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Research article

Relationship of follicle sizes and estrous manifestations with pregnancy in water buffaloes under two fixed-time artificial insemination protocols

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Abstract

The study determined the association of follicle sizes and estrous manifestations with pregnancy of the two Fixed Time Artificial Insemination (FTAI) protocols and evaluated the factors that can influence pregnancy outcome in water buffaloes. A total of 285 riverine buffaloes were subjected to either Controlled Internal Drug Release-Synch-human Chorionic Gonadotropin (CIDR-Synch-hCG) or Select AI Protocol. For CIDR-Synch-hCG protocol, buffaloes were administered with GnRH with insertion of CIDR on Day 0. Prostaglandin (PGF2α) was given on Day 7 with the removal of CIDR. The hCG was given on Day 9 and AI was performed on Day 10. For Select AI protocol, the procedure was similar to CIDR-Synch-hCG except that insemination was done either on Day 9 or Day 10 based on the size of the pre-ovulatory follicle. Follicle sizes were taken using ultrasound on Days 0, 7, 9 and 10 and estrous signs were taken on the Day of AI. Pregnancy diagnosis by ultrasound was done around Day 40 post-AI. Findings indicated that there is a significant correlation between follicle size on the day of AI, estrous signs, and FTAI protocol with pregnancy. Select AI protocol resulted in a significantly higher ($P<0.05$) pregnancy rate (55.26%) compared to the CIDR-Synch group (44.71%). Logistic regression analysis likewise revealed that Select AI protocol, pre-ovulatory follicle size on the day of AI, and estrous signs are significant factors on the success of FTAI program in water buffaloes. The present findings provide valuable information for optimizing reproductive success not only in water buffaloes but in other livestock as well.

Keywords: Buffaloes, Estrous signs, Fixed time AI protocols, Follicle size, Pregnancy

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INTRODUCTION

Water buffaloes are one of the most valuable livestock in many buffalo raising countries however, their reproductive efficiency is decreased due to various intrinsic and extrinsic factors which made buffalo raising a very challenging endeavor (Madan et al., 1996; Singh et al., 2015). In recent years, advancements in assisted reproductive technology particularly in Artificial insemination (AI) have played crucial role in the enhancement of breeding methods and the total reproductive performance of these animals. Estrus synchronization had long been widely used to maximize the use of AI technology in livestock species, but in 1995 ovulation synchronization was initiated in dairy cattle with the development of first ovulation synchronization protocol known as Ovsynch with Fixed-Time Artificial Insemination (FTAI) as part of this 10-Day Program (Pursley et al., 1995). Ovsynch is generally characterized by injection of 1st GnRH on Day 0, injection of Prostaglandin on Day 7, injection of 2nd GnRH on Day 9 with AI fixed on Day 10. Consequently, Ovsynch and FTAI have become interchangeable and become popular because of its capacity to coordinate breeding events and speed up artificial insemination program outcome (Bisinotto et al., 2014; Wiltbank and Pursley, 2014; Atabay et al., 2019).

Synchronization of ovulation by Ovsynch has been through enormous improvement particularly in water buffaloes since its initial adoption in this species (Baruselli et al., 1999). CIDR-Synch-hCG FTAI is one of the major modifications of the Ovsynch protocol which involved the incorporation of exogenous progesterone (CIDR) simultaneously with the induction of 1st GnRH on Day 0 and replacement of the 2nd GnRH by human Chorionic Gonadotropin (GnRH) as ovulatory hormone on Day 9 enhancing pregnancy in water buffaloes (Atabay et al., 2023). A meta-analysis of various research revealed that supplementing cows with progesterone increased the pregnancy rate by 5 percent (Stevenson et al., 2006; Stevenson, 2016), while using hCG as final ovulatory hormone was reported to improve conception rates in cows (Schmitt et al., 1996; De Rensis et al., 2002).

The physiological events related to CIDR-Synch-hCG protocol is mainly similar with that of the original Ovsych protocol. To describe, the injection of 1st GnRH on Day 0 induces the ovulation of the Dominant Follicle (DF) present in the ovary which is followed by the emergence of the new follicle wave and finally by the formation of the new DF on Day 7. Prostaglandin injection on Day 7 causes the regression of the corpus luteum which was formed from the ovulation of DF on Day 0. The new DF will continue to grow and injection of hCG on Day 9 supports the final maturation of the DF and the oocyte. With CIDR-Synch-hCG protocol, AI is performed 14-16 hrs after hCG injection or on Day 10, on last Day of the FTAI program. Meanwhile, Select AI, which was the proposed modification in the present study, exhibits physiological principles similar with above previous FTAI protocols, but the insemination is not fixed at Day 10 but based on the size of the pre-ovulatory follicles on the Day of AI, either at Day 9 or Day 10 of the FTAI program.

Recently, the importance of ovarian structures at various time points of the FTAI program have been given attention as possible factors influencing pregnancy. Previous studies provided evidence that follicular size at estrus influences the luteal profiles and subsequent pregnancy rates in beef (Baruselli et al., 2007; Busch et al., 2008) and dairy cows (Vasconcelos et al., 2001). Larger follicles on the day of FTAI were found associated with greater display of estrus, ovulation rate, and pregnancy following AI (Sá Filhoa et al., 2010). An optimal size of ovulatory follicles of 11-13.00 mm resulted in positive benefits on pregnancy rate in lactating beef cows (Pfeifer et al., 2012). The above-mentioned studies in cattle clearly indicate that a particular size of follicle during FTAI programs influence ovulation and pregnancy rates; however, such information is scanty and limiting in buffaloes (Pandey et al., 2011; Pandey et al., 2018).

Furthermore, the size of the follicles and estrous manifestations exhibited by FTAI protocols have been considered as fertility factors which increase the probability of a successful pregnancy. Monitoring follicle size by ultrasound can estimate the best time for artificial insemination by tracking follicle growth, ensuring that breeding attempts coincide with the buffalo's most fertile phase (Monteiro et al., 2016). Follicles grow and mature as the estrous cycle advances, with one follicle becoming dominant and finally ovulating. The size of the dominant follicle at ovulation is a strong predictor of fertility. Larger follicles are associated with a higher likelihood of successful ovulation and subsequent pregnancy. Moreover, the follicle size and growth can be determined and be used to estimate the optimal time for insemination while studying mucus discharge and uterine tone changes aids in accurate estrus detection (Monteiro et al., 2016).

Meanwhile, uterine tone is an extremely important factor in facilitating the movement of sperm and boosting the likelihood of fertilization through the process of uterine contractions. Pancarci et al. (2008) found that the degree of uterine tone contraction had a significant impact on the passage and storage of sperm in the oviduct, which in turn influenced the fertilization of eggs. The mucus discharge, which is another important element, is an indication of hormonal shifts occurring within the reproductive system of the buffalo. During the follicular phase, the mucus discharge will become more plentiful, fluid, translucent, and less thick, which will make it easier for sperm to pass through. It is essential to understand these changes in mucus composition to determine when estrus will begin and to ensure that the timing of insemination will be accurate. This way, the buffalo's reproductive efficiency can be maximized to achieve an enhanced pregnancy rate (Galvão et al., 2014; Baruselli et al., 2018).

The dual role of ovarian follicles in reproduction: production of competent oocyte for fertilization and steroidogenic functions during estrus, ovulation and pregnancy must be further explored to achieve the desired efficiencies of timed AI programs. The present study characterized the follicle growth and measurements, and signs of estrus under two Fixed Time AI protocols and evaluated their relationship with the pregnancy in buffaloes. In addition, the study determined the factors that can be used to predict the success of FTAI program in water buffalo species.

MATERIALS AND METHODS

Animal selection

The present study was carried out involving multiparous Murrah-based riverine dairy buffaloes (n =285) which were kept under similar farm management conditions to minimize environmental differences. Buffaloes were selected based on the following conditions: with body condition score greater than 3.0 (Alapati et al., 2010), confirmed not pregnant by ultrasound examination (HS-1600, Honda Electronics Co., Ltd. Japan), and with one or two ovaries with size greater than 1 cm. In addition, the presence of corpus luteum (CL) in the ovary was noted during animal selection to determine ovarian activity and animal cyclicity. All works and procedures involving the use of animals for scientific research were followed in accordance with the requirements for the protection and welfare of animals of Philippine Animal Welfare Act of 1998 and was confirmed and approved by the Institutional Animal Care and Use Committee for its compliance to experimentation requirements of the Philippine Carabao Center, Department of Agriculture, Philippines with IACUC approval number: PCC-IACUC-002-2024.

Conduct of Fixed Time Insemination and Experimental Design

Following selection, qualified buffaloes were randomly assigned to two treatment groups: either Controlled Internal Drug Release-Synch-human Chorionic Gonadotropin (CIDR-Synch-hCG) or Select AI Protocol. For CIDR-Synch-hCG (Figure 1), Controlled-Internal Drug Release (CIDR, 1.38g of micronized natural progesterone, Pfizer EAZI-Breed) was inserted in the vagina and 2 ml Gonadotrophin-Releasing Hormone (GnRH, Cystorelin, 100 ug) was administered intramuscularly on Day 0. CIDR was removed, followed by an intramuscular injection of prostaglandin (PGF2α, 2ml Bioestruvet) on Day 7. Two ml of human Chorionic Gonadotrophin (hCG, Chorulon, 10,000 units) was injected intramuscularly on Day 9. First AI was performed 14-18 to hours after hCG injection, with follow up 8 hrs after on Day 10, using frozen-thawed water buffalo sperm from bulls with proven fertility.

For Select AI protocol (Figure 2), the process is similar with that of CIDR-Synch-hCG, wherein hCG is given on Day 9, except that AI was performed on the same day, Day 9 or on Day 10 when the size of the follicle reached ≥10.0 mm. The follicle sizes were monitored and measured using Ultrasound on Days 0, 7, and on the Day of AI either on Day 9 or Day 10. Presence of mucus discharge (1 = consistency is light sticky and the color is watery, 2 = consistency is sticky and the color is glassy, 3 = consistency is very sticky and the color is glassy). For the uterine tone, the degree was characterized as medium and hard as determined by rectal palpation at the time of artificial insemination. The categorization of estrous signs was based on the manual on artificial insemination for large ruminants of the Philippine Carabao Center (Montes et al., 2019).

Ultrasonography was used to detect pregnancy on Day 40 post-FTAI. The ultrasound examinations were carried out utilizing a transrectal ultrasound scanner (Honda, HS-1600V, Japan) equipped with a 7.5 MHz linear array transducer optimized for intra-rectal placement. The dorsal and lateral surfaces of uterine horns were scanned. Cows were classified as pregnant when Corpus Luteum, uterine fluid, and an embryo with a heartbeat were present. The pregnancy rate (PR) was obtained based on the number of pregnant cows divided by all the cows inseminated.

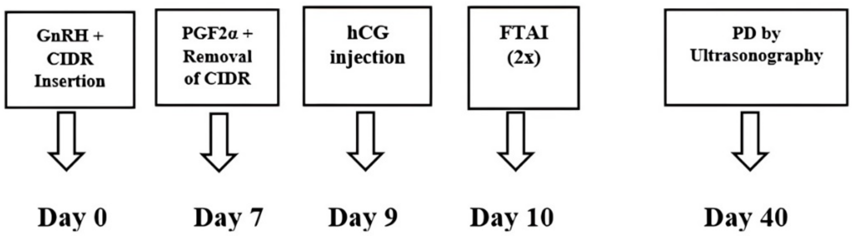

Figure 1 Schematic diagram of CIDR-Synch-hCG Protocol. On Day 0, CIDR was inserted in the vagina and 2 ml Gonadotrophin-Releasing Hormone was administered intramuscularly. On Day 7, CIDR was removed, followed by an intramuscular injection of PGF2α. On Day 9, human-Chorionic Gonadotrophin (2ml) was injected intramuscularly. On Day 10, 1st AI was performed in the morning with follow up in the afternoon, with 8 hr interval. Pregnancy detections were carried out on Day 40 post-AI by ultrasonography.

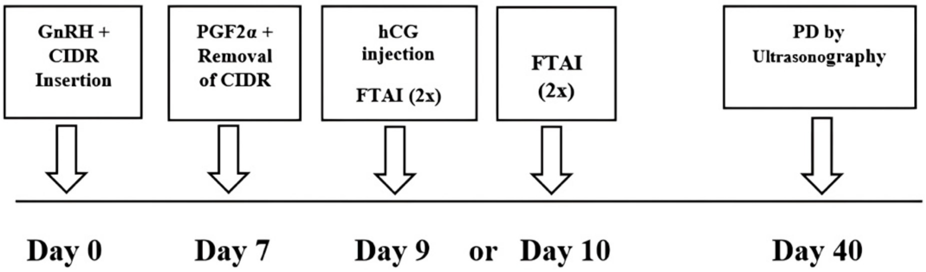

Figure 2 Schematic diagram of Select AI Protocol. On Day 0, CIDR was inserted in the vagina and 2 ml Gonadotrophin-Releasing Hormone was administered intramuscularly. On Day 7, CIDR was removed followed by an intramuscular injection of PGF2α. On Day 9, human-Chorionic Gonadotrophin (hCG, Chorulon, 10,000 units, 2ml) was injected intramuscularly. AI was performed on Day 9 or Day 10 twice, depending on the size of the pre-ovulatory follicle (≥10.00 mm). Pregnancy detections were carried out on Day 40 post-AI by ultrasonography.

Statistical analysis

All statistical analysis was done using R-software (version 4.1.0). Numerical average data on follicle sizes were presented as mean ± SE. The comparison on the average follicle sizes on Days 0, 7, and on the Day of AI and of pregnancy rates between the two FTAI protocols were done by independent sample T-test. The association of the follicle size, mucus discharge, uterine tone and FTAI protocols with pregnancy was determined using Pearson's Chi-Square test. Logistic Regression Analysis was used to determine which among the factors namely: FTAI protocol, follicle sizes, uterine tone, and mucus discharge on the day of AI can influence pregnancy outcome in water buffaloes. All statistical analyses were done using R-software (version 4.1.0). Probability of less than 5% level was considered statistically significant ($P < 0.05$).

RESULTS

Characterization of follicles sizes of buffaloes during conduct of FTAI protocols

The follicle sizes in water buffalo under two FTAI protocols are shown in Table 1. The average follicle sizes on Day 0 under CIDR-Synch-hCG (8.99 mm) and Select AI protocols (9.21 mm) are not significantly different ($P > 0.05$). Similarly, the average follicle sizes under the two protocols on Day 7 are not significantly different ($P > 0.05$). On the other hand, a significant difference ($P < 0.05$) is observed between the two protocols on the Day of AI with Select AI protocol resulting in a mean follicle size of 12.59 mm. This implies that selecting only those animals with large follicle size for insemination can improve pregnancy outcome.

Comparison on the efficiency of the two FTAI protocols showed a significant difference ($P<0.05$) in pregnancy rates between the Select AI and CIDR-Synch-hCG protocols (Table 2). These data imply that implementing Select AI which emphasizes the importance of achieving the desired follicle size before insemination could result in an increased pregnancy rate in water buffaloes.

Table 1 Follicle Sizes in Water Buffaloes under Two FTAI Protocols

Day 0		Day 7		Day of AI	
CIDR-Synch	**Select AI**	**CIDR-Synch-**	**Select AI**	***CIDR-Synch**	****Select AI**
8.99±1.51	9.214±1.78	8.79±1.17	9.83±1.81	11.61±1.79[b]	12.59±1.50[a]

Numerical average data on follicle sizes were presented as mean ± SE.
The alphabets (a, b) in the same row are significantly different with P Value of <0.05 using independent sample t-test
*Insemination was done on Day 10 under CIDR protocol, regardless of the size of pre-ovulatory follicle
**Inseminations were done based on the size of pre-ovulatory follicle, either on Day 9 or Day 10 of the FTAI program

Table 2 Comparison of Pregnancy Rates between CIDR-SYNCH and SELECT AI Protocols in Water Buffaloes

FTAI Protocol	No. of Animals	Pregnancy Rate
SELECT AI	n=185	58.26%[a]
CIDR-SYNCH-hCG	n=115	44.71%[b]

The alphabets (a, b) in the same column differ significantly (P<0.05) using independent sample t-test.

Influence of various factors with pregnancy in water buffaloes

Association of various factors with pregnancy revealed that follicle size during animal selection on Day 0 is not positively correlated with pregnancy (P >0.05), while follicle sizes on Days 7 and Day of AI are correlated with pregnancy (P<0.05), Table 3. In terms of estrous signs, uterine tone and mucus discharge are significantly correlated (P<0.05) with pregnancy. Similarly, FTAI protocols are positively correlated with pregnancy (P<0.05). In sum, the follicle sizes on Day 7, on the Day of AI, estrous manifestations and FTAI potocols influence pregnancy outcomes in water buffaloes in the present study.

Table 3 Association of Follicle Sizes, Estrous signs, and FTAI Protocols with Pregnancy in Water Buffaloes

Factors	Correlation (r)	P value
Follicle size (Day 0)	0.035	P >0.05
Follicle size (Day 7)	0.147*	P < 0.05
Follicle size (Day of AI)	0.275*	P < 0.05
Uterine Tone	0.463*	P < 0.05
Mucus discharge	0.375*	P < 0.05
FTAI protocols	0.133*	P < 0.05

* Significant at 5% level using Pearson's Chi-Square Test

Various factors as predictor variables of pregnancy in water buffaloes

Logistic regression analysis was conducted to determine which among the independent variables can be used in predicting the success of FTAI program. The logistic regression equation model was generated during the statistical analysis (Figure 3). In this equation, the predictor variables were the protocol used (Select AI), the follicle size on the day of AI, the uterine tone (hard) and the mucus discharge (2,3). In addition, the value of the Odd Ratio (OR) which provides possibilities or chances of pregnancy to occur revealed that the above factors exhibited a statistically significant positive association with the pregnancy outcome (Table 4). This suggests that the use of the Select AI protocol, follicle size on the Day of AI, hard uterine tone (Tone 3), and a mucus discharge (MD3) that is glassy and very sticky are good predictors of likelihood of pregnancy following FTAI in water buffaloes.

The logistic regression analysis revealed that the Select AI protocol had an Odds Ratio (OR) of 1.434, which is highly significant (P< 0.001). This means that water buffaloes subjected to the Select AI protocol had 1.434 times higher chance of achieving pregnancy compared to those under CIDR-Synch-hCG. The follicle size on the day of AI also emerged as a significant predictor, with an OR of 1.239 (P < 0.025). This finding indicates that for each millimeter increase in follicle size, there is a 23.9% increase in the odds of pregnancy. Uterine tone of 3 "hard" yielded a highly significant (P < 0.00) OR of 4.397, implying that buffaloes with a hard uterine tone had 4.397 times higher chance of pregnancy. It is interesting to note that no animal subjected to FTAI was observed with Uterine tone of 1 at the time of AI. Only either T2 or T3 were observed in the present study which could be due to efficient hormonal treatment with FTAI. Further, logistic regression analysis revealed that mucus discharge (M3) produced a highly significant (P < 0.001) OR of 3.159, which suggests that buffaloes with M3 mucus discharge had 3.159 times higher odds of achieving pregnancy.

$$logit[\hat{\pi}(x)] = -4.153 + 0.36Protocol_{SELECT-AI} + 0.214Size_{Day_{AI}} + 1.48Tonicity_{Hard} + 0.61Mucus_2 + 1.15Mucus_3$$

Figure 3 Logistic regression equation. The event being predicted in this case is denoted by π̂(x), which represents the probability of a specific outcome occurring given the values of the predictor variables. In this equation, the predictor variables were the protocol used (Select AI), the follicle size on the day of AI, the uterine tone, and the mucus discharge. The uterine tone was labelled as 2-medium, 3-hard, and the mucus discharge as 1-watery and light sticky, 2-glassy and sticky and 3- glassy and very sticky. Each of these variables is assigned a coefficient that represents the effect of that variable on the predicted outcome.

Table 4 Logistic Regression Analysis on various Predictor Variables on Pregnancy Outcome in Water Buffaloes.

Predictor variable	Odds Ratio (OR)	P value*
Protocol (Select AI)	1.434	< 0.001
Follicle size (day of AI)	1.239	<0.025
Uterine tone (hard,3)	4.397	< 0.001
Mucus discharge (M3)	3.159	< 0.001

* Significant at 5% level using Pearson's Chi-Square Test

DISCUSSION

Recent major research interest in ovarian functions is focused on the follicle growth and development during the induction of Timed AI protocols, particularly on the association of follicles sizes and estrous manifestations with pregnancy, and on other factors used as fertility measures in water buffaloes (Pandey et al., 2011; Neglia et al., 2016; Pandey et al., 2018) and other livestock species (Perry et al., 2007; Sá Filho et al., 2010). The present study described the follicle development and pregnancy outcome in two FTAI protocols. In terms of follicle stages and sizes, the average follicle size on Day 0 in both FTAI protocols was observed ranging from 8-9 mm which is generally considered as Dominant Follicle (DF). DF on Day 0 generally ovulates by the injection of 1st GnRH resulting in emergence of new follicle wave and subsequent formation of new DF observed on Day 7. The DF formed at Day 7 is considered as the final ovulatory follicle and its size as pre-ovulatory follicle on the Day of AI can be used to detect as well timely ovulators among the treated buffaloes (Atabay et al., 2023).

The significantly higher average follicle size observed in Select AI protocol compared with CIDR-Synch-hCG protocol could be due to selection of animals with ≥10.0 mm for AI, either on Day 9 or Day 10 of the FTAI program. The control group, CIDR-Synch-hCG; however, simply fixed the time of insemination on Day 10 without regard to the size of the pre-ovulatory follicle. Consequently, higher pregnancy rate was achieved with Select AI protocol which underscores the importance of achieving the desired pre-ovulatory follicle size before insemination. Essentially, select AI is our first work on a follicle size-based FTAI protocol through the present study.

Meanwhile, it is worthy to mention that for buffaloes in natural estrous, the dominant follicle attains a size of 13–15 mm before undergoing spontaneous ovulation (Baruselli et al., 1997a; Neglia et al., 2007). But some can go beyond 18.00 mm and said follicles can be with poor quality and aged oocytes upon ovulation compromising its developmental competence following fertilization (Atabay et al., 2023). Moreover, this large follicle can grow further and become cystic due to insufficient Luteinizing Hormone (LH) concentration. It is for this reason that hormonal induction of ovulation and determining the optimal size of pre-ovulatory follicle at AI are becoming critically important.

Meanwhile, under hormonally induced ovulation program, it was reported that follicles in Murrah × Mediterranean buffalo heifers acquired the capacity to ovulate in response to exogenous LH when they reach a diameter of 8.5–10.0 mm (Gimenes et al., 2008). These follicles sizes are widely considered Large or Dominant Follicles with ovulatory capacity. On the other hand, follicles from multiparous Mediterranean cows with diameters ranging from 4.2 to 13.0 mm ovulated when treated with hCG or GnRH agonist (Campanile et al., 2007; Campanile et al., 2008). This implies that depending on ovulatory hormones used, follicles can be induced to ovulate at a wide range of size, thus there is the need to determine the optimal size in reference to its physiological maturity. Small size can be associated with small corpus luteum with implication on subsequent progesterone production and uterine environment, while large preovulatory follicle can produce aged oocytes with compromised fertilization and subsequent developmental competence.

The association of various factors with pregnancy revealed that follicle sizes on Day 7, on the Day of AI, estrous manifestations, and FTAI protocols are positively correlated with pregnancy. Our result conforms with earlier study in which follicle size on the day of AI was directly related to the ovulation and pregnancy outcomes in buffaloes (Monteiro et al., 2016; Atabay et al., 2023). Moreover, the association has also been reported in *Bos taurus* dairy cows (Vasconcelos et al., 2001; Pereira et al., 2013; Pereira et al., 2014), *Bos taurus* beef heifers and cows (Perry et al., 2005; Perry et al., 2007), and *Bos indicus* beef heifers and cows (Sá Filho et al., 2010; Sá Filho et al., 2011). Related to this, Lopes et al. (2007) and Pfeifer et al. (2009) provided evidence that a large follicle size influences the development of a large corpus luteum (CL), resulting in elevated levels of circulating progesterone. In contrast, follicles that are smaller in size have limited number of granulosa cells which might result in a reduced number of luteal cells after ovulation, leading to a decrease in the concentration of progesterone in the plasma (Smith et al., 1994; Murdoch and Van Kirk, 1998). However, Lynch et al. (2010) did not find any evidence of an association between the follicle size and the progesterone level. Meanwhile, dominant follicles on Day 0 were found not associated with pregnancy in the present study since these were not the final ovulatory follicle that released egg for insemination. These follicles on Day 0, which ovulated following 1st GnRH injection, mainly triggered the emergence of new wave and formation of new DF observed on Day 7, which was the final ovulatory follicle and was found directly influencing pregnancy. Consequently, the association of the dominant follicle size on Day 7 with pregnancy was notable. Atabay et al. (2023) likewise emphasized the importance of the DF formed on Day 7 as indicator of animals that will undergo timely ovulation, therefore facilitating precise timing of insemination which favors pregnancy.

Furthermore, the study revealed a significant link between the estrous manifestations with pregnancy which conforms with the results reported in previous studies. Mucus discharge is a good indicator of the reproductive health and fertility potential of the animal, while uterine tone plays a vital role in facilitating sperm travel and increasing fertilization chances through uterine contractions (Pancarci et al., 2008). Uterine tone of 3, which is positively correlated with pregnancy agrees with previous findings as an indicator of fertility in water buffaloes (Atabay et al., 2023). The measurement of uterine tone serves as a diagnostic assistance to approximate the stage of oestrus. Combining mucus discharge and uterine tone data provides a comprehensive picture of estrus initiation. When these manifestations coincide, it is a significant indication that the animal is prepared to mate. These manifestations are useful tools for both natural and artificial insemination, ensuring that breeding takes place during the ideal fertile window (Bonafos et al., 1995; Pancarci et al., 2008). Finally, FTAI protocols, particularly with Select FTAI protocol, are correlated with pregnancy indicating its direct influence on pregnancy outcome in the present study.

CONCLUSIONS

Based on the present results, it can be concluded that the follicles size increases during the growth and development of ovarian follicles during FTAI program, characterized by the formation of Dominant follicle to pre-ovulatory follicle near ovulation at the time of AI. Select AI protocol which involves insemination of animals with ≥10.00 mm size pre-ovulatory follicle is superior in pregnancy outcome compared to CIDR-Synch-hCG protocol. Follicle sizes on Day 7, and on the Day of AI, uterine tone, mucus discharge, and FTAI protocols are positively associated with pregnancy. Specifically, pre-ovulatory follicle size on the

Day of AI, Uterine tone of 3, Mucus discharge of 3, and Select AI protocol are significant predictors of the likelihood of pregnancy in water buffaloes. The present study advances a follicle size-based reproductive innovation to enhance fertility, productivity, and profitability not only in water buffaloes but also in other livestock species.

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AUTHOR CONTRIBUTIONS

Zeshalyn P. Fajardo prepared the thesis proposal, spearheaded the conduct of the experiment, arranged for data analysis and interpretation, did the initial draft of the manuscript, and helped in its revisions. Eufrocina P. Atabay provided guidance and insights on the experimental design and proper conduct of the study, supervised the overall completion of the work, and did the draft, finalization and revisions of the manuscript for publication. Edwin C. Atabay and Carlito F. Dela Cruz helped in the conceptualization and design of experiments, extended technical assistance in the conduct of activities in the field, and reviewed the final paper. Ma. Elizabeth C. Leoveras served as the thesis adviser, provided guidance and supervision, and ensured that the work was on track as scheduled. Roseline D. Tadeo and Jhon Paul R. Apolinario extended technical support during the development of the thesis proposal and the conduct of the study. All authors made the final review of the manuscript following revision.

CONFLICT OF INTEREST

We hereby declare that there is no conflict of interest with respect to the publication of this manuscript.

REFERENCES

Alapati, A., Kapa, S.R., Jeepalyam, S., Rangappa, S.M., Yemireddy, K.R., 2010. Development of the body condition score system in Murrah buffaloes: validation through ultrasonic assessment of body fat reserves. J. Vet. Sci. 11, 1–8.

Atabay, E.P., Atabay, E.C., Maylem, E.R.S., Tilwani, R.C., Flores, E.B., Sarabia, A.S., 2019. Improved pregnancy in water buffaloes through synchronization of ovulation and fixed time artificial insemination technique. Philipp. J. Vet. Med. 56, 105.

Atabay, E.P., Atabay, E.C., Dela Cruz, C.F., Apolinario, J.P., Maylem, E.R.S., Flores, E.B., 2023. Influence of ovarian follicle sizes and estrous signs on pregnancy following progesterone-based fixed time artificial insemination in water buffaloes. J. Buffalo. Sci. 12, 143-150.

Baruselli, P.S., Soares, J.G., Bayeux, B.M., Silva, J.C.B., Mingoti, R.D., Carvalho, N.A.T., 2018. Assisted reproductive technologies (ART) in water buffaloes. Anim. Reprod. 15, 971–983.

Baruselli, P.S., Reis, E.L., Marquez, M.O., Nasser, L.F., Bo, G.A., 2007. Fixed time insemination in buffaloes. Ital. J. Anim. Sci. 6, 107-118.

Baruselli, P.S., Madureira, E.H., Visintin, J.A., Barnabe, V.H., Barnabe, R.C., Amaral, R., 1999. Timed insemination using synchronization of ovulation in buffalo. Rev. Bras. Reprod. Anim. 23, 360-362.

Bisinotto, R.S., Ribeiro, E.S., Santos, J.E.P., 2014. Synchronization of ovulation for management of reproduction in dairy cows. Anim. 8, 151-159.

Bonafos, L.D., Kot, K., Ginther, O.J., 1995. Physical characteristics of the uterus during the bovine estrous cycle and early pregnancy. Theriogenology. 43(4), 713-721.

Busch, D.C., Atkins, J.A., Bader, J.F., Schafer, D.J., Patterson, D.J., Geary, T.W., 2008. Effect of ovulatory follicle size and expression of estrus on progesterone secretion in beef cows. J. Anim. Sci. 86, 553-563.

Campanile, G., Di Palo, R., Neglia, G., Vecchio, D., Gasparrini, B., Prandi, A., Galiero, G., D'Occhio, M.J., 2007. Corpus luteum function and embryonic mortality in buffaloes treated with a GnRH agonist, hCG and progesterone. Theriogenology. 67, 1393–1398.

Campanile, G., Vecchio, D., Neglia, G., Di Palo, R., Prandi, A., D'Occhio, M.J., 2008. Progesterone and pregnancy status in buffaloes treated with a GnRH agonist. Livestock. Sci. 115, 242–248.

De Rensis, F., Marconi, P., Capelli, T., Gatti, F., Facciolongo, F., Franzini, S., Scaramuzzi, R.J., 2002. Fertility in post-partum dairy cows in winter or summer following estrus synchronization and fixed time AI after the induction of an LH surge with gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG). Theriogenology. 58(9), 1675-1687.

Galvão, K.N., Ribeiro, E.S., Santos, J.E.P., 2014. What is the most cost-effective breeding program for breeding heifers—timed AI, estrous detection, or a combination of both? VM199/VM199, 5/2014. Available online: https://journals.flvc.org/edis/article/view/ 131755

Gimenes, L.U., Sá Filho, M.F., Carvalho, N.A., Torres-Junior, J.R., Souza, A.H., Madureira, E.H., Trinca, L.A., Sartorelli, E.S., Barros, C.M., Carvalho, J.B., Mapletoft, R.J., Baruselli, P.S., 2008. Follicle deviation and ovulatory capacity in Bos indicus heifers. Theriogenology. 69, 852–858.

Lopes, A.S., Butler, S.T., Gilbert, R.O., Butler, W.R., 2007. Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. Anim. Reprod. Sci. 99, 34–43.

Lynch, C.O., Kenny, D.A., Childs, S., Diskin, M.G., 2010. The relationship between periovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. Theriogenology. 73, 190–198.

Madan, M.L., Das, S.K., Palta, P., 1996. Application of reproductive technology to buffaloes. Anim. Reprod. Sci. 42(1), 299-306.

Monteiro, B.M., de Souza, D.C., Vasconcellos, G.S., Corrêa, T.B., Vecchio, D., de Sá Filho, M.F., de Carvalho, N.A., Baruselli, P.S., 2016. Ovarian responses of dairy buffalo cows to timed artificial insemination protocol, using new or used progesterone devices, during the breeding season (autumn-winter). Anim. Sci. J. 87(1), 13–20.

Montes, A.R., Lapitan, J.E., Sarabia, A.S., 2019. Competency-based learning material: Artificial Insemination NC II (Large Ruminants). Philippine Carabao Center, Science City of Munoz, Nueva Ecija.

Murdoch, W.J., Van Kirk, E.A., 1998. Luteal dysfunction in ewes induced to ovulate early in the follicular phase. Endocrinology. 139(8), 3480–3484.

Neglia, G., Gasparrini, B., Salzano, A., Vecchio, D., De Carlo, E., Cimmino, R., Balestrieri, A., D'Occhio, M., 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, 2328-2333.

Pancarci, Ş.M., Öztürkler, Y., Güngö, R.Ö., Kaçar, C., Yildiz, S., Kaya, D., 2008. Use of carazolol at pre-synchronized timed artificial insemination in cows. Acta Vet. Brno. 77, 59-64.

Pandey, A.K., Ghuman, S.P.S., Dhaliwal, G.S., Agarwal, S.K., 2011. Impact of pre-ovulatory follicle diameter on plasma estradiol, subsequent luteal profiles, and conception rate in buffalo (Bubalus bubalis). Anim. Reprod. Sci. 123, 169-174.

Pandey, A.K., Ghuman, S.P.S., Dhaliwal, G.S., Honparkhe, M., Phogat, J.B., 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.

Pereira, M.H., Rodrigues, A.D.P., de Carvalho, R.J., Wiltbank, M.C., Vasconcelos, J.L.M., 2014. Increasing length of an estradiol and progesterone timed artificial insemination protocol decreases pregnancy losses in lactating dairy cows. J. Dairy. Sci. 97, 1454–1464.

Pereira, M.H.C., Sanches, C.P., Guida, T.G., Rodrigues, A.D.P., Aragon, F.L., Veras, M.B., Borges, P.T., Wiltbank, M.C., Vasconcelos, J.L.M., 2013. Timing of prostaglandin F2α treatment in an estrogen-based protocol for timed artificial insemination or timed embryo transfer in lactating dairy cows. J. Dairy. Sci. 96(5), 2837-2846.

Perry, G.A., Smith, M.F., Lucy, M.C., Green, J.A., Parks, T.E., MacNeil, M.D., Roberts, A.J., Geary, T.W., 2005. Relationship between follicle size at insemination and pregnancy success. Proc. Natl. Acad. Sci. USA. 102(14), 5268-5273.

Perry, G.A., Smith, M.F., Roberts, A.J., MacNeil, M.D., Geary, T.W., 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Dairy. Sci. 85, 684–689.

Pfeifer, L.F.M., Leal, S.C.B., Schneider, A., Schmitt, E., Correa, M.N., 2012. Effect of the ovulatory follicle diameter and progesterone concentration on the pregnancy rate of fixed-time inseminated lactating beef cows. R. Bras. Zootec. 41, 1004-1008.

Pfeifer, L.F.M., Mapletoft, R.J., Kastelic, J.P., Small, J.A., Adams, G.P., Dionello, N.J., Singh, J., 2009. Effects of low versus physiologic plasma progesterone concentrations on ovarian follicular development and fertility in beef cattle. Theriogenology. 72, 1237–1250.

Pursley, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology. 44(7), 915-923.

Sá Filho, M.F., Crespilho, A.M., Santos, J.E.P., Perry, G.A., Baruselli, P.S., 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, 23–30.

Sá Filho, M.F., Baldrighi, J.M., Sales, J.N.S., Crepaldi, G.A., Carvalho, J.B.P., Bó, G.A., Baruselli, P.S., 2011. Induction of ovarian follicular wave emergence and ovulation in progestin-based timed artificial insemination protocols for Bos indicus cattle. Anim. Reprod. Sci. 129, 132–139.

Smith, M.F., McIntush, E.W., Smith, G.W., 1994. Mechanisms associated with corpus luteum development. J. Anim. Sci. 72, 1857–1872.

Schmitt E.J-P., Diaz T., Drost M., Fredriksson E.W., Thatcher W.W., 1996. Use of a gonadotropin-releasing hormone or human chorionic gonadotropin for timed insemination in cattle. J. Anim. Sci. 74, 1084-1091.

Stevenson, J.S., 2011. Alternative programs to presynchronize estrous cycles in dairy cattle before a timed artificial insemination program. J. Dairy. Sci. 94, 205-217.

Stevenson, J.S., 2016. Synchronization and artificial insemination strategies in dairy herds. Vet. Clin. North Am. Food Anim. Pract. 32, 349-364.

Vasconcelos, J.L.M., Sartori, R., Oliveira, H.N., Guenther, J.G., Wiltbank, M.C., 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology. 56, 307-314.

Wiltbank, M.C., Pursley, J.R., 2014. The cow as an induced ovulator: Timed AI after synchronization of ovulation. Theriogenology. 81, 170-185.

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