

SOME HORMONAL AND BIOCHEMICAL BLOOD INDICES IN PREGNANT BUFFALOES BEFORE AND AFTER RETENTION OF PLACENTA

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ABSTRACT

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This study was carried out in a private dairy farm at Kaliobeia Governorate to investigate the relationship between preparturient hormonal and biochemical changes in pregnant buffaloes and buffaloes with retained placenta (RP). A total of 32 pregnant buffaloes used in this study, blood samples were collected at late stage of pregnancy (two week before calving and after parturition). Preparturient biochemical examination revealed that 25 animals were around normal levels, while blood picture of seven animals revealed anemia with leucocytosis, lymphopenia and monocytosis associated with oxidative stress as shown by obvious increase in nitric oxide (NO), lipid peroxidase (LPO) and decrease of catalase (CAT), superoxidase dismutase (SOD) ascorbic acid (ASCA) and total antioxidant capacity (TAC) values. In addition there were a significant decrease in level of selenium (Se), vitamin E with low zinc (Z), copper (Cu), iron (Fe) concentration. Hormonal changes showed that increase level of progesteron, cortisol while decrease in estradiol-17 β . Regular flow up of these animals post parturient revealed that animals considered RP (1-3 days) post calving were subjected to manual removal of there placentae. Therefore this study can put light on prevention of retained placenta is the key to avoid reproductive disturbances. A balanced, limited ration with sufficient amount of antioxidant during the 6-8 week dry period, sufficient daily exercise, sufficiently large clean and comfortable calving areas and proper sanitary procedures during the calving period minimize the chances of retention and infections of reproductive tract.

Key words: Pregnant buffaloes, LPO, Antioxidants, Hormonal changes.

INTRODUCTION

Retained placenta is the main common reproductive disorders in dairy buffaloes which induced subsequent uterine infection, endometritis, reduction of milk yield and lower conception rate beside decrease market value of animal (Hemingway, 2003 and Semacan and Sevinc, 2005). Although the etiology of retained placenta has been the subject of numerous studies the exact cause is not clear (Joosten and Heslen, 1992 and Wischral *et al.*, 2001). Meanwhile, it was found that various causes of RP have been identified i.e uterine paresis, abortion, late or pre-mature birth, dystocia infections and hormonal disorders beside some vitamin and minearal deficiencies predispose for RP (Ahmed *et al.*, 2009 and Akar and Yeldiz, 2005). According to Brzezinska-Slebodzinsk *et al.* (1994) and Kankofer (2001 a.) oxidative stress in cow is a contributory factor to increase disease susceptibility since metabolic demand associated with late pregnancy and increase milk production would be expected to increase production and uncontrolled increase reactive oxygen species (ROS) which could be a risk

factor for RP (Sordillo, 2005). RP was reported to be associated with oxidative stress in dairy cows (Kankofer, 2001 b). The antioxidant vitamins (e.g vitamin E) is important for improve the fertility of dairy cows (Allison and Laven, 2000). In late pregnancy, the production of free radicals was increased, these elevation in free radical and increasing in oxidative damage may involved in parturition, meanwhile the over increase in the oxidative process after parturition may led to RP and /or postpartum infertility (Toescu *et al.*, 2002). However vascular changes and uterine contractions has been found to play a role in placental release (Laven and Peters, 1996).

This study is a trial to highlight on some parameters as indicators of RP in dairy buffoles.

MATERIALS and METHODS

Animals and their managements: Thirty two pregnant dairy buffaloes belonging to private dairy farm in Kaliobeia Governorate were used in this

study. Animals age 4-7 years and were kept under veterinary supervision, general health condition were examined, housed in the same feeding and management condition until end of study. Case history of each animal was recorded. Buffaloes were considered to retain their placenta if it does not drop after at least 12 hrs post calving (Stephen, 2008). Buffaloes with RP showed discolored vulval membranes, foul smelling discharge, in appetite and decrease milk yield.

Sampling: Two blood samples (with and without EDTA) were collected from each animal via jugular vein puncture two week, week before parturition and one week after parturition. Uncoagulated blood samples were used for performing complete blood picture as well as for determination of selenium (Se) values. Serum was separated from coagulated blood samples by centrifugation for assaying biochemical constituents.

Analysis: Complete blood picture including erythrogram and leucogram was carried out according to (Jain, 2000). The serum levels of cortisol, progesterone and oestradiol-17 β were determined as described by (Hasler *et al.*, 1976; Xing *et al.*, 1983 and Kubosik, 1984) respectively using validated radioimmunoassay (Diagnostic Products Corporation, Los Angeles CA). Trace elements including Zn, Fe and Cu concentrations in diluted serum samples and Se in whole blood samples were

determined using atomic absorption spectrophotometry as outline by Varley *et al.* (1980). Serum calcium was determined according to Gindler and King (1972). Oxidant and antioxidant markers including LPO were estimated according to Buege and Aust (1977), NO (Montgomery and Dymock 1961), CAT (Aebi, 1984), ASCA (Haris and Ray, 1945), R-GSH (Beutler *et al.*, 1963) and TAC (Koracevic *et al.*, 2001) were determined.

Statistical analysis: Data were expressed as means \pm standard error (M \pm SE). The comparison between the groups was conducted by one way analysis of variance (SPSS, 1993).

RESULTS

The results of clinical examination of thirty two pregnant buffaloes (two week, week preparturient and week postparturient) showed that seven out of these animals suffered from RP, two of them had history of retained fetal membrane. which retained their placenta for more than 12 hrs post calving and were considered to suffer from RP and the remaining animals dropped their placenta normally within 12 hrs most of the retained groups were subjected to manual removal of their placenta. All data concerning heamatological results were presented in Table (1) while other markers of oxidant, antioxidant and some biochemical in RP showed Table (2).

Table 1: Mean values of some heamatological parameters of pregnant buffaloes before and after retained fetal membrane.

Items parameters	Control (pregnant)	2week (pre parturient)	1 week (pre parturient)	Retained fetal membrane
RBCs (10 ⁶ /mm ³)	5.9 \pm 0.14 ^b	5.6 \pm 0.16 ^b	5.4 \pm 0.12 ^b	4.8 \pm 0.17 ^a
Hb (gm/dl)	12.02 \pm 0.177 ^d	11.02 \pm 0.178 ^c	9.96 \pm 0.229 ^b	9.06 \pm 0.199 ^a
PCV(%)	34.5 \pm 0.769 ^b	32.7 \pm 0.831 ^b	30.26 \pm 0.573 ^a	29.38 \pm 0.815 ^a
WBCs(10 ³ /mm ³)	6.54 \pm 0.121 ^a	6.74 \pm 0.178 ^a	7.18 \pm 0.116 ^b	8.45 \pm 0.159 ^c
Lymphocytes (10 ³ /mm ³)	3.2 \pm 0.071 ^d	3.0 \pm 0.07 ^d	2.7 \pm 0.08 ^c	2.2 \pm 0.1 ^b
Neutrophils (10 ³ /mm ³)	2.1 \pm 0.07 ^c	2.3 \pm 0.08 ^c	2.6 \pm 0.08 ^c	3.7 \pm 0.09 ^d
Monocytes (10 ³ /mm ³)	1.1 \pm 0.08 ^b	1.3 \pm 0.07 ^b	1.7 \pm 0.10 ^b	2.5 \pm 0.08 ^c
Esinophils (10 ³ /mm ³)	0.12 \pm 0.007 ^a	0.07 \pm 0.01 ^a	0.07 \pm 0.01 ^a	0.08 \pm 0.01 ^a
Basophils (10 ³ /mm ³)	0.02 \pm 0.007 ^a	0.03 \pm 0.02 ^a	0.05 \pm 0.01 ^a	0.04 \pm 0.01 ^a

Values (a-b-c-d) at the same raw followed by different superscript letters were significantly different (P < 0.05).

Table 2: Mean values of some hormonal and biochemical parameters of pregnant buffaloes before and after retained fetal membrane.

Items Parameters	Control (pregnant)	2week (preparturient)	1 week (preparturient)	Retained fetal membrane
Progesterone(ng/ml)	2.6±0.01 ^a	2.8 ± 0.01 ^{ab}	3.1 ± 0.012 ^{bc}	3.3 ± 0.011 ^c
Estradiol-17β (Pg/ml)	93.4 ± 2.18 ^c	89.04 ± 2.22 ^c	35.10 ± 0.98 ^b	23.06 ± 0.83 ^a
Cortisol (ng/ml)	1.38 ± 0.086 ^a	1.74 ± 0.092 ^b	1.94 ± 0.067 ^b	2.54 ± 0.121 ^c
Catalase (CAT)(U/ml)	2.1 ± 0.09 ^c	1.92 ± 0.12 ^{bc}	1.72 ± 0.08 ^{ab}	1.52 ± 0.11 ^a
Superoxide dismutase (SOD) (U/ml)	32.8 ± 1.07 ^b	30.6 ± 1.17 ^b	25.4 ± 1.21 ^a	25.4 ± 1.2 ^a
Total antioxidant capacity (TAC)(mmol/l)	1.22 ± 0.12 ^c	1.12 ± 0.08 ^c	0.88 ± 0.03 ^b	0.58 ± 0.02 ^a
Selenium (ug/100ml)	8.76 ± 0.12 ^d	7.60 ± 0.24 ^c	6.1 ± 0.18 ^b	4.46 ± 0.23 ^a
Vitamin E (ug/100ml)	60.44 ± 1.03 ^d	57.16 ± 0.94 ^c	52.56 ± 0.80 ^b	49.74 ± 0.98 ^a
Lipid peroxidation (LPO) (nm/ml)	0.99 ± 0.045 ^a	1.74 ± 0.093 ^b	2.4 ± 0.071 ^c	2.56 ± 0.092 ^c
Nitricoxide(NO)(nM/ml)	22.5 ± 0.62 ^a	27 ± 0.37 ^b	29.3 ± 0.34 ^c	31.7 ± 0.52 ^d
Ascorbic acid (ug/L)	131.86 ± 1.56 ^d	118.84 ± 2.34 ^c	103.5 ± 1.82 ^b	83.0 ± 1.79 ^a
Zinc (ug/dl)	139 ± 1.69 ^d	130 ± 1.41 ^c	122 ± 1.41 ^b	115 ± 1.41 ^a
Copper (ug/dl)	78 ± 1.70 ^c	72 ± 1.70 ^b	69.8 ± 1.99 ^b	64.2 ± 1.43 ^a
Iron (ug/dl)	140.4 ± 1.63 ^c	132 ± 1.41 ^b	119.6 ± 1.63 ^a	115 ± 1.70 ^a

Values(a-b-c-d) at the same raw followed by different superscript letters were significantly different (P< 0.05)

DISCUSSION

Retained placenta is identified as major importance reproductive abnormality of cows and buffaloes. It can be substantial risk factor for toxic puerperal metritis, it causes significant economic losses, as many RP buffaloes develop metritis and suffer from infertility (Azawi and Taha 2002 and Azawi 2006). In this study animals with RP suffered from anemia as indicated by the significant decrease in RBCs, Hb and PCV, also there is a leucocytosis accompanied by lymphopenia, neutrophilia and monocytosis these may be attributed to inflammation and increase monocytes for scavenging of cell debris (Sivaraman *et al.*, 2003). Increase progesterone in preparturient period in our results (Table 2) could be a major factor contributing to the pathogenesis of retained fetal membrane (RFM) in buffaloes, however normally increasing estrogen and decreasing progesterone activities lead to multiple changes which is accompanied by increasing prostaglandin synthesis and mainly to a rise oxytocin receptor concentration in the myometrium these basic changes are necessary for subsequent placental separation and expulsion (Husslein, 1984; Sabry *et al.*, 1997 and Michal and Hanna, 2006). Increased progesterone level in RP may be due to failure of placenta to produce specific steroidal enzymes that help in progesterone metabolism and its conversion to estrogen (Matton and Dufour, 1987), which in turn may induce the

accumulation of immunosuppressive proteins in the uterine lumen and make the uterus susceptible to infection and persistence bacteria (Konigsson *et al.*, 2002).

It has been documented that RFM associated with the presence of uncontrolled elevated level of reactive oxygen species (ROS) which may disturb physiological processes leading to expulsion of the placenta (Kankofer, 2001 a,b), when free radical generation exceeds the body's antioxidant production capacity, oxidative stress develops Roth, (2000) reported that estradiol has a potent antioxidant activities. The periparturient significant increase in estradiol-17β concentration is in agreement with that results of Eissa *et al.* (1995) and Badr *et al.* (2001).

The rise in cortisol (Table 2) stimulates the conversion of placentally derived progesterone to estradiol by activating the placental enzyme 17α - hydroxylase which hydroxylates progesterone via androstenedione to estradiol 17β. The consequences of the rise in estradiol 17β and decline of progesterone in peripheral circulation have a triple effects, first estradiol has a direct effect upon the myometrium increasing its responsiveness to oxytocin, second they produce softening of cervix by altering the structure of collagen fibers, third they act upon the cotyledon-caruncle complex to stimulate the production and release of PGF_{2α} (Grunet, 1986 and Horta, 1988).

In the current study it was found that peroxidative indices (LPO and NO) are an indicator of over production of free radicals and cellular damage which were elevated during the last two weeks of pregnancy compared with non pregnant animals in the same time the level of antioxidant (CAT, ASCA, SOD TAC, selenium and vitamin E) were decreased these mean that oxidative stress and exhaustion of the antioxidant were involved in the late stage of pregnancy. These results agree with Megahed *et al.* (2006); Ahmed *et al.* (2009) and Toescu *et al.* (2002) who reported that late pregnancy was associated with the formation of susceptible oxidisable particles and an increase in oxidative damage. Antioxidative defense mechanisms are complex net work used to scavenge ROS. Kankofera *et al.* (2005) recorded that RP in buffaloes is related to imbalance between production and neutralization in ROS. Moreover, it was suggested that alterations in antioxidative capacity are related to changes in hormonal levels which appear close to parturition. Clinically important symptoms are defined as the disturbances in steroid hormones as well as prostaglandin F_{2α} concentration (Leidl and Rockel, 1980 and Heuwieser and Grunert, 1987). In this study significant decrease was recorded (Table 2) in selenium, vitamin E, Zn, Cu and Fe, similar finding were reported by Segerson *et al.* (1980); Ahmed *et al.* (1999) and EL-Khadrawy and EL-Ekhnawy (2005) who found that inadequate concentrations of selenium and vitamin E in the diet increased oxidative stress, production of lipid peroxidase and the incidence of RFM. 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 glutathione peroxidase performing the antioxidative defense of the body by eliminating hydrogen peroxides (Kommissrud and Vatn, 2005). Cu and Zn are involved in the antioxidant system via its involvement in superoxidase dismutase (SOD) and ceruloplasmin. Copper, Zn and SOD are responsible for dismutation of superoxide radicals to hydrogen peroxide in the cytosol (Halliwell and Gutteridge, 1999).

It could be concluded that retention of placenta is a serious problem resulted in uterine infection and toxic puerperal metritis. Also it has an adverse effect on some hormonal and biochemical parameters. So supplementation of dam with a balanced ration containing adequate amount of antioxidant during late stage of pregnancy beside that sufficient daily exercise, sufficient large clean and comfortable calving areas and proper sanitary procedures during the calving period is advised to minimize PR and infection of the reproductive tract.

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بعض المؤشرات الهرمونية والكيميائية في الجاموس العشار قبل وبعد الإصابة باحتباس المشيمة

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اجريت هذه الدراسة على 32 من الجاموس العشار في احدى المزارع الخاصة بمحافظة القليوبية يتراوح عمرها ما بين 4 الى 7 سنوات وكان الهدف من الدراسة استبيان العلاقة بين التغيرات الكيميائية والهرمونية للحيوانات قبل الولادة وعلاقة ذلك بحدوث احتباس المشيمة وعلى هذا تم اخذ عينات دم قبل الولادة باسبوعين واخرى قبل اسبوع ومتابعة الفحص بعينات دم اخرى بعد الولادة واوضحت النتائج ان 25 من هذه الحيوانات تراوحت قيم التغيرات الكيميائية والهرمونية حول القيم العادية بينما كانت هناك تغيرات كيميائية وهرمونية في 7 حيوانات وكانت على النحو الاتي اوضحت صورة الدم انها تعاني من انيميا مع زيادة في كرات الدم البيضاء مصحوبة بنقص في اللمفوسيت وزيادة في المونوسيت والنتروفيل واتضح من هذه الدراسة ان دلائل ضغوط الاكسدة قد ارتفعت معنويا (فوق اكسيد الدهون واكسيد النيتريك) بينما انخفضت مضادات الاكسدة (حمض لاسكروبيك والسيلينيوم وفيتامين هـ وانزيم السوبر اكسيد دسميوتيز) كما وجد نقص في عناصر الزنك والحديد والنحاس بالنسبة لمستوى الهرمونات فقد كان هناك زيادة معنوية في هرمون البروجيسترون والكورتيزون ونقص ملحوظ في مستوى الاستراديول 17ب وبتابعة هذه الحيوانات تبين عدم نزول المشيمة خلال 12 ساعة بعد الولادة مما يتضح انها تعاني من احتباس المشيمة. وتوصى الدراسة بضرورة ان تكون تغذية الحيوان كافية لاحتياجاته كما ونوعا في المراحل المختلفة من الحمل خاصة الاخيرة منها والاهتمام باضافة مضادات الاكسدة الى الاعلاف مثل السيلينيوم وفيتامين هـ كإجراء وقائي ضد اجهاد الاكسدة بهدف التغلب على العديد من الاضطرابات التناسلية المرتبطة باجهاد الاكسدة اثناء الحمل و الولادة.