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Bacterial Profile of the Genital Tract in Female-Buffalo During the Different Reproductive Stages

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Abstract: Nowadays, there is a worldwide increasing interest in buffalo breeding. The current study was planed to investigate the bacteriological aspect of the genital in female buffaloes as there is no enough data in this point. Blood samples and vaginal swabs were collected from 293 heads of buffaloes raised at Lower Egypt during the different reproductive stages. Rose Bengal test was used to check all animals for brucellosis. Progesterone level was determined by ELISA to monitor ovarian activity. Bacterial flora was isolated and identified using the standard techniques. Results showed that during the prepubertal period, Undetectable plasma progesterone level), *E.coli*, *Y. enterocolitica*, *Klebsiella* spp., *Micrococcus* spp., *C. bovis*, *S. aureus* were the most important isolated bacteria from vagina. In normal cyclic animals, *E. coli*, *E. faecalis*, *Y. enterocolitica*, *Micrococcus spp.*, *C. diversus*, *Bacillus* spp. and *P. multocida* were isolated with higher rate during the luteal, Serum progesterone level of 4.62±0.95 ng ml^{-1}) than the follicular,0.52±0.15 ng ml^{-1}) phase of the oestrous cycle. The rates of *E. coli*, *C. diversus*, *C. bovis*, *Klebsiella* spp. and *S. epidermidis* isolation were higher in anoestrous than normal cyclic animals. In pregnant animals, the most predominant isolate during the 3 stages of gestation, were *E. coli* and *Micrococcus* spp. After calving, the rate of bacterial isolation from the genital tract of buffalo-cows was the highest during the 1st week post partum followed by animals calved from 2 to 4 weeks and it was the least in animals calved since 5-12 weeks The most predominant isolates were *E. coli* followed by *S. aureus* then *S. pyogenes*. It was concluded that the genital tract of female buffaloes has its own normal bacterial flora which may play an important role in its protection against infection.

Key words: Buffaloes · genital tract · bacteria · reproductive stages · progesterone

INTRODUCTION

Buffaloes represent an integral part of the agricultural economy in Egypt and some other developing countries. According to the last FAO census, the world buffalo's population is 160 million heads. Among this, 3.920 million heads are found in Egypt, producing 3,300,000 ton of milk and 270,000 ton of meat [1]. In Egypt, buffaloes are mainly reared in small holder farms and suffer from a lot of stressful conditions such as malnutrition, bad hygiene, parasitic infestation and pollution [2]. Also, this species tends to have relatively slow rate of reproduction and more reproductive problems such as late maturity, seasonal breeding, silent heat, inactive ovaries, uterine infection and long calving interval [3-6].

Many studies were carried out on the different aspects of reproductive biology in buffaloes. However, studies concerning the bacteriological aspect of the genital tract are scarce. Therefore, the current study was designed to throw lights on the bacterial profile in the genital tract of female buffalo during the different reproductive stages.

MATERIALS AND METHODS

The current work was carried out on female buffaloes reared at Lower Egypt during the period from September 2004 to June 2007 as a part of the National Research Center Project No-7120106.

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Animals: A total number of 293 female buffaloes representing the different reproductive stages were selected. Animals were divided according to the reproductive stage into heifer-calves in the prepubertal period, 32 cases), normal cyclic buffalo-cows, 66 cases), anestrous buffalo-cows during the non breeding season, March – September, 28 cases), pregnant buffalo-cows, 84 cases) and buffalo-cows in the postpartum period, 80 cases).

Experimental design: Data pertaining to each animal including: age, case history, general health condition and the present owner complain were recorded. Animals were subjected to vaginal and rectal examinations as outlined by [7].

Sampling:

- Bood samples were collected from jugular vein of female buffaloes to assay serum progesterone level and checked animals for brucellosis.
- Vaginal swabs were collected after thorough dry cleaning of the vulva using tissue paper. Swabs were collected under aseptic conditions from the anterior vagina of all groups using the rectovaginal technique [7] and inoculated into a tube containing 10 ml Tryptic soy broth

Techniques:

Monitoring of *Brucella* antibodies: Serum samples were separated, X 1500 g, 15 minutes at 4°C), screened for *Brucella* antibodies using Rose Bengal test [8] and kept at –20°C.

Monitoring of ovarian activity: Frozen serum samples were used for analysis of progesterone level to confirm ovarian activity and to avoid misdiagnosis [9]. Kits were purchased from DIMA, Germany for quantitative determination of progesterone levels by ELISA using the micro- well method. The kit had a sensitivity of 2.0 pg/ml with the inter and intra-run precision coefficient of variations of 2.9 and 4.85, respectively [10].

Monitoring bacterial profile: Swabs were incubated at 37°C for 24 hrs and then subcultures were streaked from the enriched broth onto Nutrient, Mannitol, Blood and MacConkey agar plates. The swabs were also inoculated into Selenite-F-broth for 16 hrs then sub cultured onto SS agar medium then plates were incubated at 37°C for 48 hrs. [11]. Individual colonies from the inoculated plates were picked and separately inoculated onto slope agar and semisolid. Films from pure, suspected, fresh, young cultures were smeared, fixed and stained with Gram's Method [12]. Biochemical identifications were carried out according to [11].

Statistical analysis: Data were statistically analyzed according to [13].

RESULTS

Rose Bengal test indicated that all the examined female buffaloes were free from brucellosis.

Monitoring of bacterial profile in the genital tract of female buffaloes revealed the presence of various types of bacteria during the different reproductive stages.

1- Prepubertal period: The rate of isolation of bacteria from the genital tract of female buffaloes during the prepubertal period whereas, progesterone level is undetectable, <0.002 ng ml^{-1}) is the highest in heifers of 8 - 12 month old, 3.10±0.98 organism per animal) followed by heifers of 4 - 8 month, 2.90±0.95) then in heifer-calves < 4 month, 2.67±1.08) and the least rate of isolation was obtained from heifers above 12 month, 2.00±0.82). The most predominant isolates during this stage were *E. coli, Y. enterocolitica* and *Micrococcus* spp. as shown in Table 1.

Table 1: Bacterial profile of the genital tract in apparently healthy pre-pubertal buffalo- heifers

	Apparently healthy prepubertal buffalo heifers aged									
	< 4M(6)		4-8M(10)		8-12M(10)		>12M(6)		Total(32)	
Bacterial isolates	No	%	No	%	No	%	No	%	No	%
Gram Gram negative bacteria										
Salmonella spp.	2	33.33	0	0.00	0	0.00	0	0.00	2	6.25
Y. enterocolitica	3	50.00	2	20.00	4	40.00	2	33.33	11	34.38
E. coli	6	100.00	8	80.00	8	80.00	5	83.33	27	84.38
C. diversus	0	0.00	3	30.00	1	10.00	0	0.00	4	12.50
Klebsiella spp.	0	0.00	1	10.00	4	40.00	0	0.00	5	15.63
P. vulgaris	0	0.00	0	0.00	1	10.00	1	16.67	2	6.25
P. mirabilis	0	0.00	0	0.00	1	10.00	0	0.00	1	3.13
P. multocida	0	0.00	2	20.00	0	0.00	0	0.00	2	6.25
Gram positive bacteria										
C. bovis	2	33.33	4	40.00	2	20.00	0	0.00	8	25.00
S. aureus	0	0.00	4	30.00	3	30.00	0	0.00	7	21.88
Micrococcus spp.	3	50.00	3	30.00	0	0.00	3	50.00	9	28.13
S. bovis	0	0.00	1	10.00	5	50.00	0	0.00	6	18.75
E. faecalis	0	0.00	2	20.00	1	10.00	1	16.67	4	12.50
Mean of isolates/case±SE	2.67±1.08		2.90±0.95		3.10±0.98		2.00±0.82		2.75±0.48	

No = number of positive samples. % = was calculated according to number of examined samples ( ). SE = Standard error.

Table 2: Bacterial profile of the genital tract in apparently healthy cyclic buffalo-cows

	Phases of estrous cycle					
	Follicular phase(18)		Luteal phase (48)		Total (66)	
Bacterial isolates	No	%	No	%	No	%
Gram negative bacteria						
Y. enterocolitica	5	27.78	20	41.67	25	37.88
E. coli	13	72.22	34	70.83	47	71.21
C. diversus	6	33.33	11	22.92	17	25.75
Klebsiella spp.	0	0.00	5	10.42	5	7.58
P.multocida	1	5.56	5	10.42	6	9.09
P.mirabilis	0	0.00	3	6.25	3	4.55
Gram positive bacteria						
C. bovis	0	0.00	3	6.25	3	4.55
S. epidermidis	0	0.00	5	10.42	5	7.58
Micrococcus spp.	8	44.44	19	39.58	27	40.91
S. aureus	0	0.00	2	4.17	2	3.03
E. faecalis	5	27.78	21	43.75	26	39.39
Bacillus spp.	2	11.11	10	20.83	12	18.18
Mean of isolates/case±SE	2.17±0.51		2.83±0.41		2.70±0.33	

No = number of positive samples. % = was calculated according to number of examined samples. SE= Standard error.

Table 3: Bacterial profile of the genital tract in apparently healthy buffalo-cows during the breeding and the non-breeding seasons

	Season			
	Breeding # (66)		Non-breeding (Anoestrum, 28)	
Bacterial isolates	No	%	No	%
Gram negative bacteria				
Y. enterocolitica	25	37.88	7	25
E. coli	47	71.21	19	67.86
Salmonella spp.	0	0.00	3	10.71
C. diversus	17	24.24	10	35.71
Klebsiella spp.	5	7.58	8	28.57
P.multocida	6	9.09	0	0.00
P. mirabilis	3	4.55	0	0.00
Gram positive bacteria				
C. bovis	3	4.55	9	32.14
S. aureus	2	3.03	5	17.86
S. epidermidis	5	7.58	5	17.86
Micrococcus spp.	27	40.91	6	21.43
S. bovis	0	0.00	3	10.71
Bacillus spp.	12	18.18	4	14.29
E. faecalis	26	39.39	8	28.57
Mean of isolates/case±SE	2.70±0.33		3.10±0.59	

No = number of positive samples. % = was calculated according to number of examined samples # Mean of cyclic animals SE= Standard error.

2-Oestrous cycle: The rate of bacterial isolation was higher during the luteal phase, 2.83±0.41), whereas a mature corpus luteum was found in the ovary, with serum progesterone level of 4.62±0.95ng ml^{-1}) than in the follicular phase, 2.17±0.51), whereas a mature Graafian follicle was found, with progesterone level of 0.52±0.15 ng ml^{-1}). The average number of isolates for the normal cyclic buffalo cows was 2.70±0.33. The most predominant isolates were *E. coli*, *Micrococcus* spp. and *E. faecalis* as shown in Table 2.

3-Anoestrum: The rate of bacterial isolation was higher in anoestrous buffalo-cows (3.10±0.59) during the non breeding season, with progesterone level of < 0.002 ng ml^{-1} if compared with cyclic animals during the breeding season. The most predominant isolates were *E. coli*, *C. diversus*, *Klebsiella* spp. and *E. faecalis* as shown in Table 3.

4-Gestation periods: The rate of bacterial isolation was 3.13±0.55, 3.00±0.51and 2.11±0.50 during the early, mid and last stages of gestation, with progesterone levels of 5.40±0.57, 7.36±0.31 and res1.29±0.64, respectively (Table 4). Analysis of variance revealed significant

Table 4: Effect of stage of gestation on the bacterial profile of the genital tract in apparently healthy buffalo-cows

	Stages of gestation							
	Early stage (32) 1-3 months		Mid stage (34) 3-6 months		Last stage (18) ▸6months		Total (84)	
Bacterial isolates	No	%	No	%	No	%	No	%
Gram negative bacteria								
Salmonella spp.	2	6.25	2	5.88	0	0.00	4	4.76
Y. enterocolitica	10	31.25	10	29.41	3	16.67	23	27.38
E. coli	27	84.38	26	76.47	12	66.67	65	77.38
C. diversus	7	21.88	10	29.41	4	22.22	21	25.00
Klebsiella spp.	5	15.63	7	20.59	1	5.56	12	14.29
P. vulgaris	1	3.13	0	0.00	0	0.00	1	1.19
P. multocida	1	3.13	0	0.00	0	0.00	1	1.19
Gram positive bacteria								
S. aureus	2	6.25	4	11.76	1	5.56	7	8.33
S. epidermidis	7	21.88	7	20.59	4	22.22	18	21.43
Micrococcus spp.	23	71.88	17	50.00	8	44.44	48	57.14
S. bovis	0	0.00	2	5.88	0	0.00	2	2.38
E. faecalis	11	34.38	15	44.12	5	27.78	31	36.90
Bacillus spp.	5	15.63	2	5.88	0	0.00	7	8.33
Mean of isolates/case±SE	3.16±0.56		3.00±0.51		2.11±0.50		2.86±0.31	

Early stage up to 3m, Mid stage= 3-7m, Last stage=7-10m. No = number of positive samples.
% = was calculated according to number of examined samples. SE= Standard error. **Significant at P<0.01

Table 5: Bacterial profile of the genital tract in apparently healthy buffalo-cows during the post partum period

	Stages of post partum period							
	During 1st week pp (32)		2-4 weeks pp (24)		5-12 weeks pp (24)		Total (80)	
Bacterial isolates	No	%	No	%	No	%	No	%
Gram negative bacteria								
Y. enterocolitica	2	6.25	5	20.83	2	8.33	9	11.25
E. coli	28	87.50	20	83.33	18	75.00	66	82.50
C. diversus	3	9.38	0	0.00	3	12.50	6	7.50
P. vulgaris	7	21.88	3	12.50	2	8.33	12	15.00
P.mirabilis	1	3.13	2	8.33	3	12.50	6	7.50
P. multocida	3	9.38	0	0.00	1	4.17	4	5.00
P. aerginosa	4	12.50	0	0.00	0	0.00	4	5.00
Klebsiella spp.	7	21.88	5	20.83	1	4.17	13	16.25
Gram positive bacteria								
S.aureus	12	37.50	8	33.33	4	16.67	24	30.00
S. epidermidis	6	18.75	0	0.00	3	12.5	9	11.25
S.bovis	5	15.63	5	20.83	2	8.33	12	15.00
A. pyogenes	6	18.75	1	4.17	0	0.00	9	11.25
S. pyogenes	11	34.38	5	20.83	1	4.17	17	21.25
E. faecalis	2	6.25	2	8.33	6	25.00	10	12.50
Bacillus spp.	3	9.38	0	0.00	2	8.33	5	6.25
Micrococcus spp.	3	9.38	7	29.17	6	25.00	16	20.00
Mean of isolates/case±SE	3.21±0.57		2.63±0.54		2.25±0.46		2.78±0.31	

No = number of positive samples.

% = was calculated according to number of examined samples

SE= Standard error.

changes ($P<0.01$) in the average number of isolates with the highest number obtained during the early stage and the lowest value obtained during the last stage of gestation. The most predominant isolates during the different stages of gestation were *E. coli* followed by *Micrococcus* spp. (Table 4).

5-Post partum period: After calving, the rate of bacterial isolation from the genital tract of buffalo-cows was the highest during the 1st week post partum, 3.21±0.57 with progesterone level of0.39±0.12 ng ml^{-1}) followed by animals calved from 2 to 4 weeks, 2.63±0.54 with progesterone level of <0.002 ng ml^{-1}) and it was the least in animals calved since 5-12 weeks, 2.25±0.46 with progesterone level of 0.63±0.08 ng ml^{-1}. The most predominant isolates were *E. c*oli, followed by *S. aureus* then *S. pyogenes* (Table 5).

DISCUSSION

Every system in the body has its own normal bacterial flora which plays an important role in its protection against infection upset and enhances the host ability to compete pathogens [14]. Bacteria can enter the uterus, especially during the time of parturition and estrus, can multiply rapidly in the presence of favorable conditions, but the normal uterine defense mechanisms can counteract these bacterial invasions [15]. Uterine bacterial infections are important because they disrupt not only the function of the uterus, but also the ovary and the higher control centers in the hypothalamus and pituitary causing sub fertility and infertility [16].

In this study, the incidence of brucellosis as well as types of different bacteria in the genital tract of female-buffaloes during the different reproductive stages were investigated as a pilot study for diagnosis and controlling of infertility.

In the present work, serum examination revealed that all the examined buffaloes were free from *brucella*. In this respect, [17] and [18] recorded the lowest incidence of infection in buffaloes, 1-5%) and attributed the condition to the natural resistance of this species. Moreover, [19] isolated a gene that inhibits *in vitro* propagation of *Brucella* in buffalo cells.

As shown in Table 1, the most predominant bacteria isolated from the vagina of apparently healthy prepubertal buffaloes was *E.coli, Y. enterocolitica*, *Klebsiella* spp. and *C. diversus*, Gram negative) as well as *Micrococcus* spp., *C. bovis*, *S. aureus*, *S. bovis* and *E. faecalis*, Gram-positive). In this concern, *Staphylococci* and *Proteus* spp. were previously isolated from the genital tract of normal buffaloes. [20-22]. Moreover, [23] concluded that, *Klebsiella*, *Micrococcus* and *Proteus* spp. were opportunist contaminants transiently isolated from the uterine lumen. It is clear that, *E. coli* was isolated from apparently health pre-pubertal buffalo heifers of all age groups. Ahlers and Grunert [24] found that lochial secretions taken as early as 1 day after birth contain mostly *E. coli* and *streptococci.* So *Streptococci* appear to be members of the normal uterine bacterial flora [22].

In normal cyclic buffaloes, the rate of bacterial isolation was higher during the luteal than the follicular phase (Tables 2). Low incidence of bacteria during the follicular phase is attributed to the bacteriostatic effect of estrogens while, during the luteal phase bacteria flourished in the uterus due to the luxuriant media provided by progesterone [2]. Progesterone is generally considered as immune suppressive in livestock [25-27].

Ahmed *et al.* [28] in buffaloes and Sheldon *et al.* [29] in cows reported that uteri are more susceptible to infection during the luteal phase. Ahmed *et al.* [28] added that uterine leucocytic number and their phagocytic capacity as well as immunoglobulin contents are obviously decreased. This condition is attributed to inhibition of the myeloperoxidase H_2O_2 system [30], enhanced immunoglobulin suppression activity or production of uterine molecules, which inhibit lymphocytes proliferation [25].

The bacterial profile in anoestrous buffalo-cows during the non-breeding season was investigated and compared with normal cyclic animals during the breeding season, (Tables 3). It is clear that the rates of *E. coli*, *C. diversus*, *C. bovis*, *Klebsiella* spp. and *S. epidermidis* isolation were higher in anoestrum than normal cyclic animals. *S. aureus*, *S. bovis* and *Salmonella* spp. were isolated during anoestrum only. The condition may be related to state of ovarian inactivity and in tern absence of estrogens during the non breeding season [31].

Results in Table 4 showed that in pregnant buffalo-cows, the highest number of isolates was obtained during the early stage and the lowest value was obtained during the last stage of gestation. *E. coli, Micrococcus* spp., *E. faecalis, Y. entrocolitica, C. diversus, S. epidermidis, Bacillus* spp. and *Salmonella* spp. were isolated at all stages of pregnancy. Meanwhile, *P. multocidi* and *P. vulgaris* isolated only from early stage of pregnancy and *S. bovis* detected only from mid stage of pregnancy. During the peri-calving period, [32] isolated *S. epidermidis*, *S. pyogenes*, *S. aureus*, *A. pyogenes*, micrococci, unclassified Gram negative bacilli, *S. purans*, *S. ubris*, *E. coli* and *C. haemolyticum* from 52% of 50 freshly ruptured fetal membranes of buffaloes. Both pathogenic and non-pathogenic bacteria can enter the uterus at the time of parturition and can multiply rapidly. The normal postpartum uterus, however, has defense mechanisms to counteract this bacterial invasion [33].

Variation in the post partum interval (<35->40 days) required for uterine involution might be due to the failure of some buffaloes to eliminate postpartum bacterial infection of the uterus, which in turn, results in delayed involution [34-35]. Moreover, [36] added that the phagocytic and bactericidal capacity of bovine blood leucocytes were lower during the post partum acyclic period compared to the post partum cyclic period whereas estrogen level was high. Saad *et al.* [37] attributed such condition to the high level of plasma corticosteroids during such stressful condition. Moreover, it was recorded that the functional capacity of neutrophils is reduced after parturition in cattle [38] and buffaloes [28] and predispose to the establishment of uterine infection.

After calving the rate of bacterial isolation from the genital tract is the highest in buffalo-cows during the 1st week post partum followed by animals calved from 2 to 4 weeks and it is the least in animals calved since 5-12 weeks. The most predominant isolates were *E. coli*, *S. aureus* then *S. pyogenes* (Table 5). Gram-negative bacteria, especially *E. coli*, seem to dominate in the uterus within the first days after calving [22]. Also, *S. aureus* was recovered from the examined cases during all post partum periods. Usmani *et al.* [39] revealed that 24.1% of examined buffaloes suffered from subclinical uterine infection and They isolated *E. coli, S. aureus* and *P. vulgaris*, from infected buffaloes. These results agree with previous reports on buffaloes [34, 35] in which the incidence of delayed uterine involution due to bacterial infection was 25%. The present study also, confirmed the finding of [34] that *S. aureus* is one of the most common bacterial isolate of the uterine tract of Nili- Ravi buffaloes. It is, however, yet to be estabilished whether, the subclinical uterine infection was caused by persistence of an early postpartum infection or caused by entry of bacteria into the uterine tract post partum.

It is clear that *A. pyogenes* could be isolated from buffalo-cows at 1 and 2 - 4 weeks postpartum. Later in the early puerperium *A. pyogenes*, especially appears in lochial secretions [21, 24]. When *A. pyogenes* was isolated from the uterine fluids after day 21 postpartum, cows developed sever endometritis and were infertile at first service [40]. Later after birth, *A. pyogenes* and Gram-negative anaerobic bacteria are found in uterine secretions of animals suffering from severe puerperal endometritis, especially in combination with retention of fetal membranes [41]. The appearance of *A. pyogenes* seems to be an important indicator of a puerperal disorder resulting in a disturbed fertility [22]. High-grade contamination was associated with the presence of *E. coli* and *A. pyogenes* [38], especially after the onset of the luteal function and progesterone levels have begun to increase [42].

After parturition, but before the next pregnancy can be established, the genital tract has to return to normal non pregnant state. Required changes comprise return of ovarian cyclic activity, elimination of uterine bacterial contamination and uterine involution [16].

The presence of bacteria in the uterus causes inflammation, histological lesions of the endometrium and

delays uterine involution [43]. In addition, uterine bacterial infection or bacterial products suppress pituitary LH secretion and perturb postpartum ovarian follicle growth and function, which disrupts ovulation in cattle [44, 45]. Thus, endometritis is associated with lower conception rates, increased intervals from calving to first service or conception and more culls due to failure to conceive [22, 46]. Usmani *et al.* [39] concluded that subclinical bacterial infection of the postpartum uterus delays the cervical and uterine involution which can, in turn delay the occurrence of first postpartum oestrus and prolonged the service period in buffaloes. Furthermore ovarian function is perturbed in cattle with greater uterine bacterial infection after parturition [47, 48].

In conclusion, as any system in the body, bacteria are regularly present in the genital tract of normal buffaloes during the different reproductive stages. These bacterial flora play an important role in genital tract protection against infection. Moreover, besides the known etiological roles of heredity, nutrition, management, hormones and specific infection in infertility in buffaloes, nonspecific bacterial infection may also have a remarkable role.

REFERENCES

1. FAO, 2005. Food and Agriculture Organization, Year Book of, Production, United Nation.
2. Ahmed, W.M., H.H. El-Khadrawy and A.R. Abd El-Hameed, 2006. Applied investigations on ovarian inactivity. Proceedig of the 3rd International Conference, Veterinary Research Division, NRC, Egypt, pp: 1-16.
3. Singh, J., A.S. Nanda and G.P. Adams, 2000. The reproductive pattern and efficiency of female buffaloe. Animal Reproduction Science, 60-61: 593-604.
4. Ahmed, W.M., H.A. Sabra, E.M. Hanafi and S.I.A. Shalaby, 2002. The present situation of ovarian inactivity of cows and buffaloes in Egypt. Beni-Suef Vetrenary Medicine Journal, 12: 13-46.
5. Hussein, F.M., 2002. Reproduction in the buffalo-cow -An overview. Proceeding of the Annual 14th Congress of Egyptian Society of Animal Reproduction and Fertility, Giza, Egypt, pp: 5.
6. Ahmed, W.M., A.R. Abd El-Hameed and F.M. El Moghazy, 2008. Some reproductive and health aspects of female buffaloes in relation to blood lead concentration. International Journal of Dairy Science, 3: 63-70.
7. Youngquist, R.S., 1997. Current Therapy in Large Animals. Theriogenology, W.B. Sounders CO., USA.
8. Alton, G.G., L.M. Jones, R.D. Angus and J.M. Verger, 1988. Techniques for the Brucellosis Laboratory Institute. Institute Nationale de le Rech. Ayron., Paris., pp: 174.
9. El-Wishy, A.B., I.M. Ghoneim, H.M. Eisa and M. Dobeli, 1999. Rectal palpation and milk progesterone to monitor ovarian activity in post partum anoestrum buffaloes. Proceeding of the 20th International Congress of the Animal Reproduction, The Netherlands, pp: 1978-1980.
10. Hubl, W., T. Fehert, W. Ronde, G. Domer, H. Taubert and E. Feymann, 1982. Determination of progesterone. Endokrinologie, 79: 165.
11. Quinn, P.J., B.K. Markey, M.E. Carter, W.J.C. Donnelly and F.C. Leonard, 2002. Veterinary Microbiology and Microbial Diseases. Black well Scientific Publications, Oxford, London.
12. Cruickshank, R., T.P. Dugid, B.P. Marmion and R.H. Swain, 1975. Med. Micro. The Practice of Medical Microbiology. 12th Ed. vol., 11 Edinburgh, London and New York.
13. Snedecor, G.R. and R.G. Cochran, 1980. Statistical Methods. 7th ed. Iwoa State Univ. Press, USA.
14. Logan, A.C., A.V. Rao and D. Irani, 2003. Chronic fatigue syndrome: Lactic acid bacteria may be therapeutic value. Medical Hypothesies, 60: 915-923.
15. Madsen, S.A., P.S. Weber and J.L. Burton, 2002. Altered expression of cellular genes in neutrophils of periparturient dairy cows. Veterinary Immunopathology, 86: 159-175.
16. Sheldon, I.M. and H. Dobson, 2004. Postpartum uterine health in cattle. Animal Reproductive Science, 82-83: 295-306.
17. Refai, M.K., 2003. Brucellosis in animals and man in Egypt. Egyptian Journal of Veterinary Science, 37: 1-31.
18. Ghazi, I.A., K.A. Abd El-Razik and M.B. Kadry, 2006. Evaluation of Brucella: A diagnostic technique in the Egyptian buffaloes. Proceeding of the 3rd International Conference of Veterinary Research Division, National Research Center, Egypt, pp: 23-43.
19. Borriello, G., R. Capparelli, M. Bianco, D. Fenizia, F. Alfano, F. Capuano, D. Ercolini, A. Parisi, S. Roperto and D. Iannelli, 2006. Genetic resistance to *Brucella abortus* in the water buffalo, *Bubalus bubalis*). Infection and Immunology, 74: 2115-2120.

20. Olson, J.D., L. Ball, R.G. Mortimer, P.W. Farin, W.S. Adney and E.M. Huffman, 1984. Aspects of bacteriology and endocrinology of cows with pyometra and retained foetal membranes. American Journal of Veterinary Research, 45: 2251-2255.
21. Noakes, D.E., L. Wallace and G.R. Smith, 1991. Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms. Veterinary Record, 128: 440-442.
22. Huszenicza, G., M. Fodor, M. Gacs, M. Kulcoar, M.J.W. Dohmen, M. Vamos, L. Porkolab, T. Kegl and J. Bartyik, 1999. Uterine bacteriology, resumption of cyclic ovarian activity and fertility in post partum cows kept in large scale dairy herds. Reproduction in Domestic Animals, 34: 237-245.
23. Williams, E.J., D.P. Fischer, D.U. Pfeiffer, G.C. England, D.E. Noakes, H. Dobson and I.M. Sheldon, 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology, 63: 102-117.
24. Ahlers, D. and E. Grunert, 1993. Helicopter overflights and labor disorders in cattle, expert testimony. Dtsch Tierarztl Wochenschraft., 90: 444-447.
25. Lander-Chacin, M.F., P.J. Hansen and M. Droset, 1990. Effect of stage of the estrous cycle and steroid treatment on uterine immunoglobulin content and polymorphonuclear leukocytes in cattle. Theriogenology, 34: 1169-1184.
26. Ramadan, A.A., G.L. Johnson and G.S. Lewis, 1997. Regulation of uterine immune function during the estrous cycle and in response to infectious bacteria in sheep. Journal of Animal Science, 75:1621-1632.
27. Seals, R.C., I. Matamorosf and G.S. Lewis, 2002. Relationship between postpartum changes in 13, 14 dihydro-15-keto-PGF2α concentrations in Holstein cows and their susceptibility to endometritis. Journal of Dairy Science, 80: 984-994.
28. Ahmed, W.M., A.R. Nada and S.T.A. Shalaby, 1993. Uterine, hormonal and cellular immune response in some cases of genital disorders in buffaloes. Reproduction in Domastic Animals, 28: 298-301.
29. Sheldon, I.M., D.E. Noakes, A.N. Rycroft and H. Dobson, 2004. Effect of intrauterine administration of oestradiol on postpartum uterine bacterial infection in cattle. Animal Reproductive Science, 81: 3-23.
30. Roth, J.A., M.L. Kaeberle and L.H. Loren, 1983. Association of increased estradiol and progesterone blood values with altered bovine polymorphonuclear leukocyte function. American Journal of Veterinary Research, 44: 247-253.
31. Ahmed, W.M., 2006. Adverse conditions affecting ovarian activity in large farm animals. Proceeding of the 3rd International Conference, Veterinary Research Division, National Research Center, Egypt, pp: 251-253.
32. Bassiony, M.M., I.G.A. Ibrahim, Y.A. Farag, M.N.H. Shalaby, Z.M. Kholeaf and A. Farid, 1993. Role of miscellaneous bacteria in relation of the placenta in cows and buffaloes. Proceeding of the 5th Annual Congress of the Egyptian Society of Animal Reproduction and Fertility, Cairo, Egypt, pp: 224-231.
33. Jainudeen, M.R. and E.S.E. Hafez, 1993. Reproductive failure in females. In "Reproduction in Farm Animals", 6th ed. ESE Hafez, (Ed.), Lea and Febiger, Philadelphia, USA, pp: 261-286.
34. Ahmad, R., S.M. Amin and S.E. Kazmi, 1985. Studies on the bacterial causes of delayed uterine involution in postpartum buffaloes. Pakistan Veterinary Journal, 5: 168-170.
35. Khan, N., M. Ahmad, R. Ahmad and A. Ahmad, 1985. Post partum uterine involution and causes of its delay. Proceeding of the 1st World Buffalo Congress. Cairo, Egypt, pp: 964-966.
36. Daniel, L.R., B.P. Chew, T.S. Tanako and L.W. Tjoelker, 1987. Peripartum changes in phagocyte and lymphocyte function in daily cows. Journal of Dairy Science, 70: 166-172.
37. Saad, A.M., C. Concha and G. Astrom, 1989. Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition. Journal of Veterinary Medicine, 36: 337-345.
38. Zerbe, H., C. Ossadnik, W. Leibold and H.J. Schuberth, 2001. Influence of *Escherichia coli* and *Arcanobacterium pyogenes* isolated from bovine puerperal uteri on phenotypic and functional properties of neutrophils. Veterinary Microbiology, 79: 351-365.
39. Usmani, R.H., N. Ahmad, P. Shafiq and M.A. Mirza, 2001. Effect of sub clinical uterine infection on cervical and uterine involution, oestrus activity and fertility in post Partum buffaloes. Theriogenology, 55: 563-571.

40. Lewis, G.S., 1997. Uterine health and disorders. Journal of Dairy Science, 80: 984-994.
41. Bekana, M., P. Jonsson and H. Kindahal, 1996. Intrauterine bacterial findings and hormonal profiles in post partum cows with normal pueperium. Acta Veterinaria Scandinavia, 37: 251-263.
42. Del Vecchio, P.P., D.J. Matsas, S. Fortin, D.P. Sponenberg and G.S. Lewis, 1994. Spontaneous uterine infections are associated with elevated prostaglandin F2α metabolite concentrations in postpartum dairy cows. Theriogenology, 41: 413- 421.
43. Sheldon, I.M., D.E. Noakes, A.N. Rycroft and H. Dobson, 2003. The effect of intrauterine administration of estradiol on postpartum uterine involution in cattle. Theriogenology, 59: 1357-1371.
44. Opsomer, G., Y.T. Grohn, J. Herti, M. Coryn, H. Deluyker and A. De Kruif, 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: A field study. Theriogenology, 53: 841-857.
45. Sheldon, I.M., D.E. Noakes and H. Dobson, 2002-a. Effect of the regressing corpus luteum of pregnancy on ovarian folliculogenesis after parturition in cattle. Biology of Reproduction, 66: 266-271.
46. LeBlanc, S.J., T.F. Duffield, K.E. Leslie, K.G. Bateman, G.R. Keefe, J.S. Walton and W.H. Johnson, 2002. Denning and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. Journal of Dairy Science, 85: 2223-2236.
47. Sheldon, I.M., D.E. Noakes, A.N. Rycroft, D.U. Pfeiffer and H. Dobson, 2002-b. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction, 123: 837-845.
48. Nada, A.R., W.M. Ahmed and A.S. Abdoon, 1993. Studies on endometritis in buffalo-cows: Progesterone level in relation to infection. Egyptian Journal of Comparative Pathology and Clinical Pathology, 7: 231-238.

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