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Effect of the feeding condition and number of dominant follicles on oestrus synchronization in Swamp buffalo

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ABSTRACT

The research aimed to assess how feeding conditions and the number of the dominant follicles (DF) influence oestrus synchronization in swamp buffaloes. A total of 170 swamp female buffaloes raised in semi-grazing and housekeeping were included in the study. The synchronization used CIDR method and the follicles on the day of CIDR (Controlled Internal Drug Releasing) insert and removal were tracked by sonography. The results indicated that the age of first mating, age at first calving, and caving interval showed no significant differences in the two feeding conditions. The number of follicles at CIDR insertion and removal decreased in semi-grazing, but increased in housekeeping buffaloes. The percentage of buffalo without DF, one DF, and two DFs at CIDR withdrawal did not differ statistically. The percentage of estrus was 100% in both groups. Pregnancy rates were 53.18% in semi-grazing and 52.63% in housekeeping. The onset of oestrus was similar, but the duration of oestrus was longer in the buffaloes without DF than those with one or two DFs. The percentage of pregnant buffaloes in the group without DF was significantly lower than that in the other two groups. In summary, the efficiency of oestrus synchronization did not differ between semi-grazing and housekeeping methods. Buffaloes without DF displayed longer oestrus periods and lower pregnancy rates compared to those with one or two DFs in the ovarian system.

Keywords: Breeding condition, Dominant follicles, Oestrus synchronization, Swamp buffaloes

Swamp buffalo livestock plays a significant role in Southeast Asian countries like Southeast Asia and China. They are integral to crop production systems, providing energy for tillage, fresh organic fertilizer, and are well-suited to lowland areas and various habitats (Minervino *et al.* 2020). In Vietnam, around 2.5 million buffaloes are raised on small farms, with 1 to 4 buffalo per farm in rural and mountainous regions. Despite being valuable to smallholder farmers, their production has declined due to increased farm mechanization (Pineda *et al.* 2021). Historically, swamp buffaloes were primarily used for traction and fertilizer in agricultural farming, but as agriculture mechanized, their breeding focus shifted to meat production. In Vietnam, buffalo farming involves semi-grazing and housekeeping methods, where buffaloes graze on natural grass with supplements or are kept and provided with food and water.

Buffaloes exhibit a high rate of underground estrus, making estrus detection challenging. Assisted reproduction technologies such as oestrus synchronization, artificial insemination, and pregnancy diagnosis have been applied to buffaloes (Van Hanh *et al.* 2020). However, due to their distribution in rural areas with low feeding conditions and specific reproductive characteristics, the application of these technologies is still limited, with lower reproductive support compared to cattle (Baruselli *et al.* 2018). Nutritional status significantly impacts reproductive performance, leading to economic losses for farmers due to prolonged calving intervals (Dahiya *et al.* 2020). Inducing estrus in buffaloes is uncommon in countries with small buffalo herds, limited farmer awareness, and poor response to estrus synchronization, particularly in hot weather (Samir *et al.* 2019, Bharti *et al.* 2022).

Follicular development stage significantly affects estrus and pregnancy rates during estrous induction in buffaloes (Kavya *et al.* 2020). Additionally, factors such as nutrition, locomotion, and stress influence reproductive outcomes (Esposito *et al.* 2019). Advancements in understanding ovarian function during the buffalo oestrus cycle have led to improved synchronization of follicular development and ovulation, benefitting assisted reproductive technologies (ART). This study aimed to evaluate oestrus synchronization efficiency in swamp buffaloes, considering breeding methods and the number of dominant follicles in the ovaries at the time of CIDR removal.

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MATERIALS AND METHODS

Animals: A combined group of 170 female buffaloes from small farms in the Tuyen Quang province of North-west Vietnam, a semi-mountainous region with a hot and humid climate in the summer and cold in the winters was used in the study. Buffaloes were postpartum (from 1.5 months to 9 months), estimated by body measuring, they weighed 393.0±18 kg, aged 5.6±2.1 years old and the average number of parity was 2.5±1.1. A total of 123 swamp buffaloes were selected after ovarian screening by ultrasound, and they were accepted in the study under the following criteria: no dominant follicles, no pregnancy, and inflammation or abnormal reproduction organs. Buffaloes were raised by smallholder farmers by semi-grazing and housekeeping breeding methods. The house conditions involved natural light on a day-night cycle. The buffaloes were kept away from the bull during the study. They were fed natural grass and rice straw, agricultural by-products, mineralized salt licks, and were allowed to consume clean water.

Ultrasonography examinations: The ultrasonography examinations were performed for the examination of follicles and corpus luteum (CL) in both ovaries by ultrasound machine (Convex scanner HS-2000, Honda, Japan) with a 10-MHz endorectal transducer. Examinations were performed two times on day 0 (at time of CIDR insertion) and day 7 (at time of CIDR removal) in both groups under different breeding. The ovary visualized rectally were assessed using the electronic calipers. The number and diameter of all follicles were recorded. Follicles were categorized as either small follicles (3–8 mm) or dominant follicles (DF) (≥ 9.0 mm). On day 0, these buffaloes without corpus luteum and dominant follicles were selected for the next experiment. On day 7, the buffaloes were evaluated for the number of dominant follicles (≥ 9.0 mm).

Oestrus synchronization: Animals were monitored for estrus synchronization based on Dhami *et al*. (2015) protocol, which modified the time AI as shown in Fig. 1. The buffaloes received CIDR (Controlled Internal Drug Releasing) device impregnated with 1.38 g progesterone (Zoetis New Zealand Ltd, New Zealand). The CIDR was inserted intra-vaginally for 7 days and an intramuscular injection of 5 ml LutalyseTM (Dinoprost (prostaglandin F2α analogs) 5 mg/ml, Zoetis, Belgium) on the day of the CIDR withdrawal. Two days after, the buffaloes received a intramuscular dose of 200 mg ovurelin (Gonadotropin-releasing hormone analog –GnRH) (Zoetis New Zealand Ltd, New Zealand). After the end of the treatment, all buffaloes were observed for behavioural signs of estrus twice a day (at 12 h intervals) to estimate estrus behaviour. The buffaloes were subjected to timed AI 12 h after estrus's end and repeated again following the ruler morning and night by using frozen-thawed semen from a known fertile bull. Buffalo recorded pregnancy after 3 months by papal rectal. The oestrus synchronization efficiency is based on the feeding methods and the number of dominant follicles at CIDR removal.

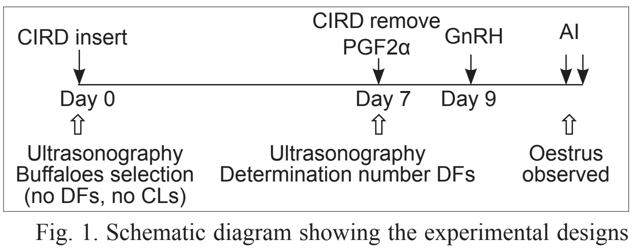

Fig. 1. Schematic diagram showing the experimental designs (DF: Dominant follicles; CL: Corpus luteum; AI: Artificial insemination).

Statistical analysis: The data of numbers of follicles and time of oestrus were presented as means±SEM (standard error of the mean). Statistical analysis was performed using the method Anova: single factor in Excel with a significant difference at least P<0.05. Pregnancy rates and percentage of dominant follicles exhibiting estrus among different treatment groups were compared using the χ^2 test which is the least significant difference at 5%.

RESULTS AND DISCUSSION

Reproductive characteristic of swamp buffalo in two breeding conditions: Reproductive characteristics of female swamp buffaloes in two breeding conditions are presented in Table 1. The results of this study indicated that the age of first mating of female buffaloes in Vietnam was 33.85 months in total and it was not significant in two breeding conditions. The age at first calving of female buffaloes ranged from 41 months to 51 months (data not shown), and it was not significantly different in the two groups. The calving intervals were the same in the two groups and it was reported as 15.71 months in total.

Table 1. Reproductive character of adult female swamp buffalo

Parameter	Semi-grazing	Housekeeping	Total
Number of buffaloes (n)	60	110	170
Age of first mating (Months)	33.82±2.53[a]	33.86±2.42[a]	33.85±2.46[a]
Age at first calving (Months)	47.20±3.23[a]	47.36±2.89[a]	47.31±3.07[a]
The calving intervals (Months)	15.70±2.34[a]	15.71±1.84[a]	15.71±2.02[a]

Means followed by different lowercase in the row letters in the lines to significant differences at P< 0.05.

The age of first mating, the age at first calving, and the calving interval of female buffaloes in this research were shorter than those in the Indonesia swamp buffalo (33.82 and 51.4 months; 47.31 and 63.4 months and 15.7 and 16.5 months, respectively) (Widi *et al.* 2021). But they were longer than those reported by Ciptadi *et al*. (2019) (33.82 and 31.12 months; 47.31 and 43.12 months and 15.7 and 14.45, respectively). Thus, the reproductive characteristics of swamp buffaloes can depend on a variety of conditions, but in the same area, the housekeeping and semi-grazing conditions did not affect these indicators.

Table 2. Relationship between feeding methods and results in oestrus synchronization

Parameter	Semi-grazing	Housekeeping	Total
Number of buffaloes (n)	47	76	123
Number of follicles at time of CIDR insert	6.68±0.20^{aA}	6.21±0.14^{aB}	6.01±0.16^{a}
Number of follicles at time of CIDR withdraw	6.38±0.18^{bA}	6.67±0.12^{bA}	6.56±0.10^{b}
No dominant follicles (n, %)	12 (25.53)	25 (32.89)	37 (30.08)
One dominant follicle (n, %)	31(65.96)	48 (63.16)	79 (64.23)
Two dominant follicles (n, %)	4 (8.51)	3 (3.95)	7 (5.69)
Rate of oestrus (n, %)	47 (100.00)	74 (97.37)	121 (98.37)
Time start oestrus (h)	42.64±1.25	43.95±1.07	43.44±0.81
Time stop oestrus (h)	74.04±1.28	75.08±1.88	74.68±1.03
Pregnancy rate (n, %)	25 (53.18)	40 (52.63)	65 (53.66)

Means followed by different lowercase letters in the columns and uppercase letters in the lines to significant differences.

Influence of feeding methods on oestrus synchronization efficient: At the CIDR insert, the number of follicles from buffaloes in semi-grazing was higher than that in the housekeeping group (P = 0.03). When the CIDR was removed, the number of follicles in the animals in the semi-grazing group was lower than that in housekeeping group. However, the difference was not statistically significant. The number of follicles in semi-grazing group reduced from the day CIDR insert to the day CIDR was removed (P<0.014), but that increased in the housekeeping (P<0.012). In total, the number of follicles were increased from the day of CIDR insert to the day CIDR was removed (P<0.001). The rate of buffaloes having one DF, two DFs or no DF varied between the groups, but there was no significant difference, as shown in Table 2. The rate of oestrus and pregnancy were similar when compared in the two groups (P<0.05).

In this study, the number of ovarian follicles expressed at the time of CIDR insert and removal did not differ in the two breeding methods but were lower in experiments of other researchers (Samir *et al.* 2019, Harun-or-Rashid *et al.* 2020). According to Samir *et al.* (2019) in Egyptian buffalo, for oestrus with CIDR-PGF2α-GnRH, at the time of CIDR withdrawal the surface follicle population was 8.20±1.20, however, at the time of estrus, the number of follicular populations was 5.00±0.45. Meanwhile, the mean number of follicles <3 mm in Murrah buffalo at the time of oestrus initiation was 10.89±0.65 for follicles < 3 mm and 1.93±0.33 for 3-10 mm follicles (Harun -or-Rashid *et al.* 2020), and the mean numbers of smaller and larger follicle sizes did not differ until day 3 after PG injection. The percentage of buffalo without dominant follicles in this study showed similarities with the one reported by Visha *et al*. (2014). When used Progesterone was impregnated with polyurethane intravaginal sponges for oestrus synchronization in Murrah buffaloes, the results showed that all the animals had no dominant follicles on the day of sponge insertion (Day 0) but all the animals had a dominant developing follicle on the day of removal of the sponges (Visha *et al.* 2014). The rate of no dominant follicles in our study showed similarities with that in the silent estrus or missing estrous group as reported by Gautam *et al*. (2017) (30.08% and 36%, respectively).

Influence of number of dominant follicles in ovary on oestrus synchronization efficient: The total number of follicles increased at CIDR insert to the day of withdrawal in all three groups (Table 3). The group without follicles exhibited the highest increase (P<0.005), while the group with 2 follicles showed the lowest increase (P<0.005). However, this difference was not statistically significant. The number of follicles was not different between the three groups on both the days of CIDR insertion and withdrawal. The estrous rate was highest in the group with 2 follicles but there was no difference compared with that in no DF and one DF group. The time of appearing of estrus was also similar in all three groups. Meanwhile, the time of end of estrus was longer in the no follicle group compared with that in one DF and two DFs groups (P<0.001). The percentage of pregnant buffaloes was significantly different between the group without follicles, reaching only 2.70% compared with 73.42% in the group with 1 follicle and 85.71% in the group with 2 follicles (P<0.05).

Table 3. Relationship between number dominant follicles in ovarian and results in oestrus synchronization

Parameter	No dominant follicles	One dominant follicle	Two dominant follicles
Number of buffaloes (n)	37	79	7
Number of follicles at time CIDR inserted	5.84±0.20^{a}	6.10±0.15^{a}	5.86±0.40
Number of follicles at time CIDR removed	6.62±0.18^{b}	6.58±0.13^{b}	6.00±0.44
Rate of oestrus (n, %)	36 (97.30)	78 (98.73)	7 (100.00)
Time starts oestrus (h)	43.68±1.27	43.38±0.95	42.86±6.34
Time stops oestrus (h)	82.68±1.12^{A}	72.23±1.17^{B}	72.00±5.86^{B}
Pregnancy rate (n, %)	1 (2.70)^{A}	58 (73.42)^{B}	6 (85.71)^{B}

Means followed by different lowercase letters in the columns and uppercase letters in the lines to significant differences.

In this study, the percentage estrus of buffalo was 100% in both models or in the group having a different number of dominant follicles at the time of CIDR removal. Campanile *et al*. (2008) also confirmed that follicle size is not the only factor affecting oestrus rate. Meanwhile, the study by Noseir *et al.* (2014) found that the diameter of the largest follicle of buffalo did not ovulate after GnRH administration was 0.97 cm. However, the difference in the presence of dominant follicles and the number of dominant follicles at the withdrawal stage of CIDR affected the duration of estrus and the pregnancy rate. In the same protocol using CIDR-PGF2α-GnRH to oestrus synchronization, the rate of estrus ranged from 65% (Azawi *et al.* 2012) to 100% (Hussein *et al.* 2012, Dhami *et al.* 2015). The duration of estrus in this study was also similar to that of the buffalo group with a normal cycle after oestrus, with a duration of 42-72 h (Hussein *et al.* 2016) and an average of 77.8±5.6 h (Azawi *et al.* 2012).

The pregnancy rate of buffalo has been shown to vary widely in different studies. As reported by Chaikhun *et al*. (2010) in swamp buffalo heifers, a pregnancy rate of only 15% in heifers and 42.9% in buffalo cows was found (Chaikhun *et al.* 2010). In our study, the pregnancy rates in total were 53.66%. The results are similar to reports in hybrid breed buffalo (Murrah × the Mediterranean) (56.5%) (De Araujo Berber *et al.* 2002), but lower than in crossbred cows (60.00%) (Dhami *et al.* 2015) or swamp buffalo (66.67%) (Mani *et al.* 2021). If only the buffalo group had dominant follicles, the pregnancy rate in the present study was 73.42 85.71%, which was similar to what was reported in Murrah buffaloes after two cycles of AI (69.3%-86.6%) (Dhaka *et al.* 2019). It can be concluded that there was no statistically significant difference in reproductive characteristics and efficiency of oestrus synchronization in buffaloes with semi-grazing and housekeeping feeding methods. The number of dominant follicles at the time of Controlled Internal Drug Releasing device removal did not correlate with the rate of estrus. However, it did affect the pregnancy rate. The group of buffaloes without dominant follicles exhibited a pregnancy rate of only 2.7%, whereas the groups with one or two dominant follicles showed pregnancy rates of 73.42% and 85.71%, respectively.

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