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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Changes in Peripheral Plasma hormone Concentrations and metabolites during the last trimester of pregnancy and around Parturition in the Egyptian Buffalo and Baladi cows.

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Manuscript Info

Abstract

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Manuscript History:

Received: 19 September 2015 Final Accepted: 25 October 2015 Published Online: November 2015

Key words:

Hormones, buffaloes, cows, energy substances, around Parturition.

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..... It is well known that pregnancy and parturition are controlled by reproductive and metabolic hormones. The present work was designed to clarify the relationship between reproductive and metabolic hormones, their metabolic effects and some biochemical parameters in blood of buffaloes and cows. plasma concentrations of progesterone, estrogen, , prolactin, Insulin like growth factor-1 (IGF-1), leptin, cortisol , triiodothyronine, thyroxin, thyroid stimulating hormone(TSH), Glucose, Total protein, Triglyceride and Cholesterol around parturition were studied on buffalo and cows. Results indicated that, the concentrations of plasma IGF-1, leptin, and, T3, T4 were decrease while cortisol and thyroid stimulating hormone (TSH) were increase with the progress in pregnancy. The concentrations of plasma estrogen and prolactin were increase around parturition, the opposite trend recorded in plasma progesterone levels. The concentrations of plasma glucose and total protein were increase around parturition, the opposite trend recorded in triglyceride and cholesterol levels. Parturition is an important period in buffaloes and cows from the point of view of physiological changes taking place, which in turn produce measurable changes in the diagnostic parameters of the blood.

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INTRODUCTION

The transition period, from 3 weeks before to 3 weeks after parturition, is critically important to health, production, and profitability of dairy cows. Most health disorders occur during this time. Compared with other stages of the lactation cycle, relatively little is known about the fundamental biological processes during the transition period. During the last three weeks of gestation, the fetus begins to release hormones from the adrenal cortex (predominantly cortisol). Cortisol from the fetus stimulates estrogen production by the placenta. The placental estrogen stimulates uterine secretion of the Iuteolytic factor, prostaglandinF2a (PGF2a), which interrupts progesterone secretion. The hormonal environment of the dam stimulates dilation of the cervix (which permits the fetus to pass through the birth canal) and uterine contractions (which expel both the fetus and placental membranes). Therefore, a combination of hormones from the fetus, placenta and dam induce a series of events leading to parturition (Rhodes, 2003). Throughout the gestation and especially during the last part, the nutrition of the pregnant animals is important. Feeding of animals should be oriented in such a way that the prepartum or parturient incidence of some of the commonly occurring metabolic disorders is minimized, a healthy viable progeny is produced and the milk production of the dairy type animals is optimum. In dairy cattle, farmers often feed their pregnant cows with concentrates only during the last few days of pregnancy and often vegetable oil is added to the concentrates. Although growth of the fetus occurs maximally during the last part of gestation, however, the value of such oil feeding is not beyond doubt. Recent suggestions for feeding of pregnant dry cows include the feeding of high-fiber

low-energy chopped straw (Roche, 2006; Wilde, 2006) and the feeding of anionic salts in combination with adequate calcium and magnesium (Beever, 2006) and restriction of rumen degradable protein (Taminga, 2006).

The metabolic and functional changes between conceptions to termination of pregnancy **are** brought about by the interaction of various hormones. A major amount of mammary development is associated with hormonal stimuli

of pregnancy. During prepartum period there is massive development of the mammary gland for lactogenesis and expulsion of fetus at parturition involved a control interaction of different hormones and absence of these

stimuli reduces the amount of mammary tissue. Many hormones and factors have an effect on the proper performance of pregnancy. Appropriate thyroid gland function and its hormonal activity are crucial to sustain the reproductive performance in domestic animals. Adequate thyroid functioning during pregnancy is a known determinant of healthy pregnancy outcomes and successful brain development in the fetus (LaFranchi et al. 2005). Thyroid hormones have wide connections with lipids (Eshratkhah and Sadaghian 2010). Lipids serve as hormones or hormone precursors, provide energy, and act as functional and structural components in cell membranes (Angelin and Rudling 2010). Studies on the lipid profile in blood of domestic animals showed the existence of wide variation between species and even within species (Nazifi et al. 2002; Eshratkhah et al. 2008). Thyroid hormones affect lipid metabolism by increasing lipolysis in adipose tissues (Chatterjea and Shinde 2005). There is a great variation in lipid and lipoprotein profiles of pregnant animals at various stages of pregnancy. The normal concentrations of serum lipids and lipoproteins of the sheep (Nazifi et al. 2002; Piccione et al. 2009; Eshratkhah and Sadaghian 2010), goats (Nazifi et al. 2002), cows (Mohebbi-Fani et al. 2012), in various physiological conditions have been investigated. Lipid profile and thyroid hormones are affected by many factors including sex, season, nutrition, age, breed, ovarian endocrine function, pregnancy, lactation, stress, and disease (Huszenicza et al. 2002; Todini 2007; Nazifi et al. 2009; Saeb et al. 2010). Leptin effects on gonadotropin-releasing hormone (GnRH) and LH secretion are mediated by neuropeptide Y (NPY) and kisspeptin, thus, leptin appears to be an important link between metabolic status, the neuroendocrine axis and subsequent fertility in farm animals, Barb and Kraeling (2004) regulates energy balance through its impact on appetite and fat metabolism, and its concentration indicates the size of body fat reserves. It acts centrally to suppress appetite by inhibiting the production of orexigenic peptides in the hypothalamus. Hammond et al (2005). These actions maintain fat stores within a narrow range in normal terrestrial mammals. Leptin is thought to have evolved as a protective mechanism to promote feeding and fat deposition when fat stores become depleted, Ahima and Flier (2000). leptin plays a role in regulating hunger and satiety, enabling the maintenance of normal weight. The neuronal target for leptin is the hypothalamus where neurons in the arcuate nucleus, ventromedial and lateral hypothalamus express high levels of leptin receptor, Popovic and Casanueva (2002).

growth factor-I has been detected in follicular fluid, Spicer and Stewart (1996).Follicular fluid levels of IGF-I increase during follicular growth and selection, Rivera and Fortune (2003), and are greater in preovulatory than subordinate follicles, Santiago et al (2005). IGFs are important in regulating fetal and placental growth in mammalian species, Han and Carter (2000). Circulating concentrations of IGF-I have been reported in foals and in the peripartum period in mares, Hess-Dudan et al (1994). The primary precursor for initiation of parturition appears to be prostaglandin in the pig, fetal cortisol in sheep and interplay of prostaglandins and fetal cortisol in the cow (Jainudeen and Hafez, 2000). In the mare oxytocin appears to be the primary parturition stimulation molecule (Liggins and Thorburn, 1994). A complex mechanism is operative in the initiation of parturition in various species.

The maintenance of pregnancy in most domestic animals is dependent on the primary corpus luteum (CL) formed on the ovary acts as a progesterone secreting endocrine gland and persists throughout pregnancy in most domestic animals except for the horse and sheep (Jainudeen and Hafez, 2000). The source of progesterone during the latter half of pregnancy is from the placenta in mare and ewes (Stellflug el al. 1997). The fetal production of progestagens increase in late gestation and consequently, progestagen concentration also increase in maternal plasma, typically peaking a few days before parturition and declining on the last day or even hours before birth in the goat although the placenta also produces some progesterone(Ousey, 2006). The initial stimulus for initiation of parturition in sheep was identified to be the fetal hypothalamo pituitary axis as birth did not occur in the sheep in the absence of fetal pituitary whether the latter was due to a genetic defect or surgical ablation (Liggins, 1973). The mechanism by which the fetal cortisol stimulates the hormonal cascade have been since then investigated in detail and it is increasingly acceptable that the fetus initiates the parturition in sheep (Jenkin and Young, 2004). It has been argued for quite some time that the initiation of parturition in the goat and cow depends on the lysis of the corpus luteum rather than the fetus. In the cow, peripheral progesterone levels fluctuate between 6 and 15 ng/mL throughout gestation (Randel and Erb, 1971) with a gradual decline in the 2-4 weeks preceding parturition. The progesterone is luteal in origin for the first 150 days of gestation (Gross and Williams, 1988). Between 150 and 250 days of gestation the placenta acts as an additional source of progesterone (Schuler et al. 2008). The exact functions of placental steroids in the bovine species continue to be poorly defined (Schuler et al. 2008). In the final month of gestation, placental progesterone

declines and pregnancy is again dependent upon luteal progesterone (Shenavai et al. 2010). Although, subtle differences do exist in the physiology of gestation and parturition between cattle and buffaloes they behave similarly in some aspects (Dobson and Kamonpatana, 1986). Nearly similar to cows the increase in progesterone in placental tissue in the buffalo is highest between days 97 and 250 (Amin and El-Sheikh, 1973). Progesterone receptors have been observed in the binu-cleate trophoblastic cells of buffalo placenta (Carvalho, 2007). Rapid decline in the plasma progesterone 3 days prepartum has been ob-served (EL-Belaly et al. 1988). The primary precursor for initiation of parturition appears to be prostaglandin in the pig, fetal cortisol in sheep and interplay of prostaglandins and fetal cortisol in the cow (Jainudeen and Hafez, 2000). In the mare oxytocin appears to be the primary parturition stimulation molecule (Liggins and Thorburn, 1994). It has been argued for quite some time that the initiation of parturition in the goat and cow depends on the lysis of the corpus luteum rather than the fetus. However, it is increasingly clear that in ruminants (cow, sheep, and goat), irrespective of whether the pregnancy is maintained by luteal or placental progesterone, the fetal pituitary-adrenal axis has the dominant role in initiating the prepartum hormonal changes in the mother (Liggins and Thorburn, 1994). A complex mechanism is operative in the initiation of parturition in various species. In the buffalo the plasma concentrations of estradiol $17-\beta$ start increasing 7 days before parturition to reach peak levels one day before parturition (Batra et al., 1982) and the progesterone decrease gradually over the last 7 days of gestation with an abrupt fall 1-2 days before parturition synchronous to peak $PGF_{2\alpha}$ concentrations at the same time (Batra et al., 1982). The maternal plasma estradiol concentrations are higher before parturition in swamp buffaloes (Kamonpatana, 1984). It can therefore be believed that the mechanism of parturition initiation in this species is similar to that in cattle. In ruminants, circulating leptin concentrations are considerably altered by changes in nutritional and physiological conditions such as parturition and lactation, and also that the susceptibility to infection insults in cows increases during periparturient period, Ingvartsen and Boisclair (2001). It is well known that mammary differentiation and milk secretion are controlled by reproductive and metabolic hormones, Neville et al (2002) and these hormones also affect the synthesis and secretion of leptin from white adipose tissue. Bradlev and Cheatham (1999).

Materials and Methods

This study was carried out at the agriculture experimental station, faculty of agriculture, Cairo University. Investigations were carried out on 16 pregnant animals (8 pregnant buffalo cows and 8 pregnant Baladi cows). Experiment was conducted during the last trimester of pregnancy (the eighth, the ninth and tenth months in buffaloes and the seventh, eighth and ninth months in cows). The experimental animals were kept under normal feeding and management conditions applied on the farm. Blood samples were withdrawn by weekly during the experimental period. Blood samples (10 mL) were collected from the jugular vein into heparinzed tubes and centrifuged at 3000 rpm for 20 minutes to separate plasma which was used for measuring hormones including Insulin like growth factor-1(IGF-1, ug/L) was assessed by radioimmunoassay technique (RIA) using ready-made kits (Immunotech SAS -130 av. kit, France). The standard curve was between 0 and 1200 ng/ml. The analytical sensitivity was reported to be 2 ng/ml. The cross reaction of the antibody with other hormones was found to be extremely low. The samples were determined in one run and the intra-assay variation coefficient was 6.3%. While leptin concentration was determined by ELISA reader (BIO TEK ELX808), using Leptin ELISA kit sandwich (DRG Instruments GmbH, Germany) according to the manufacturer's guidelines. The standard curve was between 0 and 100 ng/ml. The sensitivity of the curve was reported to be 1.0 ng/ml. Direct Ridoimmunoassay (RIA) technique was Performed the assessment of plasma thyroid stimulating hormone, **TSH(uiU/mL)**,total triiodothyronine (**ng/mL**), total thyroxin (ug/mL), and cortisol (ug/mL), concentration by using special kits delivered from IMMUNOTECH radiova, Czech Republic. Glucose (mg/dl) was determined by using enzymatic colorimetric method using Bio-Diagnostic® kit delivered from Dokki, Giza, Egypt according to Trinder (1996). Total protein (g/dl) was determined colorimetrically by using Bio-Diagnostic® kit delivered from Dokki, Giza, Egypt according to Gornal et al (1949). Cholesterol concentration (mg/dl) in plasma was determined quantitively (Colormetric Method Liquzyme) by using (STAT LAB SZSL60-SPECTRUM) according to (Finely et al, 1978). Estimation of Triglycerides (mg/dl) content in plasma was quantity (Colormetric Method Liquzyme) determined by using (STAT LAB SZSL60-SPECTRUM). according to (Fassati and Prencipe 1982). Estrogen concentrations in blood plasma were determined through ELISA by using commercially available kit (Bio Check, Inc. USA, Lot. RN-27637). The sensitivity of the estradiol ELISA assay was 10 pg/ml, intra-assay precision was less than 24.1% and interassay precision was less than 26.7%. Progesterone concentrations in blood plasma were determined through solid phase competitive ELISA by using commercially available kit (Bio Check, Inc. USA, Lot. RN-28387). The sensitivity of the progesterone ELISA assay was 0.3 ng/ml, intra-assay precision was less than 7.1 % and interassay precision was less than 12.6%. Prolactin concentrations in blood plasma were determined through ELISA measured by using enzyme linked immuno-sorbent

assay (ELISA) kit. Where the plasma prolactin levels were determined according to the method described by Goldfine (1978).

Statistical analysis of data was carried out applying SAS, package (2000). Differences among means were checked according to Duncan (1955). model

Results:

1-<u>Plasma thermogenic hormones during Pregnancy and around Parturition in Egyptian buffalo and Baladi cows</u> Results in table 1, indicated that, the concentrations of plasma thermogenic hormones were higher in plasma buffaloes than in plasma cows except cortisol around parturition. Data indicated that, the concentrations of plasma IGF-1, leptin and T3, T4 were decrease while plasma cortisol and thyroid stimulating hormone (TSH) levels were increase with the progress in pregnancy. Other studies using ruminant-specific leptin RIA showed that plasma leptin concentration was 4-7ng/ml in calves (Ehrhardt et al., 2000) or ranged between 3.4 and 13.7 ng/ml in adult cattle (Delavaud et al., 2002).

2-Plasma reproductive hormones during Pregnancy and around Parturition in Egyptian buffalo and Baladi cows

Results in table 2 indicated that, the concentrations of plasma estrogen and progesterone were higher in cows than in buffaloes around parturition. Data indicated that, the concentrations of plasma estrogen and prolactin were increase around parturition, the opposite trend recorded in plasma progesterone levels . The concentrations of plasma prolactin hormone were higher in plasma buffaloes than in plasma cows. The plasma progesterone concentrations decreased sharply 7 days before parturition, Eissa et al (1995) differed from those previously reported in buffaloes. Progesterone concentrations were found to decrease significantly 60 days before parturition (Rao et al, 1978); 21 days prepartum (Heshmat et al, 1985),or at the day of parturition (El-**Belaly** et al, 1988). Yotov et al(2013) found that, pregnant buffaloes showed basal blood progesterone levels by the 7th postpartum day and exhibited high luteal activity between postpartum days 19–34, characterized by ultra-sonographic detection of a corpus luteum different from the gestational one and blood progesterone concentrations > 0.5 ng/ml after day 34 postpartum. Buffaloes with blood progesterone concentrations < 0.25 ng/ml until the 50th postpartum day and inadequate functional activity of the corpus luteum could remain non-pregnant for a long time after parturition. Concentrations of progesterone in the plasma of cows are high throughout gestation. They diminish slowly during the final 3-4 weeks and rapidly 2-3 days before calving (Hanzen, 1986).

3-Blood biochemical parameters during Pregnancy and around Parturition in Egyptian buffalo and Baladi cows

Results in table 3 indicated that, the concentrations of plasma glucose and total protein were higher in plasma buffaloes than in plasma cows, while triglyceride and cholesterol levels were higher in plasma cows than in plasma buffaloes. Data indicated that, the concentrations of plasma glucose and total protein were increase around parturition, the opposite trend recorded in triglyceride and cholesterol levels

	IGF-1(ug/L)		Leptin(ng/ml)		Cortisol(ug/mL)		T3(ng/mL)		T4(ug/mL)		TSH(uiU/mL)	
month	buffaloes	cows	buffaloes	cows	buffaloes	cows	buffaloes	cows	buffaloes	cows	buffaloes	cows
Α	110.35± 30.36 ^a	99.17± 26.68 ^a	10.50 ± 0.92^{a}	$6.50\pm 0.52^{\rm a}$	0.33 ± 0.05^{ab}	0.31± 0.06 ^{ab}	9.16 ± 0.52^{b}	7.09± 1.67 ^{ab}	4.58 ± 0.26^{a}	3.55± 0.84 ^a	0.02± 0.33 ^b	0.07 ±0.02 ^b
В	48.04± 11.46 ^ª	83.06± 9.65 ^a	9.67± 1.35 ^a	7.00± 0.72 ^a	0.33 ± 0.03^{ab}	0.36± 0.09 ^{ab}	9.22 ± 13.29 ^b	8.09± 1.94 ^{ab}	4.61± 0.65 ^a	3.37 ± 0.98 ^a	0.14± 0.04 ^b	0.15± 0.04 ^b
С	87.09± 2.00 ^a	68.14± 10.56 ^a	7.70± 0.54 ^a	5,40± 1.25 ^ª	0.45 ± 0.08^{ab}	0.52± 0.08 ^{ab}	8.24± 1.19 ^b	6.89± 1.19 ^{ab}	4.11± 0.59 ^a	3.45± 0.59 ^a	0.10± 0.04 ^b	0.10± 0.04 ^b

Table 1 Plasma thermogenic hormones during the last trimester of pregnancy and around Parturition in Egyptian buffalo and Baladi cows

^{a,b}Means within column with group with different superscript differ significantly(p<0.05).

A,B and C = Last trimester of pregnancy.

Estrogen(Pg/mL)			Progesterone	e(ng/mL)	Prolactin(ng/l)	
month	buffaloes	COWS	buffaloes	cows	buffaloes	cows
A	23.27±	61.50±	4.73±	4.64±	232.25±	104.o2±
	51.32 ^a	16.00 ^a	1.19 ^a	3.29 ^a	179.50 ^b	187.97 ^b
В	141.47±	112.26±	4.73±	4.86±	349.31±	161.41±
	76.60 ^a	20.08 ^a	3.71 ^a	0.53 ^a	144.09 ^b	100.17 ^b
С	183.37±	248.48±	3.77±	4.77±	397.36±	238.54±
	71.50 ^a	24.39 ^a	5.52 ^a	1.9ª	169.78 ^{ab}	179.90 ^{ab}

^{a,b}Means within column with group with different superscript differ significantly(p<0.05). A,B and C = Last trimester of pregnancy.

	Glucose(mg/	'dl)	Total protein (g/dl)		Triglyceride(mg/dl)		Cholesterol(mg/dl)	
month	buffaloes	cows	buffaloes	cows	buffaloes	cows	buffaloes	cows
Α	74.33± 2.73 ^{ab}	72.33± 5.05 ^{ab}	7.82± 0.50 ^b	7.71± 0.19 ^b	118.00± 18.52 ^b	144.00± 20.18 ^b	110.33± 24.37 ^a	140.67± 9.75 ^a
В	78.00± 5.20 ^{ab}	74.67± 3.93 ^{ab}	7.98± 0.43 ^b	8.21± 0.23 ^b	93.00± 8.63 ^b	121.67± 24.39 ^b	89.67 ± 4.67 ^a	129.33 ± 14.21 ^a
С	79.67± 6.11 ^{ab}	76.00± 4.17 ^{ab}	8.17± 0.16 ^b	8.34 ± 0.28^{b}	75.00± 2.00 ^b	122.33± 17.35 ^b	86.67± 14.69 ^ª	127.00 ± 15.39 ^a

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^{a,b}Means within column with group with different superscript differ significantly(p<0.05). A,B and C = Last trimester of pregnancy.

Discussion:

1-Plasma thermogemic hormones during Pregnancy and around Parturition in Egyptian buffalo and Baladi cows

Increase in plasma triiodotheronine level in post- partum occurred to compare pre-partum might be due the increase in metabolic rate after calving as an anabolic process to rebuild destructive tissues and to compensate the deficiency of blood metabolites which could be occurred during pregnancy Pichaicharnarong et al, 1982.

Reduction in total circulating thyroid hormones during pregnancy could be due to the increase of turnover rate or the decrease in the secretion of the hormones from the thyroid gland. During pregnancy, hepatocytes increase their production of thyroid-binding globulin (TBG). High TBG and high estradiol concentration during pregnancy induced a reduction in free circulating hormones such as thyroxin (Leung and Farwell 2010). Estrogens can alter the secretion rate and dynamics of thyroid hormones. It seems that fluctuations in thyroid activity might be due to the interactions with varying concentrations of estrogens and progesterone during the pregnancy of camels (Agarwal et al. 1989). However, our findings are in agreement with different investigators who have found that free hormone levels remain unchanged or decreased in pregnancy (Rahman et al. 2007). The high cortisol level in late pregnancy means that the cow was under stress during this period and ended after calving (Pichaicharnarong et al, 1982), they demonstrated that the total serum T3 of late pregnant buffaloes markedly decreased in comparison with that of 8–9 month of pregnancy. At 1 month post-partum, T3 rose to $143.4 \pm 33.0 \text{ µg}/100 \text{ ml}$ which was significantly higher than that at late pregnancy (P < 0.001). The mean serum T3 of 1-month-old buffalo calves was relatively high, $(281.0 \pm 106.2 \mu g/100 \text{ ml})$ which was significantly higher than $143.4 \pm 33.0 \text{ ng}/100 \text{ ml}$ of their dam buffaloes (P< 0.001) at the same period of time. This conclusion could be regarded as a part of a complex multihormonal regulation of follicular steroidogenesis in cattle. Spicer and Stewart(1996), reported a direct stimulatory effect of T3 and thyroxin on thecal cell steroidogenesis. The stimulatory effect of T3 and T4 on androstenedione production (i.e. two- to fourfold increase) was similar to the influence of LH on and rostenedione production (i.e. four- to ninefold increase). Collectively, these data indicated that the stimulatory effect of T3 and T4 on bovine and rostenedione Arije et al. (1974) suggested that corticosteroids varied from 10 to 80 ng/ml between 6 and 21 days prepartum, rising gradually to116 ng/ml at parturition and falling to 25 ng/ml at 1 day postpartum levels which, did not appear to be associated with estrus activity. Habeeb et al (2002) found that the highest level of cortisol was detected at late pregnancy period in female dromedary camels. The authors attributed this result to pregnancy as a physiological process which caused the significant increase in cortisol secretion with the increase the pregnancy period. In addition, cortisol is important for surface and formation in the fetal lung to prevent hyaline membrane disease that ends in respiratory failure. Moreover, cortisol is immuno suppressive hormone that abolishes the immunological response against the fetus, consequently saving the fetal life. Cortisol also, induced gluconeogenesis that provide adequate glucose supplement necessary for the fetal nutrition (Bell, 1995). The hormone pattern established during the latter portion of gestation triggers parturition. During the last three weeks of gestation, the fetus begins to release hormones from the adrenal cortex (predominantly cortisol). Cortisol from the fetus stimulates estrogen production by the placenta ,Rhodes (2003). Exogenous glucocorticoid administered to the mother can be used to induce parturition in the cow, sheep and goat (Thorburn et al., 1977). Likewise glucocorticoid can induce parturition in the buffalo (Shukla et al., 2008) and camel.

2-Plasma reproductive hormones during Pregnancy and around Parturition in Egyptian buffalo and Baladi cows

Several investigations in buffaloes reported that plasma oestrone concentrations increased substantially during the last 2 months of gestation with maximal concentrations 5 days prior to parturition (Hung and Prakash, 1990), while total oestrogens increased markedly during the last month of pregnancy peaking 1-2 days before delivery (El-Belaly et al, 1988). The plasma progesterone was determined in cattle, Levels of approximately 3-4 ng/ml (9.5-12.7 nmol/l) were found until 3 days before parturition with a drop to 2 ng/ml (6.4 nmol/l) 1 day before calving. Less than 1 ng/ml (3.2 nmol/l) was measured on the day of parturition and during the first 3 days after calving (Terblanche and Labuschagne, 1981). In Murrah buffaloes, The PGF2 alpha concentration fluctuated before parturition and a peak was observed 1 day prior to parturition. PGFM, estradiol-17 beta and prolactin concentration increased gradually over the last 7 days with a significant peak (P less than 0.001) 1 day before parturition. The progesterone level declined gradually and an abrupt fall occurred 1-2 days post partur (Batra et al, 1982). Purohit (2011) noted that, in the buffalo the plasma concentrations of estradiol 17 β start increasing 7 days before parturition to reach peak levels one day before parturition and the progesterone decrease gradually over the last 7 days of gestation with an abrupt fall 1 – 2 days before parturition synchronous to peak PGF2a concentrations at the same

time .The maternal plasma estradiol concentrations are higher before parturition in swamp buffaloes . It can therefore be believed that the mechanism of parturition initiation in this species is similar to that in cattle.

The concentration of estradiol 17β during advanced stages of pregnancy is considerably lower as compared to an earlier report in buffalo (Rao *et al* 1978) during last 2 months of pregnancy. The peak concentration reported by these authors (720 pg/ml) in one of the buffalo was roughly 8 times

higher than the present level (82.27 \pm 9.49 pg/ml) at parturition. The higher levels obtained by these authors may be attributed to the non spedificity of the antiserum, which might have cross reacted with other estrogens (i.e., estrone, estradiol - 11a, and estriol) apart from estradiol 17 β . The antiserum used by us, however, was highly specific for estradiol 17 β and hence comparatively low values were recorded during late pregnancy and parturition. The postpartum cyclic activity depends on the synchronous activity of hypothalamic-**pituitary ovarian** axis (Perea and Inskeep, 2008). The concentrations of placental and ovarian steroids during the late gestation and the puerperium have a significant effect on hypothalamic-pituitary system, which reflects on ovarian function (El-Wishy, 2007).

(Ullah et al., 2006) recorded that, blood progesterone concentrations < 0.25 ng/ml between postpartum days 0 and 40 indicated absence of luteal activity and acyclicity. In Baladi cows El-Fouly et al. (1998) found that progesterone level was significantly increased with the progress of gestation and at the end of the third trimester of gestation, progesterone decreased and reached its minimum level during the period from 6 to 1 day before parturition. Similarly, Hashmat and Shehata (1982) observed that progesterone levels declined to 1.2-2.0 ng/ml during the last 12-24 hours before calving and were less than 1 ng/ml 24-48 hours after delivery in local Egyptian cattle. Habeeb et al. (1999) reported that during the third trimester of gestation, progesterone level decreased to reach the average of 2.5 µg/ml and after calving, the progesterone reached lowest level, less than1.0 ng/ml in Friesian cows. El-Massry et al. (1997) reported also that P4 level increased with the advance of pregnancy, showing a sharp rise in the tenth month of pregnancy (3.82 µg/ml) and then decreased to 0.96 µg/ml in lactating non-pregnant buffalo cows at day 30 post partum. Killen et al (1989), reported that a higher (P<0.05) mean progesterone concentration in heifers at d 23 before parturation to 36 d after parturition. Both mean esterone and estradiol concentrations were greater (P<0.05) in heifers at d 200. In Baladi cows El-Fouly et al (1998). reported that estrogene reached its peak on day 1 before calving. Similarly, Habeeb et al(1999, found that the highest level of estrogene was in the third trimester of pregnancy in Friesian cows and estrogene level significantly increased with advancement of pregnancy and after calving estrogene level decreased. Progesterone levels in Ethiopian Zebu cows were high (over 5 µg/ml) until the last 12 to 18 days of pregnancy. This was followed by a decline to 3.7-8.2 µg/ml one to two days before parturition ,Fao(2009) .El-Massry et al(1997). explained that the high level of estrogene and progesterone in the third trimester of pregnancy have evoked normal development of mammary alveolar tissue to bring the udder of cows into lactation processes.

3-Blood biochemical parameters during Pregnancy and around Parturition in Egyptian buffalo and cows

The findings of the current study support the previous studies of species such as cows (Nath et al. 2005), goats (Krajnicakova et al. 2003), and buffaloes (Prabhakar et al. 1999). The lower cholesterol level in these species near parturition could be attributed to the increased utilization for steroid synthesis around parturition (Saeed and Khan ,2009,2012). The increased serum triglyceride concentration during pregnancy had been reported in sheep (Nazifi et al. 2002), goats (Hussein and Azab 1998), and Shetland ponies (Kano and Sawasaki 1981). It may be due to over production of very low-density lipoprotein cholesterol (Kirsten et al. 2003). The opposite trend recorded by Kaneko et al. 2008; they found that, the higher serum levels of total cholesterol and triglyceride observed in pregnant camels were not significant. The liver is the major site of cholesterol synthesis. Cholesterol is made from acetate. Three molecules of acetyl CoA combined and produce 3-hydroxy-3-methyl-glutaryl- CoA (MHG-CoA), which is affected by different enzymes converted to malonic acid, and during different reactions, convoluted and converted to cholesterol. During pregnancy, MHG-CoA activity, acyl CoA, cholesterol acyl transferase, and other enzymes of the cholesterol synthesis are increased (Cortes et al. 2013). Higher serum total cholesterol value observed in pregnant camels was in agreement with the finding of Saeed et al. (2009) and Nazifi Habibabadi et al. (2003) in female camels and goats, respectively. They found the significant increase in serum total cholesterol concentration with advancing in pregnancy and lactation.

In conclusion

1. On the basis of comparative analysis of endocrine events during pregnancy and parturition, **it** appears that a fundamental role is played by progesterone in maintenance of pregnancy in buffalo. The **rises** in estrogen accompanied by decrease in progesterone with the approach of parturition are probably responsible for the onset of parturition.

2. Parturition is an important period in buffaloes and cows from the point of view of physiological changes taking place, which in turn produce measurable changes in the diagnostic parameters of the blood.

3. Having knowledge of the diagnostic parameters just before and just after parturition in buffaloes and cows enable one to effectively predict many varied problems occurring in the herd and to react accordingly.

Acknowledgement :

I express my gratitude to all employees of the experimental station and Research Park (CURP) the faculty of Agriculture ,Cairo University for providing facilities and kind help during the experimental work.

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