Philipp J Vet Anim Sci 2024 50(2):70-79

IMPROVED ARTIFICIAL INSEMINATION EFFICIENCY THROUGH AN OVARIAN STRUCTURE-BASED CONTROLLED BREEDING PROGRAM IN POST-PARTUM BUFFALOES

Eufrocina P. Atabay*, Edwin C. Atabay, Carlito F. Dela Cruz,
Excel Rio S. Maylem, Jhon Paul R. Apolinario, and Roseline D. Tadeo

ABSTRACT

Recently, interest in improving AI efficiency in buffaloes is on ovarian structure-based Timed AI (TAI) and was investigated in the study. In Study 1, a comparison of efficiencies between corpus luteum (CL)- and dominant follicle (DF)-based TAI was performed. Buffaloes (n=143) with CL were treated with Prostaglandin to induce its regression and estrus (T1); while buffaloes (n=157) with DF were injected with Gonadotropin-Releasing Hormone (GnRH) to induce ovulation (T2). In Study 2, a comparison among follicle-based protocols was performed, namely: Ovsynch protocol (n= 152) for T1; Controlled Internal Drug Release-Synch-human-Chorionic Gonadotropin (CIDR-Synch-hCG) protocol (n=167) for T2; and SELECT AI (n=170) for T3. Pregnancy diagnosis was conducted 35-40 days after TAI. Results in Study 1 revealed a significantly higher (P<0.05) pregnancy in follicle-based (33.41%) than in CL-based protocol (26.47%). In Study 2, a significantly higher (P<0.05) pregnancy was observed in CIDR-Synch-hCG (42.61%, T2) than in Ovsynch protocol (35.06%, T1); while SELECT AI (50.53%, T3) significantly differs (P<0.05) from T1 and T2. The study demonstrated that follicle-based- is better than CL-based protocol. While supplementing follicle-based/Ovsynch protocol with progesterone (CIDR) and replacing GnRH with hCG (CIDR-Synch-hCG) improved pregnancy. Moreover, achieving a follicle size (≥12.0 mm) on the day of AI with SELECT AI enhanced pregnancy in water buffaloes.

Keywords: buffaloes, corpus luteum, controlled breeding, ovarian structure, timed insemination

INTRODUCTION

Buffalo reproduction is generally known to be challenging if not difficult due to the extrinsic and intrinsic factors that cause reproductive concerns which limit the full genetic and economic potential of this species (Madan *et al.*, 1996). The major factor is the inherently low number of primordial follicles in buffalo heifers (Danell, 1987) compared with primordial follicles in cattle (Erickson, 1966). In addition, a high incidence of atresia in buffalo follicles (66.7%) was reported (Danell, 1987) compared with that of cattle follicles which is only 50% (Rajakoski, 1960). Moreover, a large number of follicles are lost during

Reproduction and Physiology Section, Philippine Carabao Center - National Headquarters and Genepool, Science City of Muñoz Nueva Ecija (*email: bingay2003@yahoo.com)

the regular estrus cycle of this animal with only one follicle ovulating at the end of the estrus cycle. It must be understood however, that follicle growth and development is characterized by 2-3 wave-like patterns that involve follicular recruitment, selection, and dominance with a single ovulatory follicle generated at the end of the estrus cycle, while a group of subordinate follicles regress towards the end of each wave (Baruselli *et al.*, 1997). Follicle wave emergence during the luteal phase of the estrus cycle is considered non-ovulatory resulting in a high rate of follicular wastage. Moreover, buffaloes give two births every three years, resulting in low overall reproductive efficiency while cattle deliver calves yearly.

Manipulation of ovarian function using reproductive technologies offers strategies to save these rich follicle populations from wastage and maximize their potential for calf production (Baruselli *et al.*, 2018). Exploiting ovarian structures, particularly during the estrus cycle of buffaloes has been an effective approach to elicit ovarian response. More recently, controlling the estrus cycle can be approached with the kind of ovarian structures specifically through corpus luteum at the luteal phase or dominant follicle at a follicular phase of the estrus cycle. Timed Artificial Insemination (TAI) is the widely used reproductive tool to manage controlled breeding in cattle and buffaloes. It normally involves a combination of hormones to induce physiological events during the estrus cycle. Ovarian response and dynamics by manipulating CL with Prostaglandin have been long used in cattle (Odde *et al.*, 1990) and in water buffaloes (Atabay *et al.*, 2020). Controlling ovarian activity through follicular manipulation by injecting GnRH twice, called the Ovsynch Protocol, was only started in 1995 as a procedure to restore cyclicity of the dairy cattle (Pursley *et al.*, 1995). This original ovulation synchronization protocol has been used subsequently in buffaloes with modifications for better efficiency (Baruselli *et al.*, 2018: Neglia *et al.*, 2003). The major improvement was the use of exogenous progesterone, a Controlled Internal Drug Release Device (CIDR) simultaneously given with 1st GnRH on Day 0, thus the development of CIDR-Ovsynch protocol (De Rensis *et al.*, 2005). This modification is known to promote more synchronous ovulation and enhance the developmental competence of oocytes improving pregnancy. Further modification of the CIDR-Ovsynch protocol by using hCG instead of 2nd GnRH resulted in the development of a highly efficient CIDR-Synch-hCG protocol in dairy buffaloes (Atabay *et al.*, 2020). More recently, a follicle-based TAI protocol (SELECT AI) which involved achieving an optimal size of pre-ovulatory follicle at the time of insemination constitutes an interesting innovation in various timed AI protocols in buffaloes (Pandey *et al.*, 2018). Ovarian structure-based TAI program can maximize reproductive performance and provide options for more efficient reproductive management in water buffaloes and other livestock species.

The present study aimed to explore an ovarian structure-based TAI that can harness the full reproductive potential of water buffaloes by either luteal or follicular control during the estrus cycle. Specifically, pregnancy rates were compared following hormonal treatment with Prostaglandin in the presence of corpus luteum or injection with Gonadotropin-releasing hormone (GnRH) in the presence of dominant follicle/s in the ovaries. Moreover, the physiological events and conditions associated with these ovarian structure-based timed AI protocols influencing TAI efficiencies were elucidated in the present study.

MATERIALS AND METHODS

The present work was conducted at the Philippine Carabao Center, National Gene

Pool, Lomboy Farm, San Jose City, and other Cooperatives in Nueva Ecija from September 2019 to December 2023. Buffaloes were raised in confinement and were given concentrates, forage, and/or rice straws as their regular feed ration. Water was given ad libitum. Buffaloes were used for research following the requirements of the Philippine Animal Welfare Act of 1998 and the proper management system for experimentation of the Agency.

Buffaloes of at least 60 days post-partum with a body condition score (BCS) of not less than three and with at least one of the ovaries equal or greater than two cm and with a dominant follicle size of not less than 7.0 mm were used in the study. Evaluation of BCS was done according to the method described by Alapati *et al.* (2010).

Examinations of the ovaries were conducted using an ultrasound scanner (HS-1600, Honda Electronics Co., Ltd. Japan). Sizes of the dominant follicle (DF) were measured at Day 0 of the hormonal program.

Experimental Design

Study 1. Comparison of AI efficiencies of ovarian structure-based timed AI protocols in water buffaloes. In Treatment 1, post-partum buffaloes (n=143) were subjected to a corpus luteum-based Prostaglandin protocol to regress the CL and induce estrus synchronization for TAI. Briefly, buffaloes with palpable CL and were confirmed not pregnant by ultrasound were injected with Prostaglandin ($PGF_{2\alpha}$, 5 mL, Lutalyze, IM) on Day 0 (Figure 1). Artificial insemination was performed twice on Day 3, in the morning and afternoon, at 8 hours apart. Determination of pregnancy was conducted using ultrasound on Days 35-40 after TAI.

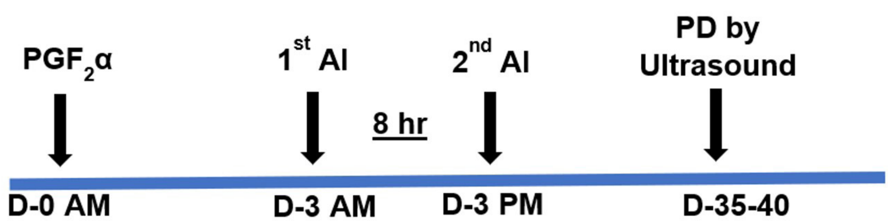

Figure 1. Schematic presentation of corpus luteum-based protocol. Day 0: Injection of $PGF_{2\alpha}$. Day 3: Conduct of 1st and 2nd AI with 8-hour interval. Days 35-40: Pregnancy detection by ultrasonography.

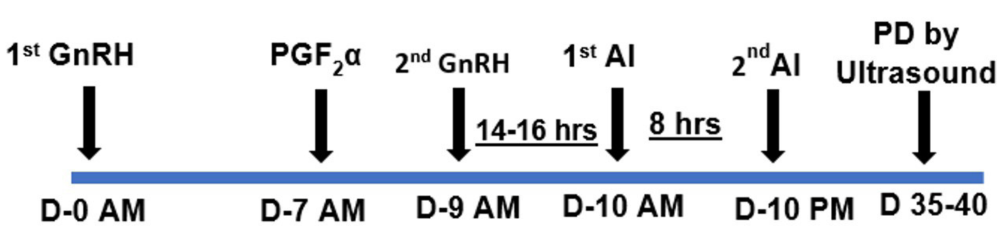

Figure 2. Schematic presentation of follicle-based protocol. Day 0: Injection of 1st GnRH. Day 7: Injection of $PGF_{2\alpha}$. Day 9: Injection of 2nd GnRH. Day 10: conduct of 1st and 2nd TAI. Days 35-40: pregnancy detection by ultrasonography.

In Treatment 2, buffaloes (n=157) were subjected to a follicle-based Ovsynch protocol to induce ovulation for TAI. Briefly, buffaloes with large follicles (7-12.0 mm) upon ultrasound were injected intramuscularly with the 1st GnRH, (Fertagyl, 2ml) on Day 0 and $PGF_2α$ (5 mL, Lutalyze) on Day 7 (Figure 2). Injection of 2nd GnRH was done on Day 9 and the conduct of 1st and 2nd AI were done on Day 10. Pregnancy was determined using transrectal ultrasonography on Days 35-40 after TAI.

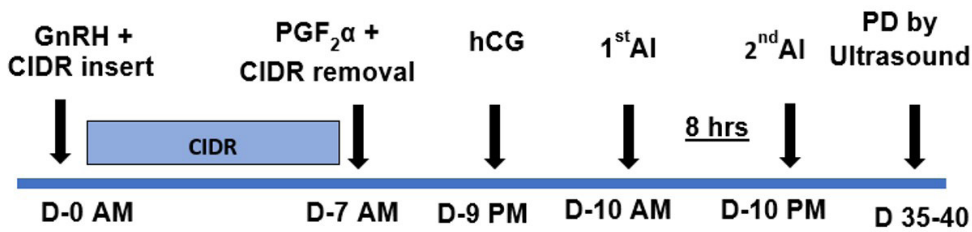

Figure 3. Schematic presentation of follicle-based CIDR-Synch-hCG protocol. Day 0: Injection of GnRH and insertion of CIDR. Day 7: Injection of $PGF_{2α}$ and removal of CIDR. Day 9: Injection of human-Chorionic Gonadotropin (hCG). Day 10: Conduct of 1st and 2nd TAI. Days 35-40: Pregnancy diagnosis using ultrasound machine.

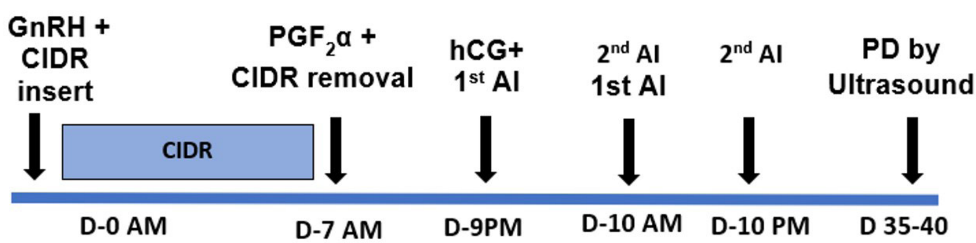

Figure 4. Schematic presentation of follicle-based SELECT AI protocol. Day 0: Injection of GnRH and insertion of CIDR. Day 7: Injection of $PGF_{2α}$ and removal of CIDR. Day 9: Injection of hCG and conduct of 1st AI when follicle size is (≥12.0 mm). Day 10: Conduct of twice TAI on Day 10 at 8 hrs interval in buffaloes with preovulatory follicles (≥12.0 mm). Days 35-40: Pregnancy detection by transrectal ultrasonography.

Study 2. Comparison of AI efficiencies among Follicle-based Time AI protocols in water buffaloes. In Treatment 1 of Study 2, post-partum buffaloes (n=152) with Dominant Follicles were subjected to the Ovsynch protocol described earlier (Figure 2). In Treatment 2, buffaloes (n=167) were subjected to follicle-based CIDR-Synch-hCG protocol to induce ovulation and TAI (Figure 3). Briefly, buffaloes were injected with GnRH (Fertagyl, 2 ml, IM) concomitant with intravaginal insertion of CIDR (Eazi-Breed, 1.38-g progesterone) on Day 0. CIDR removal and intramuscular injection $PGF_2α$ (Lutalyse, 5 mg/ml,) were done on Day 7. Two ml of hCG (Chorulon, 1,000 I.U/ml) was given on Day 9. AI was conducted twice on Day 10 with 8-hour interval. Examination for pregnancy was done using ultrasound on 35-40 Days post-TAI.

In Treatment 3, buffaloes (n=167) were subjected to a follicle-based SELECT AI protocol which is the same as follicle-based CIDR-Synch-hCG but TAI was performed simultaneously with GnRH on Day 9 or Day 10, depending on the size of the pre-ovulatory follicle of ≥12.0 mm (Figure 4). Essentially, only those buffaloes with the said size of the follicle were selected for AI at the end of the TAI program.

Pregnancy Diagnosis

Pregnancy diagnosis was carried out utilizing a transrectal ultrasound scanner (Honda, HS-1600V, Japan) equipped with a 7.5 MHz linear array transducer. The dorsal and lateral surfaces of uterine horns were scanned. Cows were classified as pregnant when corpus luteum (CL), uterine fluid, and a conceptus with a heartbeat were present. The pregnancy rate (PR) was obtained based on the number of pregnant cows divided by all inseminated buffaloes.

Transrectal Ultrasonography

The ultrasound examinations were performed using a transrectal ultrasound scanner (Honda, HS-1600V, Japan) equipped with a 7.5 MHz linear array transducer designed for intra-rectal placement. The scanning of uterine horns was performed on their dorsal and lateral surfaces. Pregnancy status was determined following the criteria described by Fricke *et al.*, (2016) with some modifications. Briefly, the criteria include the presence or absence of CL, uterine fluid, and fetus. Cows were considered pregnant when CL was present and there is presence of both uterine fluid and fetus.

Statistical Analysis

Data were presented as mean±SD with 4 replications in each Study. Data in Study 1 which compared the pregnancy rates between corpus luteum- and follicle-based TIA protocols were analyzed by t-test, while data in Study 2 which compared pregnancy rates among follicle-based TAI protocols were analyzed by One-way Analysis of Variance (ANOVA). When a significant difference was detected between dependent variables, Tukeys-Kramer's HSD was conducted as a *post hoc test*. All 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

Study 1. Comparison of AI efficiencies of ovarian structure-based timed AI protocols in water buffaloes.

The result of the study revealed a significantly higher (P<0.05) pregnancy rate in follicle-based Ovsynch protocol compared with a corpus luteum-based Prostaglandin protocol (Table 1). The ovarian structures namely: the corpus luteum and follicles significantly differ in response in terms of pregnancy rates following hormonal treatment and TAI programs in the present study.

Table 1. Pregnancy rates following ovarian structure-based timed artificial insemination in water buffaloes.

TREATMENTS (OVARIAN STRUCTURE-BASED PROTOCOL)	NO. OF ANIMALS USED	NO. OF ANIMALS PREGNANT	% PREGNANCY
T1 Corpus luteum-based (Prostaglandin)	143	38	26.47 ± 1.56[b]
T2 Follicle-based (GnRH/Ovsynch)	157	53	33.41 ± 3.33[a]

Data were presented as Mean±SD, with 4 replications.
[a,b, c] Values with different superscripts within column are significantly different at P<0.05.

Study 2. Comparison of AI efficiencies among Follicle-based Time AI protocols in water buffaloes

In this study, the AI efficiencies of the follicle-based protocols in terms of pregnancy rates were compared, with the Ovsycnh protocol as control (Table 2). Induction of follicle ovulation with the CIDR-Synch-hCG protocol yielded a significantly higher (P<0.05) pregnancy rate compared with the Ovsynch protocol. Moreover, the follicle-based SELECT AI protocol (T3) achieved a significantly different (P<0.05) pregnancy rate from follicle-based protocols (T1, T2). Select AI protocol resulted in the highest pregnancy rate among the follicle-based protocols.

Table 2. Pregnancy rates following follicle-based timed artificial insemination in water buffaloes.

TREATMENTS (FOLLICLE-BASED PROTOCOLS)	NO. OF ANIMALS USED	NO. OF ANIMALS PREGNANT	% PREGNANCY
T1 Follicle-based (Ovsynch)	152	53	35.06±2.85[c]
T2 Follicle-based (CIDR-Synch-hCG)	167	72	42.61 ± 0.47[b]
T3 Follicle size-based (≥12.0 mm, SELECT AI)	170	86	50.53±3.21[a]

Data were presented as Mean±SD, with 4 replications.
[a,b, c] Values with different superscripts within column are significantly different at P<0.05.

DISCUSSION

A controlled breeding program has been the recent reproductive strategy to exploit the estrus cycle to enhance ovarian response and maximize reproductive efficiency in water buffaloes (Baruselli *et al.*, 2018). Timed AI or fixed time AI programs are widely used reproductive tools to hormonally induce estrus or ovulation depending on the main ovarian structure at any point of the estrus cycle in cattle (Bisinotto *et al.*, 2014) and water buffaloes (Baruselli *et al.*, 1999; Baruselli *et al.*, 2007). The earlier and most common approach to control ovarian function is through the induction of CL regression and estrus synchronization using Prostaglandin (Atabay *et al.*, 2020, Odde *et al.*, 1990). A more recent and popular method of ovarian manipulation is through the induction of ovulation of the dominant follicle using Gonadotropin-releasing hormones (Pursley *et al.*, 1995). The present study compared the efficiencies of ovarian structure-based TAI protocols in buffaloes and aimed to describe physiological mechanisms and events associated with each kind of protocol. Findings in Study 1 revealed a lower pregnancy rate following CL-based estrus induction with $PGF_2\alpha$ compared with DF-based ovulation induction with GnRH. The present result is consistent with the findings that targeting CL alone for its regression without regard to the co-existence of the dominant follicle at the time of Prostaglandin injection may cause untimely or late ovulation and insemination (Atabay *et al.*, 2020). Moreover, in the presence of a DF at Prostaglandin induction, the oocyte inside the follicle could have been aged at ovulation and fertilization with low subsequent developmental competence resulting in a poor conception rate. Prostaglandin induction is found more effective with functional CL, meaning both CL and dominant follicles are present at the initial hormonal injection, achieving more precise timing of AI and improved pregnancy (Pandey *et al.*, 2011).

A major reproductive advancement pursued to address concerns about the timing of AI and oocyte quality was the induction of follicle ovulation with GnRH as the main hormone of the original Ovsynch protocol (Pursley *et al.*, 1995). GnRH is highly effective in inducing ovulation of the Dominant follicle and follicular wave emergence leading to the formation of a new Dominant Follicle with fresh oocyte at insemination, resulting in higher pregnancy outcome compared with CL regression by Prostaglandin in the present study. Furthermore, supplementation of the Ovsycnh protocol with CIDR at Day 0, as an exogenous progesterone source and replacing GnRH with hCG as the final ovulatory hormone improved the pregnancy rate following follicle-based CIDR-Synch-hCG protocol. The present result conforms with that of Sá Filho *et al.* (2010) wherein supplementation of exogenous progesterone (CIDR), prevented pre-mature ovulation and promoted more synchronous ovulation and timely insemination. On the other hand, replacing 2nd GnRH with hCG was reported to be more effective as a final ovulatory hormone than GnRH in the original Ovsynch protocol (Atabay *et al.*, 2020). HCG was reported to be more potent and act directly on the ovulation of follicles in the ovary, compared to GnRH which has yet to pass through the hypothalamus and anterior pituitary gland to release LH before it can act in the ovary to cause follicle ovulation (Baruselli *et al.*,1999).

The most recent innovation from the modifications of the follicle-based timed insemination is focused on the size of the pre-ovulatory follicle (POF) on the Day of insemination, wherein a follicle size-based TAI protocol, SELECT AI has been established in our present Study. Under this protocol only those animals with POF size of $\geq$ 12.0 mm diameter were inseminated, achieving the highest pregnancy rate so far (45-55%) from the current TAI Program in buffaloes. Limited works in this area are reported in cattle (Sá Filho *et al.*, 2010; Perry *et al.*, 2007) and in buffaloes (Pandey *et al.*, 2011; Pandey *et al.,* 2018; Neglia *et al.*, 2003). Achieving the optimum POF size before insemination is important since a small ovulatory follicle may result in poor hormonal concentrations and uterine environment that can compromise embryonic/fetal development, while too big POF may contain aged oocytes with less fertilization competence and result in failure of developmental progression (Lopes *et al.*, 2007, Vasconcelos *et al.*, 2001). Optimizing follicle sizes during TAI and their association with pregnancy are being pursued to reduce pregnancy loss and improve pregnancy not only in buffaloes but also in cattle species.

CONCLUSION AND RECOMMENDATION

The present study demonstrated efficiencies of TAI protocols wherein the hormonal programs involved are mainly based on target structures in the ovary to achieve better ovarian response and pregnancy outcome. The follicle/GnRH-based protocol is found to be superior in efficacy than the corpus luteum/Prostaglandin-based protocol. Moreover, among the Follicle/GnRH-based protocols, supplementing the Ovsynch protocol with exogenous progesterone and using human Chorionic gonadotropin for final ovulation (CIDR-Synch-hCG protocol) improved pregnancy following artificial insemination. Finally, the follicle size-based Select AI is found to be the most effective TAI protocol. The pre-antral follicle size of 12.0 mm has become a common helpful reference to conduct insemination following induced or natural estrus in buffaloes. The importance of pre-ovulatory follicle size during insemination was emphasized in the present study. Further efforts will focus on determining the optimum follicle size to do TAI in buffaloes. Specifically, research exploration will cover the optimum follicle size in adults, heifers, and among breeds of buffaloes, backed up with hormone assay and ultrasonography to enhance the precision of controlled breeding programs which can impact reproductive efficiency not only in water buffaloes but also in other livestock species.

ACKNOWLEDGEMENT

The authors are deeply indebted and grateful to individuals who extended support and various forms of assistance that led to the completion of the work; to DA-PCC for the financial and logistical support, and to DOST-PCAARRD for the financial support on the Project on the use of reproductive biotechnology for dairy production in water buffaloes.

REFERENCES

Alapati A, Kapa SR, Jeepalyam S, Rangappa SM and Yemireddy, KR. 2010. Development of the body condition score system in Murrah buffaloes: validation through ultrasonic assessment of body fat reserves. *J Vet Sci* 11(1):1-8.

Atabay EC, Atabay EP, Maylem ERS, Encarnacion EC and Salazar RL. 2020. Enhancing prostaglandin-based estrus synchronization protocol for artificial insemination in water buffaloes. *Buffalo Bull* 39(1):53-60.

Baruselli PS, Madureira EH, Visintin JA, Barnabe, VH, Barnabe RC and Amaral R. 1999. Timed insemination using synchronization of ovulation in buffalo. *Rev. Bras Reprod Anim* 23:360-362.

Baruselli PS, Mucciolo R, Visintin GA, Viana VC, Arruda RP and Madureira EH. 1997. Ovarian follicular dynamics during the oestrus cycle in buffalo (Bubalus bubalis). *Theriogenology* 47:1531–47.

Baruselli PS, Reis EL, Marquez MO, Nasser LF and Bo GA. 2007. Fixed time insemination in buffaloes. *Ital J Anim Sci* 6(Suppl 2):107-118.

Baruselli PS, Soares JG, Bayeux BM, Silva JCB, Mingoti RD and Carvalho NAT. 2018. Assisted reproductive technologies (ART) in water buffaloes. *Anim Reprod* 15(Suppl 1): 971–983.

Bisinotto RS, Ribeiro ES and Santos JEP. 2014. Synchronization of ovulation for management of reproduction in dairy cows. *Anim* 8(Suppl 1):151-159.

Danell B. 1987. Oestrus behavior, ovarian morphology, and cyclical variation in follicular system and endocrine pattern in water buffalo heifers. *PhD Thesis*. Swedish University of Agriculture Sciences Uppsala.

De Rensis F, Ronci G, Guarneri P, Nguyen BX, Presicce GA, Huszenicza G and Scaramuzzi RJ. 2005. Conception rate after fixed time insemination following ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (*Bubalus bubalis*). *Theriogenology* 63(7):1824–1831.

Erickson BH. 1966. Development and senescence of the postnatal bovine ovary. *J Anim Sci* 25(3):800-805.

Fricke PM, Ricci A, Giordano JO, and Carvalho PD. 2016. Methods for implementation of pregnancy diagnosis in dairy cows. *Vet Clin North Am Food Anim Pract* 32(1):165-180.

Lopes AS, Butler ST, Gilbert RO and Butler WR. 2007. Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. *Anim Reprod Sci* 99(1-2):34-43.

Madan ML. 1996. Application of reproductive technology to buffaloes. *Anim Reprod Sci* 42:435-446.

Neglia G, Gasparrini B, Di Palo R, De Rosa C, Zicarelli L and Campanile G. 2003. Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean buffalo cows. *Theriogenology* 60(1):125-133.

Odde KG. 1990. A review of synchronization of estrus in postpartum cattle. *J Anim Sci* 68(3):817-830.

Pandey AK, Ghuma SPS, Dhaliwal GS, Honparkhe M, Phogat JB and Kumar S. 2018. Effects of preovulatory follicle size on estradiol concentrations, corpus luteum diameter, progesterone concentrations and subsequent pregnancy rate in buffalo cows (*Bubalus bubalis*). *Theriogenology* 107:57-62.

Pandey AK, Ghuman SPS, Dhaliwal GS, and Agarwal SK. 2011. Impact of pre-ovulatory follicle diameter on plasma estradiol, subsequent luteal profiles, and conception rate in buffalo (*Bubalus bubalis*). *Anim Reprod Sci* 123(3-4):169-174.

Perry GA, Smith MF, Roberts AJ, MacNeil MD and Geary TW. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J Anim Sci* 85(3):684–689.

Pursley JR, Mee MO and Wiltbank MC. 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology* 44(7):915-923.

Rajakoski E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical, end left-right variations. *Acta Endocrinol Suppl (Copenh)* 34(Suppl 52):1-68.

Sá Filho MF, Crespilho AM, Santos JEP, Perry GA and Baruselli PS. 2010. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. *Anim Reprod Sci* 120(1-4):23-30.

Vasconcelos JL, Sartori R, Oliveira HN, Guenther JG and Wiltbank MC. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenolog*y 56(2):307-314.