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INFLUENCE OF MELATONIN TREATMENT ON PUBERTY ONSET IN BUFFALO HEIFERS FROM BULGARIAN MURRAH BREED

Vladimir Planski[1], Stanimir Yotov[2], Parvan Parvanov[1], Manol Karadaev[2], Yordanka Ilieva[3], Kalin Hristov[1], Dimiter Dimitrov[1]

[1]University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
[2]Trakia University, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria
[3]Agricultural Academy, Sofia, Agricultural Institute, Shumen, Bulgaria
E-mail planivex@abv.bg

ABSTRACT

Current study aimed to investigate the influence of melatonin treatment on puberty onset in buffalo heifers from Bulgarian Murrah breed. The experiment was carried out with eleven clinically healthy pre-pubertal Bulgarian Murrah buffalo heifers alloted in two groups – control (non treated, n=6) and experimental (melatonin-treated, n=5). According to used plan, the treatment was done three time by subcutaneous melatonin implant, containing 18 mg melatonin. Seven days after the last melatonin treatment, progesterone levels were measured by ELISA method and used as an indicator for a presence of cyclic ovarian activity. Data were processed using of a computer statistical program. The average age (12.7±1.1 months and 13.2±0.9 months), body weights (184±22 kg and 232±33 kg) and progesterone levels (0.94±0.37 ng/ml and 1.08±0.16 ng/ml) among the groups did not differ considerably. However, the minimal and the maximal progesterone values in the experimental group (0.96 ng/ml до 1.36 ng/ml) were indicative for a presence of cyclic ovarian activity in 100% of the animals versus 80% in the control group. The concluded analysis shows that melatonin implants application is connected with a trend for earlier induction of cyclic ovarian activity and hastening of a puberty onset in Bulgarian Murrah buffalo heifers.

Key words: buffalo, melatonin, puberty, sexual cycle.

INTRODUCTION

The time for a puberty onset differs and is influenced by various factors – climatic and geographical region, breed, season of birth, feeding etc. (Peeva et al., 1993; Borghese et al., 1996; Campanile et al., 2001; Singh et al. 2010; Roy et al., 2016; Planski et al., 2017). Most of the studies have been connected with an influence of the age and the body weight on the expression of cyclic ovarian activity as its determination is done by rectal palpation of the ovaries, progesterone (P_4) concentration measurement or ovarian ultrasonography (Campanile et al., 2001; Terzano et al., 2007; Roy et al., 2016; Planski et al., 2017). The obtained data among the investigation differ and sometimes are discrepant, which is a precondition for searching of others factors than abovementioned, responsible for a puberty initiation.

Melatonin is biological active substance included in the sequence of processes leading to the onset of puberty in ruminants. It is N-acetyl-5-methoxytryptamine synthesized by the pineal gland during the dark phase of the photoperiod which transfer an information to synchronize cell physiology with the dark and the light part of the day-night cycle. Besides, melatonin stimulates gene expression of antioxidative enzymes such as superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase (Reiter, 1993; Rodriguez et al., 2004; Baychev, 2008; Kanchev et al., 2010). Recently, melatonin implants have been successfully applied for stimulation of ovarian cyclicity in beef heifers and anestral buffaloes (Ghuman et al., 2010; Singh et al., 2010; Ramadan et

al., 2014). In spite of all, the precise mechanisms, the treatment plan and the melatonin dose for a puberty initiation in different buffalo breeds remain unclear.

Current study aimed to investigate the influence of melatonin treatment on puberty onset in buffalo heifers from Bulgarian Murrah breed.

Material and methods

The experiment was carried out with eleven clinically healthy pre-pubertal Bulgarian Murrah buffalo heifers, aged between 9-12 months, weighing from 160 to 220 kg and cultivated of free-penned regimen in experimental farm of Agricultural Institute, Shumen, Bulgaria.

The nutrition plan included giving of all buffalo milk until the first month after calving, followed by milk replacement unit the third month. From third to eight months the animals received alfa-alfa hay at libitum and 1.2 kg concentarate for growing-up buffaloes daily. After that the ration included vetch-barley hay and 1.8 kg concentarate daily. During experimental period all animals had unlimited approach to water. The experiment was performed between September and January.

At the begin of experiment, the animals were alloted proportionally in two groups according to their body weight - control (non treated, n=6) and experimental (melatonin-treated, n=5). The treatment was done three time (at days 0.45 and 110) by subcutaneous melatonin implants, containing 18 mg melatonin (Melovine, Ceva Animal Heath, France). The time of first melatonin treatment was accepted for day 0. Buffalo heifers with body weight <180 kg. were implanted with 1, 2 and 3 melatonin implants per animal through abovementioned periods whereas those weighing >200 kg were treated with 2–2–3 implants per animal.

Blood samples for progesterone analysis were obtained from v. jugularis of previously restrained animals, seven days after the last melatonin treatment. The blood was centrifuged at 3000 rpm for 15 min and separated sera were stored in sterile tubes at -18 ^{0}C until analysis. Hormonal concentrations were assays with a commercial Progesterone ELISA Kit (Monobind Inc, Lake Forest USA) with analytical sensitivity of 0.105 ng/ml and coefficients of variation <10%. Progesterone levels of individual samples were measured in triplicate and mean arithmetic value of the three measurements was retained as final value.

After data processing, average age and body weight, minimal, maximal and average progesterone concentrations and a percentage of animal with cyclic ovarian activity in both groups were calculated. Progesterone levels >0.71±0.24 ng/ml were accepted as an indicator for cyclic ovarian activity in this buffalo breed (Planski et al., 2017).

Data were analysed using statistical software Statistica version 7.0 (Stat-Soft 1984-2000 Inc., Tulsa, OK, USA) using of the non parametric method for a comparison of mean values and proportions, based on Student t-test. The level of significance was set at $P<0.05$.

Results and discussion

The obtained data are showed in a table 1. The values about average age and body weight of the buffalo heifers in the control (12.7±1.1 month; 184±22 kg) and experimental (13.2±0.9 month; 232±33 kg) groups did not differ significantly (P=0.43 и P=0.48). These results showed that in a case the aforementioned parameters have no considerable influence on the investigated reproductive findings (P_4 and cyclic ovarian activity) and are indicative for equalization of both groups. According to some authors (Peeva et al., 1993; Terzano et al., 2007) the age and the body weight

have a pivot role for a puberty initiation in buffalo heifers. In the contrary, other studies (Parmeggiani et al., 1992; Singh et al., 2010) reported that significant parts of animals remained acyclic in spite of gaining of the typical age and body weight for the investigated buffalo breed. This is in agreement with our hypothesis that other mechanisms (melatonin production for example) are responsible for activating of the hypotalamus-pituitary-ovarian cascade, leading to early puberty onset. It is also in correspondence with the results of different investigations in beef heifers (Tortonese and Inskeep, 1992; Jaeger et al., 1998) and anestral buffalo heifers (Singh et al., 2010), reporting for stimulation of ovarian activity after melatonin treatment.

Table 1: Age, body weight, progesterone concentration and animals with a presence of cyclic ovarian activity in the control and the experimental group.

Group	Control (n=6)			Experimental (n=5)		
Parameters	**Min.**	**Max.**	**Mean±SD**	**Min.**	**Max.**	**Mean±SD**
Age (months)	11.4	14.4	12.7±1.1	12	14.2	13.2±0.9
Body weight (kg)	170	220	184±22	180	290	232±33
P_4 (ng/ml)	0.24	1.23	0.94±0.37	0.96	1.36	1.08±0.16
	%			%		
Cyclic ovarian activity	80			100		

The present study aimed to investigate the influence of melatonin treatment on puberty onset in buffalo heifers from Bulgarian Murrah breed by using of a progesterone concentration as an indicator for cyclic ovarian activity. The obtained progesterone values (0.94±0.37 ng/ml и 1.08±0.16 ng/ml) for non treated and melatonin-treated buffalo heifers, respectively, did not differ considerably (P=0.45). However, the minimal and the maximal concentrations of P_4 within the groups showed high variation in control group (from 0.24 ng/ml to 1.23 ng/ml), whereas in treated group it was low (from 0.96 ng/ml to1.36 ng/ml) and all animals had a progesterone levels over accepted reference value for ovarian activity in Bulgarian Murrah breed. Furthermore, 20% non treated heifers had hormonal level below the reference. On this base could be accepted that melatonin implants administration is connected with a tendency for earlier initiation of cyclic ovarian activity (80% vs. 100%). It is in agreement with information by other authors (Borghese et al., 1994; Jaeger et al., 1998; Ghuman et al., 2007, 2010; Singh et al., 2010), studied effect of exogenous applied melatonin for acceleration of a puberty in beef heifers or induction of ovarian cyclicity in anestral buffalo heifers. The absence of significant differences between the values of the investigated parameters could due to low number of animals. On the other hand, the phase of estrous cycle for samples collecting can also influence progesterone results. If a part of animals is in the start or the end of estrus during blood collection, the hormonal level could also be lower and determining lack of statistical significance. In addition, there are data for shorter luteal phase, lower size of corpus luteum and decreased progesterone during the first estrus cycle after melatonin treatment than in normal estrus in anestral buffalo heifers (Ramadan et al., 2014). In our opinion, the treated of us animals are being with ovarian cyclicity, but through of previously showed reasons some of them are had decreased progesterone production.

Regardless of abovementioned, all progesterone values in melatonin-treated buffalo heifers were above the reference for the investigated breed and it could be accepted as an argument for a positive impact of the melatonin treatment on the puberty onset. In respect of that, Singh et al. (2010) reported successful induction of ovarian activity in buffaloes by melatonin implants administration.

Ramadan et al. (2014) determined the second estrous cycle after melatonin application is chracterized with greater size of the ovulatory follicle, the corpus luteum and progesterone level, compared to the same parameters in the second estrus cycle in lack of treatment. Probably at the begin, the longer melatonin exposition results in temporary drop in GnRH releasing and because of that the first corpus luteum has a short half-life with low progesterone production. After that, GnRH production and releasing are increase and it led to ovarian activity induction. However, proving of this hypothesis is need from additional investigations and will be a subject of future study.

In conclusion, the present study shows that melatonin implants administration is connected with a trend for earlier induction of cyclic ovarian activity and hastening of a puberty in Bulgarian Murrah buffalo heifers. Numerous questions about effect of melatonin on the reproductive performance in buffalo heifers remain debatable and detailed investigations with a large number of animals can clarify this area.

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