Pregnancy and Embryonic Loss Among Timed Artificial Insemination Protocols in Post-partum Dairy Buffaloes (*Bubalus bubalis*)

Eufrocina P. Atabay[1,*], Edwin C. Atabay[1,2] Carlito F. Dela Cruz[1] and
Excel Rio S. Maylem[1]

[1] *Reproduction and Physiology Section, Philippine Carabao Center, National Headquarters*

[2] *Philippine Carabao Center at CLSU, Science City of Muñoz, Nueva Ecija, 3120, Philippines*

Abstract: The aims of the study were to determine the pregnancies and embryonic losses following Timed Artificial Insemination (TAI) protocols and to generate strategic breeding management for buffaloes. In Study 1, buffaloes in natural estrus were inseminated, serving as Control (T1). In T2, buffaloes were subjected to the Controlled Internal Drug Release-Synch-human Chorionic Gonadotropin (CIDR-Synch-hCG) protocol. In T3, animals were subjected to Prostaglandin protocol, and T4 is similar to T3 except that hCG was supplemented on the Day of AI. In Study 2, buffaloes were subjected to the CIDR-Synch-hCG protocol, and the sizes of pre-ovulatory follicles were measured as the basis for the conduct of TAI. In Study 3, animals were similarly subjected to the CIDR-Synch-hCG protocol at different seasons. Results in Study 1 indicate that the CIDR-Synch-hCG protocol achieved pregnancy rates on Days 30 and 60 post-AI and embryonic loss, which are not significantly different ($P<0.05$) from those of natural estrus (T1), and that the Prostaglandin protocol supplemented with hCG (T4) resulted in significantly higher pregnancies and lower embryonic loss compared with Prostaglandin alone (T3) group. In Study 2, inseminating buffaloes with ≥12.0 mm pre-ovulatory follicle size (T3) yielded the highest pregnancies ($P<0.05$) at Days 30 and 60 with the lowest ($P<0.05$) embryonic loss among follicle size groups. In Study 3, TAI performed during January-March and October-December indicated significantly higher pregnancies ($P<0.05$) with lower embryonic loss ($P<0.05$) compared with the April-June and July-September seasons. In sum, the present study demonstrated the efficiencies of Timed AI protocols, which provided opportunities for their strategic use in breeding programs to achieve greater productivity and profitability from buffalo farming.

Keywords: Embryonic loss, follicle size, pregnancy rate, seasons, Timed Artificial Insemination, water buffaloes.

INTRODUCTION

The demand for milk and milk products in the Philippines has continued to increase over the years, which is being contributed by the growing human population of 119M as well as by an increase in dairy product per capita consumption of 27 kg, according to the Philippine Statistics Authority in 2024. Other challenges and opportunities at hand include the government milk feeding program under Republic Act 11037 for preschoolers and malnourished school children, which accounts for about 60% of local milk production. The country, however, is only 1% sufficient in local milk production, which is against the requirement and largely depends on the importation of various dairy products. Among the factors that contributed to low milk production in the country are a low population inventory of dairy cattle and buffaloes, low average daily milk production, short lactation periods of dairy animals, and a low percentage of cows in the milk line.

Cognizant of the needed development of the local dairy industry, two Agencies of the Department of Agriculture, namely the National Dairy Authority (NDA) and the Philippine Carabao Center (PCC), are mandated to lead the implementation of the National Dairy Development Program in the country. The government implements a dairy herd build-up program to ensure and accelerate the increase in local dairy stocks and local milk production. Activities include importing genetic materials from dairy animals, upgrading existing local animals to dairy breeds, producing replacement stocks through dairy breeding programs like artificial insemination and multiplier farm establishment, and preserving existing stocks. The following approaches are being implemented to hasten herd build-up: 1) stock infusion, particularly for dairy cattle and dairy goats with the target of 20,300 head and 6,750 head, respectively, from 2020-2025; 2) directed backcrossing and sexed semen usage; 3) expansion of artificial insemination services; and rearing of replacement heifers, and a buyback scheme [1, 2].

Specifically, buffalo plays a major role in food sufficiency and sustainability programs, being a major source of protein in terms of milk and meat. However, reproduction in buffalo has remained a huge challenge to many production enthusiasts and buffalo raisers in

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*Address correspondence to this author at the Reproduction and Physiology Section, Philippine Carabao Center, National Headquarters, Philippines;
Email: bingay2003@yahoo.com

the country. The reproductive performance of buffaloes is still affected by the following factors: poor estrus expression, seasonal infertility, delayed sexual maturity, low conception, and low calf production, resulting in long calving intervals, which limits the full reproduction potential of this species [3, 4]. Physiologically, reproductive efficiency is likewise affected by the quality of the sperm and oocytes before and during fertilization, the quality of the corpus luteum produced after ovulation, the condition of the uterine environment to support subsequent development and attachment of the embryos, and the endometrial condition for proper placentation [5]. What is more critical is the high incidence of embryonic or fetal loss, which is the cause of low pregnancy rate and calf drop following breeding/inseminations during non-breeding seasons in summer [6]. In most dairy and beef-developed countries, timed artificial insemination and early detection of pregnancy are incorporated into their breeding program. Not pregnant cows are subjected to early rebreeding programs to reduce the number of open dates and reduce economic loss, thus improving the productivity of the herd [7]. Recently, PCC has developed an intensified reproductive management program as part of the genetic improvement and propagation in water buffaloes [8]. The main reproductive innovation is the Fixed Time Artificial Insemination and the modifications of other timed AI techniques, which have improved the conception rates, particularly at PCC Buffalo National Genepool. However, further research efforts are warranted to address other reproductive concerns and increase the efficiencies of Timed AI technologies for calf production in buffaloes and other dairy and livestock species in general.

Thus, the present study was conducted to determine the efficiencies of Timed Artificial Insemination protocols in terms of pregnancy and incidence of embryonic loss in dairy buffaloes, evaluate the influence of follicle sizes on the day of insemination on the mentioned reproductive parameters, and determine the efficiencies of TAI protocols at various seasons of the year under the local conditions. The present work likewise aimed to define reproductive management strategies, taking into consideration breeding seasonality and defining breeding programs for water buffaloes.

MATERIALS AND METHODS

The study was conducted at various farms and cooperatives of the Philippine Carabao Center, National Headquarters, and Gene Pool, Science City of Munoz, Nueva Ecija, from September 2018 to December 2023. 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 the Philippine Animal Welfare. Act of 1998 and were approved for experimentation by the Ethics Committee of the Philippine Carabao Center, Department of Agriculture.

Selection and Treatment of Experimental Animals

Riverine buffaloes of at least 60 days post-partum with body condition score (BCS) of not less than three (3) and at least one (1) of the ovaries is equal or greater than two (2) cm in length or width, and with dominant follicle size of not less than 7.0 mm in diameter were selected and used in Study 1.

BCS was evaluated according to the method described by Alapati *et al*. [9]. Briefly, a BCS of 1 stands for emaciated animals; a BCS of 2 indicates a dorsal spine that is pointed to the touch, with the hips, pins, tail head, and ribs being prominent; a BCS of 3 represents those water buffaloes whose ribs are usually visible with little fat cover, and dorsal spine are barely visible; a BCS of 4 is for animals that are smooth and well covered, but with no marked fat deposits; and BCS of 5 is for heavy deposits of fat clearly visible on the tail head and brisket, with the dorsal spines, ribs, hooks, and pins fully covered and unable to be felt even with firm pressure.

Transrectal ultrasonographic examinations of the ovaries were conducted using an ultrasound scanner (HS-1600, Honda Electronics Co., Ltd., Japan). On Day 0 of the protocol, the dominant follicle (DF) sizes present in the left or right ovaries were measured.

Experimental Design

Study 1. Determination of Pregnancies and Embryonic Loss Following Natural or Induced Estrus and Ovulation for Timed Artificial Inseminations in Dairy Buffaloes

In Treatment 1 (Control), animals exhibiting natural estrus were artificially inseminated following the Farm's existing protocol (Figure **1**). When the animals were observed in natural estrus, which is considered Day 0, the 1st AI was conducted in the morning, and the 2nd AI was done at 8 hrs thereafter. Pregnancy diagnosis by Pregnancy-Associated Glycoprotein (PAG) assay on Day 30 and by transrectal ultrasonography on Day 60

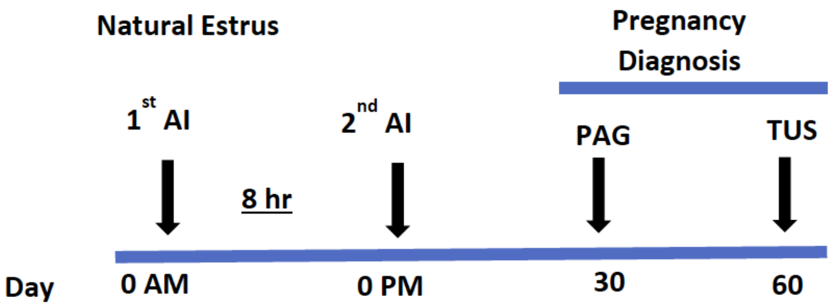

Figure 1: Schematic presentation of activities in buffaloes in natural estrus. Day 0: Conduct of 1st AI in the morning, and 2nd AI was done at 8 hrs thereafter. Day 30: Conduct of pregnancy diagnosis by Pregnancy-Associated Glycoprotein (PAG) Assay and Day 60: Pregnancy diagnosis by Transrectal Ultrasonography (TUS).

post-AI were performed to determine pregnancies and the incidence of embryonic loss.

In Treatment 2, buffaloes were subjected to Controlled Internal Drug Release-Synch- human-Chorionic Gonadotropin (CIDR-Synch-hCG) ovulation synchronization (Figure **2**). Injection of Gonadotropin-releasing Hormone (GnRH) and insertion of CIDR, which is an exogenous source of progesterone, were done on Day 0: Injection of Prostaglandin ($PGF_2α$) for lysis of corpus luteum and the removal of CIDR was done on Day 7. Injection of hCG, an ovulatory hormone, was carried out on Day 9. The conduct of 1st AI and 2nd AI at 8 hrs thereafter was performed on Day 10. Pregnancy diagnosis by PAG assay on Day 30 and by TUS on Day 60 post-AI were performed to determine pregnancies and embryonic loss following the TAI protocol.

In Treatment 3, animals with palpable corpus luteum were injected with Prostaglandin ($PGF_2α$) on Day 0 to ensure the hormone's efficacy on its target structure. All animals with mucus discharge and uterine tone of 2-3 were artificially inseminated in the morning of Day 3, with follow-up at 8 hrs thereafter (Figure **3**). Pregnancy diagnosis by PAG assay on Day 30 and by transrectal ultrasonography on Day 60 post-AI were performed to determine pregnancies and embryonic loss following the TAI protocol.

Treatment 4 is a Prostaglandin-based protocol, which is the same as Treatment 3, wherein the animals

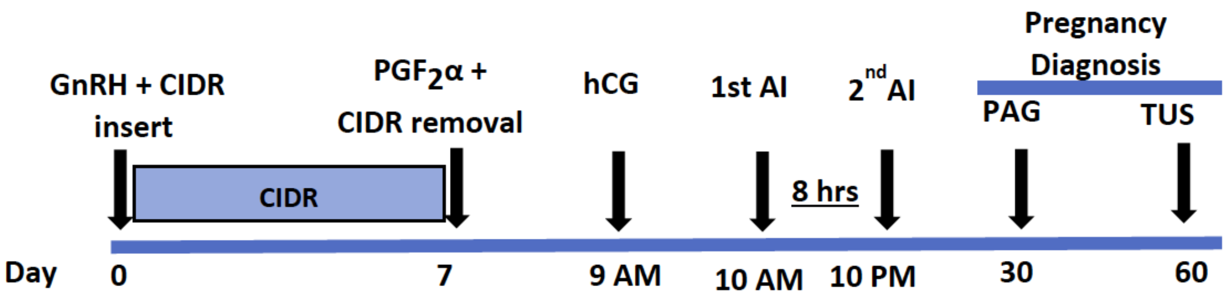

Figure 2: Schematic presentation of CIDR-Synch-hCG protocol. Day 0: Injection of GnRH and insertion of CIDR. Day 7: Injection of $PGF_{2α}$ and removal of CIDR. Day 9: Injection of hCG. Day 10: Conduct of 1st and 2nd AI at 8 hrs intervals. Day 30: Conduct of pregnancy diagnosis by PAG Assay and Day 60: Pregnancy diagnosis by TUS.

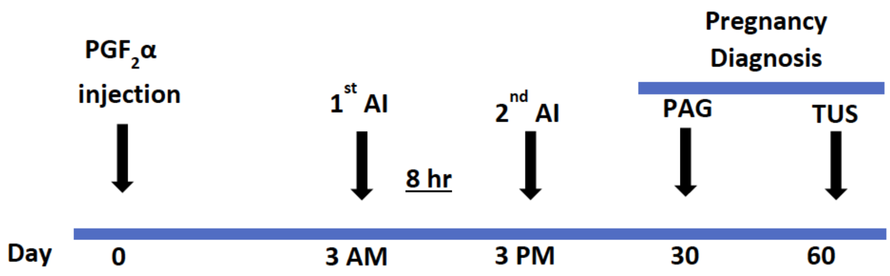

Figure 3: Schematic presentation of Prostaglandin protocol. Day 0: Injection of $PGF_{2α}$. Day 3: Conduct of 1st AI in the morning and 2nd AI in the afternoon, with 8 hrs intervals. Day 30: Conduct pregnancy diagnosis by PAG Assay. Day 60: Conduct of pregnancy diagnosis by TUS.

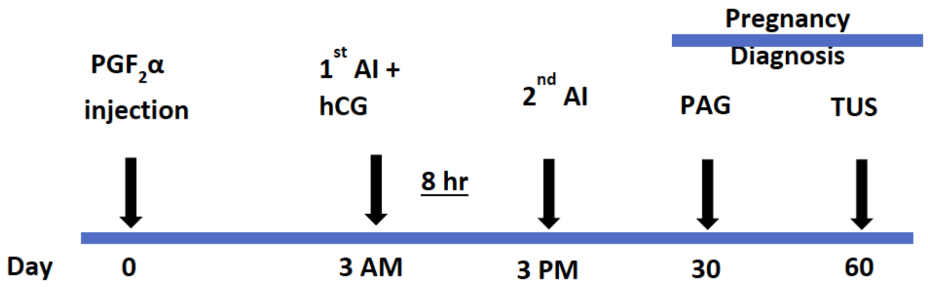

Figure 4: Schematic presentation of Enhanced-Prostaglandin protocol. Day 0: Injection of PGF$_{2\alpha}$. Day 3: Injection of hCG after conducting 1st AI in the morning and 2nd AI in the afternoon with 8-hour intervals. Days 30: Conduct of pregnancy diagnosis by PAG assay and Day 60: Pregnancy diagnosis by TUS.

were injected with PGF$_{2\alpha}$ on Day 0 but with hCG after the first AI on Day 3 to induce ovulation. A follow-up AI was done at 8 hrs thereafter (Figure **4**). Similarly, pregnancy diagnosis by PAG assay on Day 30 and by transrectal ultrasonography on Day 60 post-AI were performed to determine pregnancies and embryonic loss following the TAI protocol.

Blood Sampling and Pregnancy Diagnosis

Blood samples were collected from all cows on Day 30 post-AI by jugular venipuncture into a 10-mL vacutainer tube (BD Vacutainer, Becton, Dickinson and Company, New Jersey). They were allowed to clot, placed in a container containing crushed ice, and transported to the laboratory for plasma separation and storage. Samples were centrifuged at 1,500 x g for 15 min. Plasma was collected and stored at -20°C until the PAG assay was conducted.

All animals determined pregnant through PAG assay at Day 30 post-AI were re-examined on Day 60 using trans-rectal ultrasonography (Aloka 500V, Aloka, Wallingford, CT) with a 7.5 MHz trans-rectal linear probe. Cows diagnosed as pregnant at the first examination on Day 30 but not pregnant on Day 60 were defined to have an embryonic loss.

Assay Procedure

The plasma concentration of PAGs was determined following the procedure described in the BIOPRYN Pregnancy Test Kit. Each assay was run with duplicates of each sample: a sample from a pregnant cow approximately 60 Days in gestation and a pooled sample from non-pregnant cows to serve as controls.

Statistical Analysis

Numerical average data were presented as Mean±SD of 4 replications. Statistical analysis was done using One-Way ANOVA to compare the results of the percentages of pregnancies on Day 30 and 60 post-TAI and the percentage of embryonic loss in various studies. This was followed by the Tukey-Kramer test as a post-hoc analysis. The analysis was performed using the JMP Statistical software, Institute Inc., Cary, North Carolina, USA. The probability level of less than 5% was considered statistically significant.

Study 2. Effect of Size of Pre-ovulatory Follicle on the Day of Insemination on Pregnancy and Embryonic Loss in Dairy Buffaloes

Experimental Animals

The evaluation and selection of animals described in Study 1 were followed in the conduct of Study 2.

Timed Insemination

For this study, buffaloes were subjected to TAI following the CIDR-Synch-hCG estrus and ovulation synchronization protocol described in Study 1 (Figure **2**).

Measurement and Classification of Pre-ovulatory Follicle Sizes

On the Day of TAI on Day 10, the sizes of pre-ovulatory follicles were measured using transrectal ultrasonography and were then classified into three (3) follicle size groups: 1) < 10.0mm; 2) 10.0 to 11.9 mm; and 3) ≥ 12.0mm). Similarly, pregnancy diagnosis by PAG Assay on Day 30 and transrectal ultrasonography on Day 60 post-AI were performed to determine pregnancy rates and incidence of embryonic loss of the TAI protocol among the three pre-ovulatory follicle size categories.

Study 3. Evaluation of Pregnancies and Embryonic Loss in Dairy Buffaloes Inseminated at Different Seasons

Experimental Animals

Post-partum buffaloes for this study were similarly selected and subjected to TAI following the CIDR-

Synch-hCG protocol as described in Study 1 (Figure **2**). For this study, however, TAI was conducted during the following seasons: January–March. (Grp 1), April–June (Grp 2), July-September (Grp 3), and October-December (Grp 4). Pregnancy diagnosis by PAG assay on Day 30 and by transrectal ultrasonography on Day 60 post-AI were performed to determine pregnancies and embryonic loss following the protocol.

Blood Sampling and Pregnancy Diagnosis

The procedure of blood sampling, pregnancy diagnosis, and statistical analysis were done as described in Study 1.

RESULTS

Study 1. Determination of Pregnancies and Embryonic Loss Following Natural or Induced Estrus and Ovulation for Timed Artificial Inseminations in Dairy Buffaloes

AI efficiencies in terms of percentages of pregnancy at Days 30 and 60 post-TAI and incidence of embryonic loss thereafter are presented in Table **1**. Results revealed that buffaloes inseminated following the CIDR-Synch-hCG protocol (T2) achieved percentages of pregnancies at Days 30 and 60 post-TAI and embryonic loss, which are not significantly different (P>0.05) from those of animals in natural estrus (T1). However, the percent pregnancies achieved by the protocol (T2) at Days 30 and 60 were significantly higher (P<0.05) with significantly lower (P<0.05) embryonic loss compared with Prostaglandin-based protocols (T3 and T4). Meanwhile, buffaloes subjected to Enhanced AI, a Prostaglandin protocol with hCG supplementation (T4) revealed significantly higher (P<0.05) pregnancy rates with significantly lower % embryonic loss compared with those of Prostaglandin alone protocol (T3).

Study 2. Effect of Size of Pre-ovulatory Follicle on the Day of Insemination on Pregnancy and Embryonic Loss in Dairy Buffaloes

The efficiencies of inseminations based on the size of the pre-ovulatory follicle on the Day of TAI are presented in Table **2**. Inseminating buffaloes with pre-ovulatory follicle size of ≥ 12.0 mm (T3) revealed significantly higher (P<0.05) pregnancy rates at Day 30 and Day 60 post-TAI with the lowest embryonic loss (P<0.05) compared with inseminating with smaller follicle sizes (T2 and T1)**.** Further, the smallest follicle size group of <10.0 mm (T1) yielded the lowest pregnancy rates at Days 30 and 60 post-TAI with the

Table 1: Percentages of Pregnancies and Embryonic Loss Following Timed Artificial Inseminations in Dairy Buffaloes

Treatments	Timed AI Protocols	No. of Animals	% Pregnancy at Day 30 post-AI	% Pregnancy at Day 60 post-AI	% Embryonic loss*
1	Natural Estrus	198	49.53 ± 1.15^{a}	46.79 ± 1.78^{a}	4.20 ± 0.86^{b}
2	CIDR-Synch- hCG	210	46.17 ± 1.71^{a}	44.86 ± 1.90^{a}	2.83 ± 0.58^{b}
3	Prostaglandin alone ($PGF_{2\alpha}$)	154	21.90 ± 2.33^{b}	18.67 ± 2.70^{b}	15.00 ± 3.3^{a}
4	Enhanced AI ($PGF_{2\alpha}$ +hCG)	206	34.49 ± 0.41^{c}	31.13 ± 1.03^{c}	9.74 ± 1.96^{c}

Values are presented as Mean±SD of 4 replications.

[a,b,c] Values with different superscripts within columns are significantly different at P<0.05.

*The difference between the number of pregnant animals on Day 30 and Day 60 post-AI divided by the number of pregnant animals on Day 30, multiplied by 100.

T1. Natural Estrus: Buffaloes manifesting behavioral and external signs of natural estrus.

T2. CIDR-Synch-hCG: An Ovsynch protocol supplemented with CIDR on Day 0 and hCG on Day 9 of the TAI program.

T3. Prostaglandin protocol: Buffaloes were given $PGF_{2\alpha}$ on Day 0 and TAI on Day 3.

T4. Enhanced AI: Prostaglandin protocol supplemented with hCG on the Day of TAI.

Table 2: Percentages of Pregnancies and Embryonic Loss Following Follicle Size-based Timed Inseminations in Dairy Buffaloes

Treatments	Follicle Size Category (mm)	No. of Animals	% Pregnancy at Day 30 post-AI	% Pregnancy at Day 60 post-AI	% Embryonic loss*
1	<10.0	143	18.40 ± 2.28^{c}	14.83 ± 0.88^{c}	15.48 ± 1.37^{c}
2	10.0-11.9	260	47.92 ± 2.18^{b}	43.17 ± 0.87^{b}	9.92 ± 0.86^{b}
3	≥ 12.0	269	54.58 ± 1.13^{a}	53.38 ± 0.88^{a}	2.04 ± 0.75^{a}

Values are presented as Mean±SD of 4 replications

[a,b,c]. Values with different superscripts within columns are significantly different at P<0.05

*The difference between the number of pregnant animals on Day 30 and Day 60 post-AI divided by the number of pregnant animals on Day 30, multiplied by 100.

Table 3: Percentages of Pregnancies and Embryonic Loss in Buffaloes Inseminated at Different Seasons of the Year

Treatments	Season	No. of Animals	% Pregnancy at Day 30 post-AI	% Pregnancy at Day 60 post-AI	% Embryonic loss*
1	Jan-March	241	48.70±1.00^{a}	47.52±0.97^{a}	2.41±0.10^{a}
2	April-June	244	44.40±1.77^{b}	41.55±2.18^{b}	6.43±1.66^{b}
3	July-Sept	290	42.70±2.64^{b}	40.30±2.99^{b}	5.57±1.56^{b}
4	Oct-Dec	230	49.12±0.71^{a}	47.90±0.64^{a}	2.48±0.18^{a}

Values are presented as Mean±SD of 4 replications.
^{a,b,c}Values with different superscripts within columns are significantly different at P <0.05.
*The difference between the number of pregnant animals on Day 30 and Day 60 post-AI divided by the number of pregnant animals on Day 30, multiplied by 100.

highest incidence of embryonic loss among the Treatment groups.

Study 3. Evaluation of Pregnancy and Embryonic Loss in Dairy Buffaloes Inseminated at Different Breeding Seasons

The efficiencies of inseminating buffaloes at certain seasons of the year are presented in Table **3**. Animals inseminated during the months of January-March (Group 1) and October- December (Group 4) resulted in significantly higher pregnancies (P<0.05) on Days 30 and 60 post-AI with significantly lower embryonic loss (P<0.05) compared with those animals inseminated in the months April-June (Group 2) and July-September (Group 3). Variations in pregnancies and incidence of embryonic losses are observed in the present study.

DISCUSSION

The pregnancy outcomes and incidence of embryonic loss are valuable measures of the efficiency of every single Timed Insemination protocol and have been demonstrated in buffaloes in the present study. First, the high pregnancies and low embryonic loss achieved by the CIDR-Synch-hCG protocol imply the efficacy of said protocol in achieving the success rate of the natural estrus. The protocol is a modification of the Ovsynch protocol being supplemented with exogenous Progesterone (CIDR) on Day 0 and with hCG on Day 9, and its superior current results conform with those of previous works in enhancing follicle growth, promoting synchronous ovulation, and improving oocyte quality influencing pregnancy outcome and reducing embryonic loss [10]. Treatment with Ovsynch (GnRH/PGF2/GnRH) yielded inconsistent results, but supplementing with exogenous progesterone resulted in more consistent results in *B. indicus* cattle [11]. Moreover, the enhancement of Ovsynch protocol with CIDR at the start of the hormonal program and hCG towards the end of the treatment played a key role in inducing synchronous ovulation and the proper timing of AI, leading to improved conception rates in water buffaloes [5].

Meanwhile, using Prostaglandin (T3), which is mainly known to induce estrus, resulted in variable ovulations leading to low pregnancy rates with a high incidence of embryonic loss in the present study. Using Prostaglandin alone on Day 0 without prior use of 1st GnRH to induce ovulation and emergence of new follicular wave did not ensure the presence of new dominant follicles but with aged oocytes with poor development competence at the time of AI, resulting in embryonic loss and poor fertility in the treated animals [12]. However, the supplementation of Prostaglandin protocol with hCG on the Day of AI as a replacement for 2nd GnRH for final ovulation in the present study improved ovulation rates and pregnancy rates and minimized embryonic loss in dairy buffaloes, which corroborates with results in previous studies [12]. The superior response can be attributed to increased progesterone secretion that favors embryonic survival and successful pregnancy [13].

Meanwhile, the size of the pre-ovulatory follicle on the Day of AI has recently gained attention as a major factor in the success of timed insemination in cattle and buffaloes. The highest pregnancy rate and lowest embryonic loss in the present study were achieved when AI was conducted in buffaloes with ≥ 12.0 mm pre-ovulatory follicle size, which is considered as mature enough to sustain pregnancy by maintaining optimal CL diameter and peripheral progesterone concentration [5]. Further, it was demonstrated that a larger POF size is comprised of a higher number of granulosa cells, which have been related to the greater steroidogenic capacity of the resultant CL [14]. Pregnancy was found greater in suckled Bos indicus cows that had a larger follicle on the day of FTAI, and similarly, cows with follicles >11.1mm had an increased display of estrus and pregnancy [15]. In contrast, the low pregnancy rates and high incidence of pregnancy loss achieved by inseminating follicles with <12.0mm in the present study could be attributed to the compromised competence of small follicles for subsequent embryonic development. In beef heifers, it was demonstrated that follicles induced to ovulate at <11.0 mm size had smaller CL and secreted less progesterone than heifers ovulating in large follicles, which consequently resulted in lower pregnancy and increased embryonic loss [16]. The small size of follicles can be implicated in the quality of the corpus luteum and in the maintenance of the uterine environment during embryonic implantation. Finally, the most recent work underscores the importance of the presence of large pre-ovulatory follicles and uterine tone on the Day of AI as significant factors in the success of the CIDR-Synch-hCG FTAI program in water buffaloes [17].

In terms of the influence of seasons on AI efficiency, high pregnancy rates and low incidence of embryonic losses were observed in buffaloes following inseminations with CIDR-Synch-hCG protocol in cooler months of October to March (T4, T1), which is considered as breeding season under Philippine condition. In contrast, a reduction in both reproductive parameters was noted after inseminations from April to September (T2, T3), which is considered the non-breeding season for buffaloes under local climatic conditions. Related to the seasonal variation under the local climatic condition, reproductive performance in terms of buffalo semen motility in association with a concentration of heat shock protein 70 (HSP 70) revealed variable semen qualities among the four-season categories under the local climatic environment [18]. The said breeding seasonality mainly marked by summer infertility is characterized by anestrous, poor estrus expression, reduced fertility on breeding [19], and higher early pregnancy losses [20]. In addition, the quality of the oocytes during summer periods might be adversely affected, resulting in inefficient fertilization and subsequent development of inferior quality embryos, aside from the uterine asynchrony or uterine hormonal imbalance during implantation and subsequent maternal recognition.

Meanwhile, nutrition could be another contributory factor due to the scarcity of fresh forages during the dry season, which has an impact on the development, maturity, and ovulation of follicles [21]. Generally, however, summer anestrus is a multifactorial condition exacerbated by environmental, nutritional, hormonal, and managemental factors. Meanwhile, it was pointed out that in summer, buffaloes are particularly prone to heat stress due to their low density of sweat glands, which makes them less susceptible to cutaneous evaporative cooling [22]. Consequently, thermal stress negatively affects the fertility of high-producing cows by lowering the fertilization rates and increasing pregnancy losses [23], but Timed AI strategies reduce the impact of non-breeding season on the reproductive efficiency of water buffaloes [24], as demonstrated in the present study.

Finally, as to the strategic utility of Timed AI protocols under the local genetic improvement program in water buffaloes, the CIDR-Synch-hCG Progesterone-based protocol is recommended in the purebred buffalo production system and during non-breeding season, while a prostaglandin-hCG-based enhanced AI protocol is a practical recommended approach in national crossbreed production and during breeding season. The said breeding systems are considered cost-effective approaches to buffalo production in the country.

CONCLUSION

The present study demonstrated the efficacy of an exogenous progesterone based-TAI protocol (CIDR-Synch-hCG) similar to those achieved by dairy buffaloes inseminated under natural estrus and that enhancing the Prostaglandin-based protocol with ovulatory hormone (hCG) improved AI efficiency. Moreover, inseminating dairy buffaloes with large pre-ovulatory follicle diameters resulted in superior pregnancy outcomes. Meanwhile, TAI performed during the breeding season resulted in better AI efficiencies than during the non-breeding season, which could be attributed to the nutritional and environmental impact on buffalo reproduction. Essentially, the present work provided a strategic breeding system that considered the importance and influence of Timed AI protocols, seasonal variations, and follicle size on the Day of AI towards greater efficiency, productivity, and sustainability of buffalo farming activities.

AVAILABILITY OF DATA AND MATERIAL

Data and material for this research are available from the main author upon request.

CONFLICT OF INTEREST

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

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