Medit Vet J, 9(2): 326-332, 2024

Research Article

Evaluation of estrus detection by cervical monitoring and pregnancy rates in ovsynch and co-synch treated Anatolian buffalo heifers

İlktan Baştan[1], Fırat Korkmaz[1], Derya Şahin[2], Seher Şimşek[2], Mehmet Ali Yılmaz[2]

[1]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye
[2]International Center for Livestock Research and Training, Department of Biotechnology, Ankara Burdur, Türkiye

Key Words:
artificial insemination
Bubalus bubalis
estrus response
monitoring of cervix uteri
synchronization

Received : 05 June 2024
Revised : 04 August 2024
Accepted : 04 August 2024
Published : 31 August 2024
Article Code : 1496233

Correspondence:
İ. BAŞTAN
(ibastan@mehmetakif.edu.tr)

ORCID
İ. BAŞTAN : 0000-0001-8155-1960
F. KORKMAZ : 0000-0002-9600-908X
D. ŞAHİN : 0000-0003-1265-3618
S. ŞİMŞEK : 0000-0002-5468-0844
MA. YILMAZ : 0000-0002-4497-2894

ABSTRACT

The aim of this study was to assess the relationship between the occurrence and intensity of estrous expression and the success of artificial insemination in Ovsynch and Co-synch treated Anatolian buffalo heifers, employing a novel approach utilizing smartphone-based endoscopic inspection of the cervix uteri. Twenty-seven Anatolian buffalo heifers (n=27) were utilized for the study. In the Ovsynch synchronization protocol (n=15), GnRH (buserelin acetate, 12 µg) was administered on day 0, PGF2α (cloprostenol, 500 µg) on day 7, and GnRH (buserelin acetate, 12 µg) on day 9, with artificial insemination conducted 16-20 hours after the second GnRH application. For the Co-synch protocol (n=12), GnRH (buserelin acetate, 12 µg) was administered on day 0, PGF2α (cloprostenol, 500 µg) on day 7, and GnRH (buserelin acetate, 12 µg) along with artificial insemination on day 10. At the time of artificial insemination, estrous expression was categorized as intense, moderate, weak, or negative. Pregnancies were confirmed between 40 to 70 days after artificial insemination using either pregnancy-related glycoprotein or ultrasonography examinations.Intense, moderate, weak, and negative estrus intensities were observed at rates of 20%, 20%, 26.7%, and 33.3%, respectively, in the Ovsynch group and 41.7%, 58.3%, 0%, and 0%, respectively, in the Co-synch group ($P<0.05$). Pregnancy rates were 30% and 75% in groups exhibiting solely intense and moderate estrus expressions, respectively, in both the Ovsynch and Co-synch groups ($P<0.05$).In conclusion, utilizing a smartphone-based endoscopy apparatus for capturing images of the cervix uteri could serve as a viable alternative for classifying the intensity of estrus and determining the optimal time for artificial insemination. Moreover, in Anatolian buffalo heifers, it is recommended to employ the Co-synch protocol instead of Ovsynch.

INTRODUCTION

The Anatolian buffalo (*Bubalus bubalis*), reared in Türkiye, has 50 chromosomes, typically has a dark coat color, and originates from the Mediterranean subgroup of river buffalos (Ünal et al., 2020). In recent years, it has been established as a state policy to increase the population of Anatolian buffalo, which fell below 100,000 in 2010, through assisted reproductive technologies. (Bastan et al., 2021). The most important of these reproductive technologies is artificial insemination (AI), which is widely used worldwide. The success of AI is a very parametric phenomenon that is influenced by many factors, with the most important being the accurate timing of AI and the synchronization of estrus (Roelofs et al., 2010). A number of estrus synchronization protocols have been developed in buffaloes and applied. These protocols are based on the administration of gonadotropin-releasing hormone (GnRH), prostaglandin, and progesterone hormones. However, these protocols for synchronizing estrus in buffaloes are based on those developed for cattle (Ambarcıoglu et al., 2023; Pursley et al., 1995).

The duration of the estrus phase in buffaloes exhibits considerable variation, spanning from 5 to 72 hours. Ovulation typically transpires between 26 and 33 hours following the initiation of estrus. Consequently, both the duration of estrus and the timing of ovulation are extended in buffaloes relative to cattle breeds. This variability in estrus and ovulation times may result in suboptimal synchronization outcomes and reduced pregnancy rates in buffaloes, including the Anatolian buffalo (Küçükkebabcı and Aslan, 2002; Purohit and Rao, 2018). Additionally, the identification of estrus in buffaloes poses challenges due to the infrequency of homosexual behaviors such as mounting or standing, as well as the anatomical characteristics of the vulva that make external detection of cervical mucus difficult. This presents a substantial obstacle in pinpointing the optimal timing for artificial insemination (Neglia et al., 2020; Peralta-Torres et al., 2020).

Recent research suggests that the success of artificial insemination in cattle is closely related to the intensity of estrus expression than the synchronization protocol used (Ferraz et al., 2017; Madureira et al., 2021; Saini et al., 2023). Although many studies have shown varying pregnancy outcomes in buffaloes subjected to different synchronization protocols, few have specifically examined the relationship between the intensity of estrus expression and pregnancy outcomes (Akhtar et al., 2013; Baruselli et al., 2010; Du et al., 2021). In addition, the criteria used to define the intensity of estrus expression may vary between studies. In this study, the intensity of estrus

To cite this article:
Baştan İ, Korkmaz F, Şahin D, Şimşek S, Yılmaz MA. Evaluation of estrus detection by cervical monitoring and pregnancy rates in ovsynch and co-synch treated Anatolian buffalo heifers. Medit Vet J. 2024; 9(2): 326-332.

expression was defined by evaluating the visual appearance of estrus symptoms such as cervical mucus, vulvar edema, and vulvar hyperemia. Additionally, a smartphone-based endoscopic inspection of cervix uteri was also used to determine the intensity of estrus. This methodological approach differs from conventional techniques used in previous studies. This study aims to evaluate the relationship between the occurrence and intensity of estrous expression and the success of artificial insemination in Ovsynch and Co-synch treated Anatolian buffalo heifers, using a smartphone-based endoscopic inspection of the cervix uteri.

MATERIALS and METHODS

Animal Material

For this study, 27 Anatolian buffalo heifers (Bubalus bubalis), each at least 22 months old, were selected under field conditions (n = 27). These heifers were identified by the Ministry of Agriculture and Forestry, the General Directorate of Agricultural Research and Policies, and the National Anatolian Buffalo Breeding Project Technical Staff. Comprehensive health examinations were performed before and during the study to ensure the inclusion of only healthy animals.

Synchronization Protocols

The absence of pregnancy in the animals was confirmed using an ultrasonography examination (7,5 MHz Linear prob, Hasvet® 838, Türkiye) before initiation of the synchronization protocol. For the Ovsynch synchronization protocol (n=15), GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, intramuscular [im]) was administered on day 0, PGF2α (cloprostenol, 500 µg, Estrumate® 2 ml, im) on day 7, and GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, im) on day 9, with AI performed 16-20 hours after the second GnRH application (Neglia et al., 2016). For the Co-synch protocol (n=12), GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, im) was administered on day 0, PGF2α (cloprostenol, 500 µg, Estrumate® 2 ml im) on day 7, and GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, im) along with AI application on day 10 (Fig. 1) (Akhtar et al., 2013).

Classification of Estrous Expression

Classification of estrus expression was performed based on vulva edema and vulva hyperemia on external examination. Additionally, the cervix uteri was monitored using a smartphone-based endoscopic inspection apparatus to determine whether the ostium externum uteri was open or closed and the presence of cervical mucus. Estrus intensity was classified as follows: (Baştan 2019; Bulut 2012; Kaurav et al. 2019; Da Silva et al. 2023):

Intense: The presence of vulvar hyperemia, vulvar edema, cervical mucus, and open cervix uteri characterized intense estrus (Fig. 2).

Moderate: Moderate estrus was identified by vulvar hyperemia and vulvar edema, accompanied by open cervix uteri. However, cervical mucus was not observed.

Weak: The vulvar hyperemia and vulvar edema were observed to be mild, with an open cervix uteri noted. However, cervical mucus was not present.

Negative: Negative estrus was characterized by the absence of vulvar hyperemia, vulvar edema, and cervical mucus, along with a closed cervix uteri.

Artificial Insemination (AI)

Following the estrus examination, AI was performed using Anatolian buffalo semen on the buffalo heifers that did not have negative estrus intensity. The study utilized frozen semen in the same straw batch from Anatolian buffalo that had appropriate spermatological values (at least 15 million motile spermatozoa/straw) according to legal regulations (Bastan et al., 2021).

Pregnancy Diagnosis

The pregnancy diagnosis was performed using ultrasonography (7,5 MHz Linear prob, Hasvet® 838, Türkiye) or measuring pregnancy-associated glycoprotein in blood serum with the enzyme-linked immunosorbent assay method using the

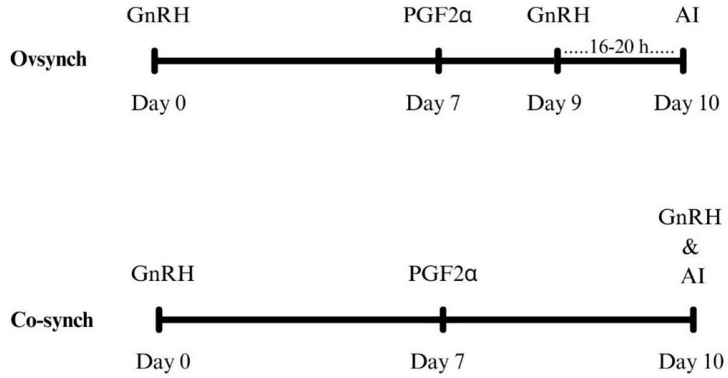

Figure 1. Ovsynch and Co-synch synchronization protocols.
GnRH: Gonadotropin-releasing hormone (buserelin acetate, 12 µg, Receptal® 3 ml), PGF2α: Prostaglandin F2-alpha (cloprostenol, 500 µg, Estrumate® 2 ml), AI: artificial insemination with frozen-thawed semen.

Figure 2. Imaging of the cervix uteri obtained through a smartphone-based endoscopic apparatus in Anatolian buffalo heifers during intense estrus.

IDEXX® Rapid Visual Pregnancy Test kit, 40 to 70 days after artificial insemination. (Arshad et al., 2022).

Statistical Analysis

Descriptive statistics were presented as percentages and numbers for qualitative variables. The relationship between estrus intensity, pregnancy rates, and the synchronization protocols used was analyzed using Fisher's exact probability test. Statistical analyses were conducted using Stata SE 15.1 statistical software, with a significance level of $P < 0.05$.

RESULTS

Table 1 presents the estrus expression of Anatolian buffalo heifers at the time of artificial insemination (AI), along with their corresponding pregnancy rates within 40 to 70 days post-AI, categorized by the synchronization protocol utilized. In the Co-synch group, the highest rate of intense estrus was observed at 41.7%. Meanwhile, buffaloes that exhibited weak and negative intensity of estrus had rates of 26.7% and 33.3%, respectively. These estrus expressions were observed only in the Ovsynch group ($P < 0.05$). The pregnancy rates for buffalo heifers in the Co-synch group were 80% for those with intense

Table 1. Estrus intensities and pregnancy rates of the ovsynch and co-synch groups.

Synchronization protocol	n	Estrus intensity (%)				Number of AIs	Number of pregnancies	P/AI (%)
		Intense	Moderate	Weak	Negative			
Ovsynch	15	20 (3/15)	20 (3/15)	26.7 (4/15)	33.3 (5/15)	10	3	30[a]
Co-synch	12	41.7 (5/12)	58.3 (7/12)	0 (0/12)	0 (0/12)	12	9	75[b]

AI: artificial insemination, P/AI: pregnancy per AI.
a,b Different letters in the same column indicate statistically significant differences ($P < 0.05$).

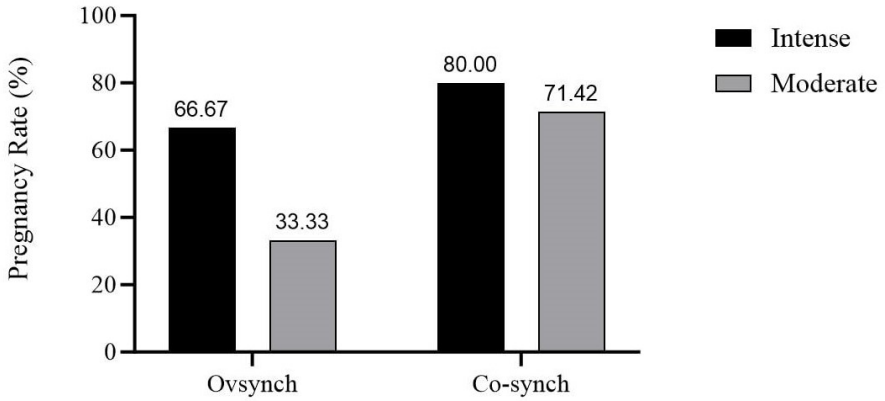

Figure 3. Pregnancy rates of the buffalo heifers with intense and moderate estrus intensities in the Ovsynch and Co-synch group ($P<0.05$).*
*: In the group exhibiting negative estrus, artificial insemination was not performed, and pregnancy was not achieved in the group displaying weak estrus.

estrus and 71.42% for those with moderate estrus (Fig. 3). In contrast, none of the four buffaloes that exhibited a weak estrus response were found to be pregnant in the Ovsynch group, while the pregnancy rates were 66.67% for buffalo heifers with intense estrus and 33.33% for those with moderate estrus. Statistical analysis revealed a significant difference ($P < 0.05$), as depicted in Figure 3.

DISCUSSION

In this study, we detected the occurrence and intensity of estrus using a novel approach that image of the cervix uteri with a smartphone-based endoscopy apparatus. Previous studies have employed various methods to assess estrus occurrence, including the observation of different behaviors (standing to be mounted, mounting, bellowing, walking fence line, increased nervousness and activity, etc), and, in part, the measurement of estradiol (E_2) and progesterone (P_4) concentrations in plasma. Several studies on artificial insemination and even embryo transfer in cattle have shown a positive relationship between the intensity of estrus and pregnancy rates (Burnett et al., 2022; Madureira et al., 2015; Madureira et al., 2022: Tippenhauer et al., 2021) Maduriera et al. (2015) reported that high physical activity during estrus was associated with the success of pregnancy per artificial insemination (P/AI) in Holstein cows.

The study investigated the relationship between estrus intensity, assessed through physical activity, and pregnancy outcomes. Findings revealed that calving ease (unassisted vs. assisted) and postpartum diseases (such as retained fetal membrane, displaced abomasum, and hyperketonemia) had adverse effects on both the intensity and duration of estrus in lactating cows (Madureira et al., 2023). For this reason, it is thought that evaluating estrus intensity through cervix uteri monitoring will provide a more objective assessment of both estrus intensity and conditions such as postpartum cervicitis and metritis, compared to determining estrus findings solely based on behavioral observations or plasma hormone (P_4 and E_2) levels.

There is limited research in the literature on the effects of the occurrence and intensity of estrus expression on the success of AI in buffaloes. Gayke et al. (2022) reported that after the Ovsynch synchronization protocol in buffalo heifers, the rates of intense, intermediate, and weak estrus were 33.3%, 41.7, and 25.00%, respectively, and the pregnancy rate was 33.33%. Sharma et al (2021) conducted a study on the Murrah buffalo and found that the Co-synch synchronization protocol resulted in 33.3% intense, 41.7% moderate, and 25% weak estrus intensities. The progesterone-supported Co-synch protocol resulted in rates of 46.7%, 33.3%, and 20% for intense, moderate, and weak intensities, respectively. These results are consistent with the estrus intensity rates observed in the current study. In Sharma et al. (2021) study, the pregnancy rate was 33.3% in the Co-synch group and 46.6% in the progesterone-supported Co-synch group. It is worth noting that hCG was used in the last stage of their synchronization protocols, unlike in the current study where GnRH was used. Kumar et al. (2016) conducted a study on Murrah buffalo and found that 38.4% exhibited intense estrus, 46.1% exhibited moderate estrus, and 15.3% exhibited weak estrus following the Co-synch protocol. The overall conception rate was 62.5%. During the study, pregnancy outcomes were observed in buffalo heifers that exhibited intense and moderate estrus expressions in both synchronization groups. The relationship between estrus intensity and pregnancy outcomes outlined in these studies aligns with the findings of the current study, despite variations in the behavioral signs used to evaluate estrus expressions. In a study examining the administration of oestradiol benzoate (OEB) on Nili-Ravi buffalo, there was no significant relation between estrous intensity and pregnancy rate (Yousuf et al., 2015). It is thought that this circumstance is due to the weak behavioral signs of estrus in buffaloes.

The efficacy of estrus synchronization protocols is closely related to the dynamics of follicular waves. In contrast to cattle breeds, buffaloes typically exhibit a two-phase pattern of follicular waves. (Jan et al., 2020; Abulaiti et al., 2022; Chaudhari et al., 2022; Manasa et al., 2022). Research on the follicular dynamics of Anatolian buffalo is limited. Additionally, while it is generally observed that Anatolian buffalo exhibit three follicular waves, there is research suggesting that two-phase follicular waves are also common in this breed. (Aksoy et al., 2002; Uçar et al., 2004; Yilmaz et al., 2014; Yilmaz et al.,7 2021). Therefore, their estrus cycle is shorter than that of buffaloes with three follicular waves, supporting the possibility of earlier ovulation after administering a second GnRH in the Ovsynch protocol. For this reason, it is thought that estrus intensity and pregnancy rate were lower in the Ovsynch group. Since biphasic follicular growth is commonly observed in bovine heifers, it is recommended to utilize the Co-synch synchronization protocol either alone or in combination with progesterone hormone supplementation. Pregnancy rates in Ovsynch and Co-synch treated buffaloes are highly variable when estrus findings are not assessed during artificial insemination. Hussein et al. (2016) achieved a 40% pregnancy rate in Egyptian buffalo heifers following the Ovsynch synchronization protocol. Chaikhun et al. (2010) stated that 15% and 42.9% pregnancy rates were obtained from treated Ovsynch swamp buffalo heifers and cows, respectively. In a study conducted by Biradar et al. (2016) on repeat breeder buffaloes aged over 10 years, the Ovsynch group exhibited a 50% pregnancy rate, whereas the Co-synch group showed a 37.5% pregnancy rate.

In the present study, significantly higher pregnancy rates were achieved in the group of Co-synch treated buffalo heifers compared to the Ovsynch group. In the Co-Synch protocol, the second GnRH application is administered during artificial insemination, distinguishing it from the Ovsynch protocol. Literature suggests that in buffaloes, the second follicular wave results in the development of a secondary follicle that is comparable in size to the dominant follicle. Additionally, it has been observed that this secondary follicle can induce simultaneous or interval ovulations with the administration of GnRH stimulation. (Wagas et al., 2016; Neglia et al., 2020). In this context, it is considered that fertilization occurs depending on the quality of the sperm used. Moreover, natural estrus and ovulation periods have been reported to be longer in buffaloes than in cattle (Neglia et al., 2020). Synthetic GnRH analogs have a longer-lasting effect than natural GnRH. On average, natural GnRH release has a plasma half-life of four minutes.

The buserelin acetate preparation utilized in this study exerts an effect that is 20-170 times stronger than natural GnRH. (Kumar et al., 2022). Therefore, the above-average pregnancy per artificial insemination (P/AI) outcomes observed in the Co-synch group could be attributed to the unique characteristics of follicular wave dynamics in Anatolian buffalo heifers and their responsiveness to exogenous GnRH treatments.

CONCLUSION

Presently, Anatolian buffaloes account for only 1% of the total population compared to cattle breeds in Türkiye. This low percentage can be attributed primarily to the difficulties associated with the care, feeding, and reproduction of Anatolian buffaloes, which are often characterized as semi-domesticated. Hence, ovarian dynamics could not be monitored using ultrasonography in this study. In conclusion, however, the study suggests that using a smartphone-based endoscopy apparatus to capture images of the cervix uteri could be a viable alternative to ultrasound applications for classifying the intensity of estrus and determining the optimal time for artificial insemination. Further studies on larger buffalo populations are needed to validate this model. Moreover, to avoid early ovulation in Anatolian buffalo heifers, it is recommended to use the Co-synch protocol instead of Ovsynch.

DECLARATIONS

Ethics Approval

This study was approved by International Center for Livestock Research and Training, Animal Experiments Local Ethics Committee at the meeting dated 20.12.2019 with the number of 66091008/1310 decisions.

Conflict of Interest

The authors have no conflicts of interest.

Author contribution

Idea, concept and design: İB

Data collection and analysis: İB, FK, DŞ, SŞ, MAY

Drafting of the manuscript: İB, FK, DŞ, SŞ

Critical review: İB, FK, DŞ, SŞ

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

Acknowledgments

We would like to thank the Ministry of Agriculture and Forestry, the General Directorate of Agricultural Research and Policies, and the National Anatolian Buffalo Breeding Project Technical Staff for their contribution to this study.

REFERENCES

Abulaiti, A., Riaz, U., Naseer, Z., Ahmed, Z., Hua, G., & Yang, L. (2022). Follicular Dynamics during Estrous Cycle of Pubertal, Mature and Postpartum Crossbred (Nili Ravi Jianghan) Buffaloes. Animals 2022, 12, 1208. https://doi.org/10.3390/ani12091208

Akhtar M. S., Ullah S., Farooq A. A., Mazhar M., & Murtaza S. (2013). Pregnancy rate in lactating buffaloes treated with or without estradiol after estrus synchronization protocols at timed AI. Buffalo Bull, 32, 366-369

Arshad, B., Shabir, A., Sagheer, M., Arshad, U., Yousuf, M. R., & Riaz, A. (2022). Validation of pregnancy associated glycoproteins-based ELISA kits to determine early pregnancy status in lactating Nili-Ravi buffaloes. Reproduction in Domestic Animals, 57(10), 1267-1276. https://doi.org/10.1111/rda.14202

Baruselli, P.S., Carvalho, N.A.T., Gimenes, L.U., & Crepaldi, G.A., (2010). Fixed-Time Artificial Insemination In Buffalo. Italian Journal of Animal Science, 6(2s), 107-118.

Badrakh, D., Yanagawa, Y., Nagano, M., & Katagiri, S. (2020). Effect of seminal plasma infusion into the vagina on the normalization of endometrial epidermal growth factor concentrations and fertility in repeat breeder dairy cows. Journal of Reproduction and Development, 66(2), 149-154. https://doi.org/10.1262/jrd.2019-148

Baştan, İ. (2019). A video-assisted intrauterine artificial insemination device for Anatolian buffalo and local cattle breed. Turkish Patent and Trademark Office. Application Number of Patent: 2019/21012.

Bastan, İ., Korkmaz, F., Şahin, D., Şimşek, S., & Satılmış, M. (2021). First Commercial Semen Cryopreservation and Main Spermatological Features of Anatolian Buffalo. Livestock studies, 61(2), 60-65. https://doi.org/10.46897/livestockstudies.610203

Biradar, S., Tandle, M. K., Suranagi, M. D., Usturge, S. M., Patil, N. A., & Babu, Y. H. (2016). Study on efficacy of cosynch and ovsynch protocols on fertility in repeat breeder buffaloes. Buffalo Bulletin, 35(4), 737-743.

Bulut, G. (2012). The ultrasonographic evaluation of ovarian function and the effects of PRID implementation on fertility parameters in dairy cattle without postpartum reproductive problems. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 52(2), 1-14.

Burnett, T. A., Madureira, A. M., Bauer, J. W., & Cerri, R. L. (2022). Impact of gonadotropin-releasing hormone administration at the time of artificial insemination on conception risk and its association with estrous expression. Journal of Dairy Science, 105(2), 1743-1753. https://doi.org/10.3168/jds.2021-20156

Chaikhun, T., Tharasanit, T., Rattanatep, J., De Rensis, F., & Techakumphu, M. (2010). Fertility of swamp buffalo following the synchronization of ovulation by the sequential administration of GnRH and PGF2alpha combined with fixed-timed artificial insemination. Theriogenology, 74(8), 1371-1376. https://doi.org/10.1016/j.theriogenology.2010.06.007

Chaudhari, D. V., Panchal, M. T., Dhami, A. J., Sarvaiya, N. P., Pathan, M. M., Hadiya, K. K., & Patel, J. A. (2022). Follicular Dynamics and Endocrine Profile during Nor-

mal Estrous Cycle and Early Pregnancy in Surti Buffaloes. Indian Journal of Veterinary Sciences & Biotechnology, 18(5), 1-8.

Da Silva, W. A. L., da Costa e Silva, E. V., Ferreira, M. G. C. R., Gheller, J. M., Poehland, R., & Melo-Sterza, F. D. A. (2023). Effect of shading on the physiological and reproductive parameters in Nelore cows. Tropical Animal Health and Production, 55(6), 374. https://doi.org/10.1007/s11250-023-03774-2

Du, C., Nan, L., Sabek, A., Wang, H., Luo, X., Hua, G., & Zhang, S. (2021). Evaluation of Ovsynch versus modified Ovsynch program on pregnancy rate in water buffaloes: A meta-analysis. Tropical Animal Health and Production, 53, 1-12. https://doi.org/10.1007/s11250-021-02828-7

Ferraz, P. A., Loiola, M. V. G., Rodrigues, A. S., Lima, M. C. C., Bittencourt, T. C. B. D. S. C. D., & Ribeiro, A. D. L. (2017). The effect of the intensity of estrus expression on the follicular diameter and fertility of Nellore cows managed under a FTAI program. Ciência Animal Brasileira, 18. https://doi.org/10.1590/1089-6891v18e-37643

Gayke, P., Razzaque, W. A. A., Patil, A., Ramteke, S. S., Gaikwad, N. Z., Ali, S. S., & Jadhav, S. (2022). Inclusion of Antioxidants in Ovsynch Protocol for Improving Fertility in Pubertal Anestrus Buffalo Heifers. Journal of Animal Research, 12(2), 273-277. 10.30954/2277-940X.02.2022.17

Hussein, H. A., Mohamed, R. H., Hossam, M., & Wehrend, A. (2016). Ovarian response and conception rate following oestrus synchronization using three protocols in Egyptian buffalo heifers. Tierärztliche Praxis Ausgabe G: Großtiere/Nutztiere, 44(05), 287-294. 10.15653/TPG-160214

Jan, M. H., Kumar, H., Kumar, S., Sharma, R. K., Gupta, A., & Mehrara, K. L. (2020). Effect of progesterone administration during growing phase of first dominant follicle on follicular wave pattern in buffalo heifers. Tropical animal health and production, 52, 1395-1402. https://doi.org/10.1007/s11250-019-02143-2

Kaurav, P. S., Shukla, S. P., Bajaj, N. K., Manjhi, V. K., & Thakur, R. K. (2019). Incidence of Postpartum Anoestrus and Oestrus Behavior and Gynecological Symptoms in Hormonal Treated Postpartum Anoestrus Buffaloes. Journal of Animal Research, 9(1), 153-157. 10.30954/2277-940X.01.2019.21

Kumar, L., Phogat, J. B., Pandey, A. K., Phulia, S. K., Kumar, S., & Dalal, J. (2016). Estrus induction and fertility response following different treatment protocols in Murrah buffaloes under field conditions. Veterinary world, 9(12), 1466. 10.14202/vetworld.2016.1466-1470

Küçükkebabçı M., & Aslan S., (2002). Evcil Dişi Mandaların Üreme Özellikleri. Lalahan Hay. Araşt. Enst. Derg. 42(2): 55-63.

Manasa Varra, G. K. V., Ramesh, H. S., Suchitra, B. R., Sudha, G., & Pooja, C. H. (2022). Cellular and molecular pathways associated with ovarian physiology and estrus cycle in buffaloes. The Pharma Innovation Journal.

Madureira, A. M., Burnett, T. A., Borchardt, S., Heuwieser, W., Baes, C. F., Vasconcelos, J. L., & Cerri, R. L. (2021). Plasma concentrations of progesterone in the preceding estrous cycle are associated with the intensity of estrus and fertility of Holstein cows. PLoS One, 16(8), e0248453. https://doi.org/10.1371/journal.pone.0248453

Madureira, A. M. L., Burnett, T. A., Marques, J. C. S., Moore, A. L., Borchardt, S., Heuwieser, W., & Cerri, R. L. A. (2022). Occurrence and greater intensity of estrus in recipient lactating dairy cows improve pregnancy per embryo transfer. Journal of Dairy Science, 105(1), 877-888. https://doi.org/10.3168/jds.2021-20437

Madureira, A. M. L., Silper, B. F., Burnett, T. A., Polsky, L., Cruppe, L. H., Veira, D. M., & Cerri, R. L. A. (2015). Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. Journal of Dairy Science, 98(10), 7003-7014. https://doi.org/10.3168/jds.2015-9672

Neglia, G., de Nicola, D., Esposito, L., Salzano, A., D'Occhio, M. J., & Fatone, G. (2020). Reproductive management in buffalo by artificial insemination. Theriogenology, 150, 166-172. https://doi.org/10.1016/j.theriogenology.2020.01.016

Neglia, G., Gasparrini, B., Salzano, A., Vecchio, D., De Carlo, E., Cimmino, R., & Campanile, G. (2016). Relationship between the ovarian follicular response at the start of an Ovsynch–TAI program and pregnancy outcome in the Mediterranean river buffalo. Theriogenology, 86(9), 2328-2333. https://doi.org/10.1016/j.theriogenology.2016.07.027

Peralta-Torres, J. A., Torres-Chablé, O. M., Segura-Correa, J. C., Ojeda-Robertos, N. F., Chay-Canul, A. J., Luna-Palomera, C., & Aké-Villanueva, J. R. (2020). Ovarian dynamics of buffalo (Bubalus bubalis) synchronized with different hormonal protocols. Tropical Animal Health and Production, 52, 3475-3480. https://doi.org/10.1007/s11250-020-02381-9

Purohit, G. N., & Rao, T. K. (2018). Estrus detection in buffaloes. International Veterinary Information Service, Ithaca NY (www. ivis. org), Last updated.

Pursley, J.,R., Mee, M.,O., & Wiltbank, M.,C., (1995). Synchronization of Ovulation in Dairy Cows Using PGF2 Alpha and GnRH. Theriogenology, 44, 915-923. https://doi.org/10.1016/0093-691X(95)00279-H

Roelofs, J., Lopez-Gatius, F., Hunter, R. H. F., Van Eerdenburg, F. J. C. M., & Hanzen, C. H. (2010). When is a cow in estrus? Clinical and practical aspects. Theriogenology, 74(3), 327-344. https://doi.org/10.1016/j.theriogenology.2010.02.016

Saini, G., Kumar, S., Pandey, A. K., Yadav, V., & Sharma, S. (2023). Intensity of estrus expression–valuable obvious determinant of fertility in Bos indicus cows. Animal Biotechnology, 1-10. https://doi.org/10.1080/10495398.2023.2234961

Sharma, K., Phogat, J. B., Pandey, A. K., Dhaka, A. P., Singh, S., & Ghadwal, S. (2021). Role of progesterone supplementation in estrus induction in Murrah buffaloes under field conditions in non-breeding season. Buffalo Bulletin, 40(1), 99-106.

Tippenhauer, C. M., Plenio, J. L., Madureira, A. M. L., Cerri, R. L. A., Heuwieser, W., & Borchardt, S. (2021). Factors associated with estrous expression and subsequent fertility in lactating dairy cows using automated activity monitoring. Journal of Dairy Science, 104(5), 6267-6282. https://doi.org/10.3168/jds.2020-19578

Uçar, M., Küçükkebapçı, M., Gündoğan M, Uğuz C, & Saban E (2004) Reproductive performance of Anatolian Water Buffaloes during postpartum period. Indian Vet J 81, 784–786.

Ünal, E. Ö., Işık, R., & Soysal, M. İ. (2020). Identification of novel single nucleotide polymorphisms in the growth hormone (GH) gene in Anatolian water buffalo (Bubalus bubalis) populations in Turkey. Revista Brasileira de Zootecnia, 49. https://doi.org/10.37496/rbz4920190260

Yilmaz, O., Yazıcı, E., Kahraman, A., Özenç, E., & Uçar, M. (2014). The relationship between ovarian follicle population and follicle size during different stages estrous cycle in Anatolian Water Buffaloes (Bubalus Bubalis). Revue de Médecine Vétérinairé (165), 111-115.

Yilmaz, O., Yazici, E., & Ucar, M. (2011). Measurements of some genital tract organ size in Anatolian Water buffalos. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 17(6).

Yilmaz, O., Yazici, E., Cicek, H., Celik, H. A., Uyarlar, C., & Ucar, M. (2021). Postpartum uterine involution and ovarian follicular dynamics in crossbreed Anatolian water buffalo (Bubalus bubalis) during summer season. Turkish Journal of Veterinary & Animal Sciences, 45(2), 299-310. 10.3906/vet-2009-26

Yousuf, M. R., Martins, J. P. N., Husnain, A., Riaz, U., Riaz, H., Sattar, A., & Ahmad, N. (2015). Effect of oestradiol benzoate on oestrus intensity and pregnancy rate in CIDR treated anoestrus nulliparous and multiparous buffalo. Animal Reproduction Science, 159, 104-108. https://doi.org/10.1016/j.anireprosci.2015.06.003