International Journal of Agricultural Technology 2021Vol. 17(5):1699-1710
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Conception rate of post-partum dairy water buffaloes (*Bubalus bubalis*) supplemented with progesterone after timed artificial insemination

Ferrer, A. V.[1], Atabay, E. C.[2,3,*], Atabay, E. P.[2], Tadeo, R. D.[2], Apolinario, J. P. R.[2] and Dela Cruz, C. F.[2]

[1]College of Agriculture, Department of Animal Science, Bulacan Agricultural State College, San Ildefonso, Bulacan 3010, Philippines; [2]Reproduction and Physiology Section, Philippine Carabao Center, National Headquarters; [3]Philippine Carabao Center at CLSU, Science City of Muñoz, Nueva Ecija, 3120 Philippines.

Ferrer, A. V., Atabay, E. C., Atabay, E. P., Tadeo, R. D., Apolinario, J. R. and Dela Cruz, C. F. (2021). Conception rate of post-partum dairy water buffaloes (*Bubalus bubalis*) supplemented with progesterone after artificial insemination. International Journal of Agricultural Technology 17(5):1699-1710.

Abstract Progesterone is an endocrine hormone naturally produced by corpus luteum which persist throughout the gestation period to maintain pregnancy. Exogenous progesterone (P4) is generally used as hormonal supplementation for synchronization of ovulation and Fixed Time Artificial Insemination (FTAI). This technique involves the use of Controlled-Intravaginal Drug Release (CIDR) in combination with Ovsynch, an Ovulation Synchronization protocol, thus CIDR-Synch (Treatment 1, Control). The effects of P4 supplementation post-artificial insemination (AI) was investigated either by reinsertion of CIDR on day 5 and its removal on day 15 post-AI (CIDR-Synch+ Reinsertion, Treatment 2) or by administration of injectable P4 on day 5 post-AI (CIDR-Synch+ P4, Treatment 3). Determination of pregnancy through Pregnancy-Associated Glycoprotein Enzyme-Link Immunoassay (PAG-ELISA) test on day 30 and Transrectal Ultrasonography on day 40 post-AI, revealed significantly higher ($P<0.05$) conception rates in Treatments 2 and 3 compared with Treatment 1. In addition, hormone assay performed from days 0 to 10 to determine the levels of P4 during the CIDR-Synch hormone treatment before insemination, yielded no significant differences ($P > 0.05$) among the three Treatments. However, P4 concentrations, on days 15 and 25 post-AI, were significantly higher ($P<0.05$) in groups with supplementations (Treatments 2 and 3), compared with Treatment 1. No significant difference in P4 concentrations was observed between Treatments 2 and 3. The present study demonstrated the beneficial effects of administration of exogenous P4 after AI which improved conception rates in post-partum dairy buffaloes. The supplementation could have enhanced the endogenous P4 level to prepare the uterine environment for subsequent embryonic development and maternal recognition of pregnancy. The results highlight important reproductive innovation that minimizes the incidence of early embryonic loss, resulting in a higher efficiency of Timed Artificial Insemination Program in water buffaloes.

Keywords: Hormone supplementation, Post-artificial insemination, Pregnancy rate, Water buffaloes

[*]**Corresponding Author:** Atabay, E. C.; **Email:** atabay22@yahoo.com

Introduction

Water buffaloes are considered as poor or difficult breeder due to long days open after parturition consequently resulting in long calving interval, a problem that reduces their reproduction and production performance. The onset of estrus and regular cyclicity of estrus cycle and initiation of ovulation during the post-calving period in parturient buffaloes are major problems that result in long postpartum anestrus and delayed breeding with consequent and serious economic losses in milk production and animal reproduction. The problems caught the attention of many researchers and farm managers. Previous results and findings revealed that the most important cause of absence or delay in the post-calving estrous cyclicity, or fertility is the predominant incidence of ovarian inactivity or non-functional ovaries.

Buffalo cows could be considered suffering from ovarian inactivity when structures could not be detected on both ovaries by two ultrasonographic examinations along with two rectal palpations with 10 days' interval between them (Ramoun and Darwish, 2006). High incidence of postpartum ovarian inactivity might be due to, several factors such as plane of nutrition, body condition score at calving, milk yield, parity, calving season and other factors (El-Wishy, 2007).

During the last few years, several studies have been done to address the prolonged postpartum anestrus in buffaloes by using hormonal treatments such as gonadotropin releasing hormone (GnRH), estrogen (E_2), prostaglandin F2α (PGF2α) and progesterone (P4) (Singh, 2003; Metwelly, 2006).

Another major issues and concern in water buffalo production and reproduction is the inherent characteristics of poor expression of estrus, thus it is very difficult to know when is the best time to do artificial insemination or bring the animal for natural breeding.

Majority (99.9%) of the water buffaloes in the Philippines are being taken cared by farmers and artificial insemination (AI) is the common practice to breed the species for upgrading and genetic improvement. To increase the number of female buffaloes to be inseminated at pre-determined time, AI technology is coupled with estrus synchronization (ES) using prostaglandin alone; however, the percent conception is very low compared to the desired efficiency. Recently, Atabay *et al.* (2020) reported an increased conception rate when gonadotropin-releasing hormone (GnRH) or human Chorionic Gonadotropin (hCG) was injected at the time/day of AI in estrous synchronized post-partum dairy buffaloes. The protocol is known as Enhanced AI as this strategy enhanced the occurrence of ovulation following prostaglandin-based estrus synchronization.

Related to the above timed insemination program, Fixed-time artificial insemination (FTAI) was introduced first time in the country, in 2014 primarily to address concerns on hard breeders after repeated insemination failures in post-partum water buffaloes (Atabay *et al.,* 2019). The technique essentially induces not just estrus but also new follicular wave emergence with GnRH, ovulation of new follicle, ensuring more precise timing of AI, leading to an improved pregnancy rate. The FTAI basically involves the ovulation synchronization protocol, Ovsynch, to ensure the production of good quality and competent oocytes for fertilization and development of good quality corpus luteum for the production of P4. The basic Ovsynch protocol was developed to improve the reproductive efficiency in post-partum lactating cows in the US (Pursley *et al.,* 1995). It is performed by hormonal treatment with first GnRH on day 0, $PGF_2\alpha$ on day 7, second GnRH on day 9, and timed performed AI at 14 to 16 h thereafter. Presently numerous modifications have been made to the original Ovsynch protocol. This includes among others the supplementation of Ovsynch with exogenous P4: Controlled Internal Drug Release, called CIDR-Synch protocol.

Water buffaloes exhibit silent heat phenomenon and are known to have short luteal phase compromising production of endogenous P4, ultimately affecting pregnancy. P4 is very important for the subsequent development of embryos in the uterus and for ensuring suitable uterine environment for implantation and recognition of pregnancy. Recently, it has been reported that P4 level is highly correlated with the size of ovulatory follicle at the time of AI. Exogenous progesterone supplementation during Ovsynch hormonal treatment improved follicular growth, oocyte development, ovulation and corpus luteum formation which produces P4.

The initial results of FTAI in water buffaloes provided evidence on the beneficial effects of programmed breeding or timed AI in this species. However, there is a need to developed timed AI protocol which will cover post-AI treatment to ensure good uterine condition during early embryo development and implantation for higher pregnancy rate. Strategic FTAI program can increase the conception rate and overall reproductive efficiency and milk productivity of water buffaloes. The present study was conducted to determine the effects of P4 supplementation post-AI on the conception rate of post-partum dairy buffaloes and to evaluate the blood P4 level before and after the administration of exogenous P4 for its influence on pregnancy.

Materials and methods

The study was conducted at the Philippine Carabao Center, National Headquarters and Gene Pool, Science City of Munoz, Nueva Ecija, from

January, 2019 to September, 2020. All procedures involving the use of animals for scientific research were approved by the Agency's Ethics Committee.

Animal selection

Dairy buffaloes of at least 60 days' post-partum, with body condition score (BCS) of not less than three (Alapati *et al*., 2010), with at least one (1) of the ovaries is equal or greater than two (2) cm in length or width, and with dominant follicle (DF) of not less than 7 mm were selected for the study. Examinations of the ovaries and pregnancy diagnosis were conducted using ultrasound scanner (HS-1600, Honda Electronics Co., Ltd. Japan). Size of DF present in the ovaries were measured at Day 0 of the protocol.

Experimental design

One hundred fifty-five (155) post-partum buffaloes were selected and subjected to fixed-time artificial insemination (FTAI) using Controlled Internal Drug Release + Ovsynch (CIDR-Synch) protocol (Atabay *et al.,* 2019) with minor modification. Briefly, water buffaloes received 2 ml intramuscular injection of gonadotrophin releasing hormone (GnRH, Cystorelin, 100 ug, Merial Ltd., GA, USA) on Day 0, simultaneous with the insertion into the vagina of Controlled Internal Drug Release (CIDR, 1.38g natural progesterone, Pfizer EAZI-Breed, DEC International, NZ. Ltd.). The CIDR inserts were removed on day 7, followed immediately by intramuscular injection of 2 ml prostaglandin (PGF2α, Bioestrovet, 500 mcg; Vetoquinol-Biowet Gorzon, WLKP, Poland). Two ml of human chorionic gonadotrophin (hCG, Chorulon, 10,000 units, Intervet Inc. Summit, NJ 07001, USA) was injected intramuscularly on Day 9. Fourteen to eighteen hours post-hCG injection (Day 10), all experimental animals were artificially inseminated in the morning and followed-up 8 hr after the first insemination. Thereafter, the inseminated animals were randomly assigned into three (3) treatments namely: Treatment 1: Without supplementation after AI (CIDR-Synch, Control); Treatment 2: CIDR reinsertion on Day 5 post-AI and its removal on day 15 post-AI (CIDR-Synch+RI); and Treatment 3: Intramuscular injection of 3 ml progesterone (IP_4, 1000 IU, Vetsfarma Ltd. Jalandhar-144001, India) on day 5 post-AI (CIDR-Synch+P_4 Injection). The study was replicated four times.

Artificial insemination

Animals were inseminated on day 10 with frozen-thawed buffalo semen from bulls with proven fertility and was done by two (2) selected AI

technicians of the Center. Presence of mucus discharge, tonicity of the uterine horn, and size of the dominant follicle were determined at the time of AI.

Pregnancy Associated Glycoproteins (PAGs) Assay

Early detection of pregnancy was conducted at Day 30 post-AI using Pregnancy Associated Glycoproteins (PAGs) assay. Blood samples were collected from inseminated animals via the jugular vein and placed into a vacutainer tube containing Heparin. Plasma was separated by centrifugation at 1500 rpm for 20 minutes, processed immediately and were stored at -20 ℃ until assay using a PAG-ELISA test kit (BioPRYN®, BioTracking).

Transrectal ultrasonography

Pregnancy was confirmed at Day 40 post AI using ultrasound scanner (Honda, HS-1600V, Japan) equipped with 7.5 MHz linear array transducer designed for intra-rectal placement. Pregnancy status was determined following the criteria described by Fricke *et al.* (2016).

Progesterone extraction

In a subset of 12 animals per Treatment, blood sample collection (10 ml) was done at days 0,7,9,15 and 25 for P4 assay. Blood samples were collected through jugular vein and were placed in a tube containing Heparin. Samples were centrifuged for 20 mins at 2000 rpm under room temperature. Plasma samples were then collected and stored in microcentrifuge tubes at -20 ℃ until analysis.

The extraction protocol was adapted from the method devised by Dr. Yojiro Yanagawa of Hokkaido University. Serum (0.5 ml) sample was pipetted into glass tube (A), added with diethyl ether and mixed for 15 minutes and then submerged into an acetone dry ice bath**.** When distinct solid portion were formed on the bottom of the tube**,** the remaining ether was decanted into another tube (B) and were left to evaporate while the solid portions were left to thaw. Tube B contained the hormone, once all the liquid has evaporated, the tubes containing progesterone were covered and kept at -20 ˚C.

Progesterone assay

Standards and samples were pipetted (20 µl) to wells and 120 µl of assay buffer to "blank" well. 100 µl of anti-body solution was dispensed on all

wells except blank; followed by 100 µl of HRP labeled hormone for all the wells. The samples were incubated for 16 to 18 hrs at 4℃. All the solutions from the wells were discarded and each plate was washed four times. Thereafter, 150 µl of substrate solution was dispensed into all wells and plate was incubated at 37 ℃ for 40 min. Finally, 50 µl of stop solution was added and plate was left to stand for 5 minutes. Absorbance (optical density) was read at 450 nm using a microplate reader.

Statistical analysis

The gathered data were analyzed by Analysis of Variance followed by Tukeys-Kramer's HSD as *post hoc test*. Data were presented as means ± SD. Analyses were performed using JMP Statistical Software (Version 11.1.1 SAS Institute, Inc., Cary, NC, USA). The minimum level of significance was set at $P < 0.05$.

Results

Conception rate after treatments

Pregnancy rates of water buffaloes subjected to FTAI with or without exogenous P4 supplementation post-AI are presented in Table 1. The study showed percent conception of 42.31%, 47.06% and 48.08% for treatment 1, 2 and 3, respectively, and Treatments 2 and 3 are significantly higher ($P < 0.05$) compared with Treatment 1. Meanwhile, there is no significant difference ($P < 0.05$) in conception rates between treatments 2 and 3. It is worthy to mention that the signs of estrus at the time of AI were observed in all experimental animals under the three treatment groups.

Table 1. Percent conception of post-partum water buffaloes with or without exogenous P4 supplementation after artificial insemination

Treatment	Number of animals used	Number of animals observed in estrus	Number of animals diagnosed pregnant	% Conception ±SD
CIDR-Synch	52	52 (100 %)	22	42.31 ± 8.60[b]
CIDR-Synch+RI	51	51 (100 %)	24	47.06 ± 9.15[a]
CIDR-Synch+P_4	52	52 (100 %)	25	48.08 ± 9.23[a]

Progesterone level

Twelve buffaloes randomly selected from each treatment for P4 level at Days 0, 7, 10, 15 and 25 showed no significant differences from days 0 to 10 indicating that all experimental animals exhibited similar responses and P4 trends during these days of hormonal treatment prior to insemination (Table 2). Increase in the P4 concentrations (> 1.0 ng/mL) from day 0 to day 7 were observed but did not differ significantly among the treatment groups. The noticeable decrease in P4 concentrations (<1.0 ng/mL) on day 10 is attributed to the removal of CIDR insert and injection of luteolytic prostaglandin (PGF2α) on day 7. On day 15, progesterone level started to increase again in all treatments indicating that all animals were at the luteal phase following ovulation, and CL was actively producing P4 around this period. Onward, marked increase in P4 concentration were observed on day 25 as compared with those on day 15 post-AI. Moreover, it is interesting to note that P4 levels in treatments 2 and 3 are significantly higher ($P < 0.05$) than that of Treatment 1. P4 concentrations between Treatments 2 and 3 are not significantly different.

Table 2. P4 concentration in post-partum dairy buffaloes supplemented with exogenous P4 post artificial insemination

Treatments	**N**	**Mean P4 level (ng/ml)**				
		Day 0 ± SD	Day 7 ± SD	Day 10 ±SD	Day 15 ± SD	Day 25 ± SD
CIDR-Synch	12	1.02 ± 0.12^{aB}	1.51 ± 0.26^{aA}	0.27 ± 0.11^{aD}	0.63 ± 0.13^{bC}	1.80 ± 0.14^{bA}
CIDR-Synch +RI	12	1.13 ± 0.31^{aB}	1.76 ± 0.27^{aA}	0.27 ± 0.11^{aD}	0.74 ± 0.11^{aC}	2.00 ± 0.21^{aA}
CIDR-Synch +P_4	12	1.16 ± 0.29^{aC}	1.66 ± 0.15^{aB}	0.29 ± 0.15^{aD}	0.76 ± 0.08^{aD}	2.20 ± 0.47^{aA}

[a,b] Values (Mean ±SD) with different superscripts within a column are significantly different ($P < 0.05$)

[A,B,C] Values (Mean ±SD) with different superscripts within a row are significantly different ($P < 0.05$)

Discussion

The main purpose of this study was evaluated the effects of P4 supplementation on the conception rates following FTAI in post-partum dairy buffaloes. The present study provided evidences that exogenous P4 either from CIDR insert or injectable commercial P4 given after insemination, improved

conception rates in dairy buffaloes. P4 supplementation post-insemination clearly demonstrated higher pregnancy outcome over the control group without supplementation. The present study conforms with previous findings that inducing exogenous P4 using progesterone-releasing intravaginal devices or progesterone injection results in the improvement in fertility (Wiltbank *et al.,* 2014) and pregnancy rates (Larson *et al.,* 2007) in cows. It is perceived that P4 supplementation could have provided more conducive environment for the subsequent development of the embryos until implantation and recognition of pregnancy resulting in better conception rates. Moreover, circulation of P4 during early pregnancy stage was reported to have supported conceptus development, conceptus attachment, and prevented early embryonic loss in beef cattle (Garrett *et al.,* 1988). Meanwhile, results from several studies on supplemental P4 were found widely varied, with some showing increase in pregnancy rates (Macmillan and Peterson, 1993; Forde *et al.,* 2011), some demonstrating no effect (Arndt *et al.,* 2009), while others ended up in decreased pregnancy rates (Van Cleeff *et al.,* 1996). Furthermore, most of the studies on P4 supplementation with increased pregnancy rate were noted in lactating cattle; while the treatment effect of treatment was not observed much in heifers. Furthermore, differing methods and duration of supplementation post-AI could have affected the results and response of cows receiving P4 treatments in the present study. Proper selection of animals and achieving perfect timing of insemination are important points to consider in order to achieve high conception rates from exogenous P4 supplementation in dairy cows.

Mounting body of evidence supports the idea that P4 plays a significant role in maintaining pregnancy by stimulating the production of different endometrial secretions necessarily for successful embryonic development (Nephew *et al.,* 1991; Bisinotto *et al.,* 2015). Several studies conducted showed that higher P4 concentration induced changes in uterine gene expression for the subsequent development of the embryos that resulted in high conception rate (Forde *et al.,* 2011). On the contrary, insufficiency of circulating P4 concentration caused embryonic loss leading to low conception rate in cattle (Mann and Lamming, 1999).

On the comparison of P4 levels among treatments in the present study, a gradual increase of P4 concentration observed from Day 0 to Day 7 is attributed to the corpus luteum formation following follicular ovulation with 1st GnRH treatment on day 0. On the contrary, the remarkable decrease of P4 level noted in day 10 in all groups is attributed to the removal of exogenous P4 source, CIDR and the action of prostaglandin injected on Day 7. This decline level of P4 near the time of AI is due to the luteolysis which was preceded by the loss of the capacity of corpus luteum to synthesize and secrete P4. Inadequate

luteolysis can result in an elevation in circulating P4 near AI and reduction in fertility (Souza *et al.,* 2007; Brusveen *et al.,* 2008; Giordano *et al.,* 2012). Subsequently, significant increase of P4 level was noticeable from day 15 until day 25 post-AI, with Treatments 2 and 3 revealing significantly higher P4 levels than the control group, without P4 supplementation. The P4 rise after estrus and AI is attributed to CIDR reinsertion and injectable P4 (Gabriel *et al.,* 2019) complementing the gradual endogenous production of P4 from the CL formed after 2nd GnRH injection. Linear increase in blood circulation of P4 from estrus to days 21 post-AI indicated successful fertilization, embryonic development and great improvements in pregnancy rate (Bakr *et al.,* 2015).

Furthermore, P4 plays various important roles during synchronization of ovulation with CIDR, as source of exogenous P4, simultaneously administered with Ovsynch protocol for FTAI. First is the improvement of oocyte quality, follicular growth, and prevention of premature ovulation improving conception. Timed AI program focuses on the follicular development and the attainment of the right size of preovulatory follicle at the time of AI (Baruselli *et al.,* 2012). Follicle ovulating at the desired diameter (13-14mm) can result in the formation of larger corpus luteum and serves as source of endogenous P4 following AI. However, some protocols may not work as expected, yielding smaller ovulatory follicle with small CL formed resulting in insufficient endogenous P4 production. The present FTAI protocol with exogenous P4 supplementation post-AI, resulted in improved pregnancy outcome. Essentially, the present study underscores the roles P4 generally played in buffalo reproduction; from oocyte development, follicular growth, early embryonic development, and maintenance of pregnancy finally leading to successful development to term of the fetus. In sum, breeding program and FTAI protocols which ensures high P4 levels post-AI support early embryonic development and uterine implantation thus is highly recommended towards greater productivity and profitability from buffalo raising and dairy-based enterprise activities.

Acknowledgements

This research work was supported by the Philippine Carabao Center, Department of Agriculture and Philippine Council for Agricultural, Aquatic and Natural Resource Research and Development, Department of Science and Technology, Philippines. Grateful acknowledgement is accorded to Joselito V. Del Rosario, Rodante V. De Vera and Mike V. Del Rosario for their invaluable help in FTAI and sample collection activities.

References

Alapati, A., Kapa, S. R., Jeepalyam, S., Moorthy, S., Rangappa, P. and Yemireddy, R. (2010). Veterinary Science Development of the body condition score system in Murrah buffaloes : validation through ultrasonic assessment of body fat reserves. Journal of Veterinary Science, 11:1-8.

Atabay, E. P., Atabay, E. C., Maylem, E. R. S., Tilwani, R. C., Flores, E. B. and Sarabia, A. S. (2019). Improved pregnancy in water buffaloes through synchronization of ovulation and fixed time artificial insemination technique. Philippine Journal of Veterinary Medicine, 56:1-9.

Atabay, E. C., Atabay, E. P., Maylem, E. R. S., Encarnacion, E. C and Salazar, R. L. (2020). Enhancing prostaglandin-based estrus synchronization protocol for artificial insemination in water buffaloes. Buffalo Bulletin, 39:53-60.

Arndt, W. J., Holle, A. J., Bauer, M. L., Kirsch, J. D., Schimek, D. E., Odde, K. G. and Vonnahme, K. A. (2009). Effect of post-insemination progesterone supplementation on pregnancy rate in dairy cows. The Canadian Journal of Veterinary Research, 701:271-274.

Bakr, M. M., Noseir, M. B. and Amrawi, G. A. (2015). Effect of Exogenous Progesterone in Treatment of Ovarian Inactivity in the Egyptian Dairy Parturient Buffalo-Cows. 47: Alexandria Journal of Veterinary Sciences, 191-200.

Baruselli, P. S., Sa Filho, M. F., Ferreira, R. M., Sales, J. N. S., Gimenes, L. U., Vieira, L. M., Mendanha, M. F. and Bo., G. A. (2012). Manipulation of follicle development to ensure optimal oocyte quality and conception rates in cattle. Reproduction in Domestic Animals, 47:134-141.

Bisinotto, R. S., Pansani, M. B., Castro, L. O., Narciso, C. D., Sinedino, L. D. P., Martinez, N., Carneiro, P. E., Thatcher, W. W. and Santos, J. E. P. (2015). Theriogenology Effect of progesterone supplementation on fertility responses of lactating dairy cows with corpus luteum at the initiation of the Ovsynch protocol. Theriogenology, 83:257-265.

Brusveen, D. J., Cunha, A. P., Silva, C. D., Cunha, P. M., Sterry, R. A., Silva, E. P. B., Geunther, J. N. and Wiltbank, M. C. (2008). Altering the Time of the Second Gonadotropin-Releasing Hormone Injection and Artificial Insemination (AI) During Ovsynch Affects Pregnancies per AI in Lactating Dairy Cows. Journal of Dairy Science, 91:1044-1052.

El-Wishy, A. B. (2007). The postpartum buffalo: Acyclicity and Anoestrus. Animal Reproduction Science, 97:216-236.

Forde, N., Beltman, M. E., Duffy, G. B., Duffy, P., Mehta, J. P., Gaora, P. O., Roche, J. F., Lonergan, P. and Crowe, M. A. (2011). Changes in the Endometrial Transcriptome During the Bovine Estrous Cycle: Effect of Low Circulating Progesterone and Consequences for Conceptus Elongation. Biology of Reproduction, 84:266-278.

Fricke, P. M., Ricci, A., Giordano, J. O. and Carvalho, P. D. (2016). Methods for and Implementation of Pregnancy Diagnosis in Dairy Cows. Veterinary Clinics of North America: Food Animal Practice, 32:165-180.

Gabriel, R. R. G., Atabay, E. P., Apolinario, J. P. R., Ortiz, J. G. M., Atabay, E. C. and Orden, E. A. (2019). Effects of Different Prostaglandin Analogues During Synchronization of Ovulation in Dairy Buffaloes (*Bubalus Bubalis*). Journal of Veterinary and Animal Science, 45:178-186.

Garrett, J. E., Geisert, R. D., Zavy, M. T. and Morgan, G. L. (1988). Evidence for maternal regulation of early conceptus growth and development in beef cattle. Journal of Reproduction and Fertility, 84:437-446.

Giordano, J. O., Wiltbank, M. C., Guenther, J. N., Pawlisch, R., Bas, S., Cunha, A. P. and Fricke, P. M. (2012). Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. Journal of Dairy Science, 95:639-653.

Larson, S. F., Butler, W. R. and Currie, W. B. (2007). Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. Animal Reproduction Science, 102:172-179.

Macmillan, K. L. and Peterson, A. J. (1993). A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation , increasing pregnancy rates and the treatment of post-partum anoestrus. Animal Reproduction Science, 33:1-25.

Mann, G. E. and Lamming, G. E. (1999). Then Influence of Progesterone During Early Pregnancy in Cattle. Reproduction in Domestic Animals, 34:239-274.

Metwelly, K. K. (2006). Treatment of ovarian inactivity in postpartum buffalo-cows with special reference to its economic evaluation. Assiut Veterinary Medical Journal, 52:214-225.

Nephew, K. P., McClure, K. E., Ott, T., Dubois, D. H., Bazer, F. W. and Pope, W. F. (1991). Relationship between variation in conceptus development and differences in estrous cycle duration in ewes. Biology of Reproduction, 44:536-539.

Pursley, J. R., Mee, M. O. and Wiltbank, M. C. (1995). Synchronization of ovulation in dairy cows using PGF2 and GnRH. Theriogenology, 44:915-23.

Ramoun, A. A. and Darwish, S. A. (2006). Ultrasound guided hormonal treatment of true anestrus in buffalo. Zagazig Veterinary Journal, 34:51-62.

Singh, C. (2003). Response of anestrus rural buffaloes (Bubalus bubalis) to intravaginal progesterone implant and PGF2α injection in summer. Journal of Veterinary Science 4: 137-141.

Souza, A. H., Gumen, A., Silva, E. P. B., Cunha, A. P., Guenther, J. N., Peto, C. M., Caraviello, D. Z. and Wiltbank, M. C. (2007). Supplementation with estradiol-17 beta before the last gonadotropin releasing hormone injection of the ovsynch protocol in lactating dairy cows. Journal of Dairy Science, 90:4623-4634.

Van Cleeff, J. V., Macmillan, K. L., Drost, M., Lucylb, M. C. and Thatcher, W. W. (1996). Effects of Administering Progesterone at Selected Intervals after Insemination of Sychronized Heifers on Pregnancy Rates and Resynchronization of returns service. Theriogenology, 46:1117-1130.

Wiltbank, M. C., Souza, A. H., Carvalho, P. D., Cunha, A. P., Giordano, J. O., Fricke, P. M., Baez, G. M. and Diskin, M. G. (2014). Physiological and practical effects of

progesterone on reproduction in dairy cattle. Animal, 8:70-81.

(Received: 10 March 2021, accepted: 18 August 2021)