

Effect of Clinical Endometritis on Ovarian Activity and Oxidative Stress Status in Egyptian Buffalo-Cows

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Abstract: This study was designed to focus on the relationship between clinical endometritis and ovarian activity with emphasis on the oxidative status of the animals. A field survey was carried out on 813 buffalo-cows in some veterinary clinics at Lower Egypt. Animals were clinically and gynecologically examined, vaginal swabs and blood samples were collected for carrying out some relevant analyses. Results revealed the prevalence of clinical endometritis in the examined buffalo cows was 16.73 % and out of which 70.59% suffered from ovarian inactivity. Vagina of buffalo-cows having clinical endometritis showed pathogenic strains that either were absent or isolated with low incidence from normal cyclic animals. *E.coli* was isolated in high incidence from healthy (71.21%) than from animals showing endometritis (18.75%), Serotyping of *E.coli* isolates showed O28, O126 and untypable strains in healthy animals versus O157, O119 and O78 stains from endometritis group. The use of multiplex PCR revealed the presence of toxic genes; Shiga toxin-II (Stx-2) and Intemin (eae-A), in *E.coli* O157 and O119 . Blood picture of endometritis group revealed normocytic hypochromic anemia, neutrophilia and monocytosis . Serum progesterone level was < 0.02 and 1.47 ng/ml in animals having inactive ovaries and persistent corpora lutea, respectively. Oxidant/antioxidant markers in the blood of buffalo-cows having uterine infection showed increased malondialdehyde (MDA) and decreased catalase (CAT), ascorbic acid (ASCA), reduced glutathione (R-GSH) and total antioxidant capacity(TAC). Also Serum iron and selenium values were lower in buffalo cows showing endometritis as compared to healthy animals. It was concluded that uterine infection disturbs ovarian function and oxidative status in buffalo cows.

Key words: Buffaloes • Endometritis • *E.coli* • Ovaries • Oxidative status • Progesterone

INTRODUCTION

Buffaloes are the main source of good quality meat and milk in Egypt and some other developing countries, despite this species is mostly reared under harsh socioeconomic conditions and shows low productive and reproductive potentials [1].

The prevalence rate of uterine infection in buffalo-cows is much higher than in cows [2]. In the same time, uterine infection is one of the most important reproductive disorders in buffalo-cows [3,4]. It causes high economic losses [1] due to medication as well as prolonged days open and calving intervals. Uterine function is often compromise by bacterial contamination of the uterine lumen during and after calving whereas, pathogenic bacteria frequently persist, causing uterine disease, a key

cause of infertility [5]. These pathogenic bacteria cause endometritis and delay uterine involution. In addition, it was reported that uterine bacterial infection, bacterial products or the associated inflammation, have negative effect on the higher centers and perturbs ovarian function and consequently fertility in bovines [6-7]. Moreover, toxic puerperal metritis can be a severe problem, which is life threatening in some cases [8].

Endometritis and metritis, as inflammatory conditions of the uterus, share common etiological factors, predispose to one another and largely share common treatment [9-10].

This study was designed to focus on the relationship between clinical endometritis and ovarian activity in buffalo-cows with special emphasis on oxidative status of the animals.

MATERIALS AND METHODS

The present study was carried out during the period from 2004-2007 as a part of the National Research Centre Project No.7120106.

Animals: A total number of 813 buffalo-cows came to some veterinary clinics at villages of Lower Egypt was investigated and followed up in this study. Buffalo-cows were mainly fed on Egyptian clover (*Trifolium alexandrinum*), few amounts of concentrates, crop residues and rice straw. A full case history and owner complains of each animal was recorded. General health condition was examined. Gynecological examination was carried out by inspecting the external genitalia and by palpating the internal genital organs through rectal examination for two successive weeks at least. Ultrasonography was performed in some cases using an Ultra sound apparatus (PiaMedical Falcs e' Saote, Netherlands) with an endorectal linear array transducer (6-8 MHz). Uterine size and texture was recorded as outlined by Ahmed *et al.* [11] and Youngquist [12]. Animals did not show estrous signs at least 6 months after calving during the breeding season (September-March) and have small non functioning ovaries have been considered to suffer from ovarian inactivity. The condition was confirmed later on by monitoring progesterone level.

Samples Collection

Bacteriological Samples: Vaginal samples were collected under possible aseptic condition using the rectovaginal technique [12]. Standardized bacteriological protocols for isolation and identification of the present bacteria were used according to Quinn *et al.* [13] and Cruickshank *et al.* [14]. Biochemical identification was carried out as outlined by Quinn *et al.* [13]. Serological tests were carried out according to Edwards and Ewing [15] using *E. coli* diagnostic antisera for pathogenic types obtained from Denka Seiken Co. Ltd. Also, multiplex PCR [16] was performed to test for the presence of toxic genes; Shiga toxin-II (Stx-2) and Intemin (*eae-A*).

Blood Samples: Samples of blood with and without EDTA were collected from all animals. Uncoagulated blood samples were used for performing complete blood picture as well as determination of R-GSH and Se values. Serum was separated from coagulated blood samples by centrifugation (x 3000g, 15 min., 4°C) and kept at -20°C until used for assaying progesterone, some oxidants antioxidant markers and some trace elements values.

Analyses

- Complete blood picture including erythrogram and leukogram were carried out and anemia indices were calculated as outlined by Jain [17].
- Serum progesterone level was assayed by ELIZA microwell technique using kits from DIMA (Germany). The Kits had sensitivity of 2.0 pg/ml with inter-and intra-run precision coefficient of variations of 2.9 and 4.85, respectively [18].
- Oxidant/antioxidant markers including MDA [19], NO [20], CAT [21], SOD [22], ASCA [23], R-GSH [24] and TAC [25] were calorimetrically assayed.
- Trace elements including Zn, Fe and Cu concentrations in diluted serum samples were determined using atomic absorption spectrophotometer (Perkin Elmer, 2380) as outlined by Varley *et al.* [26].

Statistical Analysis: Data were computed and statistically analyzed using Student's 't' Test [27].

RESULTS

Out of 813 examined buffalo-cows, 136 (16.73%) showed uterine infection. Out of which 96 animals showed clinical signs of endometritis (70.59%). These animals came to the clinics with a history of anoestrus or repeat breeding after calving. The uterus appeared large in size and hard in texture. Ultrasonography scanning revealed different grades of exudation and/or fibrosis. Both ovaries were very small sized, hard in texture and had no mature physiological structures on the surface (bilaterally inactive) in 69 cases (71.88 %) and contained persistent corpora lutea in 27 cases (28.13%).

Table 1: Oligonucleotide primer sequences used for detection of *stx2*, *eae-A* genes by PCR

Primers	Sequence (5'-3')	Specificity	Amplicon size	Annealing Temp (°C)
<i>stx2-F</i>	GGC ACT GTC TGA AAC TGC TCC	Shiga toxin type-2 (including <i>stx-2</i> variants)	255	65
<i>stx2-R</i>	TCG CCA GTT ATC TGA CAT TCT			
<i>eaeA-F</i>	GAC CCG GCA CAA GCA TAA GC	Intemin gene (encoded by <i>eae-A</i>)	384	65
<i>eaeA-R</i>	CCA CCT GCA GCA ACA AGA GG			

Table 2: Vaginal bacterial profile in buffalo cows showing clinical endometritis

Bacterial isolates	Reproductive status			
	Normal cyclic (66)		Endometritis(96)	
	No.	%	No.	%
Gram negative bacteria				
<i>Y. enterocolitica</i>	25	37.88	20	20.08
<i>E. coli</i>	47	71.21	18	18.75
<i>Salmonella</i> spp.	0	0.00	2	2.08
<i>C. diversus</i>	17	25.75	16	16.67
<i>C. freundii</i>	0	0.00	2	2.08
<i>Klebsiella</i> spp.	5	7.58	26	27.08
<i>P. multocida</i>	6	9.09	0	0.00
<i>P. mirabilis</i>	3	4.55	0	0.00
<i>P. pneumotropica</i>	0	0.00	6	6.25
<i>P. trehalosi</i>	0	0.00	2	2.08
<i>M. haemolytica</i>	0	0.00	2	2.08
<i>P. mirabilis</i>	0	0.00	4	4.17
<i>E. aerogenes</i>	0	0.00	4	4.17
Gram positive bacteria				
<i>C. bovis</i>	3	4.55	8	8.33
<i>S. epidermidis</i>	5	7.58	8	8.33
<i>Micrococcus</i> spp.	27	40.91	30	31.35
<i>S. aureus</i>	2	3.03	12	12.50
<i>E. faecalis</i>	26	39.39	4	4.17
<i>Bacillus</i> spp.	12	18.18	14	14.58
<i>Sarcina</i> spp.	0	0.00	2	2.08
<i>Streptococcus</i> spp.	0	0.00	10	10.42
<i>Diplococcus</i> spp.	0	0.00	2	2.08

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

Table 3: Values of hemogram in buffalo-cows showing clinical endometritis (Mean±SE)

Hemogram parameters	Healthy animals	Endometritis
Erythrogram		
Red cell count (10 ⁶ /ul ³)	5.57±0.11	4.44±0.36***
Hemoglobin (gm/dl)	14.90±0.34	11.21±0.13***
Packed cell volume (%)	36.62±0.18	32.57±0.27***
Mean corpuscular volume (FL)	36.60±1.93	36.9±1.50
Mean corpuscular hemoglobin concentration (%)	35.90±0.10	33.55±0.52***
Leukogram		
total white blood cell count (10 ³ /ul ³)	6.07±0.36	9.08±0.47 **
Lymphocyte (%)	60.16±1.68	53.42±1.29**
Neutrophiles (%)	39.55±2.18	41.08±1.15**
Basophiles(%)	0.24±0.14	0.59±0.11
Eosinophiles(%)	1.25±0.56	1.96±0.48
Monocytes(%)	1.25±0.60	2.95±0.47**

** p<0.01 ***p<0.001

Table 2 reveals the isolated bacteria from the vagina of investigated buffalo cows. It is clear that some pathogenic strains either were only isolated (*Salmonella* spp., *C. freundii*, *P. pneumotropica*) or isolated in high incidence(*Klebsiella* spp. *C. bovis* and *S. aureus*) from cases suffering from endometritis as compared to normal cyclic animals. *E. coli* showed higher incidence of isolation from normal cyclic animals (71.21%) in comparison with animals showing endometritis (18.75%),

Table 4: Serum progesterone level (ng/ml) in buffalo-cows showing clinical endometritis (Mean±SE)

Healthy animals		Endometritis	
Follicular	Luteal	With inactive ovaries	With persistent corpora lutea
0.44±0.13	2.89±0.17	<0.02	1.47±0.49**

**p<0.01

Table 5: Serum oxidant/antioxidant values in buffalo-cows showing clinical endometritis (Mean±SE)

Parameters	Healthy animals	Endometritis
Oxidant		
MDA(mmol/ml)	1.98±0.90	5.71±0.84 ***
NO(mmol/L)	25.55±1.58	20.17±0.58**
Antioxidant		
CAT (U/ml)	2.28±0.04	1.37±0.07 **
ASCA(ug/dl)	132.17±5.12	98.61±5.77 **
SOD (U/ml)	338.16±7.11	335.12±18.33
GSH.R(mmol/L)	6.38±0.11	1.99±0.30**
TAC (mmol/L)	1.43±0.08	0.45±0.05***

p<0.01 *p<0.001, (MDA =Malondialdehyde, NO= Nitric oxide, CAT=Catalase, ASCA=Ascorbic acid, SOD= Superoxide dismutase, GSH-R = Glutathione reduced, TAC= Total antioxidant capacity)

Table 6: Serum concentration of some trace elements s in buffalo-cows showing clinical endometritis (Mean±SE)

Element (ug/dl)	Healthy animals	Endometritis
Zinc	139.11±2.17	129.23±8.14
Copper	78.65±4.13	73.33±9.04
Iron	168.40±4.17	155.12±2.06***
Selenium	144.85±0.43	134.15±2.05***

***p<0.001

however, serotyping of representative *E. coli* (5 normal cyclic and 5 cases suffering from endometritis) showed serotypes: O28 (2), O126 (1) and 2 untypable strains, while those suffering from endometritis revealed the presence of serotypes: O157 (2), O119 (2) and O78 (1 case). Use of mPCR indicated the presence of toxic genes; Shiga toxin-II (Stx-2) and Intemin (eae-A) in *E. coli* O157 (2 strains) and O119 (2 strains) that have been isolated from buffalo-cows showing endometritis.

Erythrogram of infected animals revealed normocytic hypochromic anemia (p<0.001) while leukogram revealed enhanced cellular immunity represented by neutrophilia and monocytosis (p<0.01) as shown in Table 3.

Serum progesterone level in normal cyclic as well as affected animals is shown in Table 4. The level was 1.47 and <0.02 ng/ml in animals having endometritis with either persistent CL or bilateral smooth ovaries, respectively.

Concentrations of some oxidant/antioxidant markers in the blood of buffalo-cows having endometritis were recorded in Table 5. Increased MDA (p<0.001) and decreased NO (P< 0.01), CAT (P<0.01), ASCA (p<0.01), GSH R (p<0.01) and TAC (p<0.001) values were obvious as compared to normal animals.

Table 6 showed marked decrease in serum concentrations of iron and selenium ($p < 0.001$) in buffalo cows suffering from endometritis as compared to healthy animals.

DISCUSSION

Postpartum uterine infection is one of the most important disorders in bovines [28, 29]. It causes great economic losses [1] due to prolonged days open, long

calving intervals and culling [30]. Uterine function is often compromised in cattle by bacterial contamination of the uterine lumen after calving whereas, pathogenic bacteria frequently persist, causing uterine disease, a key cause of infertility [5].

In this study the incidence of clinical endometritis in Egyptian buffalo-cows came to clinics with complain of anoestrous was 16.73% and gynecological examinations revealed that these animals having either bilateral inactive ovaries (71.88%) or persistent CL (28.12%). The prevalence of uterine infections varies considerably among studies and the average prevalence of uterine infection is not an especially meaningful itself [31]. Variations among studies in prevalence rate is not surprising because many researchers did not describe the diagnostic methods, the classification of the uterine infections, the postpartum period during which the infection were detected, the parity of the cows, the general characteristics of the cows, or the herd management practices [32,33]. For example, in Spain the prevalence of endometritis in cows varied from 2.6 to 4.5% [33], in Denmark, 6.25% [34], in Korea, 47.6% [35], while in Australia, it varied from 5.6 to 10.9% [36], in USA 10.3% [37], in UK, 10.1% [38]. A recent study [39] found high prevalence rate of endometritis 53% among dairy herds in USA using cytological methods for the diagnosis of uterine diseases. The prevalence rate of uterine infection in buffalo cows was much higher than in cows, it was 38.54% in the Indian and 24% in Pakistan buffalo-cows [40]. In Egypt, the incidence was recorded as 38.9% [32] and 22.4% [41]. In Iran, it was recorded as 33.2% [42] and 29.4% [43].

In this work, pathogenic bacteria were isolated in high incidence from cases suffering from endometritis. Similar findings were recorded by Lopez-Gatius [33]. *S. pyogenes* and *E.coli* were associated with infertility as previously mentioned by Williams *et al.* [43]. Genital bacterial infection or bacterial products have adverse effect on the reproductive function of farm animals via

suppressed pituitary LH secretion and perturb postpartum ovarian follicular growth, function and ovulation [2, 44]. On the other hand, the current results, revealed that the rate of isolation of *E.coli* is higher from normal cyclic animals (71.21%) as compared to endometritis group (18.75%), however, serotyping of representative isolates revealed the presence of serotypes O28, O126 and untypable strains in healthy animals and O157, O119 and O78 animals showing clinical endometritis. Moreover, mPCR revealed that the later isolated stains (O157 and O119) are positive for the tested toxins (Stx-2 and eae-A) and these stains were previously isolated from buffalo-cows showing inactive ovaries [45]. Bovines act as a reservoir for *E. coli* O157 and shed it into the surrounding [46]. Also, it was recorded that *E. coli* is the most important pathogen in the genital tract of bovine [47, 48]. This investigation proved that not only the rate of isolation of bacteria could judge the severity of infection, but the presence of pathogenic bacteria is a great indication for the severity of cases. Also the presence of normal flora as *E. coli* and its incidence will never be sufficient to judge the health status of the animal as it could hinder highly pathogenic strains which have great drawbacks on the animal health status.

The current study revealed normocytic hypochromic anemia, neutrophilia and monocytosis in buffalo cows showing clinical endometritis. Bacterial toxins circulating in blood results in some deformities in the shape of red cells so that it is entrapped in the spleen network and the animals go in regenerative anemia. Also the presence of bacteria stimulate the immune system to produce more phagocytic cells such as neutrophils and monocytes [17].

Progesterone level was undetectable in animals having endometritis with inactive ovaries. The presence of pathogenic bacteria in the uterus causes endometritis, delays uterine involution and perturbs fertility [4]. In addition, uterine bacterial infection, bacterial products or the associated inflammation, suppress pituitary LH secretion and perturbs postpartum ovarian follicular growth and function, which disrupts ovulation in dairy cattle [5]. Thus, uterine disease is associated with lower conception rate, increased intervals from calving to first service or conception and more cattle culled for failure to conceive [5-8]. On the other hand, a group of animals showed persistent CL with detectable progesterone level. In this respect it was reported that uterine disorders usually associated with disturbed steroidogenesis as the diseased endometrium could not produce prostaglandin $F_2\alpha$ in the proper level that could regulate CL function and consequently progesterone production [49].

Buffalos cows having endometritis revealed disturbed oxidative status, with increased MDA and decreased NO, CAT, ASCA, GsH-R and TAC values as compared to healthy animals. It is well known that inflammatory diseases are associated with enhanced oxidative reactions and reduced antioxidant defense capabilities. These results are in agreement with Behiman *et al.* [50]. On the other hand, the current decrease in NO in cases suffering from endometritis was not expected and it needs more investigation. Oxidative stress resulting from imbalance between oxygen reactive radicals and antioxidants has a negative effect on animal health and production [51, 52] as it initiates tissue damage [53] and plays a number of significant roles in female biology [50-52]. Vitamins, trace elements and enzymes are among the antioxidants which can positively affect reproductive function in farm animals as administration of vitamin E and/or selenium have significant role in reproductive biology [53].

This study revealed decreased serum concentration of iron which may be consumed in the regenerative anemia and decreased selenium that may be due to the oxidative stress occurring in buffalo-cows having endometritis. Similar results were recorded by Kamphues [54] and Helal *et al.* [55].

In conclusion endometritis represent a serious problem in buffalo-cows, especially when associated with cessation of ovarian activity and/or oxidative stress. *E.coli*, especially the toxigenic strain O157 were only isolated from diseased animals that may act as a reservoir and shed it into the surrounding.

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