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SOME BACTERIOLOGICAL AND BIOCHEMICAL STUDIES ON BUFFALOES AFFECTED WITH RETAINED FETAL MEMBRANE IN DAKAHLIA GOVERNORATE

(With 7 Tables)

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بعض الدراسات البكتريولوجية والبيوكيميائية على الجاموس المصاب باحتباس المشيمة فى محافظة الدقهلية

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أجريت هذه الدراسة على عدد ٢٥ من الجاموس تتراوح أعمارهم من ٤- ١٠ سنوات، من القرى المحيطة بمدينة المنصورة لمتابعة نزول المشيمة بعد الولادة الطبيعية. ١٦ منهم كانت مصحوبة بنزول طبيعى للمشيمة (مجموعة ضابطة) و٩ كانت مصابة باحتباس المشيمة. أظهر الفحص البكتريولوجى لمسحات رحمية من المجموعة الضابطة أن ٢٥,٤% كانت موجبة للفحص وأسفرت عن عزل عدد ٧٤ عتره وكان الميكروب القولونى بنسبة ٣٣,٨% و١٤٤ مسحة رحمية من المجموعة المصابة باحتباس المشيمة كانت موجبة للفحص بنسبة ٧٥% وأسفرت عن عزل ٣٦٢ عتره منهم ١٧٦ (٤٨,٦%) بكتريا هوائية وأحياناً لا هوائية وكانت أعلى نسبة للعزل هو الميكروب الوتدى القيحى (١٧,٤%) والميكروب القولونى (٩,٩) وكذلك ١٨٦ (٥١,٤%) بكتريا لا هوائية وكانت أعلى نسبة للعزل هى ميكروبات البكتيرويدس (٢٤,٩%) والفيوزوباكتريم نكروفورم (١٤,٩%) وبإجراء اختبار الحساسية لتلك المعزولات وجد أن معظمها شديد الحساسية لكل من كالندامايسين، إنروفلوكساسين، سيفتيفيور، نيومايسين، تتراسيكلين والبنسلين. بالنسبة للتغيرات فى صورة ومصل الدم للحيوانات المصابة باحتباس المشيمة عند مقارنتها بالمجموعة الضابطة أوضحت الدراسة الآتى: وجود نقص معنوى فى مستوى سكر الدم ، الكلوستيرول الكلى ، الكالسيوم ، الفوسفور ، السيلينيوم ونقص معنوى عالى فى العد الكلى لكرات الدم الحمراء والبيضاء ومستوى فيتامين ج وعلى الجانب الآخر وجدت زيادة معنوية فى مستوى كل من أنزيم الأسبرتات أمينو ترانس فيريز ، الألانين امينو ترانس فيريز والدهون الثلاثية بالإضافة إلى

وجود زيادة معنوية عالية فى مستوى إنزيم اللاكتيك دى هيدروجينيز، الجاماجلوتاميل ترانس فيريز ، بولينا الدم ، الكريايتين وهرمونى الكورتيزول والبروجستيرون.

SUMMARY

A total of 25 buffaloes aged between 4-10 years, from in different villages surrounding Mansoura city to follow fetal membrane drop after normal parturition. Sixteen of them with normal dropped fetal membrane (control) and nine with retained fetal membrane were used in this study. For the first 8 weeks after parturition each buffaloe was subjected to clinical examination, bacteriological examination and haematological and biochemical studies. Bacteriological examination of 256 uterine swabs of control buffaloes showed that 65 (24.4%) swabs were bacteriologically positive. Yielded 74 isolates mainly E. coli (33.8%). Meanwhile for retained fetal membrane buffaloes 144 uterine swabs, 108 (75.0%) were bacteriologically positive with 1.9% pure single aerobic and faculitative anaerobic cultures; 14.8% pure single anaerobic and 83.3% mixed cultures. They yielded 362 isolates comprising 176 (48.6%) aerobic and faculitative anaerobic bacteria with the most predominant ones were A. pyogenes (17.4%) and E. coli (9.9%) and obligate anaerobic bacteria 186 (51.4%) mainly Bacteroides Spp. (24.9%) and Fusobacterium necrophorum (14.9%). Sensitivity test for those isolated bacteria revealed that the most of isolates were highly sensitive to clindamycin, enrofloxacin, ceftioufur, neomycin, tetracycline and penicillin. The changes in blood and serum of retained fetal membrane buffaloes when compared with control group, revealed that: Presence of significant decrease in the level of glucose & total cholesterol & calcium & phosphorus & selenium and high significant decrease in the total count of erthrocytes, leucocytes and vitamin C level. On the other side significant increase was observed in the level of S. AST, S.ALT and triglyceride. High significant increase were noticed in S.LDH, γGT, blood urea, Creatinine, cortisol and progesterone levels.

Key words: *Placenta, fetal membrane, buffaloes, bacteriology, antibiotic sensitivity.*

INTRODUCTION

Retained fetal membrane (RFM) with subsequent uterine infection, endometritis, reduction in milk yield and lower conception rates are a common problems in dairy animals. (Hemingway, 2003). Such problems associated with activities of microorganisms, the intrauterine microorganisms which isolated during disturbances of the

post partum uterus have been described in several studies (Fredriksson *et al.*, 1985 and Cohen *et al.*, 1996). The bacteriological examination of the uteri of dairy cattle with RFM has been studied aerobically, but most of aerobic bacteria have little effect on fertility and Actinomyces pyogenes, E. coli, Bacteroides spp. and Fusobacterium necrophorum were the major isolated bacterial pathogens (Konigsson *et al.*, 2001 and Maarouf and El–Bealawy 2004). Since now bacteriological surverys of both human and bovine uteri indicated that, they are anaerobic environment in which mixed bacteria populations are common. (Ruder *et al.*, 1981). More recently research workers suggested that there were a synergistic relationship between A. pyogenes, Bacteroides spp. and F. necrophorum in producing infertility (Cohen *et al.*, 1996 and Mateus *et al.*, 2002). The condition is characterized by a large number of fetal chorionic epithelial cells undergoing apoptosis immediately after the expulsion of the fetus a consequence of diminished blood supply to the uterus (Boos *et al.*, 2003). Retained fetal membrane in dairy animals depend on some factors as age of the dam, season of calving, twining, hormonal impalance and nutritional deficinces (El-hanafy, 1998 and Ahmed *et al.*, 2004). Some researchers have concluded that this condition is a result of metabolic disturbances during the preparturition period. These disturbances are related to the negative energy balance (Bertics *et al.*, 1992). Moreover vascular changes and utrine contractions has been found to play a role in placental release (Laven and Peters 1996).

There fore the present study was planned to:

- Determine some aerobic and anaerobic bacteria that could be recovered from the post partum uterus of buffaloes with RFM and their relationship to uterine infections.
- Invitro antibiotic sensitivity against the isolated strains.
- Variations in some haematological and biochemical constituents associated with RFM in buffaloes.

MATERIALS and METHODS

Animals:

A total number of 25 clinically healthy buffaloes aged between 4-10 years, reared in different villages surrounding Mansoura city were closely observed after normal parturition for placental drop. All animals were not treated with any drug. sixteen of them with normal dropped fetal membrane within 12 hours post partum (control) Roberts (1986) and nine buffaloes with RFM kept for used in this study for the first 8-weeks after calving each buffaloe was subjected to clinical

examination vaginally and rectally (RFM buffaloes showed discolored vulval membranes, foul smelling discharge, inappetence and decreased milk yieid). Two uterine swabs were collected weekly from each buffaloe Noakes *et al.,* (1989).

Bacteriological examination:

For bacteriological examination 256 swabs from normal control buffaloes and 144 from RFM ones. These swabs were immediately aseptically inoculated in thioglycolate medium and nutrient broth and directly transported to the laboratory with 2-4 hours for bacteriological examination. The broth was incubated aerobically and anaerobically at 37°C for 24 hr., then subcultures were made by streaking on the following media: nutrient agar, MacConkey agar, blood agar and chocalate agar. The plates were incubated at 37°C aerobically and anaerobically for 2 to 7 days then subcultured for colonies purification. The purified colonies were identified morphologically and biochemically according to Holt *et al.,* (1994) and Quinn *et al.,* (1994).

In vitro antibiotic sensitivity test:

The disc diffusion technique was performed on isolated bacteria from infected cases according to Finegold and Martin (1982). Ten chemotherapeutic discs kindly supplied by Oxoid and namally, Clindamycin, Enrofloxacin, Neomycin, Ceftiofur, Penicillin, Tetracycline, Ciprofloxacin, Ampicillin, Streptomycin and Gentamycin.

Blood samples:

Two blood samples were collected from each animal via jugular vein puncture. The first samples were whole blood collected in vacutainer tubes containing EDTA as anticoagulant and were used for haematological studies according to Jain (2000). The second samples were collected in centrifuge tubes and allowed to clot at 73°C and then non haemlysed blood serum was separated used for measuring some biochemical constituents such as glucose, LDH, γGT, ALT, Blood urea, creatinine, total cholesterol and triglyceride using commercial diagnostic kits accoroding to Trinder (1969); Kachmar and Moss (1976); Tietz (1994); Reitman and Frankel (1957); Fawcett and Scott (1960); Husdan and Rapoport (1968), Allain *et al.,* (1974); Fossati and prencipe (1982) respectively. Vitamin C was determined after the method described by (Lowry et al 1945) by using spectrophotometer. Progesterone was measured by RIA using coat-A count progesterone kite (diagnostic products, Los Angeles CA) according to (Kubasik, 1984). Selenium concentration was determined using atomic absorption spectrophotometer according to (Mestek *et al.,* 1997). Cortisol level was

also assayed RIA (Abraham, 1981). Serum calcium and inorganic phosphorous was determined by using commercial diagnostic kits according to (Gindler and king, 1972) and Goldenberg, 1966) respectively.

Statistical analysis:

The statistical analysis T test according to (Snedecor and Cochran 1982) by using slide write plus for windows version 3.0 WSWP.

RESULTS

The results of the present study were illustrated in Tables (1-7).

Table 1: Bacteriological examination of both normally dropped fetal membrane and R.F.M. buffaloes.

Case of Animals	No. of animals	No. of swabs	Negative swabs		Positive swabs		Pure single cultures				Mixed cultures	
							Aerobic and faculitative anaerobic bacteria		Obligate anaerobic bacteria			
			No.	%	No.	%	No.	%	No.	%	No.	%
Normal	16	256	191	74.6	65	25.4	7	10.8	0	0.0	58	89.2
RFM	9	144	36	25.0	108	75.0	2	1.9	16	14.8	90	83.3

Table 2: Percentage of bacterial species isolated from 65 positive swabs of buffaloes with normal dropped fetal membrane and 108 positive swabs of RFM buffaloes.

Bacterial species	Buffaloes with normal dropped F.M.		Buffaloes with R.F.M.	
	No.	% *	No.	% *
I- Aerobic and faculitative anaerobes:	62	83.8	176	48.6
A. pyogenes.	0	0.0	63	17.4
E. coli.	25	33.8	36	9.9
Staph. Aureus	7	9.4	11	3.0
Strept. Pyogenes	0	0.0	22	6.1
Strept. Faecalis	15	20.3	0	0.0
Proteus spp.	15	20.3	21	5.8
Citrobacter freundii	0	0.0	13	3.6
Klebsilla pneumonae	0	0.0	10	2.8
II- Obligate anaerobe:	12	16.2	186	51.4
Bacteriodes spp.	9	12.2	90	24.9
Fusobacterium necrophorum	0	0.0	54	14.9
Clostridium perfringenes	3	4.0	8	2.2
Peptostreptococcus spp.	0	0.0	25	6.9
Eubacterium lentum	0	0.0	9	2.5
Total	74	100.0	362	100.0

* Percentage in relation to total organisms in each group alone.

Table 3: Incidence of bacterial species isolated from examined buffaloes during first 8 weeks post partum.

Bacterial species	Weeks post partum																Total			
	1		2		3		4		5		6		7		8		N.		R.	
	N.	R.	N.	R.	N.	R.	N.	R.	N.	R.	N.	R.	N.	R.	N.	R.	No.	%	No.	%
I- Acrobic and facultitative anaerobes:	44	44	18	76	0	35	0	13	0	3	0	3	0	1	0	1	62	83.8	176	48.6
A. pyogenes.	0	12	0	20	0	16	0	7	0	3	0	3	0	1	0	1	0	0.0	63	17.4
E. coli.	19	11	6	14	0	8	0	3	0	0	0	0	0	0	0	0	25	33.8	36	9.9
Staph. Aureus	5	2	2	7	0	2	0	0	0	0	0	0	0	0	0	0	7	9.4	11	3.0
Strept. Pyogenes	0	8	0	12	0	2	0	0	0	0	0	0	0	0	0	0	0	0.0	22	6.1
Strept. Faecalis	11	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	15	20.3	0	0.0
Proteus spp.	12	5	3	12	0	3	0	1	0	0	0	0	0	0	0	0	15	20.3	21	5.8
Citrobacter freundii	0	3	0	7	0	2	0	1	0	0	0	0	0	0	0	0	0	0.0	13	3.6
Klebsilla pneumonae	0	3	0	4	0	2	0	1	0	0	0	0	0	0	0	0	0	0.0	10	2.8
II- Obligate anaerobe:	8	28	4	65	0	46	0	24	0	11	0	8	0	2	0	2	12	16.2	186	51.4
Bacteroides spp.	6	12	3	28	0	25	0	11	0	6	0	6	0	1	0	1	9	12.2	90	24.9
Fusobacterium necrophorum	0	8	0	18	0	11	0	9	0	4	0	2	0	1	0	1	0	0.0	54	14.9
Clostridium perfringenes	2	2	1	4	0	1	0	1	0	0	0	0	0	0	0	0	3	4.0	8	2.2
Peptostreptococcus spp.	0	4	0	12	0	7	0	2	0	0	0	0	0	0	0	0	0	0.0	25	6.9
Eubacterium lentum	0	2	0	3	0	2	0	1	0	1	0	0	0	0	0	0	0	0.0	9	2.5
Total	52	72	22	141	0	81	0	37	0	14	0	11	0	3	0	3	74	100.0	362	100.0

N.= Normal dropped fetal membrane. R.= Retained fetal membrane.

Table 4: Antibiogram of the most isolated microorganisms recovered from buffaloes with RFM.

Organisms / Antimicrobial agent and its potency	A. Pyogenes (N = 63)		E. coli (N = 36)		Bacteroides spp. (N = 90)		Fusabacterium necrophorum (N = 54)	
	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %
Clindamycin 2 ug	54	85.7	28	77.8	79	87.8	48	88.9
Enrofloxacin 5ug	56	88.9	30	83.3	81	90.0	47	87.0
Tetracycline 30 ug	50	79.4	26	72.2	78	86.7	45	83.3
Penicillin 10 ug	42	66.7	20	55.6	73	81.1	44	81.5
Neomycine 10 ug	53	84.1	29	80.6	78	86.7	47	87.0
Ampicillin 10 ug	40	63.5	22	61.1	69	76.7	38	70.4
Ceftiofur 30 ug	54	85.7	30	83.3	78	86.7	45	83.3
Ciprofloxacin 30 ug	36	57.1	19	52.8	58	64.4	30	55.6
Gentamycin 10 ug	22	34.9	12	33.3	34	37.8	26	48.1
Streptomycin 10 ug	12	19.0	8	22.2	18	20.0	14	25.9

N.= Number of isolates.

Table 5: Mean values of some haematological parameters of buffaloes with retained fetal membrane in comparison with normally dropped ones.

Item / Parameters	Control group	Retained fetal membrane group
Total RBCs ($x10^6$ /mm^3)	6.63 ± 0.17	5.78 ± 0.13 ↓**
Hb (g/dL)	11.09 ± 0.21	10.95 ± 0.26 ↓ N.S
PCV (%)	33.11 ± 0.81	30.26 ± 1.14 ↓ N.S
Total WBCs ($x10^3$ /mm^3)	9.63 ± 0.20	8.32 ± 0.27 ↓**

** Significant at $p < 0.01$.
N.S: Non significant.

Table 6: Mean values of some liver and kidney functions of buffaloes with retained fetal membrane in comparison with normally dropped ones.

Item / Parameters	Control group	Retained fetal membrane group
Glucose (mg/dl)	51.37 ± 1.42	45.13 ± ↓*
LDH (IU/L)	151.81 ± 1.88	161.14 ± 2.37 ↑**
γGT (IU/L)	9.60 ± 0.25	11.20 ± 0.32 ↑**
AST (IU/L)	11.19 ± 0.33	12.89 ± 0.50 ↑*
ALT (IU/L)	7.29 ± 0.37	8.94 ± 0.44 ↑*
Blood urea (mg/dL)	24.48 ± 1.35	31.19 ± 1.69 ↑**
Creatinine (mg/dL)	0.97 ± 0.08	1.82 ± 0.21 ↑**

* significant at $p < 0.05$
** Significant at $p < 0.01$.

Table 7: Mean values of some biochemical parameters of buffaloes with retained fetal membrane in comparison with normally dropped ones.

Item / Parameters	Control group	Retained fetal membrane group
Total cholesterol (mg/dl)	84.27 ± 1.45	78.11 ± 1.77 ↓*
Triglyceride	28.52 ± 1.02	33.27 ± 1.41 ↑*
Cortisol (ng/ml)	0.82 ± 0.05	1.25 ± 0.10 ↑**
Progesterone (ng/ml)	0.77 ± 0.11	1.38 ± 0.16 ↑**
Calcium (mg/dl)	10.91 ± 0.20	9.86 ± 0.30 ↓*
In organic phoshprus (mg/dl)	5.37 ± 0.19	4.33 ± 0.31 ↓*
Selenium (mg/dl)	3.63 ± 0.26	2.53 ± 0.31 ↓*
Vitamin C (mg/l)	2.61 ± 0.13	1.50 ± 0.25 ↓**

* significant at $p < 0.05$
** Significant at $p < 0.01$.

DISCUSSION

Uterine infections in dairy animals with retained fetal membrane were common problems as recorded by konigsson *et al.,* (2001).

The obtained results for bacteriological examination (Table 1) of normal dropped fetal membrane buffaloes (control) showed that 65 (25.4%) biopsies were positive which yielded 74 isolates (Table 2) mainly E. coli (33.8%) and the isolated bacteria rapidly decreased after the first week and disappeared after the second week (Table 3). Nearly similar results were reported by Maarouf and El-Bealaw (2004). The results of bacteriological examination of RFM buffaloes (Table 1) showed that 108 (75.0%) samples were bacteriologically positive with 1.9% pure single aerobic and faculitative anaerobic cultures, 14.8% pure single anaerobic and 83.3% mixed cultures. The positive samples (Table 2) yielded 362 isolates, comprising 176 (48.6%) aerobic and facultative anaerobic bacteria and 186 (51.4%) obligate anaerobic bacteria. The most predominant aerobic isolates were A. pyogenes (17.4%) and E. coil (9.9%) where in anaerobic bacteria, Bacteroides spp. (24.9%) and Fusobacterium necrophorum (14.9%) were the predominant types (Table, 2). These results agreed with those previously recorded by Holt *et al.,* (1989) Laven and peters (1996) and Dhaliwal *et al.,* (2001). The obtained results (Table 3) revealed that the number of isolated bacteria from RFM buffaloes were firstly increased during the first two weeks, this attributed to decreased in No. of neutrophils during this time. (Abd El–Aziz *et al.,* 2002), then progressively decreased during the subsequent weeks and no bacteria were isolated after the end of the 6^{th} week, except A. pyogenes, Bacteroides, spp. and Fusbactarium necrophorum were isolated in a mixed culture in 7^{th} and 8^{th} week. This result agreed with that reported by Dhaliwal *et al.,* (2001). Therefore this findings showed that these organisms could be classified as primary pathogens in dairy cattles with RFM while other pathogens may be due to unhygienic conditions during the handling of parturient buffaloes. These result agreed with that reported by Dhaliwal *et al.,* (2001). On other hand, Holt *et al.,* (1989) cited that E. coli, haemolytic streptococci and A. pyogenes were the predominant pathogens responsible for this problem.

In vitro, the susceptibility distribution of most isolated pathogens to different antibiotic is represented in Table (4), most of isolates were highly sensitive to clindamycin, enrofloxacin, ceftioufur, neomycin, tetracycline and penicillin. These results similar with those mentioned by

Van den Bogaard *et al.*, (1992), Cohen *et al.*, (1996), Konigsson *et al.*, (2001) and Scott *et al.*, (2005).

The present findings of the erythrograme parameters, (Table 5) revealed that the RFM group showed highly significant ($P<0.01$) decrease in total RBCs count, while non significant decrease in haemoglobin and packed cell volume values were noticed. These results were supported by those of (Srinivas *et al.*, 1999 and Abdel Aziz *et al.*, 2002). This reduction in RBCs count may be due to depressed appetite. (Table 5) showed that presence of high significant ($P<0.01$) leucopenia in RFM group, than control ones. This leucopenia may be due to high concentration of leukocytes that infiltrate the endmetrium post calving. This result agreed with the result of (Kudlac *et al.*, 1995) in cows. Dealing with the glucose mean values Table (6) showed that there was significant ($P<0.05$) decrease below the control level. The decreased glucose level in (RFM) group could be attributed to energy deficiency causing weakness and inadequate strength to normal calving including normal expulsion of the placenta. This result was supported by those of (Sabry *et al.*, 1997 and Patel *et al.*, 1999).

It is evident from Table (6) that there was high significant ($P<0.01$) increase in LDH and γGT activity in (RFM) group when compared with the control group. This result was in accordance with (Kudlac *et al.*, 1995 and Kandil *et al.*, 2002). Peter *et al.*, (1987) found that cows with afterbirth retention had, significantly higher activity of LDH, which lead excessive lactic acid production results in metabolic acidosis. Concerning γGT (Kankfer and Maj 1997) said that the changes in this enzyme may indicate to an imbalance in free radicals generation and neutralization. Table (6) revealed that there were significant ($P<0.05$) increase in AST and ALT level over the control level. These results coincided with those reported by (Laven and Peters 1996) and (Srinivas *et al.*, 1999) who attributed these changes to bacterial infection and their toxins.

In the present study there were high significant ($P<0.01$) increase in blood urea and creatinine levels in RFM Table (6). These results were similar to those reported by (Maarouf and El–Bealawy, 2004) whose attributed such changes perhaps due to presence of several pathogenic bacteria which may caused nephritis in cows.

As shown from the results given in Table (7) we found that serum cholesterol had an opposite patteren to triglyceride and showed significant ($P<0.05$) decrease in its levels in comparison to control group. The reduction in cholesterol level could be attributed to its

utilization for synthesis of progesterone which more available in retained fetal membrane. This agreed with the results reported by (Kandeil *et al.*, 2002). while the elevation in triglycerides level could be explained by the negative energy balance caused mobilization of the body fat. As consequence the animal accumulated greater amount of triacylglycerol in the liver. This accumulation was associated with an increase in the interval from parturition to the first ovulation (Rukkuramusk *et al.*, 1999).

With regard to cortisol and progesterone levels Table (7) indicated high significant ($P<0.01$) increase in RFM group than control ones. Similar results were reported in cows with placental retention, regarding progesterone (Sabry *et al.*, 1997), cortisol (Laven and Peters, 1996). This noticeable increase may be due to the endocrine imbalance in prepartum period and this predispose for myometrial dysfunction and placental retention. The results displayed in Table (7) revealed that calcium and in organic phosphorus levels decreased significantly ($P<0.05$) in (RFM) group when compared with control one. These results closely fitted with results in cattle obtained by (Younis, 1990 and Ahmed *et al.*, 1999). Arthur *et al.*, (1992) concluded that the lower levels of calcium and phosphorus in (RFM) group may be due to excessive mobilization of these cations to the fetus during the late stage of pregnancy as well as due to the increased draing of calcium through colostrum at the onset of lactation. The results of the present investigation showed significant ($P<0.05$) decrease in selenium level in RFM cases which present in Table (7). This result goes hand in hand with those recorded previously by (El–Khadrawy and El–Ekhnawy 2005). Ahmed *et al.*, (1999) recorded that inadequate concentrations of selenium and vitamin E in the diet increased oxidative stress, increased production of lipid peroxidase and in turn increased the incidence of RFM.

The data illustrated in Table (7) indicated highly significant ($P<0.01$) decrease of vitamin C level in (RFM) group if compared to control group. The marked reduction in vitamin C level may be due to the use of this vitamin during the pathogenesis of this disease, where vitamin C may be consumed as antioxidant against the oxidative stress that elevated during inflammation (Friedrich, 1988). Moreover vitamin C may be used for biosynthesis of collagen in the damaged tissues (Barnes 1975). The result in the present work agreed with those reported by (Kandeil *et al.*, 2002).

Finally, we can concluded that: RFM in buffaloes is a serious problem, A. pyogenes, E. coli, F. necrophorum and Bacteroides spp. are the predominant bacteria and primary pathogens in buffaloes with RFM during the post partum period and the bovine uterus is anaerobic environment. So the selection of antibacterial agents for controlling RFM should be based on their effectiveness in anaerobic environment also their inhibitory effect on these bacteria beside good hygiene and good cleaning. There is a correlation between the concentration of blood constituents especially calcium, phosphorus, selenium and vitamin C and the occurrence of this syndrome. So this permitting correction of these deficiency.

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