

Effect of ovarian characteristics and propylene glycol supplementation on pregnancy rates per AI in suckled beef cows subjected to a 7-day CO-Synch fixed-time AI protocol across seasons, body condition scores, and postpartum intervals

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Abstract

The present study evaluated the independent effects of ovarian characteristics and oral propylene glycol (PG) supplementation on pregnancy rates in suckled beef cows subjected to the 7-day CO-Synch + CIDR fixed-time AI (FTAI) protocol across different seasons, body condition scores (BCS; 5-point scale), and parturition-to-FTAI intervals. Cows received a CIDR insert and 10 µg GnRH on day -10, followed by 25 mg PGF2α at CIDR removal on day -3, and another 10 µg GnRH 72 h later (day 0). In Study 1, the ovarian characteristics of 36 cows were assessed transrectally on days -10 and 0 relative to the FTAI. In Study 2, cows were assigned to a control (n = 118) and a treatment group (n = 57). The treatment group received 1 ml PG/kg body weight mixed with 1 kg rice bran from days -10 to -1, whereas the control group received the same amount of rice bran without PG. Season, BCS, and the parturition-to-FTAI interval were included as covariates. In Study 1, cows with a dominant follicle diameter ≥ 9.00 mm at AI had a higher pregnancy rate than those with a diameter < 9.00 mm (50.0 % vs. 7.1 %, $P = 0.014$). In Study 2, PG supplementation increased pregnancy rate compared with the control group (68.42% vs. 51.40%, $P = 0.036$). Pregnancy rates were lower during the hot season in cows with BCS < 2.5, and in those with a parturition-to-FTAI interval < 90 days ($P < 0.05$). The numerically highest pregnancy rates were observed during the cool season and among cows with a BCS ≥ 2.5, whereas the lowest rate occurred in control cows with a parturition-to-FTAI interval < 90 days ($P < 0.05$). In conclusion, the pregnancy rate in cows subjected to the 7-day CO-Synch + CIDR FTAI protocol was influenced by follicle diameter and propylene glycol supplementation. Variation in pregnancy outcomes among PG-supplementation cows was further affected by season, BCS, and the parturition-to-FTAI interval. Propylene glycol supplementation enhanced pregnancy rates, particularly during the cool season and in cows with a BCS ≥ 2.5.

Keywords: fixed-time AI, ovarian characteristics, pregnancy rate, propylene glycol, suckled beef cow

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Introduction

In beef cattle production, artificial insemination (AI) protocols combined with hormonal treatments, specifically fixed-time AI (FTAI), have been implemented to enhance reproductive efficiency. FTAI synchronizes follicular development, *corpus luteum* regression, and ovulation, thereby allowing insemination without the need for estrus detection (Taponen, 2009). The 7-day CO-Synch protocol incorporating a controlled internal drug release (CIDR) device for progesterone is one of the most widely used FTAI methods worldwide (Lamb *et al.*, 2001; Wilson *et al.*, 2010). This protocol has been shown to be effective, achieving pregnancy rates ranging from 59% and 75% (Wilson *et al.*, 2010).

In commercial beef cattle production systems, calves are typically weaned at an age that aligns with operational schedules or market requirements. However, weaning age may be adjusted according to factors such as forage availability (Enriquez *et al.*, 2011). Under rural conditions, smallholders often delay weaning to allow calves extended access to their dams' milk, because forage quality and availability fluctuate across seasons and years (Mtengeti *et al.*, 2008; Orihuela, 2021). During the suckling period, both the act of suckling and maternal bond inhibit the resumption of postpartum ovarian activity in beef cows by suppressing the frequency of luteinizing hormone (LH) pulses, which are required for estradiol synthesis and induction of the preovulatory LH surge (Deffy *et al.*, 2000; Crowe *et al.*, 2014; Bayemi *et al.*, 2015). This inhibition results in postpartum anestrus and prolongs the calving-to-conception interval. Previous research demonstrated that ovarian activity and the LH surge were suppressed before weaning (Madej *et al.*, 1992). In contrast, when suckling was restricted, allowing calves to nurse only once or twice daily beginning on day 30 postpartum, ovulation occurred earlier (Stagg *et al.*, 1998). In suckled cows, the timing of initiating FTAI protocols at different stages of the estrous cycle influences the diameter of the largest follicle at CIDR removal (Scarpa *et al.*, 2019). This variation in follicular size may affect fertility by altering the ovarian response to the hormones used in the FTAI protocols. To the authors' knowledge, limited information is available on how the 7-day CO-Synch FTAI protocol influences ovarian characteristics and, consequently, fertility in suckled cows. A better understanding of the effects of this protocol on ovarian dynamics and fertility is therefore essential for improving reproductive management in suckled beef cows.

Nutrition, especially energy intake, plays a crucial role in the reproductive performance of cattle, particularly during the lactation period. Insufficient carbohydrate levels in the diet are linked to negative energy balance (NEB) (Garnsworthy *et al.*, 2008). A NEB adversely affects reproductive processes in cows, impacting oocyte maturation, embryo development, and follicular development (Jorritsma *et al.*, 2003; Satitmanwiwat *et al.*, 2017; Zhao *et al.*, 2021), as well as impairing GnRH secretion, reducing LH production, and delaying ovulation (Nigussie, 2018). Propylene glycol is a precursor of pyruvate and propionate,

which are essential for the gluconeogenesis pathway in the liver (Zhang *et al.*, 2020). A comprehensive review of the effect of propylene glycol on reproduction in dairy cows has been published (Kaewlamun *et al.*, 2020). A supplement of propylene glycol during NEB showed satisfactory results in relieving metabolic stress by increasing blood glucose, decreasing ketosis (Butler *et al.*, 2003; McArt *et al.*, 2013; Mecitoğlu *et al.*, 2017), and enhancing cow reproductive performance (Madoz *et al.*, 2020). Recently, it was found that the Ovsynch FTAI protocol, accompanied by a daily propylene glycol drench, decreased NEB metabolites and increased the pregnancy rate in dairy cows (Cakircali *et al.*, 2023). However, to date, studies on the effect of propylene glycol supplementation associated with the 7-day CO-Synch + CIDR FTAI protocol on beef cow fertility have never been reported.

It is well known that the reproductive performance of cows subjected to FTAI can be influenced by various factors, including season, the interval between parturition and initiation of the protocol (Lamb *et al.*, 2001; Echternkamp and Thallman, 2011), and body condition score (BCS) (Lamb *et al.*, 2001; Vedovatto *et al.*, 2022). The pregnancy rates of suckled beef cows subjected to three different 7-day CO-Synch-based FTAI protocols varied during Spring but not in Autumn in Ireland. Additionally, the overall pregnancy rate of cows (59%) following the three protocols in Spring was significantly higher than in Autumn (52%) (Randi *et al.*, 2021). In multiparous cows given an FTAI between 46 and 50 days postpartum, the pregnancy rate reached 70.8% and remained stable when FTAI was performed within 60 days postpartum. However, the pregnancy rate to FTAI decreased as the postpartum interval increased beyond this time (Araújo *et al.*, 2022). Beef cows with a BCS above 5 (9-point scale) after calving, which were subjected to FTAI, showed higher pregnancy rates (54.8%) compared to those with a BCS below 5 (34.2%) (Carvalho *et al.*, 2022).

Therefore, the present study aimed to assess the impact of (1) ovarian characteristics and (2) oral propylene glycol supplementation across various seasons, body conditions, and parturition intervals on the pregnancy rate of suckled beef cows subjected to the 7-day CO-Synch + CIDR FTAI protocol.

Materials and Methods

The experiment was performed according to the procedure that was approved and licensed by the School of Agricultural Resources, Chulalongkorn University, animal care and use committee (protocol number IACUC002/2567) under the Ethical Principles and Guidelines for the Use of Animals, National Research Council of Thailand.

Animals and farm management: The study was conducted in a beef cattle community enterprise for smallholders under a large agricultural extension system covering a thousand Thai-native crossbred cows in a province located in the North of Thailand, during November to February (cool season) and March to October (hot season). General farm management by smallholders in the beef cattle community enterprise

was performed using a conventional rearing system, including free grazing with feed supplementation. The cattle were allowed to graze freely for around 6-8 h per day, and they were given commercial concentrate feed and typical forages such as corn silage, Napier grass, or rice straw. Cows had free access to water and salt licks. Natural insemination and artificial insemination are used in the general breeding management system for cattle production. Open cows and pregnant cows were kept in a conventional open-housing system. The pens consisted of a concrete or beaten-earth floor for both the indoor and outdoor areas. Calves were allowed to stay with their dams until they were naturally rejected by their dams. Herd health management (vaccination program, deworming) was performed according to the Provincial Livestock Department Service recommendations.

Experimental design: Study 1 aimed to investigate the effect of ovarian characteristics in suckled cows on the success of FTAI. A total of 36 Thai native crossbred suckled cows, 132.3 ± 57.0 days postpartum, were included in the FTAI protocol. The bodyweight and body condition score (BCS) of the cows were 343.8 ± 53.8 kg and 2.25 ± 0.53 (5-point scale), respectively. The ovarian characteristics of the cows were measured on day -10 ($n=36$) and day 0 (a random sub-sample, $n=26$) of the FTAI protocol. Thereafter, the effect of ovarian characteristics on the pregnancy rate of the suckled cows was evaluated. Study 2 was performed to evaluate the effect of oral propylene glycol supplementation in relation to factors including season, BCS, and the parturition to synchronization interval (between the day of parturition and the day of CIDR insertion) on the pregnancy rate of suckled cows to FTAI. A total of 175 suckled cows were included in the FTAI protocol. Two experimental groups were formed: control ($n = 118$) and treatment ($n = 57$). In the control group, rice bran (without propylene glycol) was given, 1 kg per female daily, from day -10 to day -1. In the treatment group, propylene glycol (1 ml per kg body weight) was mixed with 1 kg of rice bran per female and given daily from day -10 to day -1. In both the control and treatment groups, rice bran was fed at 8.00 to 9.00 am separately from the typical forage. The pregnancy rate of the females in the control and treatment groups was measured and compared.

Measurement of ovarian characteristics and pregnancy diagnosis: The ovarian characteristics and pregnancy of suckled cows synchronized with the FTAI protocol were evaluated using transrectal ultrasonography (HS-1600, Honda Electronics Co., Ltd., Toyohashi, Japan) equipped with a 7.5 MHz linear probe. In Study 1, the suckled cows' ovarian characteristics were measured on day -10, and subsequently, 26 were randomly selected for the measurement of ovarian characteristics on day 0 of the FTAI protocol. On day -10 and day 0, the ovarian characteristics, including the length, width of the ovaries, and the diameters of the largest follicles on both ovaries, were measured. The average of the left and right ovary measurements is representative of the length and width of the ovary. Pregnancy diagnosis was performed on day -10 of the FTAI protocol and

between day 60 and day 65 post-insemination by detecting the appearance of a fetus in the uterus. The same measurements were performed on the suckled cows in Study 2.

Synchronization protocol and artificial insemination (AI): The synchronization protocol was the same for both studies 1 and 2. It was performed as previously described by de Graaff and Grimard (2018). On day -10, non-pregnant females were given an intramuscular injection of 10 µg (2.5 ml) (Taponen *et al*, 2009) GnRH analogue (Receptal®, Merck Animal Health, Madison, NJ, U.S.A.) and a CIDR was inserted into the vagina. Thereafter, on day -3, the CIDR was removed and 25 mg (5 ml) of PGF_{2α} (Lutalyse®, Zoetis Inc, Kalamazoo, MI, USA) was administered intramuscularly. Artificial insemination was performed within 66 to 72 h after CIDR removal. The day of insemination was considered to be day 0. In addition, all females received an intramuscular injection of 10 µg (2.5 ml) GnRH analogue (Receptal®, Merck Animal Health, Madison, NJ, U.S.A.) immediately after artificial insemination. A conventional AI technique was employed to deposit frozen-thawed semen (0.25 ml straw) into the uterine body. The frozen semen used in this study was produced by a certified semen production unit under the Armed Forces Development Command, Supreme Command Headquarters. The AI procedure was carried out by a highly trained, certified, and professional technician.

Statistical analysis: Statistical analyses were conducted using SAS (SAS Inst. Inc., Cary, NC, USA). Continuous data were evaluated using the MEANS procedure, and the frequency of categorical parameters was analyzed using the FREQ procedure. In Study 1, to evaluate the effect of ovarian characteristics on the pregnancy rate of the cows, the independent variables were defined as width of the ovary on day -10 (< 15 mm and ≥ 15 mm), length of the ovary on day -10 (< 21 mm and ≥ 21 mm), largest follicle diameter on day -10 (< 7 mm and ≥ 7 mm), width of the ovary on day 0 (< 15 mm and ≥ 15 mm), length of the ovary on day 0 (< 21 mm and ≥ 21 mm), largest follicle diameter on day 0 (< 9 mm and ≥ 9 mm). The effect of independent variables on pregnancy rate was analyzed by the Chi-squared test using the FREQ procedure. In Study 2, the independent variable, propylene glycol supplementation, was considered as a main effect. The independent variables included season [hot ($n = 92$) and cool ($n = 83$)], BCS [< 2.5 ($n = 63$) and ≥ 2.5 ($n = 110$)], and the interval between parturition and initiation of the FTAI protocol were considered as covariates. The normality of the variables was checked by using the Univariate analysis procedure. The parturition-to-FTAI interval was not distributed normally and was categorized as < 90 days ($n = 39$), 90–149 days ($n = 65$), and 150–365 days ($n = 62$). The effect of independent variables on the pregnancy rate of the cows was analyzed by logistic regression using the GLIMMIX procedure of SAS. Least-square means $\pm$ SEM were obtained from each variable class and compared using the least significant differences (LSD) test. For all analyses, a significant difference was defined at $P < 0.05$.

Result

Study 1

Description of the ovarian characteristics of cows on the day of CIDR insertion and on the day of AI: The reproductive characteristics of the suckled cows synchronized with the FTAI protocol on the day of CIDR insertion and on the day of AI are presented in Table 1. The width of the ovary on the day of CIDR insertion and on the day of AI was 14.2 ± 3.3 mm and 15.4 ± 2.1 mm, respectively. The length of the ovary on the day of CIDR insertion and on the day of AI was 20.9 ± 4.1 mm and 20.8 ± 2.9 mm, respectively. Lastly, the largest follicle diameter on the day of CIDR insertion and on the day of AI was 7.5 ± 4.0 mm and 8.4 ± 2.8 mm, respectively.

Effect of ovarian characteristics on the day of CIDR insertion and the day of AI on the pregnancy rate of cows: The ovarian characteristics on the day of CIDR insertion did not affect the pregnancy rate of cows ($P > 0.05$, Table 2). In addition, the width and the length of the ovaries on the day of AI did not affect the pregnancy rate of cows ($P = 0.116$ and $P = 0.658$, respectively, Table 2). Suckled cows with the largest follicle diameter, at least 9.0 mm, on the day of AI had a higher pregnancy rate than those where the largest follicle diameter was below 9.0 mm on the day of AI (50.0 % vs. 7.1 %, $P = 0.014$, Fig. 1).

Table 1 A description of the ovarian characteristics on the day of CIDR insertion and on the day of AI for suckled cows undergoing a FTAI protocol.

Variables	Means $\pm$ SD	Range
Day of insertion (n = 36)		
Width of the ovary (mm)	14.2 ± 3.3	9.2–22.3
Length of the ovary (mm)	20.9 ± 4.1	13.0–28.6
Follicle diameter (mm)	7.5 ± 4.0	1.0–23.9
Day of AI (n = 26)		
Width of the ovary (mm)	15.4 ± 2.1	12.5–22.5
Length of the ovary (mm)	20.8 ± 2.9	13.1–28.3
Follicle diameter (mm)	8.4 ± 2.8	5.0–15.4

Table 2 Effect of reproductive characteristics on the day of CIDR insertion and on the day of AI on the pregnancy rate of suckled cows undergoing a FTAI.

Characteristics	Class	Pregnancy rate (%)	P value
On the day of CIDR insertion (n = 36)			
Width of the ovary (mm)	< 15 mm	11.1	0.211
	≥ 15 mm	30.0	
Length of the ovary (mm)	< 21 mm	20.0	0.750
	≥ 21 mm	15.4	
Follicle diameter (mm)	< 7 mm	12.5	0.453
	≥ 7 mm	23.1	
On the day of AI (n = 26)			
Width of the ovary (mm)	< 15 mm	14.3	0.116
	≥ 15 mm	41.7	
Length of the ovary (mm)	< 21 mm	23.1	0.658
	≥ 21 mm	30.8	

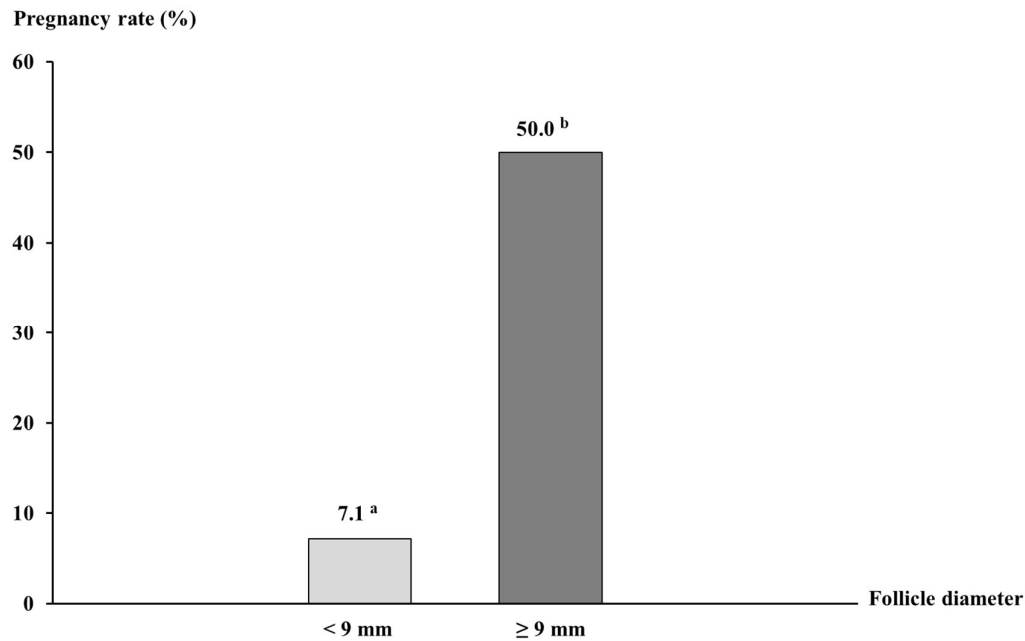


Figure 1 Effect of follicle diameter on the day of AI on the pregnancy rate of suckled cows undergoing a FTAI. ^{a, b} means with different superscript letters differ significantly ($P < 0.05$).

Study 2

Effect of oral propylene glycol supplementation, season, body condition score, and parturition-to-FTAI interval on the pregnancy rate of suckled cows synchronized with the FTAI protocol: Overall, the pregnancy rate of the suckled cows undergoing FTAI in the present study was 56.9% (99/174). The pregnancy rate of the suckled cows was affected by propylene glycol supplementation, season, body condition score, and the parturition-to-FTAI interval ($P < 0.05$, Table 3). The pregnancy rate of suckled cows in the treatment group was significantly higher than in the control group (respectively, 68.4% vs. 51.4%, $P = 0.036$). The suckled cows synchronized for FTAI in the hot season had a lower pregnancy rate than those synchronized in the cool season (respectively, 44.6% vs. 70.4%, $P = 0.0007$). The suckled cows with a BCS less than 2.5 had a lower pregnancy rate than those with a BCS above 2.5 (35.5% vs. 70.6%, $P < 0.001$). Lastly, suckled cows with a parturition-to-FTAI interval of less than 90 days had a lower pregnancy rate than those with a parturition-to-FTAI interval of 90–149 days (38.5% vs. 64.6%, $P = 0.024$). The parturition-to-FTAI interval 150–365 days (61.7%) was not different from the groups with a parturition-to-FTAI interval below 90 days (38.5%, $P = 0.056$) and 90–149 days (64.6%, $P = 0.939$).

The interaction between oral propylene glycol supplementation and season on the pregnancy rate of suckled cows subjected to FTAI: The interaction between propylene glycol supplementation and season on the pregnancy rate of cows is presented in Fig. 2. In the cool season, the pregnancy rate of suckled cows in the treatment group was not different from that of the control group (75.0% vs. 65.8%, $P = 0.393$, Fig. 2). In the hot season, the pregnancy rate of suckled cows in the treatment group was not different from that of the control group (52.9% vs. 42.4%, $P = 0.423$, Fig. 2). The

pregnancy rate of suckled cows in the treatment group in the cool season (75.0%) was not significantly different from suckled cows in the treatment group in the hot season (52.9%, $P = 0.391$, Fig. 2) and cows in the control group in the cool season (65.8%, $P = 0.872$, Fig. 2). However, the pregnancy rate of cows in the treatment group in the cool season was higher than cows in the control group in the hot season (75.0% vs. 42.4%, $P = 0.005$, Fig. 2).

The interaction between oral propylene glycol supplementation and BCS on the pregnancy rate of suckled cows undergoing FTAI: The interaction between propylene glycol supplementation and BCS on the pregnancy rate of suckled cows is presented in Fig. 3. The pregnancy rate of suckled cows in control group with BCS < 2.5 (30.2%) was lower than that of the cows in the treatment group with BCS ≥ 2.5 (78.9%, $P < 0.0001$) and cows in the control group with BCS ≥ 2.5 (65.6%, $P = 0.0009$). However, the pregnancy rate of suckled cows in the control group with BCS < 2.5 (30.2%) was not different from that of suckled cows in the treatment group with BCS < 2.5 (47.3%, $P = 0.539$). Likewise, the pregnancy rate among suckled cows in the treatment group with BCS ≥ 2.5 (78.9%) and those in the treatment group with BCS < 2.5 (47.3%) and in the control group with BCS ≥ 2.5 (65.6%) were not different ($P > 0.05$).

The interaction between oral propylene glycol supplementation and the parturition-to-FTAI interval on pregnancy rate of suckled cows undergoing FTAI: The interaction between propylene glycol supplementation and the parturition-to-FTAI interval on the pregnancy rate of suckled cows is presented in Fig. 4. The lowest pregnancy rate was observed in suckled cows in the control group with a parturition-to-FTAI interval of less than 90 days (22.2%) compared to suckled cows in the control group with a parturition-

to-FTAI interval of 90–149 days (58.1%, $P = 0.027$) and suckled cows in the control group with a parturition-to-FTAI interval of 150–365 days (64.8%, $P = 0.006$) and suckled cows in the treatment group with a parturition-to-FTAI interval of less than 90 days (75.0%, $P = 0.019$) and suckled cows in the treatment group with a parturition-to-FTAI interval of 90–149 days (77.2%, $P = 0.001$). The pregnancy rate of suckled

cows in the treatment group with a parturition-to-FTAI interval of 150–365 days (56.5%) was not significantly different from that of the control group for the same interval ($P > 0.05$, Fig. 4).

Table 3 Effect of oral propylene glycol treatment, season, BCS, and the parturition-to-FTAI interval on the pregnancy rate of suckled cows undergoing a FTAI.

Independent variables	P value	Class	Pregnancy rate (%)
Main factor			
Propylene glycol supplementation	0.036	control treatment	51.4 ^a 68.4 ^b
Covariates			
Season	< 0.001	hot cool	44.6 ^a 70.4 ^b
BCS	< 0.001	< 2.5 ≥ 2.5	35.5 ^a 70.6 ^b
Parturition-to-FTAI interval (days)	0.022	< 90 90–149 150–365	38.5 ^a 64.6 ^b 61.7 ^{ab}

^{a, b} means that the different superscripts within a covariate class differ significantly ($P < 0.05$).

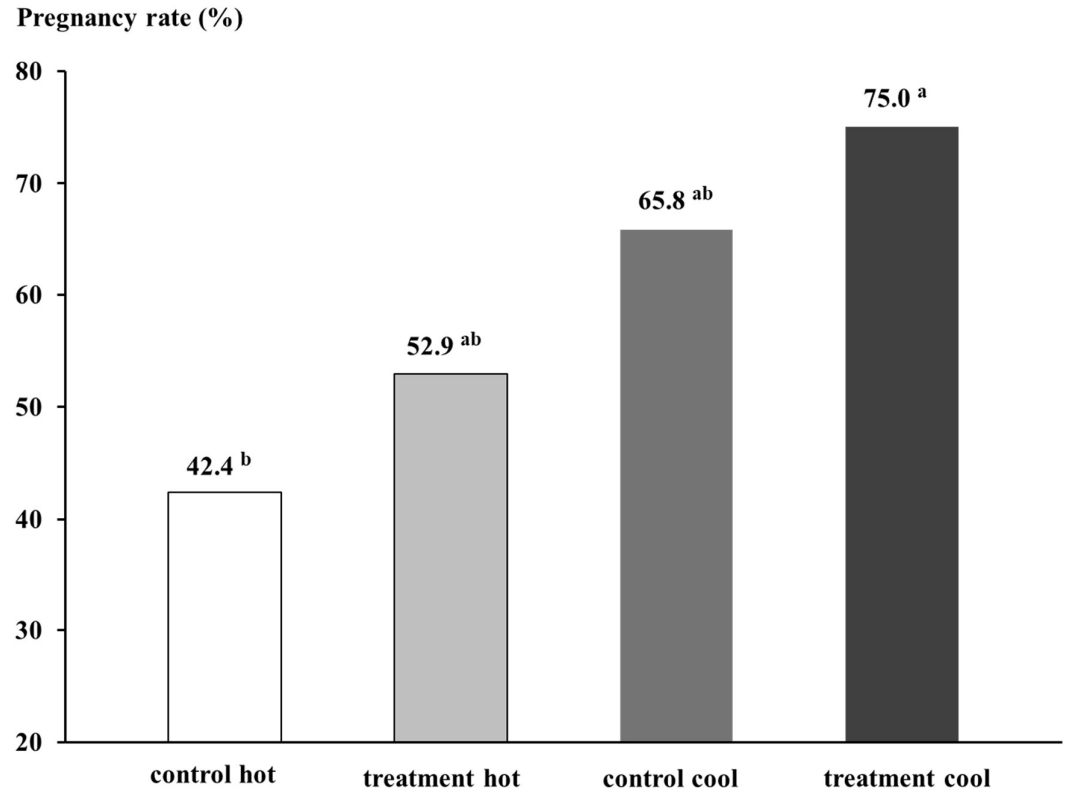


Figure 2 Interaction between oral propylene glycol treatment and season on the pregnancy rate of suckled cows undergoing FTAL. ^{a, b} means with different superscript letters differ significantly ($P < 0.05$).

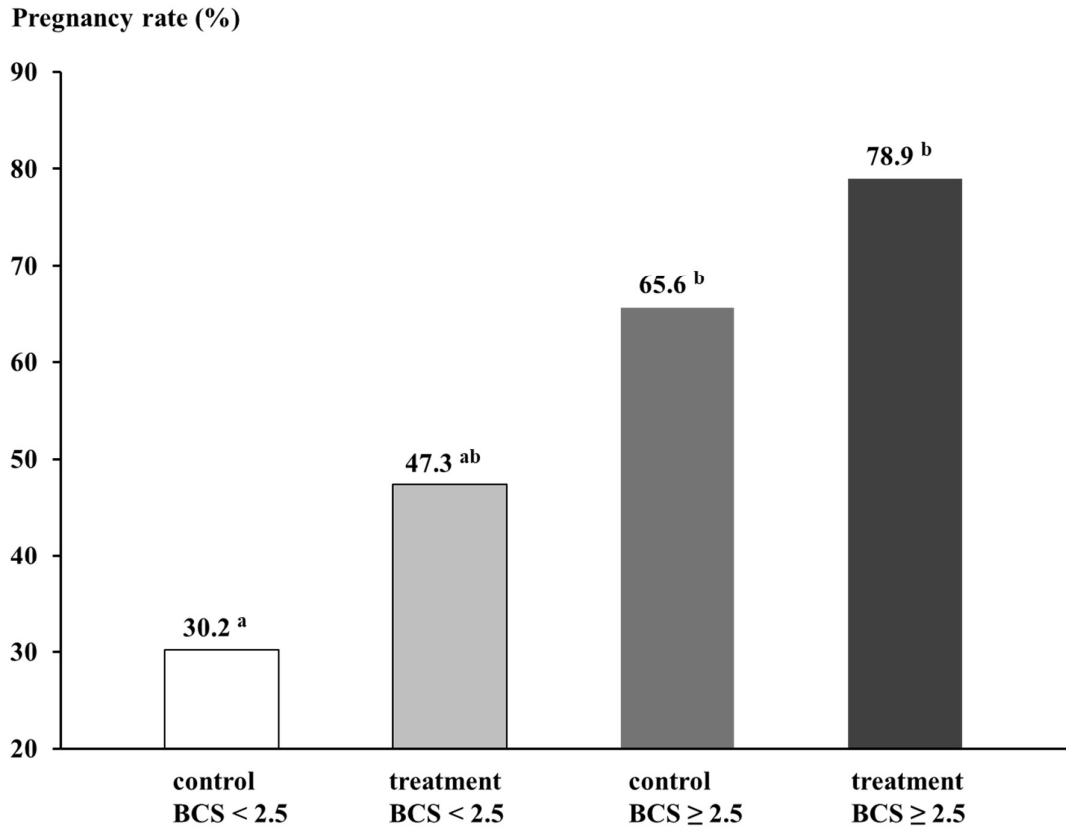


Figure 3 Interaction between oral propylene glycol treatment and body condition score on the pregnancy rate of suckled cows undergoing FTAL. ^{a, b} means with different superscript letters differ significantly ($P < 0.05$).

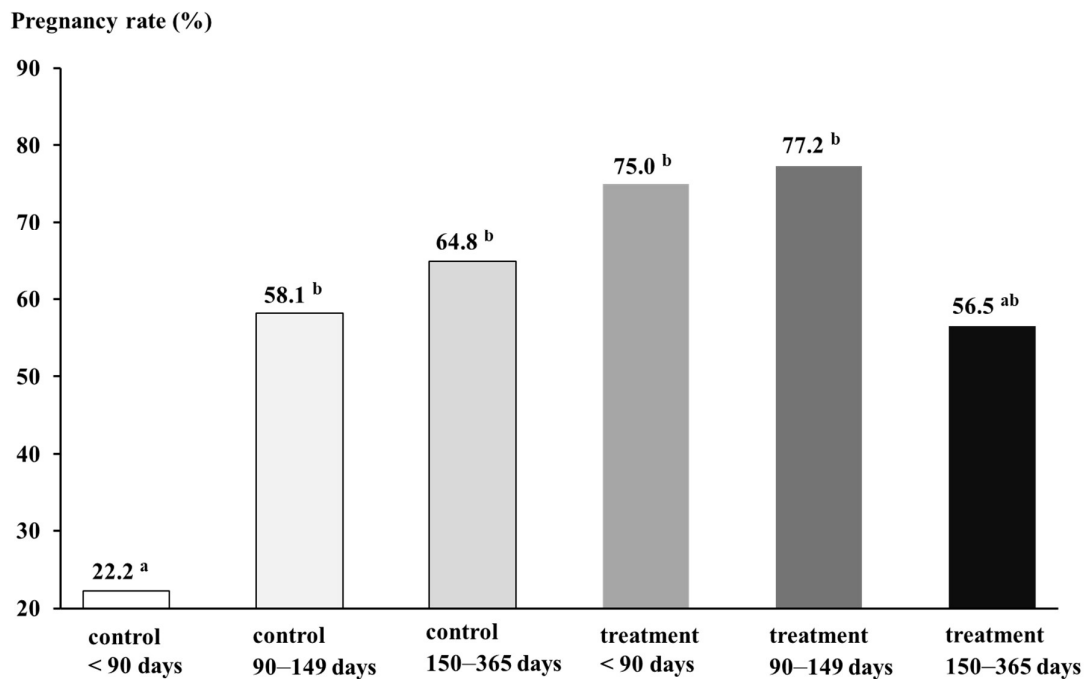


Figure 4 Interaction between oral propylene glycol treatment and the parturition-to-FTAI interval on the pregnancy rate of suckled cows undergoing a FTAI. ^{a, b} means with different superscript letters differ significantly ($P < 0.05$).

Discussion

In this study, the overall average pregnancy rate for cows in both Study 1 and Study 2 was 38.1%, with a range of 19.4% to 56.9%. This was notably lower than the 45% to 68% pregnancy rates reported in previous studies that used the CO-Synch + CIDR protocol, where artificial insemination was conducted 66 to 72 h after CIDR removal (Dobbins *et al.*, 2009; Bridges *et al.*, 2008). This may be due to a breed effect since in the present experiment we used native crossbred Thai cattle, whereas previous publications used Angus crosses.

In Study 1, the overall ovarian characteristics, including the average width, length, and follicle diameter of the ovary on the day of CIDR insertion and on the day of AI, appear to be consistent with published literature. We recorded an average follicle diameter on the day of AI (8.4 mm), which was slightly smaller compared to earlier findings using the 7-day CO-Synch AI protocol, which induced ovulation with GnRH 48 to 66 h after CIDR removal. The latter experiment recorded follicle diameters ranging from 12.7 to 13.3 mm (Tadesse *et al.*, 2011). Furthermore, a previous study using the CO-Synch AI protocol showed that cows with smaller-than-average follicles, which were induced to ovulate with GnRH after the removal of the intravaginal progesterone-releasing device, had a lower likelihood of becoming pregnant (Lamb *et al.*, 2001). In the present experiment, we found that the size of the largest follicle on the day of AI affected the pregnancy rate of cows synchronized with the FTAI protocol. In postpartum anestrus cows that had ovulation induced with GnRH, a positive correlation was observed between conception rate and ovulatory follicle diameter (Perry *et al.*, 2007). Additionally, larger follicle diameters at FTAI were

associated with higher ovulation rates, estrus expression, and improved pregnancy rates in the cows that had ovulated (Filho *et al.*, 2010). These findings agree with the current results regarding the impact of follicle diameter on the day of artificial insemination (AI) and pregnancy rates in suckled cows. The larger follicle diameter (> 9 mm) and the higher pregnancy rates observed in the present study may be attributed to the increased sensitivity of larger follicles to ovulation induction by luteinizing hormone (LH) following GnRH administration. The granulosa cells in follicles with a diameter close to 10 mm show an increase in mRNA levels for the LH receptor (Xu *et al.*, 1995; Bao *et al.*, 1997). Follicles reach ovulatory capacity at approximately 10 mm in diameter. Around 80% of cows with follicles of this size ovulate in response to LH treatment (Sartori *et al.*, 2001), while smaller growing follicles, measuring 7 mm or 8.5 mm diameter, did not ovulate after LH treatment (Sartori *et al.*, 2001).

To date, few studies have reported the impact of oral propylene glycol supplementation before insemination on the reproductive response of cows undergoing a 7-day Co-Synch FTAI protocol. In postpartum cows undergoing the Ovsynch FTAI protocol, daily oral supplementation with 300 ml of propylene glycol from the start of the Ovsynch protocol until insemination did not affect follicle size on the day of service of dairy cows (Cakircali *et al.*, 2023). In addition, although 500 ml of propylene glycol given daily to dairy cows from parturition until ovulation increased oocyte quality and maturation compared to controls, the differences were not statistically significant (Rizos *et al.*, 2008). Further research is needed to determine the effect of oral propylene glycol supplementation on oocyte developmental competence.

The effect of oral propylene glycol supplementation on the pregnancy rate of cows undergoing a FTAI protocol has produced interesting results. For example, cows that received a propylene glycol supplementation had a higher pregnancy rate compared to controls (46.1% vs. 30.6%, $P = 0.05$) on day 30 after FTAI using the Ovsynch protocol (Cakircali *et al.*, 2023). However, in the same experiment, when the pregnancy rate was measured on day 60, there was only a trend for an improvement in pregnancy rate, PG > controls ($P = 0.07$, 42.1% vs. 27.8%, respectively) (Cakircali *et al.*, 2023). Oral propylene glycol administration did have a clear positive impact by lowering serum levels of metabolic parameters associated with fertility, such as beta-hydroxybutyrate (BHB) (Cakircali *et al.*, 2023) and non-esterified fatty acids (NEFA) (Rizos *et al.*, 2008; Cakircali *et al.*, 2023), compared to the control group. Ospina *et al.* (2010) showed that pregnancy rate in cows decreased by 0.8% to 1.2% when over 15% of the herd had serum BHBA levels exceeding 12 mg/dl and serum NEFA concentrations above 0.3 mEq/l from two days before calving to two weeks postpartum. In cattle, BHBA has been found to activate the nuclear factor-kappa B (NF- κ B) signaling pathway, which in turn increases the expression of inflammatory cytokines in endometrial cells, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor α (TNF α). The inflammatory response triggered by BHBA is considered a crucial link between high BHBA levels during ketosis and the rise in uterine inflammation and poor reproductive outcomes in cows (Li *et al.*, 2019). Therefore, the improvement in pregnancy rates observed with an oral propylene glycol supplement in the present study might be due to its effect on mitigating NEB-related metabolic parameters, which are associated with reproductive performance in cows.

Previous studies have described the impact of seasonal heat stress on the reproductive performance of cows undergoing FTAI, with varying results (Pegorer *et al.*, 2007; De Rensis *et al.*, 2015; Ungerfeld *et al.*, 2021). The pregnancy rates for cows undergoing either GnRH or hCG-based FTAI protocols were similar in both the summer and the winter (De Rensis *et al.*, 2002). In contrast, a seasonal effect on pregnancy rate was observed in cows undergoing fixed-time artificial insemination (FTAI) with a CIDR, estradiol benzoate, and GnRH-based protocol, with pregnancy rate higher in autumn than in spring (Ungerfeld *et al.*, 2021). The variation in seasonal effects observed may be linked to the different FTAI protocols employed in various studies. To date, there is no reported data on how seasonality affects the pregnancy rates of cows undergoing a 7-day CO-synch + CIDR FTAI protocol. Nonetheless, it is well-established that heat stress is associated with increased pregnancy losses in cows. Heat stress lowers uterine blood flow, leading to elevated uterine temperatures (Roman-Ponce *et al.*, 1997), which creates an unfavorable environment for fertility (Gwazdauskas *et al.*, 1973).

Following insemination, heat stress adversely affected embryonic development (Bigger *et al.*, 1987). The reduction in embryonic development may be linked to a decline in interferon-tau production, which is crucial for preventing corpus luteum regression and

sustaining pregnancy (Spencer *et al.*, 2007). Heat stress in lactating cows altered their metabolism independently of their energy balance or nutritional intake (Rhoads *et al.*, 2009). In warm conditions, cows relied on β -oxidation to generate metabolic heat rather than carbohydrate oxidation (Baumgard *et al.*, 2011). This led to elevated levels of serum NEFA and BHBA in heat-stressed cows (Ingvarsen and Anderson, 2000; Tian *et al.*, 2015). The current findings suggest that oral propylene glycol, a glucogenic precursor, may not successfully reduce serum NEFA and BHBA levels in heat-stressed cows during hot weather. This is consistent with a previous study by Erisir and Yildiz (2016), which found no significant improvement in pregnancy rates among heat-stressed cows supplemented with propylene glycol (250 ml daily from day 3 to 15 postpartum) compared to a control group. However, in winter, lactating cows with high milk yields and poor body condition scores are likely to experience a more pronounced negative energy balance (Thammacharoen *et al.*, 2021). Therefore, propylene glycol supplementation might help mitigate negative energy balance in lactating cows during cooler seasons.

In the current study, the influence of BCS at fixed-time AI on pregnancy rates agrees with previous research. A higher BCS before insemination is crucial for successful pregnancies following FTAI (Sá Filho *et al.*, 2010; Junior *et al.*, 2017). Cows with optimal BCS tend to have better pregnancy outcomes due to improved hormonal balance, reproductive tract health, and more effective responses to synchronization protocols. Postpartum cows with reduced BCS often have lower serum IGF-1 levels (Carvalho *et al.*, 2022) and higher serum BHBA and NEFA (Barletta *et al.*, 2017). In the later study, cows with a BCS of ≥ 2.5 (5-point scale) had the highest pregnancy rates. In the present study, propylene glycol-treated cows with a BCS < 2.5 had a numerically higher pregnancy rate compared to control cows with a BCS < 2.5; however, the difference was not statistically significant. Our results show that oral propylene glycol supplementation (1 ml per kg body weight) improved reproductive performance in cows with an optimal BCS undergoing FTAI. However, the current dose may be inadequate to enhance reproductive performance in cows in poor BCS undergoing FTAI. Further research is needed to determine if adjusting the propylene glycol dose can improve pregnancy outcomes for cows with lower BCS.

In this study, the impact of the interval from parturition to FTAI on pregnancy rates agrees with findings from previous studies (Pursley *et al.*, 1998; Taponen, 2009; Randi *et al.*, 2021; Tomazi Filho *et al.*, 2022). Cows with a short interval from calving (50–70 days) and subjected to the Ovsynch protocol exhibited higher pregnancy rates compared to those with longer intervals (> 70 days) (77.7% vs. 37.5–50.0%, respectively) (Taponen *et al.*, 2009). Conversely, when cows were treated with a GnRH and PGF2 α -based protocol, early postpartum cows (50–75 days) had a lower pregnancy rate compared to later postpartum cows (76–100 days and > 100 days) (36% vs. 47% and 43%, respectively) (Pursley *et al.*, 1998). For cows treated with the 7-day CO-Synch FTAI protocol, the

pregnancy rate was lowest (49%) in cows ≤ 50 days postpartum and highest (63%) in cows 60–80 days postpartum (Randi *et al.*, 2021). Additionally, in protocols based on P4 and estradiol analogs, cows with a calving-to-service interval < 52 days had a lower pregnancy rate than those with a calving-to-service interval ≥ 52 days (46% vs. 56%, respectively) (Tomazi Filho *et al.*, 2022). Previous research reveals inconsistent findings on how the calving-to-service interval impacts pregnancy rates, with variations attributed to factors such as different synchronization protocols (Geary *et al.*, 1998) and cow parity (Araújo *et al.*, 2022). Typically, the uterus is fully involuted by 40 days postpartum (Lin *et al.*, 2021). In cows where calves were weaned at 180 days postpartum, high NEFA levels were still observed at days 30 and 81 postpartum (Alforma *et al.*, 2022). The decreased pregnancy rate observed in the control group with a calving-to-FTAI interval of < 90 days may be due to incomplete uterine involution and/or metabolic imbalance within 90 days postpartum. Supplementation with propylene glycol might improve the pregnancy rate in cows with a calving-to-FTAI interval of < 90 days by reducing serum NEFA levels.

In conclusion, the 7-day CO-synch + CIDR FTAI protocol was effectively applied to suckled beef cows in a rural setting, achieving a promising pregnancy rate. The pregnancy rate depended on the diameter of the largest follicle at the time of FTAI and the use of propylene glycol supplementation during the protocol. Suckled cows with follicle diameters greater than 9.0 mm had a higher pregnancy rate compared to those with smaller diameters. Propylene glycol supplementation (1 ml per kg body weight) during the FTAI protocol was found to improve pregnancy rates. However, lower pregnancy rates were observed: when insemination occurred in hot weather, in cows with a body condition score below 2.5, or with a parturition-to-FTAI interval of less than 90 days. Conversely, propylene glycol supplementation was more effective in enhancing pregnancy rates when insemination was performed in the cool season compared to the hot season and in cows with a body condition score of at least 2.5 compared to cows with a lower BCS. In the present study, a propylene glycol supplementation did not counterbalance the factors that had a negative effect on reproductive success.

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