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EFFECT OF MELATONIN IMPLANTATION PRIOR TO OVSYNCH PLUS CIDR PROTOCOL ON SUBSEQUENT OVARIAN ACTIVITY AND PREGNANCY RATE IN SUMMER ANESTROUS MURRAH BUFFALOES

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ABSTRACT

For assessing the impact of melatonin pre-treatment in summer anestrous Murrah buffaloes on subsequent reproductive activity of a fixed time artificial insemination (FTAI) protocol, 83 anestrous Murrah buffaloes were subcutaneously inserted 2x4 mm size absorbable melatonin (MT) implants (18 mg/50 kg b wt) at the base of left ear and 38 Murrah buffaloes were used as non-implanted controls. On day 35 post-MT, the Treatment and Control group Murrah buffaloes were subjected to FTAI protocol (Ovsynch plus CIDR). Ovarian ultrasonography and jugular vein blood sampling was carried out on day 7 and 10 of protocol and on day 7-post AI. Overall pregnancy rate on day 90 post-AI in MT group was higher compared to Control group (55.4 vs 31.6%, $P<0.05$), along with the higher ($P>0.05$) plasma progesterone on day 7 of FTAI protocol in Murrah buffaloes of the former group which conceived subsequently compared to their non-pregnant counterparts. Moreover, MT group had higher ($P>0.05$) increase in dominant follicle diameter between day 7 and 10 of protocol compared to Control group. On day 7 post-AI, the luteal profile in pregnant Murrah buffaloes of MT group was better ($P<0.05$) compared to their counterparts in Control group (CL: 14.80±0.70 vs 10.82±0.46 mm, P_4: 1.54±0.07 vs 1.04±0.08 ng/ml). In conclusion, melatonin pre-treatment in summer anestrous Murrah buffaloes could be associated with better luteal activity and higher pregnancy rate in the Ovsynch+CIDR protocol.

Keywords: *Bubalus bubalis*, buffaloes, melatonin, ovarian activity, pregnancy, summer

INTRODUCTION

The seasonal variation in ovarian activity and conception rate of buffaloes breed is caused by exogenous (photoperiod, climate, nutrition, and management) and endogenous (hormones and genotype) factors (D'Occhio *et al*., 2020). Compared to winter season, a major proportion of Murrah buffaloes not only fail to exhibit estrus during summer (22 to 26 vs 74 to 86%; Singh *et al*., 1989), but also pregnancy rate falls from 54% in winter to 27% during summer (Ghuman and

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Dhami, 2017). To counteract poor estrus exhibition by buffaloes during summer season, various estrus induction protocols such as administration of hCG hormone (Abdulkareem *et al* 2021)/ synchronization or fixed time artificial insemination (FTAI) protocols were proposed, however, the conception rate remained very low (7%) using protocols like Ovsynch (Baruselli *et al*., 2007). Other protocols which had modifications in Ovsynch protocol along with administration of intravaginal progesterone and equine chorionic gonadotropin were able to achieve 69.8% conception rate compared to 25% with Ovsynch in non-breeding season (Ghuman and Dhami, 2017). Thus, it is important to explore various alternatives which can improve the success rate of FTAI protocols in summer season.

An increase in plasma melatonin during summer season by the use of exogenous implants was shown to alleviate seasonal suppression in reproductive activity of buffaloes through melatonin-induced stimulation of hypothalamus-pituitary-ovary axis (Ghuman *et al*., 2010; Pandey *et al*., 2018). Moreover, in summer season, melatonin can increase the pregnancy rate in Murrah buffaloes through antioxidant impact on ovarian function, oocyte quality and developing embryo competence (Vazquez *et al*., 2010; Singh *et al*., 2016). In a study, the melatonin pre-treatment for 45 days followed by intravaginal progesterone device placement for 9 days successfully improved the diameter of largest follicles and the ability to maintain corpus luteum (CL) at day 21 post-AI in buffalo heifers during out-of-breeding season under tropical conditions (Ramadan *et al*., 2014).

Therefore, the objective of this study was to assess the efficacy of melatonin implants pre-treatment for 35 days on success rate of subsequent Ovsync plus CIDR protocol in terms of improved ovarian activity and pregnancy rate in summer anestrous Murrah buffaloes.

MATERIALS AND METHODS

Animals

This study was conducted on anestrous Murrah buffaloes (mean age ≥36 month, mean body weight ≥350 kg) housed at various small holder dairy farmers near to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (latitude 30°56’N; longitude 75°52’E), Punjab, India, during the hot-humid month from May to August (referred to as the ‘summer season’ with maximum ambient temperatures and relative humidity index (THI) ranging from 74 to <78; Habeeb *et al* 2018). Feeding of buffaloes done routinely including concentrates (4-6 kg; CP-19.8%; TDN-76%) and green roughages (28-30 kg; CP-9%; TDN- 55%). For the confirmation of anestrous buffaloes, during 60-day period before the start of experiment, all the buffaloes were subjected to three observations per day for the detection of overt estrus and the buffaloes failing to show overt estrus were included in the study.

Experimental design

Anestrous Murrah buffaloes were randomly allocated to Control (n=38) and MT (n=83) groups. Using an implanter, MT group buffaloes were administered 2×4 mm size absorbable melatonin implants (18 mg melatonin/implant, Regulin, CEVA Animal Health Limited, France) at the base of left ear. Total implants inserted to each buffaloes were calculated on basis of their body weight (one implant/50 kg, Ghuman *et al*., 2010). These implants were designed to induce high plasma melatonin for at least 60 days in ewes (Forcada *et al*., 2002). On day 35 after melatonin priming, the

MT and Control group buffaloes were subjected to fixed-time artificial insemination protocol (FTAI).

FTAI protocol

Ovsynch plus Controlled Internal Drug Release (CIDR) protocol involved administration (i/m) of gonadotropin releasing hormone (GnRH, 0.02 mg) on day 0 and 9, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 500 µg cloprostenol sodium) on day 7. In addition, the buffaloes were inserted CIDR device per-vaginally (Eazi-breed; 1.38 g natural progesterone, Zoetis) from day 0 to day 7. On day 10 of protocol, AI was done using frozen semen, followed by per-rectal pregnancy diagnosis on day 90 post-AI.

Ovarian ultrasonography, blood sampling and hormone analysis

Ultrasonography was carried out in each buffalo with a battery-operated B-mode ultrasound scanner equipped with linear-array rectal transducer (Sonosite Vet M Turbo, USA) on day 7 and day 10 of FTAI protocol to assess the diameter of dominant follicle and on day 7 post-AI for the diameter of corpus luteum (CL). Blood was sampled from the jugular vein in a heparinized vial after each scan. Plasma was separated immediately and kept in two aliquots at -20°C until analysis. Plasma progesterone was assayed with a solid-phase radioimmunoassay using antisera raised in our laboratory (Ghuman *et al*., 2009). Sensitivity of the assay was 0.1 ng/ml; intra- and inter-assay variation coefficients were 4.8% and 9.1%, respectively.

Statistical analysis

Numerical data are represented as mean ± SEM, and differences were considered significant at $P<0.05$. Chi-square test was employed for assessing within and between group differences in pregnancy rate. Two sample Student's t-test was employed for assessing the differences within/between control and implanted buffaloes for a) plasma progesterone, and b) diameters of dominant follicle/CL. Statistical analyses were performed using MINITAB release 13.2 statistical software (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

The summer anestrous Murrah buffaloes of MT group had a considerably higher pregnancy rate compared to Control group (55.4 vs 31.6%, $P<0.05$, Table 1). Furthermore, within Control group, a considerably higher proportion of buffaloes remained non-pregnant (68.4%, $P<0.05$, Table 1). In a previous study, the overall conception rate following Ovsynch protocol in Control group was lower as compared to melatonin-primed buffaloes subjected to Ovsynch (20 vs 50%; Kavita *et al*., 2018). The beneficial impact of melatonin treatment on pregnancy rate in summer anestrous buffaloes can be ascribed to shielding of oocytes from oxidative stress, improved embryo viability, improved uterine expression of progesterone receptors and an improvement in fertilization rate (Forcada *et al*., 2006; Ramadan, 2017).

Nevertheless, regardless of the significance, MT group buffaloes which conceived subsequently had higher (P0.05) plasma progesterone on day 7 of Ovsynch plus CIDR protocol compared to their non-pregnant counterparts as well as pregnant and non-pregnant buffaloes of Control group (Table 1). Also, in a previous study on buffaloes, following the start of Ovsynch protocol on day 28 post-implant insertion, plasma progesterone was higher on day 7 of protocol in Melatonin-treated group as compared to Control (Kavita *et al*., 2018). This suggested that melatonin might have induced better follicular development in the priming period which subsequently lead to better luteal profile. Thus, the growth of ovulatory follicle, destined to ovulate at the end of FTAI protocol, in an optimal progesterone environment could have been the reason for successful conception. In fact, on the day of first GnRH injection of Ovsynch protocol, most of melatonin-primed buffaloes already had CL which might have also contributed progesterone (Kavita *et al*., 2018).

The dominant follicle diameter of the pregnant buffaloes in the Control group (13.80±1.69 mm) is numerically lesser than those of pregnant buffalo in the MT group (12.50±0.6 mm) at day 7. There was no significant difference in the dominant follicle diameter of the pregnant buffalo in Control and MT group (Table 1). In a previous study on summer anestrous buffaloes, following Ovsynch protocol, Melatonin-treated group had larger diameter of preovulatory follicle compared to their controls on the day of AI (Kavita *et al*., 2018). The administration of slow-release melatonin implants is known to elevate plasma melatonin (Pandey *et al*., 2019), and the latter through its stimulatory action on reproductive axis leads to an increase in circulating GnRH / LH concentrations followed by the higher growth rate of dominant follicles (Viguie *et al*., 1995; Misztal *et al*., 2002).

On day 7 post-AI, the luteal profile in pregnant buffaloes of MT group was better ($P<0.05$) compared to their pregnant counterparts in Control group (CL: 14.80±0.70 vs 10.82±0.46 mm, P_4: 1.54±0.07 vs 1.04±0.08 ng/ml, Table 1). In similar studies in buffaloes subjected to FTAI protocol during out of breeding season, the diameter of CL and plasma progesterone during post-AI period was higher in Melatonin-primed group compared to Control (Ramadan *et al*., 2014; Kavita *et al*., 2018). It was further suggested that melatonin-induced increase in dominant follicle diameter subsequently generated a larger CL with higher steroidogenic capacity (Kavita *et al*., 2018). Moreover, the preovulatory follicle diameter has positive correlation with CL diameter, plasma progesterone and subsequent pregnancy rate in buffaloes (Pandey *et al*., 2018).

There was significant difference ($P<0.05$) in plasma progesterone concentration at day 7 post-AI between pregnant and non-pregnant buffaloes of Control and MT group (Table 1). This is a well-

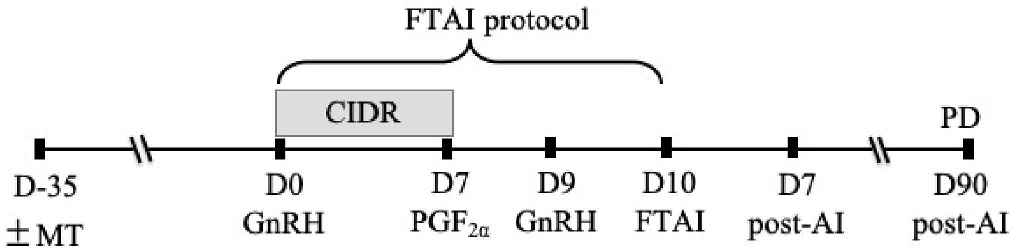

Figure 1. Experimental design for summer anestrous buffaloes with or without melatonin (MT) implants treatment subjected to Ovsynch plus CIDR protocol. CIDR-Controlled internal drug release, D-Day, GnRH- Gonadotropin releasing hormone, PD-Pregnancy diagnosis, $PGF_{2\alpha}$-Prostaglandin $F_{2\alpha}$.

Table 1. Pregnancy rate and ovarian activity following an Ovsynch plus CIDR protocol with or without pre-treatment of melatonin (MT) implants in summer anestrous Murrah buffaloes (n=121). CL: Corpus luteum, DF: Dominant follicle, P_4: Progesterone.

Group	Pregnancy status	n	Day of Ovsynch + CIDR protocol				Day 7 post-AI	
			Day 7		Day 10			
			Plasma P_4, ng/ml	DF diameter, mm	Plasma P_4, ng/ml	DF diameter, mm	CL diameter, mm	Plasma P_4, ng/ml
Control, n=38	Non-Pregnant	26 (68.4%)^{a,c}	0.70±0.25	14.80±2.15	0.39±0.05	14.50±0.63	12.30±0.48	0.58±0.07^{a}
	Pregnant	12 (31.6%)^{b,e}	0.85±0.48	13.80±1.69	0.38±0.03	13.57±0.87	10.82±0.46^{a}	1.04±0.08^{b,e}
MT, n=83	Non-Pregnant	37 (44.6%)^{d}	0.65±0.19	12.33±0.67	0.29±0.04	13.24±0.49	13.76±0.55	0.54±0.10^{c}
	Pregnant	46 (55.4%)^{f}	1.11±0.18	12.50±0.66	0.38±0.06	13.84±0.51	14.80±0.70^{b}	1.54±0.07^{d,f}

^{a} *vs.* b, c *vs.* d, e *vs.* f $P<0.05$: within a column

established fact that a higher plasma progesterone during post-AI luteal phase is associated with better pregnancy rate in buffaloes (Pandey *et al*., 2018). The combination of melatonin with select-synch and co-synch can also be explored in anestrous buffaloes during summer in order to investigate the effect on ovarian and luteal profiles.

In brief, melatonin priming prior to an Ovsynch plus CIDR protocol in summer anestrous buffaloes might be associated subsequently with better luteal activity and higher pregnancy rate. Thus, melatonin pre-treatment can improve reproductive efficiency of buffaloes subjected to Ovsynch plus CIDR protocols in summer season.

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