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EFFECTS OF MELATONIN AND CONTROLLED INTERNAL DRUG RELEASE DEVICE TREATMENT ON BLOOD METABOLITES OF BUFFALO HEIFERS DURING OUT-OF-BREEDING SEASON UNDER TROPICAL CONDITIONS

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SUMMARY

Sixteen Murrah buffalo heifers, divided into control and treatment groups of eight animals each, were used to study the effect of melatonin and controlled internal drug release (CIDR) device treatment on the resumption of ovarian activity during out-of-breeding season (summer solstice). Treated group were implanted with melatonin (18 mg melatonin /50 kg body weight) for 45 days, then heifers of both groups received CIDR for 9 days. All heifers received im 500 IU eCG, at day before CIDR removal, and 10 µg GnRH at day after CIDR withdrawal. All animals were subjected to estrous detection regime daily. Blood samples in conjunction with transrectal ultrasonography were performed twice weekly to determine blood metabolites concentrations as well as to monitor the ovarian follicular activity. During out-of-breeding season melatonin treatment resulted in a significant increase in plasma concentrations of albumin, ALT ($P \leq 0.01$), glucose, HDL, Mg and Ca ($P \leq 0.05$), while ALP concentrations was decreased ($P \leq 0.05$).The effect of day after melatonin treatment exhibited increases in glucose concentrations ($P \leq 0.01$) during day 0, 7 and 35, and creatnine concentrations in day 21 compared other days. Melatonin and CIDR treatment resulted in an increase in plasma concentrations of albumin, glucose, ALT, Ca ($P \leq 0.0$ 1), HDL and Mg ($P \leq 0.05$), and reductions in plasma ALP ($P \leq 0.01$). The effect of day after melatonin and CIDR treatments resulted in decreases ($P \leq 0.05$)in the concentrations of plasma total protein, albumin and Ca but glucose increased ($P \leq 0.05$) after melatonin with CIDR treatment .It is concluded that melatonin and CIDR treatment successfully improved reproductive performance and blood metabolites of buffalo heifers during out-of-breeding season .

Keywords: Melatonin, CIDR, heifers, blood metabolites

INTRODUCTION

Seasonal reproductive activity is regulated by an endogenous rhythm. This rhythm is synchronized with the geophysical year by environmental stimuli, the most important of which is photoperiod. Unlike temperature, availability of food, rainfall or other environmental cues, photoperiod provides information about the season and remains constant from year to year. Photoperiodic stimuli are transmitted via neuroendocrine pathways into hormonal signals that regulate gonadal activity. Reproductive activity is not a direct function of daylength, but is affected by the photoperiodic history of the animal, the direction of photoperiodic changes and the stage of the circannual rhythm at which a photoperiodic signal is received (Gorman and Zucker, 1995).

Buffalo is alleged to have poor reproductive efficiency owing to delayed puberty and prolonged inter-calving intervals (Zicarelli, 2010). A major factor associated with the latter is occurrence of repeat breeding (5–30%; Kumar and Singh, 2010). Hormonal aberrations are contributors toward failure of conception in 26% of repeat breeder animals (Singh *et al.*, 2009). Moreover, a majority of the embryonic loss (30%) occurs between days 8 and 16 post-breeding in dairy cattle (Sreenan *et al.*, 2001), while similar studies are lacking in dairy buffalo.

The effects of photoperiod on reproduction can be modified to a certain extent by temperature, nutrition, body condition or age. Under subtropical conditions in India, poor reproduction in buffaloes during summer season was projected as the combined effect of *elevated* ambient temperature and poor nutrition. However, detailed analysis of literature has suggested that the regulation of reproductive axis in buffaloes by melatonin could be central to the seasonal decline of ovarian cyclicity in buffaloes. Circulating concentrations of melatonin increase during hours of darkness. In fact, during long daylength, keeping buffaloes in artificial darkness reduces occurrence of cyclicity in buffaloes (Parmeggiani *et al.*, 1993).

Melatonin, a pineal gland hormone, is implicated in the sequence of events leading to the onset of puberty in cow heifers (Tortonese

and Inskeep, 1992). Recently, melatonin implant treatments were successfully exploited for initiating ovarian cyclicity in true anestrus buffalo heifers (Ghuman *et al.,* 2007; and Ramadan *et al.,* 2014). Because of the interaction of melatonin with various endocrine systems (Zieba *et al.,* 2007), it was thought that melatonin treatment may initiate ovarian cyclicity in true anestrus buffalo heifers through its influence on body metabolism (Darul and Kruczynska 2004,).Melatonin (N-acetyl-5-methoxytryptamine) is synthesized by the pineal gland during the dark phase of the photoperiod and it is rapidly released to the blood stream where the highest levels reach nano molar concentrations (Reiter, 1993).

Melatonin is a signal that conveys photoperiodic information to synchronize cell physiology with the dark–light cycle (Reiter, 1993). In addition, it is a lipophilic agent that crosses lipid bilayers (Bongiorno*et al.,* 2005), and acts as a free radical scavenger, neutralizing hydroxyl and peroxyl radicals among others, preventing lipid membrane peroxidation and apoptosis (Jou *et al.,* 2004), and protecting the DNA from the damage induced by free radicals (Majsterek *et al.,* 2005). Moreover, melatonin stimulates gene expression of antioxidative enzymes including superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase (Rodriguez *et al.,* 2004).

Applying estrus/ovulation synchronization protocols along with fixed-time breeding in buffalo provides a potential alternative for increasing the productive period (Ghuman *et al.,* 2012). However, the protocols used have achieved variable success with respect to conception rate in buffalo (Ghuman*et al.,* 2012; and Ramadan *et al.,* 2014). This justifies the need to develop measures for improving the conception rate of buffalo subjected to fixed-time breeding protocols.

In lactating animals, blood biochemical variables including total protein, triglycerides, FFA, and thyroid hormones are important indicators of metabolic activity (Karapehlivan *et al.,* 2007).Metabolic profiles were found to be a practical tool for auxiliary feeding evaluation and assessment of milk production of dairy cows (Kida, 2003), but biochemical attributes during various physiological stages have not been reported adequately in buffalo heifers. This study was performed to evaluate blood metabolic concentrations as indicators of sustainability of buffalo heifers to the expenditure of melatonin implants and CIDR treatment for preventing summer-induced decline in ovarian activity in true anestrus buffalo heifers.

MATERIAL AND METHODS

The present study was conducted at the buffaloes' farm (29° 10′ N, 75° 41 E), Central Institute for Research on Buffaloes (CIRB), Hisar, India. All procedures and experimental protocols were conducted in accordance with the "Guide for the Care and Use of Agricultural Animals in Research and Teaching", Federation of Animal Science Societies (2010).

Animals and management:

Sixteen Murrah buffalo heifers (aged 36.06 ± 0.69 months and weighted 348.81 ± 8.54 kg) were used in the present study. The study was conducted during the hot-humid months from last week of April to July when ambient temperatures and relative humidity ranged from 33 to 45°C and 35 to 80%, respectively. Heifers were confined for the entire period of study to a barn with access to an open sheltered space. All heifers in this study had attained the pubertal age. They were subjected to teasing twice daily for estrous detection, but failed to exhibit estrus. They were fed on roughage and concentrate supplement according to their body weight requirements (NRC, 2001). Chaffed green fodder and wheat straw were offered in summer. Water was offered in excess to animals at all times. Heifers were free from diseases and were clinically normal with a healthy appearance. They were subjected to gynecological examination before inclusion in the study, and those diagnosed with any pathological condition of the reproductive tract were excluded after ultrasonography.

Experimental design:

Heifers were randomly allocated to melatonin non-implanted (control) and implanted (treated) groups (n = 8 each). In melatonin-treated group, heifers were administered 2 × 4 mm absorbable melatonin implants (18 mg melatonin ⁄ implant, Regulin®, CEVA Animal Health Limited, Chesham, Buckinghamshire, UK) at the base of left ear using an implanter. Total implants inserted to each heifer were calculated on the basis of their body weight (one implant⁄ 50 kg, Papachristoforou *et al.,* 2007).These implants were designed to release melatonin for at least 60 days, although their functionality can extend to more than 100 days without disturbing the endogenous secretion of melatonin as seen in ewes (Forcada *et al.,* 1995, 2002). On day 45 after melatonin implantation, all implanted heifers were treated with Eazi- Breed CIDR (1.38 g of progesterone ; Pfizer Animal Health , New Zealand) for 9 days (removed at day 54) and were i.m. treated with 500 IU eCG (Folligon®, Intervet, International, Boxmeert, Netherlands) at the day before CIDR removal (day 53). All animals were examined daily for estrous detection and were im treated with 10 µg GnRH (Receptal, Intervet, International,

Boxmeert, Netherlands) at the time of insemination (day 55).

Ultrasonography and blood sampling:

Ovarian ultrasonography was carried out with a B-mode ultrasound scanner (Toshiba, SSA 220, Just Vision, Medical Systems Corporation, Tochigi-ke, Japan) equipped with 5 ⁄ 7.5 MHz linear-array rectal transducer (ALR 575 probe, ECM). Blood samples were collected via jugular venipuncture in a heparinized vial after each scan. Plasma was obtained by centrifugation of samples at 700 × *g* for 20 min at 4°C. Plasma was separated immediately and kept in two aliquots at -20°C until analysis. Ultrasonography and blood sampling were conducted twice a week throughout the period of melatonin treatment then at days of CIDR insertion (day 45) and removal (day 53) and each day of hormonal injection (day 53 for eCG and day 55 for GnRH, respectively). After insemination (which was carried out at days 55, 56), ultrasonography and blood sampling were achieved at days 75, 84 (21, 30 days after AI) to follow up formation and maintenance of CL.

Plasma biochemical parameters

Plasma total protein was measured by the Biuret method as described by (Armstrong and Carr, 1964) and total albumin concentration was determined by the method of Doumas*et al*, (1972).Glucose was determined according to Barham and Trinder (1972). Total cholesterol concentration was measured calorimetrically as described by Watson (1960). High density lipoprotein (HDL) concentration was measured according to Burstein *et al.* (1970). Transaminase activities [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] were measured colorimetrically (Reitaman and Frankel, 1957). Alkaline phosphatase (ALP) and Mg were measured as performed by Zilva and Pannall (1979). Creatinine concentration was measured by a colorimetric method (Myers *et al.*, 2006) and Ca determination was performed by the method of Beeler and Catrou (1983).

Statistical Analysis

All data records were tested for normality with the Shapiro-Wilk (W) test from the UNIVARIATE procedure (SAS, 2000), and results indicated that all data were distributed normally [W > 0.90]. Data for the effect of melatonin was analyzed using PROC MIXED of SAS (SAS Inst., Inc., Cary, NC) for repeated measures. Treatment and days were used as fixed effects and individual buffalo heifers as random effects. Data for the effect of melatonin on plasma blood metabolites were analyzed by adapting the following model:

$$Y_{ijk} = \mu + T_i + D_j + (T*D)_{ij} + e_{ijk}$$

Where Y_{ijk} = observed value of the dependent variable determined from a sample taken from each animal; μ = overall mean; T_i = fixed effect of the i^{th} treatment (i = 1,2); D_j = fixed effect of the j^{th} day (j = d0:d45); $(T*D)_{ij}$ = first order interaction between treatments and days; e_{ijk} = the residual error. Secondly, to compare the effect of melatonin followed by CIDR with CIDR only on plasma blood metabolites, the following model was used

$$Y_{ijk} = \mu + T_i + D_j + (T*D)_{ij} + e_{ijk}$$

Where Y_{ijk} = observed value of the dependent variable determined from a sample taken from each animal; μ = overall mean; T_i = fixed effect of the i^{th} melatonin + CIDR and CIDR treatments (i = 1, 2); D_j = fixed effect of the j^{th} day (j = d45:d84); $(T*D)_{ij}$ = first order interaction between treatments and days; e_{ijk} = the residual error. Significant differences among means within each classification were tested using $LSD_{0.05}$.

RESULTS

Effect of melatonin on biochemical and enzymatic properties:

Biochemical and enzymatic properties in anestrus buffalo heifers as affected by melatonin treatment are displayed in Tables (1) and (2). Melatonin treatment increased plasma concentrations of albumin, alanine aminotransferase (ALT), ($P \leq 0.01$) and glucose, HDL, Mg and calcium ($P \leq 0.05$), while alkaline phosphatase (ALP) concentration reduced ($P \leq 0.01$) in treated than control groups. Other components; total protein, cholesterol, aspartate aminotransferase (AST) and Creatinine were not significantly affected with melatonin treatment. Plasma concentrations of creatinine ($P \leq 0.05$) recorded significant increase, but all other biochemical and enzymatic parameters are not affected by day advancement in the melatonin treated heifers.

Effect of melatonin and CIDR on biochemical and enzymatic properties:

Biochemical and enzymatic properties in anestrus buffalo heifers as affected by melatonin and CIDR treatments are displayed in Tables (3) and (4). Melatonin and CIDR treatment caused significant increases in plasma concentrations of albumin, glucose, ALT, and Ca ($P \leq 0.01$), and in plasma concentrations of HDL and Mg ($P \leq 0.05$). On the other hand, melatonin and CIDR treated heifers showed significant reduction in plasma concentrations of ALP ($P \leq 0.01$) (Table 3).Plasma concentrations of total protein, albumin and calcium revealed the greatest values ($P \leq 0.01$) on day 45 after melatonin and CIDR treatment, while plasma concentration of glucose recorded the highest values on days 53, 55 and 75 after the treatment.

Table 1. Effect (LSM±SEM) of melatonin treatment on blood biochemical parameters of heifers during the out-of-breeding season

	Treatment		SEM	*P*-Value		
	Control	Melatonin		Group	Day	Group × Day
Total protein (g/dl)	8.66	8.64	0.18	0.941	0.975	0.935
Albumin (g/dl)	3.36 [b]	3.60 [a]	0.05	0.001	0.696	0.358
Glucose (mg/dl)	61.86 [b]	67.98 [a]	2.07	0.054	0.008	0.892
Cholesterol (mg/dl)	72.36	74.67	1.59	0.290	0.059	0.967
HDL (mg/dl)	41.89 [b]	64.10 [a]	6.83	0.028	0.722	0.689
AST (U/L)	161.23	162.27	6.93	0.918	0.708	0.963
ALT (U/L)	43.75 [b]	90.62 [a]	11.88	0.008	0.747	0.571
ALP (U/L)	186.86 [a]	128.5 [b]	16.73	0.018	0.246	0.699
Creatinine (mg/dl)	1.67	1.80	0.06	0.189	0.018	0.601
Mg (mg/dl)	2.66 [b]	3.78 [a]	0.33	0.023	0.556	0.631
Ca (mg/dl)	8.98 [b]	9.33 [a]	0.11	0.039	0.401	0.925

[a-b] Within a row, means with different superscripts differ ($P< 0.05$).

Table 2. Effect (LSM±SEM) of day after melatonin treatment on blood biochemical parameters of heifers during the out-of-breeding season

	Day								*P*-Value		
	0	7	14	21	28	35	42	SEM	Group	Day	Group × Day
Total protein (g/dl)	8.44	8.92	8.54	8.74	8.54	8.69	8.73	0.37	0.941	0.975	0.935
Albumin (g/dl)	3.38	3.50	3.44	3.61	3.46	3.51	3.48	0.09	0.001	0.696	0.358
Glucose (mg/dl)	73.18[a]	71.43[a]	53.66 [b]	62.67[ab]	58.00[b]	71.22[a]	64.31[ab]	4.12	0.054	0.008	0.892
Cholesterol (mg/dl)	72.03	80.63	75.92	75.67	69.50	69.50	71.42	2.88	0.290	0.059	0.967
HDL (mg/dl)	42.26	44.32	60.68	60.41	61.38	40.83	61.12	13.68	0.028	0.722	0.689
AST (IU/L)	163.10	166.70	151.59	179.03	151.67	170.17	150.00	13.27	0.918	0.708	0.963
ALT (IU/L)	53.93	45.90	76.03	89.91	80.92	49.80	73.85	22.67	0.008	0.747	0.571
ALP (IU/L)	192.75	209.10	135.35	128.92	130.87	187.20	119.71	31.06	0.018	0.246	0.699
Creatinine (mg/dl)	1.37[c]	1.50[bc]	1.81[ab]	1.92[a]	1.84[ab]	1.86[ab]	1.84[ab]	0.12	0.189	0.018	0.601
Mg (mg/dl)	2.45	2.73	3.38	3.52	3.64	2.78	4.05	0.60	0.023	0.556	0.631
Ca (mg/dl)	9.03	9.36	8.74	9.22	9.12	9.24	9.43	0.21	0.039	0.401	0.925

[a-c] Within a row, means with different superscripts differ ($P< 0.05$).

Table 3. Effect (LSM±SEM) of melatonin and CIDR treatments on blood biochemical parameters of heifers during the out-of-breeding season

	Treatment		SEM	*P*-Value		
	CIDR	Melatonin+ CIDR		Group	Day	Group × Day
Total protein (g/dl)	8.58	8.60	0.17	0.950	0.047	0.908
Albumin (g/dl)	3.09 [b]	3.41 [a]	0.05	0.001	0.017	0.597
Glucose (mg/dl)	55.21 [b]	65.16 [a]	1.48	0.001	0.033	0.816
Cholesterol (mg/dl)	68.88	71.86	7.52	0.258	0.555	0.886
HDL (mg/dl)	41.32 [b]	62.29 [a]	6.61	0.040	0.808	0.843
AST(IU/L)	131.82	134.33	7.36	0.821	0.958	0.983
ALT (IU/L)	44.20 [b]	98.72 [a]	12.60	0.007	0.764	0.737
ALP (IU/L)	163.48 [a]	97.99 [b]	15.53	0.009	0.265	0.586
Creatinine (mg/dl)	1.62	1.84	0.11	0.126	0.989	0.876
Mg (mg/dl)	2.65 [b]	4.09 [a]	0.45	0.032	0.913	0.875
Ca (mg/dl)	8.59 [b]	9.09 [a]	0.11	0.006	0.038	0.548

[a-b] Within a row, means with different superscripts differ (P< 0.05).

Table 4. Effect (LSM±SEM) of day after melatonin and CIDR treatments on blood biochemical parameters of heifers during the out-of-breeding season

	Day					SEM	*P*-Value		
	45	53	55	75	84		Group	Day	Group × Day
Total protein (g/dl)	9.24 [a]	8.71[ab]	8.3[b]	8.59[ab]	8.0[b]	0.27	0.950	0.047	0.908
Albumin (g/dl)	3.45 [a]	3.25[abc]	3.15[bc]	3.32[ab]	3.08[c]	0.08	0.001	0.017	0.597
Glucose (mg/dl)	53.03[b]	63.78[a]	61.04 [a]	63.46 [a]	59.61[ab]	2.04	0.001	0.033	0.816
Cholesterol (mg/dl)	73.33	67.67	70.10	72.40	68.37	3.01	0.258	0.555	0.886
HDL (mg/dl)	54.27	52.48	54.06	58.26	39.92	11.10	0.040	0.808	0.843
AST (IU/L)	139.23	131.03	131.71	126.80	136.59	12.35	0.821	0.958	0.983
ALT (IU/L)	75.87	69.40	71.77	89.98	50.31	20.62	0.007	0.764	0.737
ALP (IU/L)	113.87	119.23	112.51	122.05	185.00	26.05	0.009	0.265	0.586
Creatinine (mg/dl)	1.78	1.73	1.75	1.75	1.66	0.16	0.126	0.989	0.876
Mg (mg/dl)	3.70	3.56	3.41	3.46	2.72	0.69	0.032	0.913	0.875
Ca (mg/dl)	9.34[a]	8.92[ab]	8.77[b]	8.69[b]	8.49[b]	0.19	0.006	0.038	0.548

[a-c] Within a row, means with different superscripts differ (*P*< 0.05).

DISCUSSION

Treatment melatonin implants was successfully exploited for initiating ovarian cyclicity in true anestrus buffalo heifers (Ghuman *et al.,* 2007; and Ramada n*et al.,* 2014). However, the precise mechanisms involved remain unknown. Alterations in melatonin release with changes in photoperiod are associated with changes in lactation, body growth and composition in cattle (Dahl *et al.,* 2002). Because of the interaction of melatonin with various endocrine systems (Zieba *et al.,* 2007), it was thought that melatonin treatment may initiate ovarian cyclicity in true anestrus buffalo heifers through its influence on body metabolism (Darul and Kruczynska, 2004).

Present data showed that plasma total protein were not affected by treatment with melatonin and CIDR. Our results are consistent with previous reports showing no significant change in the concentration of follicular fluid total protein either with the stage of the estrous cycle or during development of the follicle in buffaloes (Arshad *et al.,* 2005; and AbdEllah *et al.,* 2010). Optimum total serum proteins are essential for the expression of estrus (Tandle *et al.,* 1998). In addition, the oxidation of a protein typically results in an increase in carbonyl contents. This increase is due to the oxidation of lysine, arginine, proline or other amino acid residues. Protein carbonyl groups are the biomarkers of oxidative stress (Dalle-Dome *et al.,* 2003). In human plasma, all amino acids in the protein are susceptible to oxidative modification by oxidants such as hydroxyl radicals and hypochlorous acid (Levine *et al.,* 1994).

The significant ($P \leq 0.01$) increases in plasma albumin concentration with treatment of melatonin and CIDR in the present study agree with previous findings in anestrus buffalo heifers (Singh *et al.,* 2010). Albumin is the most abundant plasma protein; it could play a major role as an antioxidant in plasma at least by thiol oxidation and carbonyl formation (Himmelfarb and Mcmonagle, 2001). In the present study, it's expected that the characterization of oxidative status of serum albumin would provide, not only useful information regarding the redox state of the animal body, but also alterations in the conformation and function of animal serum albumin which may result in modification of its biological properties.

In the present study, plasma concentrations of glucose were significantly increased ($P \leq 0.01$) by melatonin and CIDR treatment. Glucose is the primary energy source for the ovary. It is stimulatory to follicular growth and is possibly metabolized by the ovary through anaerobic pathways (Rabiee *et al.,* 1997). Furthermore, plasma glucose is a metabolic signal providing information for the central control of GnRH release (Foster and Nagatani, 1999). High plasma glucose was previously reported in cycling dairy cattle compared to anestrus (Singh and Singh, 2006).The variation in glucose concentration across different follicle sizes indicates its relation with the growth and development of the follicle, possibly due to its function as an energy source and utilization of its intermediate metabolites in extracellular matrix expansion (Nandi *et al.,* 2008).

Increased lipolysis during lactation is hormonally regulated and not an expression of energy deficiency. There is a strong reduction in lipogenesis and esterification and an increase in nor-epinephrine- and epinephrine- stimulated free fatty acid release (Holtenius and Hjort, 1990). In the present study, plasma concentrations of HDL were significantly increased ($P\leq 0.01$) by melatonin treatment. Also, following melatonin treatment, no alterations were observed in cholesterol profile of buffalo heifers suggesting non-significant role of lipids in melatonin treatment-induced onset of ovarian cyclicity. Cholesterol has a significant role in the ovarian function as it is the precursor molecule for steroid biosynthesis (Arshad *et al.,* 2005). Also, Expression of estrus is more likely in dairy animals with higher plasma cholesterol (Westwood *et al.,* 2002). On the other hand, the lower cholesterol concentration in reproductively acyclic buffalo indicates yet again the contribution of nutrition to the development of reproductive acyclicity (Das and Khan, 2010).

Regarding plasma enzyme activities, the concentration of transaminase enzyme ALT was increased ($P \leq 0.01$) with treatment of melatonin and CIDR (Table1 and 3). Previous studies are lacking to substantiate the present findings of plasma activities of AST and ALT with respect to the melatonin in the dairy buffalo. Fischbach (2000) reported that deranged enzymatic actions affect normal reproductive behavior of the animal, while Singh *et al. (*2010) found no alterations in plasma concentration of AST and ALT enzyme activity following melatonin treatment for buffalo heifers. Thus, minor alterations in AST during treatment period in spite of elevated plasma activities of ALT suggested that hepatic functions were not impaired in buffalo (*Bubalusbubalis*) (Randhawa *et al.,* 2009). Follicular growth is a dynamic process in which follicular development is continuous but accelerates during the later stages of the estrous cycle (Wise, 1987). Therefore, biochemical composition of serum may change during the estrous cycle. ALP is a lysosomal enzyme that catalyzes various reactions in the body, including synthesis of proteins and DNA turnover within the nucleus (Mishra *et al.,* 2003), and the stage of estrus is known to affect the level of ALP (Wise, 1987). Plasma concentrations of Mg and Ca increased significantly with melatonin and CIDR treatment ($P \leq 0.05$).In previous studies, there were no alterations in plasma concentrations of creatinine following melatonin implants treatment though initiation of ovarian cyclicity has significant positive correlations with plasma Ca (Singh *et al.,* 2010)

In conclusion, the responsiveness of out-of-breeding season heifers for melatonin and CIDR treatment showed significant increase in plasma blood concentrations of albumin, glucose, HDL, ALT, Mg and Ca, while melatonin treatment showed significant decrease in plasma concentration of ALP.

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تأثيرات المعاملة بالميلاتونين وتحاميل البروجسترون المهبلية على نواتج الأيض في الدم لعجلات الجاموس خارج موسم التناسل تحت الظروف الاستوائية

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تم استخدام ١٦ من عجلات الجاموس وقسمت الي مجموعتين الاولي كنترول والثانية المعاملة (ثمانية حيوانات لكل مجموعة) وذلك لدراسة تأثير المعاملة بالميلاتونين وتحاميل البروجسترون المهبلية علي اعاده النشاط المبيضي خارج موسم التناسل (موسم الصيف). تم زرع (١٨ مللي جرام ميلاتونين /٥٠ كجم وزن جسم) للحيوانات المعاملة لمدة ٤٥ يوم وبعد ذلك تم وضع تحاميل البروجسترون المهبلية لكلا المجموعتين لمدة تسعة أيام وقبل ازالة تحاميل البروجسترون المهبلية بيوم واحد تم اعطائها ٥٠٠ وحدة دولية من الفوليجيون (eCG) تحت الجلد لكل العجلات ، وبعد نزع تحاميل البروجسترون المهبلية تم اعطاء ١٠ميكروجرام من الريسبتال (GnRH) . تم متابعة الشياع يوميا لكل العجلات. تم أخذ عينات الدم بالتزامن مع الفحص المبيضي بواسطة جهازالسونار والذي تم اجراؤه مرتين في الاسبوع لتحديد تركيز نواتج الايض ورصد النشاط الحويصلي للمبايض. أظهرت النتائج خلال خارج موسم التناسل زيادة معنوية (احتمال ٠.٠١) في تركيزات الالبيومين و نشاط الإنزيمات الناقلة لمجموعة الأمينALT وكذلك زيادة معنوية (احتمال ٠.٠٥) للجلوكوز والماغنسيوم والكالسيوم وLDH بينما أدت المعاملة الي انخفاض معنوي لتركيزالالكالين فوسفاتيز (احتمال ٠.٠٥) . أما بالنسبة لتاثير اليوم أدت المعاملة بالميلاتونين الي زيادة في تركيزات الجلوكوز (احتمال ٠.٠١) في الايام ٠ و ٧و ٣٥ وكذلك الكرياتنين عند اليوم ٢١ مقارنة بالايام الاخري. أظهرت المعاملة بالميلاتونين و تحاميل البروجسترون المهبلية معا الي زيادة معنوية في تركيزات كلا من الالبيومين والجلوكوز وALT والكالسيوم (احتمال ٠.٠١) و LDH والماغيسوم (احتمال ٠.٠٥) بينما أدت المعاملة الي انخفاض معنوي (احتمال ٠.٠١) في تركيز انزيم الالكالين فوسفاتيز. أدت المعاملة بالميلانونين و تحاميل البروجسترون المهبلية معا الي انخفاض معنوي (احتمال ٠.٠٥) في تركيزات كلا من البروتين الكلي والالبيومين والكالسيوم بينما ادت الي زيادة معنوية في تركيزالجلوكوز (احتمال ٠.٠٥). تشير النتائج الي ان المعاملة بالميلاتونين و تحاميل البروجسترون المهبلية ادت الي تحسين الاداء التناسلي وتركيزات نواتج الايض في الدم لعجلات الجاموس خارج موسم التناسل.