

Investigations on Retained Placenta in Egyptian Buffaloes

W.M. Ahmed, Amal R. Abd El Hameed, H.H. ElKhadrawy and Emtenan M. Hanafi

Department of Animal Reproduction and AI, Veterinary Research Division,
National Research Centre, Dokki, Giza, Egypt

Abstract: This study was carried out to investigate the phenomenon of retained placenta (RP) in buffaloes, since there is no enough data on this reproductive disorder in buffaloes under the prevailing Egyptian condition. Also, comparison of some relevant blood constituents in relation to RP was another target. A total number of 813 heads of buffalo-cows was examined in Veterinary Clinics at lower Egypt during 2004-2007. Animals were gynaecologically examined and blood samples were collected for analysis of some relevant constituents in relation to RP. Treatment of affected animals with systemic antibiotic was tried. Results revealed that 3.3% of the examined buffaloes had history of RP for 1-3 days post calving since 1-3 months. The affected animals had inferior body condition scores (BCS), endometritis with persistent corpora lutea in 88.89% of the cases, serum progesterone level averaged 1.20 ± 0.14 ng/ml in RP group versus 0.51 ± 0.11 and 2.87 ± 0.39 ng/ml during the follicular and luteal phases of the estrous cycle in the control group, respectively. Blood picture revealed anemia with leucocytosis, lymphopenia and monocytosis. RP was associated with oxidative stress as shown by the obvious increase of blood malondialdehyde (MDA) and nitric oxide (NO) and decreases of catalase (CAT), superoxide dismutase (SOD), ascorbic acid (ASCA), glutathione reduced (R-GSH) and total antioxidant capacity (TAC) values with low zinc (Zn), copper (Cu), iron (Fe) and selenium (Se) concentrations. In conclusion, RP is associated with stressful condition in buffaloes and the condition lead to high incidence of infertility and culling, especially when it was associated with uterine fibrosis.

Key words: Buffaloes • Retained placenta • Progesterone • Hematology • Oxidative stress • Trace elements

INTRODUCTION

Retained placenta is among the main reproductive disorders in dairy cattle. It causes considerable economic losses in the herd due to decreased milk production, illness and treatment cost, beside a decreased market value of the animal [1, 2].

The incidence of RP in cattle varied between 5 and 10 % [3]. In buffaloes, wide variations were reported (2.89-12.23%) and the incidence gradually rose with parity , reached a maximum at the fifth parity (30%) and associated with malnutrition [4,5]. Meanwhile, it was found that Iraqi buffaloes developed RP by 54%[6]. Various causes of RP have been identified i.e. uterine paresis, abortion, stress, late or premature birth, dystocia, infections and hormonal disorders. Also, some vitamin and mineral deficiencies predispose for RP [7, 8].

Oxidative stress in cows is a contributory factor to increase disease susceptibility [9], since metabolic demands associated with late pregnancy, parturition and

initiation of lactation would be expected to increase the production of reactive oxygen species (ROS) [10]. RP was reported to associate with oxidative stress in dairy cows [11].

Large number of investigations were carried out on RP in cows , however, in Egyptian buffaloes, available literature on this subject are few, so, the present work was designed to investigate the phenomenon of RP in buffaloes. Also, comparison of some relevant blood constituents in relation to RP was another target.

MATERIALS AND METHODS

Animals: The present study was carried out as a part of the National Research Centre Project No. 7120106. A total number of 813 heads of buffalo-came to some veterinary clinics at Lower Egypt during the period from 2004 to 2007 was included in this study. A full case history and owner complain of each animal was recorded. General health condition was examined and BCS was recorded on scale

of 1 (very thin) to 5 (very fat) as outlined by Ahmed *et al.* [12]. Buffalo cows were considered to retain their placentae if it does not drop after at least 12 hrs post calving [3]. Animals were gynaecologically examined using rectal palpation and Ultrasonography (in some cases) using an Ultra sound apparatus (PiaMedical Falcs e' Saote, Netherlands) with an endorectal linear array transducer (6-8 MHz) and reproductive status and/or disorders were recorded.

Collection of Blood Samples: Samples of blood (with and without EDTA) were collected from animals. Uncoagulated blood samples were used for performing complete blood picture as well as for determination of R-GSH and Se values. Serum was separated from coagulated blood samples by centrifugation ($\times 3000$ g, 15 minutes at 4°C) and kept at -20°C for assaying progesterone level as well as some oxidant/antioxidant markers.

Analysis

- Complete blood picture including erythrogram and leukogram was carried out according to Jain [13].
- Serum progesterone level was assayed by ELISA microwell technique using kits from DIMA (Germany). The kit had a sensitivity of 2.0 pg/ml with inter-and intra-run precision coefficient of variations of 2.9 and 4.85, respectively [14].
- Oxidant/antioxidant markers including MDA [15], NO [16], CAT [17], SOD [18], ASCA [19], R-GSH [20] and TAC [21] were colorimetrically assayed using kits from Biodiagnostic (Egypt).
- Trace elements including Zn, Fe and Cu concentrations in diluted serum samples and Se in whole blood samples were determined using atomic absorption spectrophotometry (Perkin Elmer, 2380) as outlined by Varley *et al.* [22].

Treatment Trial: Affected animals were treated with the recommended dose of Long Acting Terrmycin (Pfizer).

Statistical Analysis: Data were computed and statistically analyzed using Student's 't' test [23].

RESULTS

Twenty seven out of 813 buffalo cows (3.3%) have a history of retained placentae, since 1-3 months and came frequently to clinics for follow up. These animals retained their placentae for more than 12 hrs post calving

(1-3 days) and were considered to suffer from RP and the remaining animals dropped their placentae normally within 12 hrs. Most of the retained groups were subjected to manual removal of their placentae.

RP group mostly have poor body condition score (2.11 ± 0.20) as compared to normal group (2.88 ± 0.28).

Gynaecological examination by rectal palpation and/or ultrasonography revealed that all RP group (100% of 27 vs. 13.87% of 786 which did not retained their placentae) suffered from chronic endometritis with different grades of fibrosis, some animals showed severe metritis and owners were advised to cull them. Ovaries contained persistent corpora lutea in 24 cases (88.89%) of the RP group and showed no cyclic activity in the 3 cases (11.11%). Serum progesterone level was 1.20 ± 0.14 ng / ml in RP group with retained CL and <0.02 ng/ml in those animals showing ovarian inactivity, while it was 0.51 ± 0.11 and 2.87 ± 0.39 ng/ml during the follicular and luteal phases of the estrous cycle in the group which dropped their placentae in the proper time, respectively.

Buffalo cows with RP showed a decreased ($p < 0.01$) RBCs, Hb and PCV. On the other hand, leukogram showed leucocytosis accompanied with lymphopenia with monocytosis as compared to animals dropped their placentae in the proper time (Table 1).

Concentrations of some oxidant/antioxidant markers and trace elements of buffalo-cows in relation to placental dropping are shown in Table 2 and 3, respectively. Increased value of MDA and NO ($p < 0.05$) and decreased values of CAT, SOD and ASCA, R-GSH and TAC ($p < 0.01$; Table 2) as well as low values of Zn, Cu, Fe and Se ($p < 0.01$; Table 3) were detected in the blood of buffalo suffering from RP as compared to normal animals.

Table 1: Effect of placental retention on blood picture of buffalo cows (Mean $\pm$ SE)

Parameters	Control (N=10)	Retained placenta (N=10)
Erythrogram		
RBCs ($10^6/\text{ml}^6$)	5.57 ± 0.11	$4.21 \pm 0.11^{**}$
Hb (g/dl)	14.90 ± 0.34	$11.51 \pm 0.28^{**}$
PCV (%)	36.62 ± 0.18	$31.80 \pm 0.37^{**}$
Leukogram		
Total WBCs ($10^3/\text{ml}^3$)	6.07 ± 0.36	$8.24 \pm 0.9^*$
Lymphocytes (%)	60.16 ± 2.68	$53.42 \pm 0.29^*$
Neutrophils (%)	39.55 ± 2.18	41.08 ± 1.15
Monocytes (%)	1.24 ± 0.60	$3.45 \pm 0.26^{**}$
Esinophils (%)	1.25 ± 0.56	1.96 ± 0.48
Basophils (%)	0.24 ± 0.14	0.59 ± 0.11

* $p < 0.05$ ** $p < 0.01$

Table 2: Effect of placental retention on concentration of some oxidant /antioxidant markers in buffalo-cows (Mean±SE)

Oxidants/ antioxidants	Control(N=10)	Retained placenta(N=10)
Oxidants		
Malondialdehyde (MDA,mmol/ml)	0.98±0.09	2.27±0.44*
Nitric oxide (NO, µmol/L)	15.55±1.58	22.29±2.17*
Antioxidants		
Catalase (CAT,U/ml)	2.28±0.04	0.88±0.15**
Superoxide dismutase (SOD,U/ml)	338.16±7.11	271.0±17.39**
Ascorbic acid (ASCA, µgm/L)	132.17±5.12	84.84±4.25**
Glutathione reduced (R-GSH,mmol/L)	6.38±0.11	1.67±0.07**
Total antioxidant capacity (TAC, mmol/L)	1.43±0.08	0.456±0.05**

* p<0.05 ** p<0.01

Table 3: Effect of placental retention on concentration of some trace elements in buffalo-cows (Mean±SE)

Trace elements	Control(N=10)	Retained placenta(N=10)
Zinc (Zn, µg/dl)	139.11±2.17	118.93±2.66**
Copper (Cu, µg/dl)	78.65±3.13	69.36±2.04*
Iron (Fe, µg/dl)	145.83±8.3	118.40±4.17**
Selenium (Se, µg/L)	144.85±0.43	128.55±1.25**

* p<0.05 ** p<0.01

DISCUSSION

In every survey of the factors associated with reproductive disorders in buffaloes such as endometritis, metritis and toxic puerperal metritis, RP is identified as of major importance [24, 25]. In the same time, it was reported that RP is a reproductive abnormality unique to cows and buffaloes among other domestic ruminants [26]. Also, RP can be a substantial risk factor for toxic puerperal metritis in buffaloes [25]. It causes significant economic losses, as many RP buffaloes develop metritis and suffer from infertility [24]. Ideally, the postpartum period is a noninfectious event. At parturition, the contracting uterus expels the calf and the placenta. In buffaloes with RP, these membranes keep the cervical canal open and the uterine lumen distended, hence inhibiting normal uterus contractions. At the same time, the foetal membranes hang out of the vestibulum readily exhibited for faeces and other environmental contaminants and predispose for uterine infections and it was associated with toxic puerperal metritis in 52.4% of affected buffalo cows [27]. Moreover, endometritis occurred in 100% of the current examined animals having RP with different grades of, this condition agreed with the finding of [28] as they found 100 vs. 10.17% incidence of endometritis in RP and nonretained group of cows.

Confirming previous reports on bovine RP and concomitant high progesterone [29, 30] level in the blood, animals in this study showed a homogeneous increase in the progesterone. Increased progesterone level in RP group may be due to failure of the placenta to produce specific steroidal enzymes that help in progesterone metabolism and its conversion to estrogen [31], which in turn may induce the accumulation of immunosuppressive proteins in the uterine lumen and make the uterus susceptible to infection and persistence of bacteria [32]. On the other hand, no significance difference was found in progesterone values between RP and non RP groups, with marked high progesterone/estrogen ratio in the RP group [33]. Moreover, the PGF₂α from the diseased endometrium to lyses CL is another cause [31].

In this study, buffalo cows with RP suffered from anemia as indicated by the significant decrease in the RBCs, Hb and PCV. Also, there is a leucocytosis accompanied with lymphopenia and monocytosis. The condition may be attributed to inflammation and increase of monocytes for scavenging of cells debris. RP condition in cows exerted a significant decrease on hemoglobin content and PCV [34]. On the other hand, [33] found no differences in PCV, total and differential leucocytes between dairy cows with and without RP. While, [35] found a lower level of monocytes in RP cases.

In the current study, buffalo cows with RP showed increased MDA and NO and decreased CAT, SOD, ASCA, R-GSH and TAC values, proving that these animals were under oxidative stress. These results are in line with those of Kankofer *et al.* [36] who found that cows with RP had lower glutathione peroxidase activity in maternal and placental tissues than cows without RP. Antioxidative defense mechanisms are complex networks used to scavenge ROS. There is evidence that some parameters of oxidative stress are altered in cases of RP as compared to properly released placenta [37] and they added that RP in cows is related to imbalance between production and neutralization of ROS. Moreover, it was suggested that alterations in antioxidative capacity are related to changes in hormonal levels which appear close to parturition, calving itself due to changes in oxygen use and pressure may induce the increase in ROS production. Clinically important symptoms are defined as the disturbances in steroid hormones, as well as prostaglandin F₂α concentration [38, 39].

In this study, Zn, Cu, Fe and Se concentrations decreased in animals with RP as compared to normal

buffalos. Similar results were obtained by Zhang *et al.* [40] in dairy cows with RP. Also, [29, 34] noticed decrease of Fe in cows suffer from RP. Concentrations of selenium were significantly decreased in cows with RP [41, 42]. Se, Cu and Zn are trace minerals required for functioning of enzymes involved in the antioxidant defense system. Se is known to be incorporated in the enzyme glutathione peroxidase performing the antioxidative defense of the body by eliminating hydrogen peroxides [43]. Cu and Zn are involved in the antioxidant system via its involvement in the SOD and ceruloplasmin. Copper-Zn SOD is responsible for dismutation of superoxide radicals to hydrogen peroxide in the cytosol [44].

It could be concluded that retention of placenta is the most important factor leading to uterine infection and toxic puerperal metritis occurring during the early postpartum period. Prevention of retained placenta, of course, is the key. The optimum is to maintain a healthy, contented and active cow prior to, during and after parturition. A balanced, limited ration during the 6-8 week dry period; sufficient daily exercise; sufficiently large, clean and comfortable calving areas (preferably on pasture); and proper sanitary procedures during the calving period minimize the chances of retention and infections of the reproductive tract.

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