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CERVICO-VAGINAL BACTERIA OF RETAINED FOETAL MEMBRANE COWS AFTER PARTURITION WITH SPECIAL REFERENCE TO SOME BLOOD BIOCHEMICAL CHANGES AND REPRODUCTIVE PERFORMANCE IN GHARBIH GOVERNORATE.

G.R. Hassb El-Naby and kh. I. El-Ekhnawy

Animal health Research institute (Tanta. Lab).

ABSTRACT

The study aimed to through a light on the bacteria of the servico-vaginal mucous from retained foetal membrane cases , measurement of some blood constituents and hormones and its relationship to reproductive performance in parturited cows in modern farm near Tanta. 10 retained placental cases and other 10 cases with normal dropped placenta (control). The bacteriological investigation of uterine swabs in control group revealed that (21.9%) were positive, yielded 49 bacterial isolates. The most higher percent was *E. coli* (32.65%) and followed by *Staphylococcus aureus* (20.40%), from obligate anaerobic pathogens, *Bacteriodes spps* (4.08%) and *Fusobacterium necrophorum* (2.04%).In retained placenta cases there were (68.8%) positive swabs yielded 169 isolates. The higher percent was E.coli (18.39%),followed by *Staphylocacus aureus* (14.69%), *Corynebacterium pyogens* (11.25%), *Proteus spps* (8.87%), and *Enterococcus faecalis* (7.69%). From obligate anaerobic pathogens, there were *Bacteriodes spps* (4.78%), *Fusobacterium necrophorum* (3.55%) and *Clostridium perfringens* (2.95%). Although it was evidence that *Escherichia.coli*, *Staphylococcus aureus* and *Corynebacterium pyogens* shared in most mixed infected cultures. Yet most isolates were very sensitive to Ceftiofur, Enrofloxacin and moderately sensitive to Oxyteteracyllin and ampicillin and weakly sensitive to Amoxycillin and Pencillin.The blood analysis showed significant lower level of calcium, phosphorous, magnesium and oesteradiol- 17 B and higher level of progesterone than normal dropped placental cows.Subsequently reproductive performance was affected as it had long time of uterine involution ,appearance of 1st post partium heat , calving interval as well as high number of services per conception as compared with normal ones.

Keywords: Bacteria- Retained placenta- reproductive performance – antibiotics.

INTRODUCTION

Retention of foetal membrane and post partium endometritis are common problem in dairy cows. , frequently diagnosed and considered of a major economic impact due to negative effect on reproduction and milk production (**Goshen and Shigel. 2006**). The condition is basically due to failure of the villi of the foetal cotyledones to separate from the maternal crypts of the

caruncles (**Laven and Peters, 1996**).Foetal membranes were considered to be retained if not dropped within 12hrs after calving **(Grunert., 1986**) The economic viability of dairy herd depend on normal reproduction in those farm animals where pathological changes in their reproductive tract caused by microorganisms to be the main factor of infertility **(Krishnan, et al, 1994).** And when endometritis developed, conception rates will impaired. **(Takacs,et.al., 1990).** The bacteriological examination of dairy cows with R.F.M have been studied aerobically but most of anaerobic bacteria have little effect on fertility where *Corynebacterium pyogens, E.coli, Bacteriodes spps*, and *Fusobacterium necrophorum* were the major pathogens, **(Konigesson, et. al., 2001).** Also, **(William,et. al., 2005)** identified *Corynebacterium pyogens, Proteu*s and *Fusobacterium necrophorum* associated with mucopurulant vaginal mucous, while *Corynebacterium pyogens, E.coli*, non heamolytic *Streptococci* and *Pasteurella haemolytica* were associated with a fetid mucous odour in retained placental cases. R.F.M was also due to farm, year, parity, season of calving, milk production, twinning and sex of calf **(Chassagne, et. al., 1998).** The aim of the study was conducted to investigate:- The relationships between cervicovaginal bacterial contamination by some aerobic and anaerobic bacteria. In vitro, sensitivity of the bacterial agents to some antibiotics. The development of endometritis in cows that experienced retained their placenta and reproductive performance, also changes in some heomatological and biochemical blood constituent in friesian cows under the egyption conditions.

N.B.:-R.F.M. (Retained foetal membrane)

MATERIAL and METHODS

MATERIAL:-

1. Animal:

10 dairy imported friesian cows with R.F.M aged (3- 8 years) at El-Gemmiza farm (near Tanta) at a period of two years (2006- 2007)., from 75 cows closely observed after parturition for placental drop. And were not treated with any drug. Also 10 dairy cows with normal dropped F.M. within 12 hrs. postpartium **(Robert.,1986)** used as control. The farm was free from *T.B* and *Brucella*.according to farm documents .

2. Gas- pack anaerobic Jar BBl- 814-12)**.&** gas generating kits ,code No. BR55(Oxoid)
3. Kits for progesterone ,estradiol - 17B detection (los anglos., U.S.A .)
4. Kits for determination of calcium ,phosphorous, magnesium (stambio-Texas.U.S.A.)
5. Blood samples from each examined animals via jugular vein for biochemical analysis

Methods:

1. Clinical examination: according to :- **Drillich, et. al., (2003).**

Cows that retained F.M more than 12 hrs and showed discoloured vulvar membranes, foul smelling were assigned to be examined in this work.

Rectal palpation were carried out weekly during the puerperium until the occurrence of uterine involution and monitoroing of the first postpartium heat. Also, data including number of service**/** conception, calving intervals and milk production were recorded.

2.Bacteriological Examination:

2.1**.Samples:** A total of 320 cervicovaginal swabs (160 from each group) where . Two sterile swabs were collected weekly for investigation microbiologically during the first **8** weeks post partium for each dairy cows**. (Noakes, et. al., 1991).** After cleaning the perineum and vulva with 70% ethanol, asterile swabs were interoduced into the vagina up to cervix, contamination was avoided by opening vaginal cleft and placing the swab cranial to external uretheral opening, and slightly pulled back for several times to ensure saturation **(Shum. 1987).**

2.2.**Isolation and Identification of the isolates:** according to **(Erich and Morrow 1980)**

The swabs were immediately aseptically placed directly into scrow capped bottles containing sterile nutrient broth and sent without delay to Tanta. Vet. Lab.within (2-4hrs) for bacteriological examination. For aerobic isolates a loopful from each sample was streaked on Nutrient agar, MacCkonky agar, Blood agar, S.S agar(Oxoid.L.td) and incubated at 37C for 24- 48 hrs.The purified colonies were streaked on slope agar for further identification by studying the culture characters, pigment production, staining reaction, motility and biochemical activity according to **(Finegold and Martin. 1976).**For anaerobic isolates, a looful from each sample was inoculated into thioglycolate broth medium (oxoid G M10) and incubated anaerobically for 24hrs then streaked on Cooked meat medium (Most. DM 120), Neomycin blood agar and incubated anaerobically at 37C for 24- 48hrs .The purified colonies were identified morphologically, (culture characters, motility) and by biochemical tests according to **(Koneman, et. al 1992 and Holt, et. al., 1994).**

2.3.**In vitro, sensitivity test,** antibacterial susceptibility test were performed on random isolates from each species of isolated bacteria according to **(Qunin, et al., 1994).** 9 chemotheraputic discs kindly supplied by (Oxoid.L.td) and namely: Amoxycllin (10 ug,) Ampicillin (30ug,) Cephaloxin (10 ug,) Ceftiofur (30ug,) Enrofloxacin (10ug,) Gentamycin (10ug,) Oxyteteracycllin (30ug,) Pencillin (30ug) and Streptomycin (15ug).

3.**Blood plasma analysis:**

Blood samples were taken at calving day, centrifuged (1500/ 15min)for separation of plasma and kept at – 20C, pending biochemical analysis. Progesterone, oestradiol- 17B were assayed by radioimmunoassay **(Abraham, 1981).** Using kits from diagnostic products corporation (Los Anglos. U.S.A). calcium, inorganic phosphorous, magnesium were colormetrically determined using chemical kits from stambio (Texas. U.S.A).

4.**Stastistical analysis:**

Data were computed and stastistically analysed as outlined by **(Snedecor and Cochran. 1980).**

(Table :1) : Results of Bacteriological examination of both normally dropped and retained foetal membrane cows.

Cases N= 10	No. of examined cows N= 75		-ve swabs		+ve swabs		Single pure isolates		Mixed isolates	
	No.	%	No.	%	No.	%	No.	%*	No.	%*
Normal dropped F.M cows	10	13.3	125	78.1	35	21.9	25	71.43	10	28.57
Retained F.M cows	10	13.3	50	82.3	110	68.8	73	66.36	37	33.64

% according to total swabs number in both cases .

*% according to total number of +ve swabs recovered from both cases.

F.M. : foetal membrane

(Table :2) : Types and incidence of bacterial isolates from positive swabs recovered from cows normaly dropped and retained foetal membranes during the first 8th weeks post partium .

Types of isolates	Normaly dropped placenta				Cows with retained foetal membranes			
A (aerobic and facultative anaerobic bacteria)	No.	%	Persistance per weeks from : to		No.	%	Persistance per weeks from : to	
G+Ve								
- *Staphylococcus aureus*	10	20.40	1	2	25	14.79	1	8
- *Corynebacterium pyogens*	-	-	-	-	19	11.25	1	7
- *Enterococcus faecalis*	6	12.24	1	2	13	7.69	1	3
- *Streptococcus pyogens*			-	-	9	5.32	1	5
Total	16	32.65			66	39.05		
G-Ve								
- *Escherichia.coli*	16	32.65	1	8	32	18.93	1	8
- *Citrobacter.spps*	4	8.16	2	3	10	5.91	1	3
- *Klbsiella genitalium*	3	6.12	2	-	8	4.73	2	8
- *Proteus spps*	5	10.20	1	3	15	8.87	1	8
- *Salmonella. spps*	-	-	-	-	2	1.18	3	-
- *Enterobacter colaeca*	2	4.08	2	-	2	1.18	2	-
- *Pseudomonas aeruginosa*	-	-	-	-	9	5.33	3	5
- *Pasteurella multocida*	-	-			6	3.55	2	4
Total	30	61.22			84	49.68		
(B) Obligate anaerobic								
- *Bacteriodes . spps*	2	4.08	2	-	8	4.78	5	7
- *Fusobacterium necrophorum*	1	2.04	3	-	6	3.55	5	7
- *Clostridium perfringens*	-		-		5	2.95	2	4
Total	3	6.12			19	11.24		
Cummulative totals	49	100			169	100		

* % according to total number of bacterial isolates in each two cases.

(Table :3) : Types and incidence of mixed bacterial isolates recovered from normal and retained foetal membrane cases .

Cases	Types of mixed isolates	No.	%
Normal dropped placental cows	- *E. coli – Staphylococcus aureus – Streptococcus faecalis*	3	6.1
	- *E. coli – Staphylococcus aureus*	3	6.1
	- *E. coli – Citrobacter spps*	2	4.08
	- *E. coli – Klbsiella genitalium*	1	2.04
	- *E. coli – Proteus spps – Entrobacter colaeca*	1	2.04
		10	28.57*
Cows suffered from retained foetal membrane	- *Corynebacterium pyogens + Staphylococcus aureus – E.coli*	3	1.77
	- *Corynebacterium pyogens + Streptococcus faecalis – E.coli*	3	1.77
	- *Corynebacterium pyogens + Staphylococcus aureus + Klbsiella genitalium*	2	1.18
	- *E. coli + Staphylococcus aureus + Proteus. spps .*	4	2.36
	- *E. coli + Streptococcus faecalis + Corynebacterium pyogens*	5	2.95
	- *E. coli + Bacteriodes. spps.*	1	0.59
	- *E. coli + Proteus spps + Pseudomonas aeruginosa*	1	0.59
	- *E. coli + Fusobacterium necrophorum + Pseudomonas aeruginosa*	1	0.59
	- *E. coli + Pseudomonas aeruginosa*	1	0.59
	- *E. coli + Pseudomonas aeruginesa + Streptococcus pyogens .*	1	0.59
	- *Citrobacter spps + Klbsiella genitalium + Proteus. spps.*	2	1.8
	- *Clostridium perfringens + Pasteurella multocida*	3	1.77
	- *Bacteriodies spps + Pseudomonas aeruginosa*	2	1.18
	- *Streptococcus pyogens + Citrobacter. spps .*	5	2.95
	- *Staphylococcus aureus + Salmonella. spps.*	1	0.59
	-Fusbacterium necrophorum + Bacteriodes. spps.	2	1.18
Total		37	33.64*

- % according to total bacterial isolates in each two cases .
- *%according to total number of +ve swabs recovered from both cases.
- *E. coli* :-Escherichia.coli

(Table :4) : Results of sensitivity of the randum isolates of obtained bacteria to antibiotics

Antibiotics / isolates	Amoxycillin	Ampicillin	Cephaloxin	Enrofloxacin	Erythromycin	Gentamycin	Oxytetracyline	Pencillin	Streptomycin
-Staphylococcus aureus	SS	SSS	SS	SSS	SS	SSS	S	S	S
- Streptococcus pyogens	S	S	SS	SSS	SS	SS	S	R	R
- Enterococcus faecalis	S	S	SS	SS	SSS	SS	R	R	R
- Corynebacterium pyogens	R	S	S	SS	SS	SS	R	R	R
Escherichia -coli	S	SS	SS	SS	SS	SSS	S	R	R
Citrobacter. spps.	S	S	S	SSS	SSS	SS	S	R	R
- Klbsiella genitalium	SS	SS	SS	SSS	SS	SS	S	S	R
Proteus. spps.	R	R	SS	SSS	SSS	SS	S	R	S
Salmonella. spps.	R	R	R	SS	SS	SS	R	R	R
- Enterobacter colaeca	R	R	S	SS	SS	SS	R	R	R
- Pseudomonas aeruginosa	S	SS	S	SSS	SS	SSS	R	R	R
- Pasteurella multocida	S	SS	S	SSS	SSS	S	R	R	R
- Bacteriodes . spps	S	SS	SS	SSS	SSS	S	SS	SS	R
- Fusobacterium necrophorum	S	SS	SSS	SSS	SSS	S	SS	SS	R
- Clostridium perfringens	S	S	SS	SSS	SSS	S	SS	SS	R

R = resistant strain
S = intermediate sensitivity
SS = Sensitive
SSS = highly sensitive

(Table :5) : Effect of placental retention on the subsequent reproductive performance of friesian cows (Mean ± SE) .

Parameters	Cows. D.F.M N= 5	Cows R.F.M N= 5
Time for postpartium Uterine involution (day)	35.09 ± 1.71	59.91 ± 3.28
First observed heat (day)	41.72 ± 1.72	89.55 ± 3.05**
No. of services/ conception	1.30 ± 0.15	2.90 ± 0.23**
Calving interval (days)	401.17 ± 12.30	534.46 ± 33.60**
Milk production (Kg/cow/day	7.71 ± 1.21	6.35 ± 0.40**

(Table :6) : Effect of placental retention on steriod hormone level in the blood of friesian cows on the day of calving (Mean ± SE) .

Normal level	Cows dropped placenta <12 hrs (N= 5)	Cows retained placenta >12 hrs (N= 5)
Progesteron (mg/ml)	0.067 ± 008	6.630 ± 0.13**
Oestradiol – 17B (pg/ml)	144.35 ± 2.49	118.64 ± 6.58 **

(Table :7) : Effect of Retained foetal membranes on some blood biochemical constituents in the day of calving in friesian cows (Mean ± SE) .

Constituents	Cows D.F.M (N= 5)	Cows R.F.M (N= 5)
Calcium (mg/dl)	10.90 ± 0.12	8.25 ± 0.63**
In.organic phosphorous (mg/dl)	5.46 ± 0.28	3.75 ± 0.15 **
Calcium/phosphorous	2.01 ± 0.09	2.24 ± 0.11 *
Magnesium	2.91 ± 0.14	2.58 ± 0.09 *

* P < 0.05) D.F.M. dropped foetal membrane

** P < 0.01) R.F.M retained foetal membrane

RESULT & DISCUSSION

Retention of placenta in cattle is predispose to purperial infection, endometrites, meteritis and salpengities. Affected cows showed genereal health disturbance **(Sosa and Nasr, 1995).** And the condition was mainly related to putrifaction of R.F.M. which is a rich media for bacterial growth, multiplication and toxins production leading to toxaemia and illness **(Grunert, 1986).**In this study 18.8% of Friesian cows retained their placenta > 12 hrs postcalving (Table.1) Although higher incidence (30%) was observed by **Sabry, et. al.,(1997)** and similar result (13- 1%) recorded by **Goshen, et al., (2006).** Variation in the incidence of R.F.M were related to calving season **(Kaneto, et. al., 1997),** parity **(Choudhury, et. al., 1997),** calf sex **(Garcia- Munize, et. al., 1998).** Our bacteriological investigation revealed that out of 10 normal dropped F.M. cows, 35+ve samples yield 49 isolates (Table. 2) *Staphylococcus aureus* was (20.40%) and *Enterococcus faecalis* (12.24%) and this was lower than **Kaczmarowski, et. al(2004).** who isolated them with percentages of 30% and 16% respectively,Also our result were in agreement to **(Osman, et. al., 1991)** who recorded that *Staphylococcus aureus* and *Streptococcus spps* were the predominant pathogens in buffaloe cows infected purperium, While among G-ve bacteria, *E.coli* was the predominatnt organism (32.65%). followed by *Proteus. Spps* (10.20%), *Citrobacter. Spps* (8.15%), *Klbsiella genitalium* (6.12%), and *Enterobacter coleaca* (4.08%) **Zerbe, et. al (2001) and Metwally. (2004)** recorded that *E.coli, Citrobacter, Proteus, spps and Klbsiella spps* were isolated from uteri of normal cows and buffaloes postpartium. Regarding to obligatory anaerobic bacteria, the *Bacteriodes spps* was (4.08%) which was lower than that mentioned by **El – Dessouky, et. al., (2006)** who recovered it with a percentage of (12.2%)**.** The perssistance of microorganisms was from 1st to 3rd weeks in this group of animals except *E-coli* which found till 8th week postpartium due to that most healthy cow are able to clear the uterus from other bacteria with first 2nd : 3rd weeks after calving **(Bondurant, 1999).** Also, the negative bacterial isolation inspite of perssistance of purulent vaginal or uterine discharge may be due to the fact that isolation of microorganisms from inflamed cow uterus depend on the stage of healing process during which micro organisms would disappear first and later of inflammatory signs resulting in sterile discharge **(Bois. 1986).** Concerning the bacteriological examination of R.F.M cows, 110 (68.8%) +ve swabs yielded 169 isolates. *Staphylococcus aureus* was the predominant organism (14.79%) and this was higher than that mentioned by **El.Dessoukey, et. al (2006)** who isolated it with a percentage of 30%**.** The higher percentage and its persistence from the 1st week till 8th week were due to the fact that Staphylococcal disease was arised when defensive mechanism of cow is breached by hormonal imbalance - estrogen with bacteriostatic action giving rise to Staphylococcal endometritis (**Lammines; et.al., 1981**). Also, it is

well established that Staphylococcal coagulase +ve strains are more virulence than others. Its virulence cannot be explained in terms of single factor invading organisms and low defensive mechanism of the host to face this invasion which magnify the organism virulence, (**Anderson, 1986**), In the second place *Corynebacterium pyogens* isolated with percentage of (11.25%) and found till 7th week .Although **kudryavtsev, et.al., (1991**) cited that cows recovering from *Corynebacterium pyogens* endometritis may require 1 month after clearance of organisms for fertility to be restored. High percentage reached 57.9% was recorded by **Kaczmarowski, et. al., (2004**). *Streptococcus pyogens* and *Enterococcus faecalis* isolated with percentage of (5.32%) , (7.69%) and persist from 1st week till 5th and 3rd week respectively. This was agree with **El- Dossouky, et.al., (2006**).Regarding G-ve bacteria, *E-coli* was the predominant bacterium (18.93%) followed by *Proteus. spps* (8.87%), *Citrobacter spps* (5.9%) and still recovered till the 3rd and 8th week postpartium. like **Kask, et.al., (1988**), while *Pseudomonas aeruginosa* (5.33%), *Pasteurella multocida* (3.55%), *Salmonella. Spps* and *Enterobacter coleaca* (1.18%), were recovered during 2nd till 5th week postpartium like that mentioned by **Konigesson, et.al. (2001**), also, **Ahmed, et.al., (2004**) who isolated *Salmonella. spps* specially in selenium treated group.Concerning the obligatory anaerobic bacteria, our investigation revealed that *Bacteriodes spps*, *Fusobacterium necraphorum* were (4.08%) and (2.04%) respectively in normally dropped F.M. cows and recovered at 2nd and 3rd week only. While in R.F. M cows were (4.78%) and (3.55%) and recovered from 5th till 7th week postpartium and these results were agreed with **Dhaliwal, et.al (2001**). While *Clostridium perfringens* was (2.95%) and recovered during 2nd to 4th week and this result achived by **Dohman, et. al (2000**). Our results revealed that the aerobic isolates from R.F.M predominant during the three first week., this attributed to decrease in number of neutrophils during this time (**Abd El.Aziz, et.al., 2002**). While the obligatory anaerobic organisms recovered from 5th to 7th week showed that these organisms were originally thought to be secondary invader requiring previous infection, wound or other predisposing cause to gain entery into the host (**Itman, et.al, 1991**). **Holt, et.al (1989**). cited that *E. coli and heamelytic Streptococci and Corynebacterium pyogens* were the predominant pathogens responsible for this problem. On other hand **Dhaliwal, et.al., (2001**) showed that these obligatory anaerobes could be classified as primary pathogens in dairy cattle with R.F.M while other pathogens may be due to unhygienic condition during handling of parturient buffaloes.Concerning mixed isolates (Table.3*) E.coli* was the predominant pathogen, sharing with *Staphylococcus aureus, Streptococcus faecalis, Citrobacter spps, Proteus spps and Klbsiella genitalium* in cow cases with normal dropped F.M, like that recorded by **Williams, et.al., (2005**).who isolated *E. coli, Streptococcus spps* in mixed cultres with other bacterial pathogens, also **Kaczmarowski, et.al., (2004**) isolated *E. coli* and other species of Enterobacteriacae from 47.6% of healthy cow while **Kask, et.al., (1998**) isolated *E.coli,*

Streptococcus spps, Staphylococcus spps; Proteus spps in mixed cultures.

In the same time in cows with R.F.M also *E.coli***,** *Staphylococcus aureus and Corynebacterium pyogens* were the most pathogens sharing in different mixed cultures than other pathogens like that recorded by **Zerbe, et.al., (2001**) who isolated *E.coli* with *Corynebacterium pyogens* in mixed cultures also **Konigesson, et.al., (2001**) said that the predominant were *E.coli, Streptococcus spps, Fusobacterium necrophorum, Corynebacterium pyogens, Bacteriodes. Spps, Pasteurella spps and Proteus spps,* shared in several mixed cultures and added that *E.coli, Pasteurella and Proteus spps,* could be isolated for 2-3 weeks in mixed cultures postpartium. On the other hand **Dohman, et.al., (2000**) isolated *E.coli, and Clostridium spp*s in mixed cultres. It is interested that **Azawy. (2008**) suggested that peripartium complication followed by R.F.M with the dominance of *E-coli* in uterine lumen might favour the colonization of other bacteria including facultative anaerobies and strictly anaerobic in uterine wall of buffaloes.

The most active antibiotics (Table. 4) against bacterial isolates (in vitro) were Ceftiofur, Enrofloxacin, and Gentamycin, while Oxytetracycllin, Ampicillin and Cephaloxin had moderate effect, other antibiotics such as Amoxycellin, Pencillin and Streptomycin had lower effectiveness against most pathogens and these results were similar to that recorded by **Scott, et.al., (2005**) who cited that Ceftiofur sodium intrauterine administration on cow reduce the risk of culling in cow with R.F.M, also, (**Drillich, et.al., 2006**) used systemic treatment of antibiotics with Ceftiofur 3- 5 consective days, while (**Farce, 1997**) used Oxyteteracyllin as early treatment of R.F.M. Concerning reproductive performance of cow (Table. 5) following R.F.M, were take significantly longer time for completion of uterine involution and appearance of the first postpartium heat ($P < 0.01$), had higher number of services per conception ($P < 0.01$), longer calving interval ($p < 0.01$) and lower milk production ($p < 0.05$) compared to control cows which dropped their F. M in the proper time (2- 8hrs after calving), The deleterious effect on the fertility of affected cows were reported by **Abdo; (1988**)**.**Problems arised as a sequellae of the severe inflammatory process of endometrium leading to delayed uterine involoution (**Zaiem, et.al., 1997**), failure of synthesis and / or release of PGF2α (**Slama, et.al., 1994**) and consequently cessation of ovarian activites, increased number of service/ conception decreased pregnancy rate and increased calving interval (**Laven and Peters, 1996**).Also, the affection of general health and weight loss can not be denied. Another goal of the current study was to evaluate the steroid hormone levels following R.F.M (Table. 6) which indicated that cows suffering from placental retention had got significantly ($P < 0.01$) higher level of progesterone and lower level of oesteradiol -17B as compared to cows dropped their foetal membrane in the proper time, these results were not completely consistent with those obtained by **Wischral, et.al., (2001**) who reported that progesterone levels were not differ from animals with retained placenta or not, while lower esterogen level was found in cow with placental retention

compared to control.Progesterone levels in the prepartium and calving periods were contraversial because higher progesterone values were observed by **Bosu, et.al., (1984**).Lower values were recorded by **Wischral, et.al., (2001**) and no significant changes were found between animals with and without retention of placenta (**Peters and Bosu, 1987**). Actually, it is known that a high progesterone concentration is harmful to the uterine defense mechanism as it causes immunosuppression due to the estimation of PGF2α production by progesterone (**Wango, et.al., 1992**) **.** The lower esterogen at calving in cow with retained placenta was observed by **Kaneto, et.al., (1997**). and this fact could be considered as a symptoms of placental immaturity rather than its cause (**Thomas, et.al., 1992**) or a deficiency in the Delta 5 ways of steroid production (estrogen synthesis) in the bovine placenta. Moreover, estrogen deficiency induces a diminished leucocytic activity **(Gilbert, et al., 1993)** and low levels of PGF2α at calving. Concerning the other blood constituent (Table 7) cows with R.F.M showed significant decrease in calcium, phosphorous and magnesium after calving compared with cows dropped foetal membranes in proper times. In balance of calcium and phosphorous metabolism was correlated positively with R.F.M **El. Hanfy, (1998**)., **Beri, et.al., (1996**) indicated low calcium, magnesium and phosphorus values during the pericalving period. In the same time **Sabry, et.al., (1997**) found that low magnesium and phosphorous values during the post calving period predispose for R.F.M.

So, from the previous results it is concluded that R.F.M in dairy cows is a serious problem. *E.coli, Staphylococcus aureus* and *Corynebacterium pyogens* were the predominant pathogens and there were synergism between its occurrence with anaerobic bacteria which considered as a secondary invader under predisposing factors,.**Sosa and Nasr, (1995**) concluded that prepartium calcium supplementation prevent the problem mainly due to imporoving health condition and enhancing of the myometrial sensitivity.

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