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BLOOD CORTISOL, TRIIODTHYRONINE (T_3) AND THYROXINE (T_4) LEVELS AND THEIR RELATION TO REPRODUCTIVE PERFORMANCE IN PRE AND POSTPARTUM PERIODS IN EGYPTIAN BUFFALOES

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ABSTRACT

The present study was carried out of the Experimental station belonging to the Department of Animal Production, Faculty of Agriculture, Al- Azhar University. Blood samples were taken from 23 pregnant buffaloes at 60 days prepartum every 15 days, at parturition and until 90 days postpartum. Serum cortisol, T_3 and T_4 were assayed. Cortisol level was almost constant and increased ($P< 0.05$) steadily at parturition, After parturition was declined then increased until 90 days, postpartum. Concentration of cortisol was lower in buffaloes which had did not exhibit estrus than buffaloes which had exhibit estrus. Statistically non-significant. Triiodothyronine (T_3) and thyroxine (T_4) showed no significant from 60 days prepartum until 90 days postpartum. However, the levels of T_3 and T_4 were increased gradually towards parturition. After parturition were decreased and fluctuated until 90 days postpartum. In buffaloes which had exhibit estrus, The levels of T_3 and T_4 were higher, non-significant, than buffaloes which had did not exhibit estrus.

Keywords: Buffalo, late pregnancy, parturition, postpartum, Cortisol, T_3 and T_4.

INTRODUCTION

A number of important hormonal changes have been reported during the late pregnancy, parturition and postpartum period of several domestic animals (Smith *et al.*, 1973, Mehta *et al.*, 1991, Agarwal *et al.*, 1992, Garg *et al.*, 1997 and El-Belely *et al.*, 2000). Cortisol is the major glucocorticoid produced by the adrenal cortex in bovines, involved in glucogenesis, protecting the animal from stress and enabling it to adapt to changing circumstances (McDonald, 1982). Increase in serum cortisol concentration before parturition occurs in ewes (Okab *et al.*, 1992 and Mahmoud, 1993), in goats (Ashour and Badr, 2000) in cows (Smith *et al.*, 1973 and Chaiyabutr *et al.*, 2000), in buffaloes (Sarvyiya *et al.*, 1993 and Garg, *et al.*, 1997) and in camel (Leon *et al.*, 1990). The plasma level of cortisol was lower in buffalo cows with inactive ovaries as compared with cyclic animals (Ezzo *et al.*, 1992).

Triiodothyronine (T_3) and thyroxine (T_4) hormones affect the metabolic pool of nitrogen and available energy necessary for the reproduction system and the developing embryo. Therefore, abnormal decrease in thyroid hormones may interfer with normal pregnancy (Hafez, 1980). Thyroid hormones have been observed to increase in several species in late pregnancy (Soliman *et al.*, 1963) including the cows (Blum *et al.*, 1983),buffaloes (Garg *et al.*, 1997), goat (El-Sayed, 1986) and camel (Heshmat *et al.*, 1984 and Agarwal *et al.*, 1992). A decline in serum T_3 and T_4 concentration postpartum occurred in the cow (Soliman *et al.*, 1963, Willard *et al.*, 1995 and Stojice *et al.*, 2001) in buffaloes (Garg *et al.*, 1997) and in camel (Leon, *et al.*, 1990). The levels of thyroid hormones in the blood of cow with inactive ovaries were comparatively low when compared with its

level in the blood of normal healthy animals (Soliman *et al.*, 1964) . Kumer *et al.*, (1991) reported that the circulating levels of thyroid hormone were high during estrus than anestrus.

The present study was carried out to investigate the levels of cortisol and thyroid hormones in the blood of buffaloes during prepartum, parturition and postpartum periods and their relation to reproduction performance.

MATERIAL AND METHODS

Experimental Animals:

Twenty three healthy Egyptian buffaloes from the Experimental Farm of the Faculty of Agriculture Al-Azhar University, located at Mustorod, Kalubia Governerate, 5-11 years old with different parities were used in this experiment 60 days prepartum to 90 days postpartum.

Animals were fed according to their live body weight and production, on concentrate mixture containing 17% crude protein, 2.5 fat and 15% crude fiber, in addition to rice straw or wheat straw and berssem hay. Egyptian clover (*Trifolium alexandrinum*) was offered at 20 to 25 kg/animal/day. At the end of 90 days postpartum, the buffaloes were divided into two groups according to their estrus behaviour, those which exhibited estrus during 90 days postpartum (group 1) and those which did not exhibit estrus (Group 2). Buffaloes were checked for estrus sings three times daily at morning mid day and late afternoon using a fertile bull.

Blood sampling:

Blood samples were taken via jugular vein venipuncture early in the morning and were allowed to clot, then centrifuged and the serum was obtained and stored at – 20°C. Samples were collected every 15 days prepartum, at parturition and 90 days postpartum.

Hormone assay:

Serum cortisol, triiodothyronine (T_3) and thyroxine (T_4) were estimated by direct radioimunoassay (RIA) using coat - A – Count Kit. Products by DPC – Dianostic, Corporation, Los Angles CA, U.S.A. according to Foster and Dunn, (1974), Larsen (1972) and Chopra, (1972) for the three hormones respectively.

Statistical Analysis:

Statistical analysis was carried out to test the significant differences in blood constituents in prepartum and postpartum periods and between the two groups using GLM procedure of SAS (1988). Analysis of variance and Duncan's multiple range test were used to compare means.

RESULTS AND DISCUSSION

Cortisol:

The changes in the concentration of serum cortisol from 60 days prepartum to 90 days postpartum are shown in table (1) and figure (1). From 60 days prepartum cortisol level was almost constant to 15 days then increased ($P< 0.05$) at parturition. At 15 and 30 days postpartum, the cortisol concentration was similar for prepartum periods. From 45 days postpartum, value of cortisol was increased and fluctuated until 90 days postpartum. Level of cortisol was insignificant higher in the buffaloes which exhibited estrus

(group 1) than buffaloes which did not exhibit estrus (group 2), table (1) and figure (1).

Change in serum cortisol concentrations reported in this study are similar to those reported for the ewes (Mahmoud, 1993 and El-Belely *et al.*, 2000), goat (Ashour and Badr 2000), camel (Leon *et al.*, 1990 and Agarwal, *et al.*, 1992), cows (Adams and Wagner, 1970) and buffaloes (Soliman *et al.*, 1981 Mehta, *et al.*, 1991 and Sarvyiya, *et al.*, 1993). In most mammalian species, the fetus dominates the mechanism stimulating the onset of parturition. A significant increase in the fetal plasma concentration of cortisol occurs during final stages of gestation in sheep, goat, cattle and pig (Thorburn, *et al.*, 1977). An earlier study had shown that the maternal adrenal gland is activated during the last 2 to 3 days of pregnancy (Smith *et al.*, 1973 and Agarwal, *et al.*, 1992). It had also been documented previously that the fetal pituitary – adrenal axis plays a primary role in determining the time of parturition by secreting increased amount of corticosteroids (Adams and Wagner, 1970 and Thorburn and Challis, 1979). It may be that in sheep, the ovine foetus secretes large amount of corticosteroids earlier than 6 to 8 days prior to parturition (Challis, *et al.*, 1985). El-Belely, *et al.*, (2000) demonstrated that the temporal change in cortisol during the 10 days prepartum period correlated with significant alterations in components of the blood coagulation system.

There are different speculations about the role of cortisol during the final stage of pregnancy. Adams (1969) reported that the increased glucocorticoids observed prior to parturition may be related to the initiation of parturition and lactation. Glucocorticoids can be inhibited by progesterone adminstration (Jochle *et al.*, 1972) and increased glucorticoids is accompanied by a decline in progesterone (Schams *et al.*, 1972). Mahmoud, (1993) found that parturition might be induced by ascending amounts of glucocorticoids. Ashour and Badr (2000) observed that during pregnancy, the maternal cortisol levels increase due to its placental transfer from fetus to the mother during late pregnancy. Hoffmann, *et al.*, (1977) indicated that, the ability of the foetal adrenal to synthesize corticoids increases dramatically possibly at the final phase of gestation as a result of increased receptor sensitivity to corticotrophin and increased activity of 17α – hydroxlase.

The higher circulating cortisol levels at parturition (Table 1) was possibly as a result of rather than the cause of parturition and was stress-induced response (Jain and Madan, 1985). Surge in corisol level during parturition may indicate the adrenal association with maternal milk secretion (Convey, 1974), Parturition (Prakash and Madan, 1986). However, in cows and buffaloes parturition can be induced experimentally by injecting synthetic corticosteroids (Mehta *et al.*, 1991).

Results of the present study indicate an excellent antagonistic pattern between serum cortisol and progesterone recorded in the same animals by Badr, *et al.*, (2001) during the last phase of pregnancy where the decrease in serum cortisol coincides with an increase of progesterone level and vice versa. This agrees with the observation reported by Boulfekhar and Brudieux (1980) in sheep. Hoffmann, *et al.*, (1977) reported that the increase in

endogenous corticosteroids before parturition might be a direct cause of corpus luteum regression in the cow.

Triiodothyronine (T_3) and Thyroxine (T_4):

During the last 60 days of pregnancy, serum concentrations of T_3 and T_4 increased gradually until day of parturition, the increase was non-significant. After parturition, levels of T_3 and T_4 declined and fluctuated until 90 days postpartum. The present study showed that concentrations of T_3 and T_4 were high in buffaloes which had exhibited estrus than those which did not exhibit estrus, the difference was statistically non-significant (Table 1 and Figure 1).

These results are in agreement with earlier reports on sheep (Jacob and Vadodaira 2000 and Viswanthal, *et al.*, 2000), goats (Maharajan *et al.*, 1982 and El-Sayed, 1986), camel (Heshmat, *et al.*, 1984 and Agarwal *et al.*, 1992), cows (Soliman *et al.*, 1963, and Stojic *et al.*, 2001) and buffaloes (Garg, *et al.*, 1997). Contradictory results were reported by Okab *et al.*, (1993) who found that the levels of T_3 and T_4 in ewes were significantly lower during late pregnancy than in early and mid pregnancy.

Table (1): Means ± SE of serum cortisol, Triiodothyronine (T_3) and Thyroxine (T_4) during late of pregnancy, parturition and postpartum period in Egyptian buffaloes.

Blood values / Days	Cortisol (µg/dl)	Triiodothyronine (T_3) (ng/dl)	Thyroxine (T_4) (µg/dl)
Prepartum			
-60	0.32±0.03 b	119.13±4.51 a	4.28±0.12 a
-45	0.37±0.04 b	120.52±3.78 a	4.33±0.17 a
-30	0.31±0.07 b	121.73±4.92 a	4.41±0.21 a
-15	0.37±0.03 b	121.18±5.63 a	5.11±0.18 a
Parturition	2.15±0.11 a	123.12±6.52 a	5.29±0.23 a
Postpartum			
15 Group 1	0.38±0.05	117.18±3.18	4.42±0.18
Group 2	0.35±0.06	116.92±4.22	4.29±0.15
Mean	0.37±0.04 b	117.10±3.07 a	4.36±0.12 a
30 Group 1	0.36±0.08	114.22±4.28	4.31±0.22
Group 2	0.31±0.07	111.10±3.32	4.03±0.17
Mean	0.33±0.05 b	112.65±3.11 a	4.16±0.16 a
45 Group 1	0.87±0.13	116.12±5.11	4.83±0.13
Group 2	0.38±0.08	109.27±6.26	4.13±0.20
Mean	0.62±0.11 b	112.70±4.38 a	4.49±0.14 a
60 Group 1	0.91±0.11	118.33±4.32	4.67±0.17
Group 2	0.32±0.05	108.72±5.80	4.28±0.16
Mean	0.61±0.09 b	113.53±3.72 a	4.47±0.12 a
75 Group 1	0.86±0.15	118.63±7.38	4.88±0.18
Group 2	0.39±0.03	108.41±6.72	4.15±0.21
Mean	0.62±0.12 b	113.51±5.34 a	4.52±0.17 a
90 Group 1	0.95±0.14	117.38±4.48	4.92±0.22
Group 2	0.37±0.04	109.16±5.20	4.32±0.19
Mean	0.65±0.11 b	113.26±3.28 a	4.63±0.16 a

a, b Means with the same letter within each column are not significantly different.

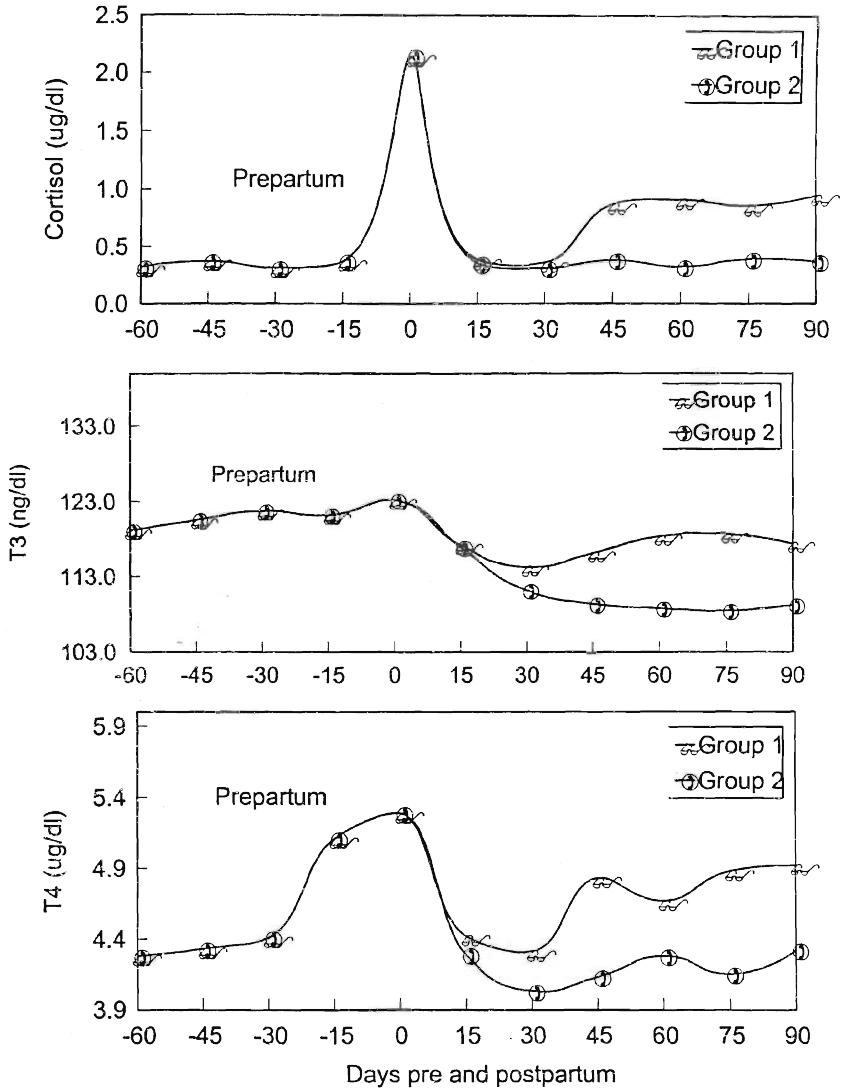

Fig.(1): Average of serum cortisol, T3 and T4 in late pregnancy and postpartum period, including buffaloes exhibit estrus (Group 1) and did not exhibit estrus (Group 2) during the first 90 days postpartum.

Riis and Madsen (1985) found that the level of thyroxine concentrations in the goat decreased 1-2 days before parturition, perhaps as a result of nutrient redistribution to the mammary gland in preparation for lactation.Willard *et al.*, (1995) reported that blood T_3 increased towards parturition, while T_4 level

decrease towards parturition. Garg, *et al.*, (1997) reported that the increase in T_3 and T_4 were found between 271 and 301 days of gestation. Increased thyroid activity during late pregnancy coincided with placental oestrogen. Secretion increased thyroid activity during pregnancy is necessary as this may utilize the nitrogen metabolic pool of an available source of energy, thus meeting the requirements of the tissues of the reproductive system and of the growing embryo (Hafez, 1968). In addition, it has been suggested that high level of estrogen during late pregnancy might have activated the thyroid in cattle, buffaloes, camel and goats (Abdo, 1962; Soliman *et al.*, 1963; Garg, *et al.*, 1997, Heshmat *et al.*, 1984 and El-Sayed, 1986).

Data of the present work showed that levels of T_3 and T_4 were lower in postpartum in comparison to prepartum. These results agree with previous studies on buffaloes (Mehta, *et al.*, 1991). Garg, *et al.*, (1997) found that T_3 and T_4 were low from day 1-28 days after calving and showed increases on days 35-42 indicating a role for thyroid activity in the resumption of postpartum follicular activity in the buffalo.

Vanjonack and Jahnson (1975) have suggested that because thyroid hormones are excreted by the mammary gland, cows with high milk production lose greater amounts of these hormones through the udder, thus, resulting in lower plasma concentrations. These authors also discussed the possibility of an enhanced uptake of thyroid hormones by target organs.

The results of the present study, indicated that the concentrions of T_3 and T_4 were high in buffaloes which had exhibited estrus than those which did not exhibit estrus. The trends obtained in the present work are in agreement with the finding of Soliman *et al.*, (1981), Borady, *et al.*, (1985), Younis *et al.*, (1989), Kumar, *et al.*, (1991) and Fadlallah, *et al.*, (1999). It seems that thyroid hormones play a role in ovarian activity and the manifestation of estrus (Borady, *et al.*, 1985). While Andersen, *et al.*, (1980) and Benjaminsen (1981) did not notice any differences in serum T_4 between cows showing strong estrus and those with weak/silent heat. They suggested that a minimum level of T_4 is necessary for the cow into heat and that the sensitivity to T_4 level perse may vary between cows. Also, Trembly (1976) reveald that no correlation was present between T_4 and T_3 and the fertility problems in cows. Al-Azab, *et al.*, (1984) found no significant variation present in TSH levels between repeat – breeder and normal buffaloes. Younis, *et al.*, (1989) concluded that deficiency of thyroid hormones may help in the incidence of repeat breeder syndrome in buffaloes besides other factors. D'Angelo and Fisher (1969) fount that estrogen might also stimulate TSH release from pituitary gland, causing elevation of thyroid hormones in buffaloes which had exhibit estrus as observed in the present study. Thyrotrophic stimulating hormone (TSH) influences reproduction via the production of thyroxine and triiodothyroxine which regulate the metabolic pools of nitrogen producing available energy necessary for the reproductive system (Magdub, *et al.*, 1982). Soliman, *et al.*, (1981) recorded that the level of T_3 was lower in the serum of buffaloes during the 1st 75 days postpartum than that observed at estrus, the T_3 level was very low in the serum of buffaloes with prolonged postpartum period. These authors suggested that T_3 is more important for reappearance of postpartum estrus. The prolonged

postpartum period in buffaloes may be due to hypofunction of T_4 to T_3. Nasr *et al.*, (1963) found that infertility in cattle was usually accompanied by hypofunction of the thyroid gland.

From this study it could be concluded that certain blood hormones, such as cortisol, T_3 and T_4 can be measured and used as indicated of reproductive status of buffaloes during the postpartum stages and not only the evaluation of progesterone concentration.

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مستويات هرمونات الكورتيزول والترايايودوثيرونين والثيروكسين وعلاقتها بالأداء التناسلي في فترة ما قبل وبعد الولادة في الجاموس المصري.

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اجري هذا البحث في مزرعة تجارب كلية الزراعة بجامعة الأزهر. استخدم في هذا البحث عدد ٢٣ جاموسة عشار، أعمارها تتراوح بين ٥ – ١١سنة وذلك في الفترة ابتداء من ٦٠ يوم قبل الولادة وحتى ٩٠ يوم بعد الولادة وكان الهدف من هذا هو دراسة التغير في مستويات هرمونات الكورتيزول والترايايودوثيرونين والثيروكسين وعلاقتها بالأداء التناسلي. تم اخذ عينات دم من الحيوانات كل ١٥ يوم خلال فترة الدراسة وتم الحصول على سيرم الدم وحفظه حتى إجراء التحليلات المختلفة.

أظهرت النتائج أن هرمون الكورتيزول كان ثابتا تقريبا في فترة ما قبل الولادة ثم زاد زيادة معنوية عند الولادة ثم انخفض حتى ٦٠ يوم من الولادة. ثم تتجه الزيادة تدريجيا حتى ٩٠ يوم من الولادة وكانت هذه الزيادة غير معنوية. كما أن تركيز هرمون الكورتيزول في سيرم دم الجاموس التي ظهرت عليها علامات الشياع أعلى منها في الجاموس الذي لم يظهر اأعليه علامات الشياع.

أوضحت النتائج أن هرموني الترايايودوثيرونين والثيروكسين زادا زيادة تدريجية من ٦٠ يوم قبل الولادة وحتى الولادة ثم اتجه إلى الانخفاض بعد ذلك. كما أوضحت النتائج أن تركيز هذين الهرمونين في دم الجاموس التي ظهرت عليه علامات الشياع أعلى منها في الحيوانات التي لم تظهر عليه علامات الشياع وكانت هذه الزيادة غير معنوية.

نستنتج من هذا البحث انه يمكن قياس بعض الهرمونات مثل هرمون الكورتيزول والترايايودوثيرونين و الثيروكسين في فترة ما بعد الولادة كدليل على الحالة التناسلية للجاموس وليس فقط قياس هرمون البروجسترون.