epidermids* , *Enterobacter cloacae* for progesterone hormone. There were significant differences (P<0.05) for estrogen levels between each of women infected with *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* and women infected with *Staphylococcus epidermids* , *Enterobacter cloacae* as existence significant differences (P<0.05) between the level of estrogen hormone for women infect with *Staphylococcus aureus* and women infect with *Escherichia coli*.While the level of prolactin hormone ranged from normal to high with all infection except *Staphylococcus epidermids* , *Enterobacter cloaceae* its presence was accompanied with normal levels of prolactin hormone.Observed precence of significant differences (P<0.05) between the level of this hormone for women infect with *Staphylococcus aureus* and each of women infect with *Gardnerella vaginalis* , *Klebsiella pneumonia*,as precence significant differences (P<0.05) between the level of this hormone for women infect with *Gardnerella vaginalis* , *Klebsiella pneumonia* and each of women infect with *Staphylococcus epidermids* , *Enterobacter cloaceae*.

Table (2) The relationship between hormones and infections in follicular phase of menstrual cycle

Hormons / Pathogens	Progsteron(ng/ml) mean±SD	Prolactin(ng/ml) mean±SD	Estrogen(pg/ml) mean±SD
Candida albicans	2.46 ± 1.85^{a}	17.60 ± 12.0^{bcd}	186.03 ± 12.30^{ab}
Staphylococcus aureus	1.86 ± 0.95^{ab}	13.06 ± 2.65^{cd}	174.10 ± 6.90^{b}
Escherichia coli	1.26 ± 0.35^{bc}	21.56 ± 3.35^{abc}	196.96 ± 5.15^{a}
Gardnerella vaginalis	0.76 ± 0.15^{bc}	26.36 ± 2.05^{a}	191.40 ± 2.20^{ab}
Klebsiella pneumonia	0.70 ± 0.10^{c}	25.10 ± 1.70^{ab}	179.66 ± 11.75^{ab}
Staphylococcus epidermids	0.30 ± 0.10^{c}	15.56 ± 5.75^{cd}	71.10 ± 25.40^{c}
Enterobacter cloaceae	0.40 ± 0.10^{c}	12.50 ± 7.20^{d}	43.26 ± 11.65^{d}
L.S.D.	1.15	8.66	18.42

table (3) shows that the diversity of infections was lower in women in the ovulation stage, where they were isolated only *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , the results also showed a rise in both estrogen and progesterone hormones , the progesterone elevation was slight while the rise in estrogen was apparent ,whereas prolactin had no apparent effect,all the infections were accompanied by normal levels of this hormone . The study recorded simple significant differences ($p<0.05$) between the level of estrogen and progesterone hormones for women infected with *Staphylococcus aureus* and the level of this hormones for women infect with *Escherichia coli* for estrogen and progesterone hormones , while there were no significant differences between other infections.

Table (3) The relationship between hormones and infections in mid (Ovulation phase) of menstrual cycle

Hormones / Pathogens	Progsteron(ng/ml) mean±SD	Prolactin(ng/ml) mean±SD	Estrogen(pg/ml) mean±SD
Candida albicans	9.96 ± 2.95^{ab}	19.0 ± 3.80^{ab}	544.50 ± 40.90^{ab}
Staphylococcus aureus	10.46 ± 1.05^{a}	11.76 ± 4.95^{b}	594.10 ± 72.90^{a}
Escherichia coli	7.16 ± 0.65^{b}	21.20 ± 6.66^{a}	517.0 ± 6.0^{b}
Gardnerella vaginalis	9.0 ± 0.30^{ab}	14.16 ± 3.35^{ab}	552.50 ± 54.50^{ab}
L.S.D	3.20	7.35	75.89

Candida albicans , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Staphylococcus epidermids* ,*Proteus mirabilis*, *Pseudomonas aeruginosa* isolated from women with leuteal phase ,table (4) shows that the precence of *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* was associated with elevated estrogen and progesterone levels while prolactin was within normal limits and the growth of *Staphylococcus epidermids* , *Proteus mirabilis*, *Pseudomonas aeruginosa* was associated with normal range of each hormones, A significant differences ($p<0.05$) were observed between the level of both estrogen and progesterone hormones for each of women infect with *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* and women infect with *Staphylococcus epidermids* , *Proteus mirabilis*, *Pseudomonas aeruginosa* for both estrogen and progesterone hormones , there were no significant differences ($P>0.05$) between the level of prolactin hormone for women infected with the types of infection.Pregnancy was accompanied by high levels of estrogen, progesterone and prolactin hormones,this is accompanied with existence *Candida albicans* , *Staphylococcus aureus*, *Escherichia coli*, *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Streptococcus*

agalactiae ,*Trichomonas vaginalis*, the study recorded no significant differences between the level of progesterone in women infect with all type of isolates,as for prolactin hormone , there were significant differences (P<0.05) between the level of this hormone for women infect with *Candida albicans* and each of women infect with *Escherichia coli* ,*Trichomonas vaginalis* , and the absence of significant differences (P>0.05) between the level of prolactin hormone for women infect with other infections . as for estrogen the results showed significant differences (P<0.05) between the level of this hormone in women infect with *Escherichia coli* and women infect with each of *Candida albicans* , *Klebsiella pneumonia* , *Gardnerella vaginalis* .There was also a significant difference (P< 0.05) between the level of estrogen hormone in women infect with *Streptococcus agalactiae* and *Klebsiella pneumonia* for estrogen hormone as shows in table (5).

Table(4) The relationship between hormones and infections in leuteal phase of menstrual cycle.

Hormones / Pathogens	Progesterone(ng/ml) mean±SD	Prolactin(ng/ml) mean±SD	Estrogen(pg/ml) mean±SD
Candida albicans	25.50 ± 2.10^{b}	18.99 ± 9.35^{ab}	264.50 ± 44.50^{a}
Staph. aureus	30.30 ± 1.80^{a}	11.70 ± 3.90^{ab}	287.0 ± 11.0^{a}
E.coli	26.06 ± 4.45^{b}	20.16 ± 7.35^{a}	254.50 ± 20.50^{ab}
Gardnerella vaginalis	23.56 ± 1.45^{b}	12.36 ± 3.05^{ab}	268.50 ± 43.50^{a}
Klebsiella pneumonia	26.20 ± 2.30^{ab}	13.56 ± 6.25^{ab}	221.50 ± 4.50^{b}
Staphylococcus epidermids	17.20 ± 1.40^{c}	9.66 ± 2.85^{b}	115.16 ± 35.85^{c}
Proteus mirabilis	16.40 ± 3.30^{cd}	16.96 ± 9.05^{ab}	57.70 ± 9.80^{d}
Pseudomonas.aeruginosa	12.30 ± 4.50^{d}	19.30 ± 6.10^{ab}	105.36 ± 18.45^{c}
L.S.D.	4.15	9.88	39.59

Table (5) The relationship between hormones and infections in pregnant women:

Hormones / Pathogens	Progesterone(ng/ml) mean±SD	Prolactin(ng/ml) mean±SD	Estrogen(pg/ml) mean±SD
Escherichia coli	43.46±5.05^{a}	70.26±29.75^{a}	2872.66±78.50^{a}
Staph. aureus	34.26±5.75^{a}	66.86±33.15^{ab}	2517.66±465.50^{ab}
Streptococcus agalactiae	85.96±64.05^{a}	59.80±40.20^{ab}	2215.0±785.0^{ab}
Trichomonas. Vaginalis	88.86±60.15^{a}	70.36±29.65^{a}	1822.0±477.0^{abc}
Gardnerella vaginalis	83.06±56.95^{a}	65.36±34.65^{ab}	1610.0±539^{bc}
Candida albicans	34.50±12.50^{a}	26.36±2.05^{b}	1555.66±420.0^{bc}
Klebsiella pneumonia	87.27±59.70^{a}	33.06±1.35^{ab}	1079.0±489.0^{c}
L.S.D.	65.48	40.97	1201.05

4.Discussion:

There are a many studies on several aspects specific to infection with microorganism , but we did find a few studies on the relationship between the incidence of infection with microorganisms and influence of female sex hormones most studies have been carried out on laboratory animals . This study showed that among from not pregnant women the proportion of women with vaginitis in the leuteal phase of the menstrual cycle was more than ovulation phase and the follicle phase this result agrees with (Kalo-Klein and Witkin,1989), according to our study , the high estrogen level was associated with fungal vaginitis in each of the three stages of menstrual cycle in addition to pregnancy, estrogen plays a role in increasing the adhesion of Candida albicans to the vagina epithelial cells (VEC). Thus encouraging the transformation from the yeast shape to the filamentous shape (King *et al*.,1980 ; Chaffin,and Stocco, 1983; Granger,1992; Vazques and Sobel , 2002) ,. the study also showed that the fungal infection was associated with high progesterone level , with respect to innate defenses , polymorphonuclear leukocytes (PMNs) were recently shown to have minimal effects on vaginal

fungal burden in vivo (Black *et al*., 1998; Fidel *et al* .,1999) despite their ability to kill *C. albicans* in vitro (Odds,1988) and their frequent presence in vaginal lavage fluid during infection. Interestingly, while PMN anti-Candida activity , in vitro showd that reduce in the presence of progesterone , but not estrogen (Nohmi *et al*.,1995) , the result of our study agrees with (Kalo-Klein and Witkin ,1989)Which proved that the incidence of fungal infections was associated with elevated level of estrogen and progesterone and also the bacterial infection was companion with elevated estrogen and progesterone , this agrees with (Galask , 1988) , the estrogen imbalance, especially in pregnant women, this will lead to a change in the acidity of the vagina, thus allowing infect with microbes.In normal women ,estrogen supports vaginal epithelium in glycogen accumulation which in turn helps in maintenance of vaginal pH(Lehman,2009). the female genital tract infection attributed to changes in the secretion levels of the steroid hormones estradiol and progesterone and the ability of some microbial species to use these hormones as growth factors. As that the Reproduction of lactobacilli depends on oestrogen levels (Galask,1988;Miller *et al* .,2000) the numbers of these bacteria may change during the menstrual cycle depending on change of estrogen level "as acidification by vaginal secretion stimulates the growth of lactobacilli" , if the level of this hormone is changed, the number of lactobacilli will be reduced and vaginal environment changes to become an appropriate place for pathogens(Kornman and Loesche, 1982). Female genital tract (uterus, cervix, and vagina) mucosal tissues are regulated by several factors commonly produced in the mucosa, as well as by estrogen and progesterone(Witkin *et al*., 2007). Innate and adaptive mucosal immunity in the FGT are regulated by the female sex hormones esterogen and progesterone and have been reviewed in(Bouman *et al* .,2005; Butts and Sternberg,2008) hormones regulate the transport of Igs, the levels of cytokines, the expression of TLR genes(Aflatoonian *et al*.,2007; Aflatoonian and Fazeli ,2008) and the distribution of immune cells and antigen presentation in the genital tissues during the reproductive cycle(Wira *et al*,2005) .In some studies oestrogen has been showed to have a protective effect in autoimmunity, particularly in T cell mediated autoimmune conditions, through activation of ER-α signalling (Yang *et al*., 2010).The study found that the parasite *Trichomonas vaginalis* was isolated only from pregnant women ,where the parasite presence was accompanied by a rise in estrogen, progesterone and prolactin ,the role of estrogen in influencing the parasite is unclear and controversial, according to an earlier study (Sugarman and Mummaw,1988) the estrogen decrease the growth of parasite, and their attachment to mammalian cells in vitro, and acted as a chemo repellent, therefore this study didn't agrees with the results of the current study. While the results of the current study agreed with the findings of the researcher(Inceboz *et al* .,2012). who has studied the relationship between the estrogen and *T. vaginalis* in vitro, which he found the high concentrations of estrogen promoted the reproduction of *T. vaginalis* , the researcher instructed the reason for this situation estrogen can change in pH and make it suitable for the growth of the parasite , increased parasite growth at the high concentration of this hormone is that it affects directly in its own hormone receptors on the surface of the parasite, which may play an important role in the growth and reproduction of the parasite, as previously proposed by(Ford *et al*.,1987). The results also agreed with the findings of (Martinotti and Savoia.,1985) who found that the estrogen(17-α –Estradiol, 17-β-Estradiol) ,they were motivated for the growth of the parasite. As the results also agreed with (Markel and Voges, 2006)who suggested that the greater the concentration of progesterone inhibits *T. vaginalis* growth in vitro as well as for prolactin hormone (Baeten *et al*., 2001).

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