

PREDICTIVE VALUE OF SERUM HORMONES AND BIOCHEMICAL PROFILES FOR RETAINED PLACENTA IN BUFFALOES

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ABSTRACT

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Retained fetal membrane (RFM) is one of the most important calving related disorders affecting economics of the farmer and the herd health. The aim of the study was to compare some hormonal and biochemical parameters level in blood serum of buffalo-cows with retained fetal membranes (RFM) and normal non retained animals (C). Blood samples were collected from buffalo-cows (n=55) for three times (after four months, seven months of gestation and 24 hour after parturition), after parturition only nine buffalo-cows affected with RFM (16.36%). The obtained results, showed significance decrease ($P < 0.05$) in Calcium, Zinc and Selenium between RFM and C groups after four months of gestation, while, in RFM group after seven months of gestation results revealed significance decrease ($P < 0.05$) in 17- β Estradiol, calcium and selenium and significance increase ($P < 0.05$) in progesterone, cholesterol and linolinic free fatty acids. But after 24 hour postpartum there was significance decrease ($P < 0.05$) in cholesterol, linolinic free fatty acids and calcium, with significant increase ($P < 0.05$) in progesterone and Cortisol. The obtained results concluded that Progesterone and Cortisol were significantly increased remarkably just after 7th month of pregnancy, in addition to significantly decreased of estradiol 17- β and biochemical profiles extending till 24 hrs after calving.

Key words: Buffalo-cows, retained placenta, hormones, biochemical parameters.

INTRODUCTION

One of the most common problems seen during the post –parturient period of high–yielding dairy buffaloes is RFM. It causes important economic losses due to decreased pregnancy rates, increased calving – conception intervals and a rapid drop in milk yield (Semacan and Sevinc, 2005; Seifi *et al.*, 2007 and Rabbani *et al.*, 2010). Most buffaloes usually expel the fetal membranes within 8 hours following parturition. Retention of fetal membranes for 12 hours or more after parturition indicates abnormal condition which is one of the most important calving related disorders affecting economics of the farmer and the herd health (Laven and Peters, 1996). The productive and reproductive performance of the buffalo is negatively influenced by the calving related reproductive disorders, especially retention of fetal membranes (El-Wishy, 2007). The incidence of retention of fetal membranes and genital prolapse is high in the buffalo compared to cattle (Samad *et al.*, 1987; Arthur *et al.*, 1989; Laven and Peters, 1996 and Rabbani *et al.*, 2010).

The incidence of retained fetal membranes increases with parity, twins and premature births and varies from country to country, year to year and from herd

to herd (Hussain, 1983). Physiologically, steroids are fundamental in pregnancy maintenance and parturition where it regulate prostaglandin synthesis, which stimulated by low level of progesterone (Wango *et al.*, 1992 and Wooding *et al.*, 1996) and estrogens function to increase the prostaglandine F2 α (PGF2 α) levels in the uterus pre-parturition (Ham *et al.*, 1975 and Rasmussen *et al.*, 1996). But progesterone levels in animals with retained fetal membrane are higher (Elecko *et al.*, 1982 and Matton *et al.*, 1987), estrogen levels are lower (Thomas *et al.*, 1992 and Kankofer *et al.*, 1996) than normal. Decreased level of estrogen and delay in lyses of the corpora lutea resulting in increased level of progesterone are suggested to be associated with calving related disorders like dystocia and retention of fetal membrane in dairy cows (Zhang *et al.*, 1999 and Ali *et al.*, 2009) with a higher significant levels of Cortisol throughout the peri-partum period (Peter and Bosu, 1987). Energy disturbances can start a few weeks before calving reported that cows with RFM showed a high level of ketone bodies (KB) and free fatty acids (FFA) in the last week before parturition. Hypoglycaemia was not observed in RFM cows with high blood concentration of FFA (Kuzma *et al.*, 1996 and Kaczmarowski *et al.*, 2006). It can be related to the high level of Cortisol (Weber, *et al.*, 2000) which

is a cause of RFM (Subandrio and Noakes, 1997). Overfeeding during the non-lactating stage reduces feed intake and stress near parturition accelerates the release of NEFA from adipose tissue, resulting in an excess uptake of NEFA by the liver (Rukkwamsuk *et al.*, 1998). Circulating NEFA is absorbed by the liver and re-esterified to Triacylglycerols (TAG) which are then secreted in the blood (Herd *et al.*, 1988). Excess hepatic lipids are stored as TAG and are associated with decreased metabolic functions of the liver (Drackley, 1999) and lead to increase the incidence of health disorders (Jorritsma *et al.*, 2000). Abnormalities in trace element regulation have been reported in various obstetric pathologies in animals (Graham *et al.*, 1994; McSparran *et al.*, 1977; Naziroglu *et al.*, 1998 and Olujohungbe *et al.*, 1998).

The aim of the study was to compare the serum levels of hormones (progesterone, 17- β oestradiol and Cortisol); biochemical parameters (Total cholesterol and FFA) and some macro elements (Ca, P) and some micro elements (Cu, Zn and Se) at different stages during gestation period and just after parturition as a predisposition effect on the development of retained fetal membrane in apparently healthy pregnant buffaloes.

MATERIALS and METHODS

Animals and management

A total of fifty five pregnant Buffaloes were used for the current experiment without any interference during gestation period or parturition. This study was conducted at El Gharbyia Province (Qtoor city). The buffaloes were confined to partly roofed structures with open sides and free-stall barns with a concrete floor. They were fed a ration consisting of hay of barseem (*Trifolium Alexandrium*); silage (Green Maize) and concentrates (prepared mainly from maize, soybean, and wheat bran). All buffaloes were artificially inseminated from sound healthy bulls and were milked by machine twice daily along seven months, then dried off milk for the rest days of gestation. At 10 days prior to expected day of parturition, every buffalo kept in separate calving pen until 2 days after parturition.

Blood collection

Blood samples (approximately 10 ml) were collected from Jugular veins into plain vacutainer tubes using its special needles. The blood samples were collected

once, after four months, seven months of gestation and 24 hrs after parturition. The blood was centrifuged and the obtained serum samples were stored at -20°C until hormones and biochemical analyses were done.

Biochemical analysis

Calcium (Ca), inorganic phosphorus (P) and total cholesterol were estimated by commercial kits calorimetrically according to Gindler and King (1972), Goldenberg (1966) and Watson (1960), respectively. Copper (Cu), zinc (Zn) and selenium (Se) concentrations were analyzed by atomic absorption (spectrophotometry) as described by Bauer, (1982). Free fatty acids "Linolenic and Linoleic FA" were estimated by Gas chromatography according to Hollender *et al.* (2002).

Hormonal analysis

Serum concentrations of progesterone, 17- β estradiol and cortisol were determined by immunoenzymatic methods (Biodata Diagnostic tests). The sensitivity of these tests were: progesterone 0.15 ng/ml, C.V.% - 3.9; 17- β oestradiol 5 pg/ml, C.V.% - 3.9 and cortisol 0.36 ng/ml C.V.% - 3.7.

Definitions, assumptions, and groups

Buffaloes were intensively observed for expulsion of fetal membranes until 12 h after calving. Buffaloes expel their fetal membranes within 12 h after calving were considered normal parturition (n= 46). The fetal membranes were not expelled within 12 h after calving were considered a RFM (n= 9).

Statistical analysis:

All obtained data was subjected to "T. test between Retained foetal membrane and normal groups" according to Snedecore and Cochran (1987).

RESULTS

Out of 55 parturitions in buffalo-cows, 9 buffaloes (16.36%) took a long time for expelling the fetal membranes (18.5-21.0 hrs). While, 46 buffaloes (83.64%) showed a normal averages of time (10.13 hours) for expelling the fetal membranes. Tables 1, 2 and 3 showed significances in hormonal and biochemical value levels of blood serum examinations during four, seven months of gestation and 24 hr. after parturitions.

Table 1: Mean (\pm SE) blood serum level of hormonal and chemical values after four months of gestation in Buffalo-cows with retained fatal membrane (RFM) or without (control, C)

Item	RFM	Control (C)
E ₂ (ng/ml)	11.70 \pm 0.83	11.31 \pm 0.05
P ₄ (ng/ml)	7.36 \pm 0.53	7.12 \pm 0.24
Cortisol (ng/ml)	15.92 \pm 0.24	15.78 \pm 2.22
T.cholesterol (mg/dl)	106.17 \pm 5.39	104.895 \pm 1.64
Linolinic FA (ng/ml)	74.64 \pm 3.82	74.73 \pm 1.03
Linoleic FA (ng/ml)	102.47 \pm 1.68	95.12 \pm 1.77
Ca (mg/dl)	7.16 \pm 0.11	8.48 \pm 0.25**
Ph (mg/dl)	3.89 \pm 0.19	4.44 \pm 0.13
Cu (μ g%)	168.33 \pm 6.32	161.02 \pm 3.20
Zn (μ g%)	191.89 \pm 5.97	212.04 \pm 3.03**
Se (μ g%)	223.11 \pm 9.22	246.22 \pm 4.59**

** = significance (P < 0.05).

Regarding to the studied parameters in Table 1 of RFM and normal animals after four months of gestation, only there were significance decrease (P < 0.05) in the level of Calcium, Zinc and Selenium in RFM than normal, while there were no significant differences in the others parameters.

Table 2: Mean (\pm SE) blood serum level of hormonal and chemical values after seven months of gestation in Buffalo-cows with retained fatal membrane (RFM) or without (control, C)

Item	RFM	Control (C)
E ₂ (ng/ml)	24.42 \pm 1.13	93.29 \pm 2.42**
P ₄ (ng/ml)	8.20 \pm 0.65**	5.42 \pm 0.13
Cortisol (ng/ml)	29.10 \pm 1.38**	7.11 \pm 0.09
T.cholesterol (mg/dl)	126.89 \pm 3.96**	103.80 \pm 1.43
Linolinic FA (ng/ml)	55.14 \pm 3.05**	69.31 \pm 1.28
Linoleic FA (ng/ml)	96.50 \pm 6.33	98.53 \pm 2.28
Ca (mg/dl)	6.73 \pm 0.35	9.13 \pm 0.11**
Ph (mg/dl)	4.10 \pm 0.26	4.397 \pm 0.12
Cu (μ g%)	178.78 \pm 7.96	180.93 \pm 2.95
Zn (μ g%)	206.89 \pm 6.94	208.30 \pm 3.43
Se (μ g%)	226.78 \pm 18.62	290.93 \pm 10.72**

** = significance (P < 0.05)

As shown in Table 2, buffaloes that showed RFM had a significance decrease (P < 0.05) in the level values of estradiol. But contrarily there were significance increase (P < 0.05) in the level values of progesterone; Cortisol; T.cholesterol; Linolinic FA in RFM than control group. In the mean while, significant decrease (P < 0.05) in the level values of Calcium and Selenium between the two groups respectively. Other parameters (linoleic FA; phosphorus; copper and Zinc) showed no significant in the level values during this stage of pregnancy.

Table 3: Mean (\pm SE) blood serum level of hormonal and chemical values 24 hour postpartum in Buffalo-cows with retained fetal membrane (RFM) or without (control, C)

Item	RFM	Control (C)
E ₂ (ng/ml)	76.10 \pm 4.48	73.97 \pm 2.55
P ₄ (ng/ml)	2.52 \pm 0.27**	1.66 \pm 0.13
Cortisol (ng/ml)	50.62 \pm 6.22**	30.10 \pm 1.81
T.cholesterol (mg/dl)	102.28 \pm 3.22	135.89 \pm 1.85**
Linolinic FA (ng/ml)	62.36 \pm 2.53	70.10 \pm 1.26**
Linoleic FA (ng/ml)	94.80 \pm 3.05	86.57 \pm 2.06
Ca (mg/dl)	6.69 \pm 0.40	8.42 \pm 0.08**
Ph (mg/dl)	3.71 \pm 0.20	3.71 \pm 0.06
Cu (μ g%)	162.33 \pm 3.40	175.30 \pm 2.34**
Zn (μ g%)	207.44 \pm 7.83	202.87 \pm 3.25
Se (μ g%)	215.44 \pm 13.59	305.65 \pm 9.20**

** = significance ($P < 0.05$)

After 24 hrs postpartum (Table 3) the mean level values of Progesterone and Cortisol still increase significantly ($P < 0.05$) as in the seventh month of pregnancy in RFM than control group, while, the level values of T.cholesterol; Linolinic FA; Calcium; Copper and Selenium showed significant decrease ($P < 0.05$) in RFM than control group than just after the 7th months of pregnancy. But, other parameters (Estradiol; Phosphorus and Zinc, also, Linoleic FA) after 24 hrs postpartum showed no significance in the mean level values of blood serum in buffaloes with RFM or without.

DISCUSSION

The present findings for the incidence of RFM (16.36%) were similar to these reported in buffaloes (16.18%) by Oresnik (1995) and Sabry *et al.* (1997). The reciprocal changes in estradiol and progesterone synchronize the parturition process. Asynchrony of this hormonal mechanism can lead to calving problems (Olujohungbe *et al.*, 1998). The results of the present study of buffalo-cow were significantly increase ($P < 0.05$) in mean values of blood serum progesterone before and after calving in the buffaloes with RFM than without, these results were parallel to that obtained by Ali *et al.* (2012) in Pakistan.

The reason behind this increased level of progesterone concentration is reduced level of Estradiol-17 β and delayed regression of corpora lutea in dystocia (Zhang *et al.*, 1999) or in spontaneous parturition (El-Wishy, 2007). In this study, serum estradiol-17 β concentrations were found to be lower in buffaloes affected with RFM compared with buffaloes without RFM, but appear significantly ($P < 0.05$) just after the 7th month of pregnancy. This lower level of estrogen is also reported earlier (Erb *et al.*, 1981) and these concentrations caused reduced synthesis of PGF2 α resulting in calving difficulty (Wischnal *et al.*, 2001) and consequently RFM.

Contrarily, higher estradiol and lower progesterone concentration in dystocia cattle as compared to controls were reported by Yokus *et al.* (2010).

Increased progesterone level in RFM may be due to failure of the placenta to produce specific steroidal enzymes that help in progesterone metabolism and its conversion to estrogen (Matton *et al.*, 1987), which in turn may induce the accumulation of immunosuppressive proteins in the uterine lumen which make the uterus susceptible to infection and persistence of bacteria (Königsson *et al.*, 2002).

El-Nemer *et al.* (2000) observed that during the week before parturition, the level of estradiol reaches its maximum level and this help the uterus to get rid of any remnant of fetal membranes and also to prevent endometriosis. Therefore, a decreased level of estrogen may be indicated as a factor enhancing RFM.

Similar to the results that were obtained by Kornmatitsuk *et al.* (2000); Sabry *et al.* (1997); Michal *et al.* (2006) and Kankofer *et al.* (1998) in RFM cows, buffaloes with RFM in present study showed a homogeneous increase in the progesterone and Cortisol and a decrease in the estradiol 17 β levels. The increased cortisol may be related to the

stress in buffalo-cows with RFM (Dobson and Smith 2000). Similar data were previously recorded by Farzaneh *et al.* (2002) and Kandeil *et al.* (2002).

The frequency of RFM can increase as a result of insufficient feeding of high-efficient animals, also, the energetic mineral and the wrong balance of an alimentary dose lead to metabolic disorders, loss of acid-base equilibrium, hormonal system changes and even to suppress activity of the immune system (Levis, 1997). It was reported that prepartum FFA (Laszlo *et al.*, 2009 and Ospina *et al.*, 2010) was significantly associated with development of clinical diseases in dairy cows including retained placenta (Ospina *et al.*, 2010). Serum levels of FFA may be more useful to identify buffalo-cows with a metabolic abnormality or energy imbalance that might predispose them to RFM (Quiroz-Rocha *et al.*, 2009). In this study, T triglycerides level significantly decreased in buffalo-cows with RFM than control, similar to the data in RFM cows obtained by Macak *et al.* (1999). Furthermore, significant changes in serum cholesterol concentrations could be simply due to decrease of feed intake, because most cholesterol in ruminants is of intestinal origin (Uchide *et al.*, 1997). It is well known that feeding problems during the peri-parturient period may affect susceptibility of cows to metabolic disorders (Dann *et al.*, 2005) including RFM. The decreased cholesterol level may be owing to the persistence utilization of cholesterol for progesterone synthesis or due to increase breakdown of cholesterol (Kandeil *et al.*, 2002).

Minerals are important in the prevention of RFM (Wilde, 2006). Most minerals act as co-factors or activators of enzymes systems and elements. Calcium sensitizes female tubular genitalia for the action of hormone like oxytocin. So, calcium deficiency can act as a predisposing factor for uterine inertia leading to dystocia, retention of fetal membranes and metritis (Dabas *et al.*, 1987 and Mohanty *et al.*, 1994). So, the significant decrease of serum calcium level was parallel to the results observed by Mandali *et al.* (2002) in cattle and buffalo affected with retention of fetal membrane. Another works by Patel *et al.* (1999); Akar *et al.* (2002) and Melendez *et al.* (2004) reported significantly low level of blood serum calcium and inorganic phosphorus four weeks post partum in buffaloes which had retention of fetal membrane during parturition. In accordance with the present study, they recorded a non significant decrease in Zn, P and decrease in Ca, Cu in dystocia at the prepartum period as compared to non dystocia ones, which implies evaluation of these elements in 7th month of pregnancy, does not appear to be useful in the assessment of dystocia. Others as Hattab and Abdel Moghney, (1994); Markiewicz *et al.* (2001); Farzaneh *et al.* (2002) and Roche, (2006) were recorded that RFM animals show concurrent decreased zinc and selenium, this complex factors

might lead to RFM. While the results of a similar studies on the predispose to RFM in buffalo-cows pre and post-calving periods by Bari *et al.* (1996); Sabry *et al.* (1997); Akar and Yeldiz, (2005) and Tillard *et al.* (2008) showed significant decrease level in blood serum values of Ca, P, Zn and Se in accordance with the present results. This decrease of Se, Zn and Cu is attributed to the high demand of these elements in enzyme involved in the antioxidants defense system in dairy cows, they added that these elements incorporated in glutathione peroxidase performing the antioxidative defense mechanism by eliminating hydrogen peroxide (Kommisrud *et al.*, 2005). Free radicals which increased towards parturition have a detrimental effect on cellular membrane integrity and compromising the cellular function of steroidogenic tissues and embryos (Arechiga *et al.*, 1998).

Conclusion: the obtained results indicate that the biochemical profiles of T. Cholesterol, linolonic free fatty acids, Calcium, and Selenium mean level values of blood serum were similarly significantly decrease in RFM than control buffalo-cows just after 7th month of pregnancy or just 24 hrs after calving in the same farm. Also, the hormonal profiles of increased significance of progesterone and cortisol, with lower decreased significance of estrogen seem to be the main predictive changes in retained placental buffalo-cows.

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الصورة الهرمونية والبيوكيميائية بمصل الجاموس كقيم تنبؤية بحدوث احتباس المشيمة

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يعد الاحتباس المشيمي أحد أهم الاضطرابات التي تحدث بعد الولادة ، والتي تؤثر على اقتصاديات المربي وعلى صحة القطيع . لذا فان الهدف من تلك الدراسة هو مقارنة مؤشرات الدم لمستويات بعض الهرمونات والعناصر البيوكيميائية في الجاموس المصاب أو الغير مصاب بالاحتباس المشيمي. وقد تم جمع عينات الدم ثلاث مرات من عدد خمسة وخمسون جاموسة (بعد أربعة شهور من الحمل ، سبعة شهور من الحمل و بعد أربعة وعشرون ساعة من الولادة) وكانت نسبة الجاموس المصاب بالاحتباس المشيمي هي 16.36%. بعد أربعة شهور من الحمل كان هناك تناقص معنوي ($P < 0.05$) في مستوى الكالسيوم والزنك والسيلينيوم في مجموعة الاحتباس المشيمي مقارنة بالمجموعة الغير مصابة ، بينما في مجموعة الاحتباس المشيمي وبعد سبعة شهور من الحمل ظهر تناقص معنوي ($P < 0.05$) في هرمون الاستروجين والكالسيوم والسيلينيوم ، مع زيادة معنوية ($P < 0.05$) في مستوى كلا من هرموني البروجيسترون والكورتيزول والحمض الدهني اللينولينييك. أما بعد 24 ساعة من الولادة فقد تناقص معنوياً ($P < 0.05$) مستوى الكوليستيرول والحمض الدهني اللينولينييك وظل مستوى كلا من هرموني البروجيسترون والكورتيزول في تزايد معنوي ($P < 0.05$) لمجموعة الاحتباس المشيمي عن المجموعة الغير مصابة في نفس المزرعة. وتشير نتائج تلك الدراسة الى أن زيادة مستوى البروجيسترون والكورتيزول منذ الشهر السابع من الحمل وحتى بعد 24 ساعة من الولادة مع نقص هرمون الاستروجين والعناصر الكيميائية المذكورة ربما تبدو مؤشراً للتغيرات التي تؤدي الى الاحتباس المشيمي بالجاموس.