

## Effect of Melatonin and Its Combination with CIDR on Reproductive Performance in Anestrus Lactating Iraqi Buffaloes (*Bubalus bubalis*)

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### ABSTRACT

Because of the little studies about the reproductive performance in postpartum anestrus lactating Iraqi buffaloes, this study was designed to investigate the effect of melatonin alone or with progesterone control internal drug release (CIDR) for induction of fertile estrus in anestrus lactating Iraqi buffaloes. The present study was performed on 30 lactating Iraqi buffaloes suffered from ovarian inactivity, which was diagnosed by trans-rectal palpation during 60-90 days postpartum. The age of cows was between 3-6 years. The study was performed at Al-Thahab Al-Abiad village, Abu-Graib, Baghdad province during the period from February-June 2020. Animals were divided into three equal groups (10 buffaloes for each one). The 1<sup>st</sup> group was treated with melatonin implants (90 mg/animals/SC) for 30 days, the 2<sup>nd</sup> group was treated with melatonin implants conjugated with CIDR (1.38 gm) in day 20 for 10 days, and the 3<sup>rd</sup> group was kept without treatment (control group). The results reported animal responses of 90%, 100%, and 50% in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> groups, respectively, with a higher significance for treated groups compared with control group, and the readings of  $8.26 \pm 1.33$ ,  $7.15 \pm 0.97$  and  $160.5 \pm 14.31$  days represented the duration of response in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> groups, respectively with significant differences relative to the control. Moreover, the number of services per conception recorded insignificant findings in all groups, while the pregnancy rate recorded significant differences in the treated groups compared with the control group. Finally, the open days recorded  $153.6 \pm 6.72$ ,  $142.8 \pm 7.45$ , and  $217.3 \pm 19.56$  in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> groups, respectively, with a higher significance seen in the treated group compared with control group. It can be concluded that the role of melatonin could improve the reproductive performance of anestrus lactating Iraqi buffaloes suffering from ovarian inactivity. Furthermore, the melatonin followed by CIDR protocol could be safe and effective.

**Keywords:** Buffaloes, Reproductive performance, Melatonin, CIDR

### Introduction

Buffaloes are characterized by polyestrous continuous species and able to breed throughout the year but they show a seasonal breeding pattern with faraway from the equator (1, 2). The consideration as a seasonal polyestrous is related with many factors represented by ambient temperature, period of lightening, and nutrition (3). To severe seasonality and decreased milk production, it is necessary to influence suitable management conditions, particularly for out season breeding. Buffaloes are considered the main sources of good quality of milk and meat in

Iraq and some other countries in spite of it mostly shows low productive and reproductive potentials (4). The stress and environmental effects represent a direct effect on the disturbance of neuro endocrine physiology, which results in hyperprolactinemia leading to reduce the secretion of gonadotropin then poor maturation of follicles and reproduction of estradiol, many reproductive problems anestrus either temporary or deep due to lactation (4, 5). Various studies indicated to the relationship between plasma melatonin level and seasonal activity related with cow buffaloes (6, 7). Moreover, decreasing melatonin level in blood serum was concerned with a low seasonal ovulatory activation and this decrease is related with long days although present of ovulatory activity and follicles (6). Many mammals characterized by seasonal reproductive activities are synchronized by the secretion of melatonin from pineal gland. These cells act as

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Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Iraq. Received: 20 September 2020, Accepted: 12 December 2020, Published: 28 December 2020.

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neuroendocrine transducer of environmental changes by activation of N-acetyltransferase, which converts serotonin to N-acetyl serotonin. The production of melatonin by the pineal gland showed circadian rhythm characterized by low level of production during day time and increasing concentrations during the night, but the process of melatonin synthesis includes hydroxylated tryptophan to 5-hydroxytryptophan and subsequently into serotonin. Serotonin acetylation to form N-acetylserotonin is finally converted into melatonin by methylation of hydroxyl group (7, 8). Melatonin has free radical scavenging properties as well as stimulating several other antioxidant enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione catalase) (9). Melatonin synthesis is stimulated by the release of norepinephrine from fibers extending from the superior cervical ganglion, but finally the conversion of N-acetyl serotonin to melatonin and the release into the circulation increase markedly during the hours of darkness (10). Murrah buffaloes reached puberty between the ages of 33.1 and 36.5 months, whereas in cross breeds such as Haryana and Sahiwal, reared under same management and environmental conditions, reached puberty at 24.6 months.

Nutritional efficiency improves in crossbred beef heifers by elongating the photoperiod during winter. Estrous behavior increased by progesterone, estradiol-17  $\beta$ , and declined plasma melatonin in buffalo heifers exposed to 4 h of artificial light have been reported during autumn and winter seasons after sunset (9). Ramadan et al. (8) reported that lactating buffaloes treated with melatonin implants showed an increase in estradiol concentration in blood serum compared with animals without treatment. Many studies found that using of hormonal treatment including eCG, GnRH, progesterone and hCG during postpartum indicated to improvement in fertile estrus induction and pregnancy rates, while the recent studies suggest using melatonin hormone alone or in conjugation with progesterone (CIDR or PRID) this represented a successful program to induce fertile estrus in lactating anestrus buffaloes (7, 9-11). Because of few studies about the reproductive performance in postpartum anestrus lactating Iraqi buffaloes (*Bubalus bubalis*), this study was designed to investigate the effect of

melatonin alone or with CIDR for induction of fertile estrus in anestrus lactating Iraqi buffaloes.

## Materials and Methods

### Animals of Study

This study was performed on 30 healthy adult anestrus lactating Iraqi buffaloes 60-90 days postpartum that suffered from ovarian inactivity. The females were diagnosed by trans-rectal palpation, aged between 3-6 years during February-June 2020, in Al-Thahab Al-Abiad village, Abu Graib, Baghdad province.

### Chemicals and Hormones

Melovine (melatonin implants/ one implant contain 18 mg melatonin/ Ceva Co. Sante animal/ France), and CIDR (Control Internal Drug Release) containing 1.38 gm of Medroxy acetate progesterone/ Intervet- Holand, were used in this study.

### Experimental Design

The animals were divided randomly into three equal groups (10 buffaloes for each) according to the type of treatment. The 1st group was treated with 5 melatonin implants (90 mg/S.C) for 30 days (persisted implants for 30 days), the 2nd group was treated with 5 melatonin implants conjugated with CIDR (1.38 gm Medroxy acetate progesterone) for 10 days in day 20 and the 3rd group was kept without treatment as a control group. All animals were completely isolated from the males during the treatment period.

### Reproductive Parameters

The reproductive parameters examined in this study included: animal response, duration of response, number of services per conception, pregnancy rate, and open days.

### Statistical Analysis

The statistical analyses of the data of this study included mean, standard error, Chi-square, and F-test (12).

## Results and Discussion

Results of animal response and duration of response of anestrus lactating Iraqi buffaloes

subjected to melatonin with or without CIDR are presented in Table 1. The results showed that the animal response was 90% (9/10 animals), 100% (10/10 animals), and 50% (5/5 animals) in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> groups, respectively, with a higher significance ( $P < 0.01$ ) in the treated groups than in the control group, and these findings agree with (7, 8, 11). These findings mean the important role of melatonin in increasing the response of animals to estrus behavior due to liberating the

gonadotropic hormones by its effect on GnRH secreted from the hypothalamus.

The duration of response was  $8.26 \pm 1.33$ ,  $7.15 \pm 0.97$ , and  $160.5 \pm 14.31$  days in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> groups, respectively, with higher significant differences ( $P < 0.01$ ) in the treated groups compared with the control group. These results agree with those of other authors (9, 10, 13), and this could be due to the high level of hormones in responses to treatment.

**Table 1. Animal response (Number, %) and duration of response (days) of anestrus lactating Iraqi buffaloes subjected to melatonin with or without progesterone control internal drug release (CIDR)<sup>1</sup>**

Reproductive Parameters	Type of treatment		
	Melatonin		Control
	90 mg/SC (30 days)	90 mg/SC (30 days) +CIDR in day 20 for 10 days	Without treatment
Animal Response (No., %)	9/10, 90% <sup>a</sup>	10/10, 100% <sup>a</sup>	5/10, 50% <sup>b</sup>
Duration of Response (days)	$8.26 \pm 1.33$ <sup>a</sup>	$7.15 \pm 0.97$ <sup>a</sup>	$160.5 \pm 14.31$ <sup>b</sup>

<sup>1</sup>Mean $\pm$ SE, n=10

<sup>a-b</sup> Means within a row lacking the same superscript differ significantly at  $P < 0.01$

**Table 2. Number of services per conception, pregnancy rate, and open days in anestrus lactating Iraqi buffaloes subjected to melatonin with or without progesterone control internal drug release (CIDR)<sup>1</sup>**

Reproductive Parameters	Type of treatment		
	Melatonin		Control
	90 mg/SC (30 days)	90 mg/SC (30 days) +CIDR in day 20 for 10 days	Without treatment
No. of services/conception	$1.63 \pm 0.11$ <sup>a</sup>	$1.48 \pm 0.18$ <sup>a</sup>	$1.51 \pm 0.21$ <sup>a</sup>
Pregnancy rate (No., %)	8/10, 80% <sup>a</sup>	9/10, 90% <sup>a</sup>	5/10, 50% <sup>b</sup>
Open days	$153.6 \pm 6.72$ <sup>a</sup>	$142.8 \pm 7.49$ <sup>a</sup>	$217.3 \pm 19.56$ <sup>b</sup>

<sup>1</sup>Mean $\pm$ SE, n=10

<sup>a-b</sup> Means within a row lacking the same superscript differ significantly at  $P < 0.01$

While there was no significant the number of services per conception (Table 2) reported insignificant values ( $P > 0.01$ ) of  $1.63 \pm 0.11$ ,  $1.48 \pm 0.18$  and  $1.51 \pm 0.21$  in the three groups, respectively. These results were in agreement with the studies of (9, 10 and 11), and these findings refer to the fact that the number of services per conceptions are not affected by the type of hormonal treatment. The pregnancy rate revealed significant differences ( $P < 0.01$ ) in treated groups (80% and 90%) compared with the control group

(50%). These results agree with (6, 7 and 11) studies. These findings mean that the melatonin gives a highly fertile estrus, which is represented by increasing in pregnancy rates. Finally, the open days recorded  $153.6 \pm 6.72$  days,  $142.8 \pm 7.45$  days and  $217.3 \pm 19.56$  days in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> groups, respectively, with a highly significant difference  $P < 0.01$  in the treated groups compared with the control group and these results were in agreement with (8, 9, 10, and 11). This means that the reduction of the duration of response leads to

reduction in open days. It could be concluded that melatonin improves the reproductive performance in anestrus lactating Iraqi buffaloes suffering from ovarian inactivity.

### Acknowledgments

The authors are greatly appreciable to the owners of the buffalos that have been included in our study

### Conflict of Interest

The authors declare that there is no clear conflict of interest

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## تأثير الميلاطونين لوحده او مع البروجسترون (CIDR) على الأداء التناسلي في الجاموس العراقي الحلوب عديم الصراف

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### الخلاصة

نظرا لقلة الدراسات المتعلقة بالأداء التناسلي في الجاموس العراقي الحلوب عديم الصراف بعد الولادة فقد صممت هذه الدراسة وذلك للتحري عن تأثير استخدام هرمون الميلاطونين لوحده او مع البروجسترون (CIDR) لأحداث الشبق الخصب في هذه الحيوانات. أجريت الدراسة الحالية على 30 جاموسة حلوب عراقية تعاني من حالة خمول المبايض خلال فترة 60-90 يوم بعد الولادة وشخصت بطريقة الجس عبر جدار المستقيم. تراوحت أعمارها ما بين 3-6 سنوات وتمت الدراسة في قرية الذهب الأبيض / ابو غريب / محافظة بغداد للفترة من شباط إلى حزيران 2020. قسمت حيوانات التجربة عشوائيا إلى ثلاث مجاميع متساوية ضمت كل مجموعة 10 حيوانات. عولجت المجموعة الأولى بهرمون الميلاطونين على شكل غرز تحت الجلد وبجرعة 90 ملغم / حيوان لمدة 30 يوم ، أما المجموعة الثانية عولمت بالميلاتونين بنفس الجرعة والوقت أعلاه إضافة إلى استخدام البروجسترون (CIDR) في اليوم 20 ولمدة 10 أيام وتركت المجموعة الثالثة بدون معاملة ومثلت مجموعة السيطرة و عزلت الحيوانات لمدة 30 يوما عن الذكور. سجلت نتائج الاستجابة للمعاملات الهرمونية 90%، 100% و 50% للمجاميع الأولى والثانية والثالثة على التوالي مع فارق معنوي بمستوى  $P < 0.01$  يميل لصالح المجاميع المعالجة مقارنة مع مجموعة السيطرة أما فترة الاستجابة فقد كانت 1.33+8.26، 0.97+7.15 و 14.31+160.5 للمجاميع الأولى والثانية والثالثة على التوالي وبفارق معنوي  $P < 0.01$  لصالح المجاميع المعالجة. فيما كانت نسبة التلقيحات اللازمة للحمل بدون فارق معنوي لجميع المجاميع في الدراسة. سجلت نسبة الحمل فارقا معنويا لصالح المجاميع المعالجة مقارنة مع مجموعة السيطرة وكذلك فترة الأيام المفتوحة التي سجلت 6,72+153,6 ، 7,45+142,8 و 19,56+217,3 في المجاميع الأولى والثانية والثالثة على التوالي . وعليه نستنتج من الدراسة الحالية الدور المهم للميلاطونين في تحسين الكفاءة التناسلية في الجاموس العراقي الحلوب عديم الصراف والذي يعاني من حالة خمول المبايض.

الكلمات المفتاحية: جاموس، كفاءة تناسلية، ميلاتونين، بروجسترون.