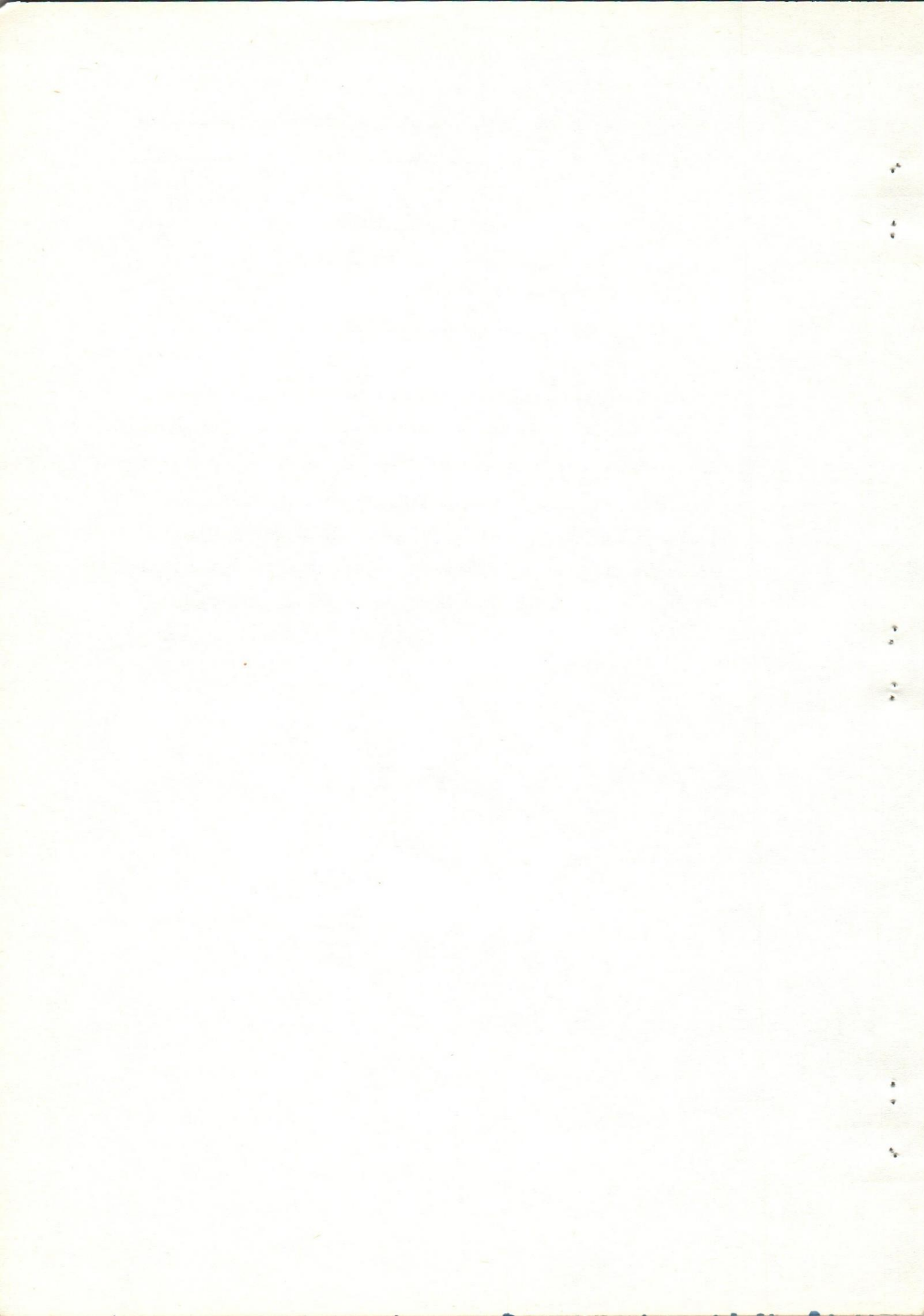


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البكتريا الطبيعية للجهاز التناسلى  
للماشية العشر وعلاقة وجودها بمراحل الحمل المختلفة

منير عفيفى ، مجاهد أبو العطا ، طلعت شومان ، السيد الصواف ، كمال زكى

جمعت ٦٢ عينة من عنق الرحم لماشية عشر وسليمة مطهريا ( ٣٤ بقرة و ٢٨ جاموس )  
حيث وجد أن ٥٠ عينة كانت ايجابية للفحص الجرثومى كان منها ٢٧ من الأبقار بنسبة ٧٩.٤% ،  
٢٣ من الجاموس بنسبة ٨٣.١% وكانت نسبة الحالات السلبية ٢٠.٦% و ١٧.٩% على التوالى  
كانت نسبة الجراثيم المعزولة اثناء المرحلة الأخيره من الحمل مرتفعة وذلك يرجع الى  
زيادة معدل المروجسترون اذ وصلت الى ٤٣.٤٦% فى الأبقار و ٤٠.٤٣% فى الجاموس تليها  
المرحلة الوسطى حيث كانت النسبة ٣٢.٩٦% فى البقر والجاموس على التوالى . ولكن لاحظ  
أن الجراثيم كانت قليلة وبعضها اختفى تماما اثناء الفترة الاولى من الحمل حيث لم تتجاوز النسبة  
فى الأبقار عن ٢٢.٦٤% وفى الجاموس ٢١.٢٧% .



MICROFLORA OF THE GENITALIA OF THE PREGNANT COWS AND BUFFALOES  
AND THEIR CORRELATION TO THE DIFFERENT PHASES OF PREGNANCY  
(With Two Tables)

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SUMMARY

Out of 62 samples (34 from cows and 28 from buffaloes) derived from clinically healthy pregnancy bovines, 50 samples (27 from cows and 23 from buffaloes) were found to be bacteriologically positive. While the incidence of sterile samples was 20.6% in cows and buffaloes respectively.

The percentages of the isolated microorganisms in pregnant animals were higher towards late pregnancy, due to the increase of progesterone level, reaching 40.43% and 43.4% in buffaloes and cows; followed by the mid stage of pregnancy with an incidence of 38.3% and 33.96% in buffaloes and cows respectively. The early stage of pregnancy showed lower frequency of bacterial isolates (21.27% in buffaloes and 22.64% in cows).

INTRODUCTION

Many investigators performed a lot of researches on the flora of pregnant animals by collecting uteri from slaughter houses (WALL, 1915; ROEHL, 1920; CLARK and STEVENSON, 1949; GEISSLER, 1954; PLOZAS-MOROLES, 1955 and DAWSON, 1959).

In Egypt, EL-SAWAF et al. (1961) applied bacteriological study on the uterine flora of 22 pregnant buffaloes, where they secured a variety of organisms like *C. pyogenes*, *Str. pyogenes*, *Staph. aureus* and *E. coli*. While ZAKI et al. (1961) on the examination of the external os of 15 pregnant buffalo-cows and 9 pregnant heifers, isolated *Corynebacteria*, *Anthracoïdes*, *Streptococci* and *Micrococci*, but recovered no *Sarcina*, *E. coli* and *proteus* from pregnant buffalo-cows. On the other hand, BARAKAT (1965) studied the flora of 50 pregnant uteri collected from slaughter house. He proved that 9 cases were sterile and isolated from the rest different species of *Corynebacteria* (72%), *Anthracoïdes* (62%), *Streptococci* (40%), *Coliform* (26%), *Yeasts* (18%), *Sarcina* (16%), *Clostridium* (12%), *Gaffkya* (10%), *Staphylococci* (4%) and *Micrococci* (2%).

The aim of this work was to find a relation between the microflora of the genital tract of the living cows and buffaloes and the different stages of pregnancy and clarify any changes present.

MATERIAL and METHODS

Sixty two vaginal swabs were collected from pregnant cows and buffaloes (34 and 28 respectively) in the Veterinary Clinic at Batanon, Monefia, Egypt. The examined animals were grouped according to the stage of pregnancy into:

- Early pregnancy: are those animals in the 1st three months (45-100 days) of pregnancy.
- Mid pregnancy: are those animals in 4-7 months (101-200 days) of pregnancy.
- Late pregnancy: are those animals above 7 months pregnancy (up to 201 days) or approaching parturition.

Bacterial samples were collected from external os of the examined animals. The swabs collected were placed directly in 1% sterile glucose broth, incubated at 37°C for 24 hours and then inoculated on the following media plates (DIFCO, 1964):- Nutrient agar, 5% Sheep-blood agar, MacConkey's liver infusion agar with gentian violet (1/200,000) and Sabaroud's agar with actidione (1/2000). The inoculated plates were incubated at 37°C for 24-72 hrs. at least.

Growing colonies were picked and purified by further subculturing on selective media and the isolates were identified morphologically by Gram's stain and biochemically. All biochemical reactions applied were recorded finally after five days incubation at 37°C at least.

The members of the genera *Micrococcus*, *Staphylococcus*, *Sarcina*, and *Gaffkya* were identified to species

according to ABDEL MALEK and GIBSON (1948), BREED *et al.* (1957) and WILSON and MILES (1964). While the members of genus *Streptococcus* were recognised according to SEELEMAN (1954), BREED *et al.* (1957) and MERCHANT and PACKER (1967).

The isolates belonging to genus *Corynebacterium* were identified according to schemes of REED *et al.* (1957), KIELSTEIN and KOTSCHKE (1963), COWAN and STEEL (1965) and MERCHANT and PACKER (1967). Gram-negative bacilli were differentiated according to BREED *et al.* (1957), MACKIE and MACCARTNEY (1960), COWAN and STEEL (1965) and EDWARDS and EWING (1969). Yeasts and moulds were identified according to the cultural character and morphological appearance.

## RESULTS

Bacteriological examination of the collected samples revealed that 50 cases were positive, from which 27 cases of cows (79.4%) and 23 from buffaloes (82.1%). While 7 cows and 5 buffaloes proved to be bacteriologically negative with an incidence of 20.6% and 17.9% respectively.

In cows, out of 27 positive cases, 7 animals revealed one type of bacteria, while 14 cases showed 2 types of bacterial flora and the remaining 6 samples harboured three types of mixed infection. The incidence, frequency and species of the isolates from cows in different stages of pregnancy are tabulated in Table (1).

Table (1): Incidence of isolated bacterial species from pregnant cows as regard stages of pregnancy

Species of isolates	No. of isolates in pregnancy						Total	
	Early		Mid		Late		No.	%
	No.	%	No.	%	No.	%		
<b>MICROCOCCUS SPECIES</b>	3	5.7	6	11.7	4	7.6	13	24.7
Staph. aureus	-	-	4	7.6	-	-	4	7.6
Staph. epidermidis	-	-	1	1.9	1	1.9	2	3.8
Sarcina lutea	-	-	1	1.9	1	1.9	2	3.8
M. flava	1	1.9	-	-	1	1.9	2	3.8
M. luteus	2	3.8	-	-	-	-	2	3.8
M. varians	-	-	-	-	1	1.9	1	1.9
<b>STREPTOCOCCUS SPECIES</b>	-	-	-	-	2	3.8	2	3.8
Str. durans	-	-	-	-	2	3.8	2	3.8
<b>CORYNEBACTERIUM SPECIES</b>	-	-	2	3.8	3	5.6	5	9.4
C. bovis	-	-	-	-	2	3.8	2	3.8
C. unclassified	-	-	2	3.8	1	1.9	3	5.6
<b>ANTHRACOIDSS</b>	2	3.8	5	9.4	3	5.6	10	18.8
<b>YEASTS</b>	4	7.6	3	5.6	5	9.4	12	22.6
<b>GRAM-NEGATIVE RODS</b>	3	5.6	2	3.8	6	11.4	11	20.6
E. Coli	3	5.6	2	3.8	4	7.6	9	17.0
K. genitalium	-	-	-	-	1	1.9	1	1.9
Pr. rettgeri	-	-	-	-	1	1.9	1	1.9
<b>Total</b>	12	22.64	18	33.96	23	43.4	53	100.0

Generally speaking, the total bacterial flora of pregnant cows were 53 strains, where Micrococci constituted 27.7%, Yeasts 22.6%, Gram-negative rods 20.6%, Anthracoides 18.8%, Corynebacteria 9.4% and Streptococci 3.8%. The most prevalent organisms isolated in all stages of pregnancy were Yeasts, Anthracoides and *E. coli*,

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where the remainders were recovered in one stage only.

In regarding the distribution of the flora in the three stages of pregnancy in cows, it was noticed that the most predominating organisms were isolated at the late stage, as 22 strains were recovered in an incidence of 43.4%, followed by mid stage where 19 strains were isolated (33.96%). A marked decrease in the incidence was noticed at the early stage of pregnancy (22.64%).

From 23 positive buffalo cases, 47 strains were recovered from which 17 Micrococci (36.3%), 9 Gram-negative bacilli (19.0%), 8 Anthracoïdes (17.2%), 5 Streptococci (10.5%) and 4 strains from each of Corynebacteria and Yeasts (8.5% each). The distribution of the isolates flora from the 23 positive cases was as follows: only one type was isolated from 3 buffaloes, two types of microorganisms from 16 cases and three species of bacteria from 4 samples. The species of the isolates, their presence in different stages of pregnancy and their incidence was summarised in Table 2. From such results, it was found that 10 strains (21.27%) were recovered in the early stage of pregnancy in buffaloes and the most prevalent organisms were Micrococcus species (4 strains). Regarding the mid pregnancy, 18 isolants were identified (38.3%) of which 8 Micrococcus species were predominant, while at late stage 19 strains were isolated (40.43%). The most prevalent organisms of this stage were Gram-negative bacilli (6) followed by Micrococci (5).

Table (2)  
The incidence of bacterial species isolated from pregnant buffalo samples with regards stages of pregnancy

Species of isolates	No. of isolates in pregnancy						Total	
	Early		Mid		Late			
	No.	%	No.	%	No.	%	No.	%
MICROCOCCUS SPECIES	4	8.4	8	17.2	5	10.7	17	36.3
Staph. aureus	1	2.1	-	-	-	-	1	2.1
Staph. epidermidis	-	-	1	2.1	3	6.5	4	8.5
Sarcina lutea	1	2.1	1	2.1	-	-	2	4.2
M. luteus	1	2.1	3	6.5	-	-	4	8.6
M. colpogenes	1	2.1	-	-	-	-	1	2.1
M. flava	-	-	3	6.3	-	-	3	6.5
M. caseolyticus	-	-	-	-	1	2.1	1	2.1
Gaffkya tetragena	-	-	-	-	1	2.1	1	2.1
STREPTOCOCCUS SPECIES	1	2.1	2	4.2	2	4.2	5	10.5
Str. durans	-	-	-	-	2	4.2	2	4.2
Str. bovis	1	2.1	1	2.1	-	-	2	4.2
Str. zymogenes	-	-	1	2.1	-	-	1	2.1
CORYNEBACTERIUM SPECIES	2	4.2	-	-	2	4.2	4	8.4
C. haemolyticum	-	-	-	-	2	4.2	2	4.2
C. bovis	1	2.1	-	-	-	-	1	2.1
C. unclassified	1	2.1	-	-	-	-	1	2.1
ANTHRACOÏDES	2	4.2	3	6.5	3	6.5	8	17.2
YEASTS	-	-	3	6.5	1	2.1	4	8.6
GRAM-NEGATIVE BACILLI	1	2.1	2	4.2	6	12.7	9	19.0
E. coli	-	-	2	4.2	3	6.4	5	10.6
K. genitalium	-	-	-	-	1	2.1	1	2.1
Aerobacter sp.	-	-	-	-	1	2.1	1	2.1
Pr. vulgaris	1	2.1	-	-	1	2.1	2	4.2
Total	10	21.27	18	38.3	19	40.43	47	100.0

It has been noticed, that the significant important species isolated in the late stage of pregnancy either in cows or buffaloes, were E. coli; K. genitalium, Pr. vulgaris, Pr. rettgeri, Str. durans and C. haemolyticum.

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## DISCUSSION

The bacteriological examination of vaginal swabs from pregnant cows and buffaloes revealed the presence of sterile samples in 17.9% and 20.6% respectively. Similar results were obtained by BARAKAT (1965) who found that out of 50 pregnant uteri, 9 cases were sterile.

A variety of microorganisms were isolated in the different stages of pregnancy in both cows and buffaloes. The most prevalent species isolated were: *E. coli* (14), *Staph. epidermidis* (6), *M. luteus* (6), *Staph. aureus* (5), *Sarcina lutea* (4), *Str. durans* (4), Unclassified *Corynebacterium* (4) and *C. bovis* (4) in addition to different species of *Anthracooides* and Yeasts. Nearly similar findings were presented by GEISSLER (1954), PLOZAS-MORALES (1955), EL-SAWAF *et al.* (1961), ZAKI *et al.* (1961) and BARAKAT (1965).

In contrast to EL-SAWAF *et al.* (1961) who isolated a high incidence of *Str. zymogenes* (28%) and *C. pyogenes* (15%), these pyogenic organisms were not isolated in this work. Although ZAKI *et al.* (1961) recorded that *E. coli* and *Proteus* species were not isolated from the pregnant mature buffaloes, but in this work *E. coli* was recovered from all stages of pregnancy from both cows (17%) and buffaloes (10%). These results agreed with that of ROEHL (1920), GEISSLER (1954), PLOZAS-MORALES (1955) and EL-SAWAF *et al.* (1961). In case of *Proteus* species 2 strains of *Pr. vulgaris* (4.2%) were recovered from buffaloes and one strain of *Pr. rettgeri* (1.9%) from cows. This agreed with GEISSLER (1954) and PLOZAS-MORALES (1955).

It was evident that the percentage of the isolated strains in pregnant cattle and buffaloes was higher towards late pregnancy. This may be due to the fact that the progesterone level is becoming higher after the first trimester (ROBERTS, 1956; ABDO, 1962; HAFEZ, 1974). It is universally accepted that progesterone has no bactericidal effect like oestrogen (RAWSON *et al.* 1953; MOURSI, 1961 and SHARAF *et al.* 1963). Thus it is not surprising that the number of the isolates is less in the first than in the second and third trimesters.

In late pregnancy, although the oestrogen level is high, especially before parturition (ROBERTS, 1956), yet the number of isolates was high. This may be attributed to the fact that, in this period, relaxation of the genitalia and liquefaction of the cervical mucous plug occurs, thus giving a way to bacteria to gain entrance to the genital tract.

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