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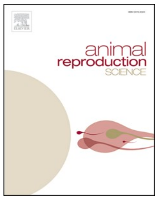

Comparison of three reproductive management strategies for lactating dairy cows using combination of estrus detection or ovulation synchronization and Fixed-Timed Artificial Insemination

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A B S T R A C T

The objective of this study was to compare the reproductive performance of lactating dairy cows submitted to first AI after combination of estrus detection and fixed timed AI (**FTAI**) and FTAI only. Cows were randomly assigned to receive AI at detected estrus between 50 and 70 d in milk (**DIM**), if not detected in estrus, were enrolled in either Ovsynch **(ED-Ov,** n = 485) or PRIDsynch (**ED-PR,** n = 505) protocols; or received FTAI at 80 DIM after Double-Ovsynch protocol (**DO,** n = 501). Cows were body condition scored (**BCS**) at calving and at 43 DIM; and evaluated for postpartum disorders within 7 d postpartum; clinical mastitis, lameness and bovine respiratory disease were recorded until first AI. Ovarian cyclicity was monitored at 43 and 50 DIM, and at 70 and 77 DIM. Pregnancy diagnoses (**PD**) were performed at 32 and 63 d after AI. Overall prevalence of postpartum anovulation was 7.8%. Pregnancy per AI (**P/AI**) did not differ between reproductive strategies at 32 d PD (ED-Ov = 43.2%; ED-PR = 41.7%; DO= 45.3%). Primiparous cows had greater P/AI than multiparous cows (53.7% *vs* 36.8%). Cows on farm 1 had lower P/AI compared with their counterparts on farm 2 (42.1% *vs* 45.4%). Cows with BCS > 2.5 at 43 DIM had greater P/AI compared with cows with BCS ≤ 2.5 (44.5% *vs* 34.7%). Similar P/AI for cow's receiving AI at detected estrus and FTAI, low prevalence of disease anovulation may have contributed to the similar performance of ED-Ov, ED-PR and DO.

1. Introduction

Reproductive performance is strongly associated with dairy herd profitability and sustainability (Bekara and Bareille, 2019; Cabrera, 2014; Giordano et al., 2011). Reproductive strategies are designed to maximize milk production (Pecsok et al., 1994), produce enough replacement heifers (Giordano et al., 2012b), and minimize reproductive management costs (Giordano et al., 2012b). The rate at which pregnancies can be achieved after the voluntary waiting period (**VWP**) is a combination of both insemination risk (**IR**) and pregnancy per artificial insemination (**P/AI**) (Bekara and Bareille, 2019; Cabrera, 2014). In herds using 100% synchronization

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of ovulation for fixed-timed artificial insemination (**FTAI**), all cows will receive AI at the end of the program (IR= 100%), so the success of these programs depends on achieving a high P/AI. Conversely, strategies reliant on detection of estrus (**ED**) are subject to variations in both IR and P/AI.

Although hormonal synchronization of ovulation and FTAI is widely used to improve reproductive performance, many dairy farms still prefer to AI cows after ED (Caraviello et al., 2006; Ferguson and Skidmore, 2013). Several factors may lead to sub-optimal reproductive performance in reproductive strategies using ED, for example, ED depends on the display of estrous behavior, milk yield (Lopez et al., 2004), lameness (Somers et al., 2015) and other environmental factors (Palmer et al., 2010). In addition, approximately 20% of lactating dairy cows are anovular before first postpartum AI (Bamber et al., 2009; Walsh et al., 2007a, 2007b), meaning they are unlikely to express estrus (Wiltbank et al., 2002). Cows suffering from negative energy balance, diagnosed with postpartum diseases and primiparous cows are at further increased risk of anovulation (Monteiro et al., 2020; Ribeiro et al., 2013).

A common reproductive strategy in dairy herds involves a combination of submitting to AI cows detected estrus at the end of the VWP, and the remaining cows treated with Ovsynch protocol for FTAI (Ferguson and Skidmore, 2013), fixing a time for the first postpartum AI. Using Ovsynch in dairy cows resulted in a reduction of the median days to first AI and days open compared to cows receiving AI after ED (Pursley et al., 1997). In addition, cows submitted to AI after Ovsynch achieve similar P/AI to that of cows receiving AI after ED (39% *vs* 37%) (Pursley et al., 1997) with limited hormone use. However, P/AI to FTAI after Ovsynch may be affected by variable synchronization rates (Moreira et al., 2000a; Vasconcelos et al., 1999; Wiltbank et al., 2014) and poor fertility of anovular cows at FTAI (Gümen et al., 2003).

Ovsynch with progesterone (**P4**) supplementation and presynchronization with GnRH and $PGF_{2\alpha}$ (Double-Ovsynch) are examples of two of several modifications to the original Ovsynch protocol to overcome synchronization rate challenges and improve response to the hormonal treatments in cyclic and anovular cows.

Progesterone supplementation between first GnRH and $PGF_{2\alpha}$ injections of Ovsynch improved P/AI at FTAI compared to Ovsynch alone (Chebel et al., 2010; Lima et al., 2009; Stevenson et al., 2006) since cows lacking a corpora lutea (**CL**) and with low circulating P4 concentrations at initiation of the synchronization protocol may benefit from exogenous P4 (Bisinotto et al., 2015; Sangsritavong et al., 2002). Adequate circulating P4 concentrations are important for optimal oocyte and embryo development (Rivera et al., 2011) and reduction of embryo losses (Bisinotto et al., 2015; Walsh et al., 2007a, 2007b).

Double Ovsynch (**DO**) uses a complete Ovsynch protocol as a presynchronization procedure (Souza et al., 2008) and improved fertility by optimizing the time of the estrous cycle for initiation of the breeding Ovsynch and possibly by inducing ovulation in anovular cow. Initiating breeding Ovsynch between d 5 and 12 of the estrous cycle was shown to optimize P/AI in lactating dairy cows (Cartmill et al., 2001; Moreira et al., 2000a; Vasconcelos et al., 1999). This was associated with an increased probability of a dominant follicle ovulating following first GnRH injection, leading to formation of a CL with subsequent elevated circulating P4 concentrations, and successful CL regression after $PGF_{2\alpha}$ administration (Cartmill et al., 2001; Moreira et al., 2000b; Vasconcelos et al., 1999). Double Ovsynch enhances P/AI compared with other reproductive strategies without the need for ED (Denis-Robichaud et al., 2017; Rial et al., 2022; Stangaferro et al., 2018), and restores ovarian activity in most anovular cows (Ayres et al., 2013; Herlihy et al., 2012; Souza et al., 2008). However, DO is a more complex protocol that increases the number of hormonal treatments and associated labor.

Although synchronization protocols are widely used by veterinarians in the UK to improve fertility performance in dairy cattle, to our knowledge, there has not been any research comparing DO with more traditional reproductive strategies that combine ED and FTAI under UK housing and management conditions.

The objective of this study was to compare three reproductive management strategies for lactating dairy cows: i) combination of ED and Ovsynch for FTAI; ii) combination of ED and PRIDsynch for FTAI; iii) Fixed timed AI after DO protocol. The primary hypothesis was that DO would result in greater P/AI at the time of first AI when compared with strategies reliant on estrus detection combined with hormonal synchronization.

2. Materials and methods

2.1. Animals housing and management

Cows were enrolled between October 2018 and February 2020 from two commercial Scottish dairy farms serviced by the Scottish Centre for Production, Animal Health and Food Safety, University of Glasgow (under University of Glasgow ethics number 44a/18). All cows were milked three times a day and housed all year round in free-stall sheds. Primiparous and multiparous cows were housed separately, and multiparous cows were separated into high and low yielding groups. During the study, farms 1 and 2 milked 765 and 580 cows, respectively, and mean farm 305d mature-equivalent milk production (from the Cattle Information Service (**CIS**) monthly milk recordings) was 13,279 and 10,770 kg, respectively. Cows were fed once a day in a single rail feedline barrier with a total mixed ration (**TMR**) based on grass silage, cereals and a concentrate-mineral mix, meeting or exceeding the requirements for maintenance and milk production (NRC, 2001), with *ad libitum* access to water.

2.2. Experimental design

Pregnant heifers, primiparous and multiparous cows were blocked by parity and randomly assigned to one of three different reproductive strategies and enrolled at parturition: ED-Ovsynch (**ED-Ov;** n = 485), ED-PRIDsynch (**ED-PR;** n = 505) and Double Ovsynch (**DO;** n = 501). Cows enrolled in ED-Ov and ED-PR groups were eligible for insemination at detected estrus between 50 ± 3 and 70 ± 3 DIM. Trained farm personnel performed ED three times a day based on visual observation (for 30 min each time). Cows received a single AI 12 h if estrus was observed. If cows in ED-Ov and ED-PR groups were not detected in estrus between 50 ± 3 and 70 ± 3 DIM, they were subjected to one of the following hormone protocols (Fig. 1):

a) Ovsynch: GnRH (G1) at 70 ± 3 DIM, $PGF_{2\alpha}$ (PG1) 7 d later, $PGF_{2\alpha}$ (PG2) 24 h later, GnRH (G2) 32 h later and FTAI 16 h later.
b) PRIDsynch: GnRH (G1) + PRID at 70 ± 3 DIM, $PGF_{2\alpha}$ (PG1) 7 d later, $PGF_{2\alpha}$ (PG2) + PRID removal 24 h later, GnRH (G2) 32 h later and FTAI 16 h later.

Cows enrolled in the DO group, received GnRH (G) at 53 ± 3 DIM, 7 d later $PGF_{2\alpha}$ (PG), GnRH (G) 3 d later, GnRH (G1) 7 d later, $PGF_{2\alpha}$ (PG1) 7 d later, $PGF_{2\alpha}$ (PG2) 24 h later, GnRH (G2) 32 h later and FTAI 16 h later with no opportunity for mating at observed estrus. Trained technicians performed all inseminations (two technicians for farm 1; one technician for farm 2), using frozen-thawed commercial semen from high fertility sires. Sire selection (beef or dairy) was a farmer decision conducted using a commercial software package designed to optimize each mating based on each farm's specifications.

Insemination risk (**IR**) was defined as the number of cows receiving AI out of the total number of cows available for AI. Pregnancies per AI at first AI was defined as the percentage of cows that became pregnant after first postpartum AI out of the total number of cows receiving AI in each reproductive strategy. For all ovulation synchronization protocols, GnRH treatments consisted of 100 μg of Gonadorelin diacetate administered intramuscularly (Ovarelin®, Ceva Sante Animale, Libourne, France), and $PGF_{2\alpha}$ treatments consisted of 25 mg of Dinoprost Thromethamine Sodium administered intramuscularly (Enzaprost®, Ceva Sante Animale, Libourne, France). Progesterone supplementation consisted of 1.55 g progesterone, administered *via* a progesterone intravaginal device (PRID® Delta, Ceva Sante Animale, Libourne, France). Cows were excluded from the study if they were sold, classified as do not breed (**DNB**), died before AI, received AI before the end of the voluntary waiting period (**VWP**), non-compliance of the hormone protocols, or if they had missing data. Cows with uterine infection (**UI**) were also excluded as the farm's treatment protocols included $PGF_{2\alpha}$, which may have acted as a presynchronization and influenced the trial results.

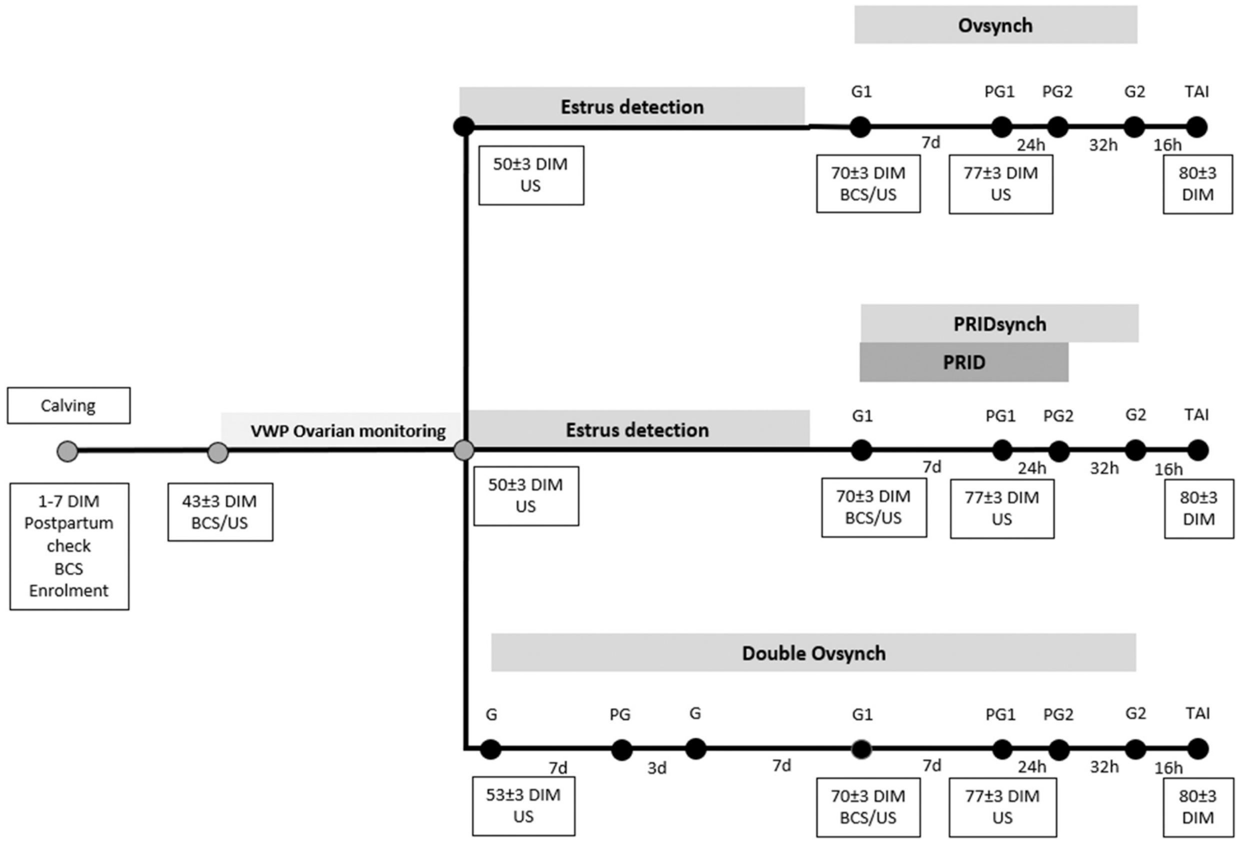

Fig. 1. Schematic representation of the reproductive strategies and procedures performed in the current study. All cows were randomized and allocated to receive AI after a combination of estrus detection and Ovsynch (ED-Ov), combination of estrus detection and PRIDsynch (ED-PR) and Double Ovsynch (DO). Cows in Double Ovsynch started the protocol at 53 ± 3 DIM and received fixed time AI at 80 ± 3 DIM. Postpartum check was performed between 1 and 7 days after calving; and body condition score (BCS) was measured at calving and 43 ± 3 DIM. Ovarian cyclicity monitoring was performed by transrectal US at 43 ± 3 and 50 ± 3 DIM and pregnancy diagnoses (PD) were performed at 32 ± 3 and 63 ± 3 d after AI.

2.3. Body condition score and milk production

Body condition score was performed at calving, at 43 ± 3 DIM and at 70 ± 3 DIM using a scale of 1–5 with 0.25 increments (Edmonson et al., 1989). Cows were classified as BCS ≤ 2.5 and BCS > 2.5 (Carvalho et al., 2014) and BCS 'loss' was attributed when ≥ 0.5 points were lost between measurements. Fourth week milk yield (**W4MK**) and cumulative milk yield 30 days after calving (**MK30**) data were retrieved from the dairy farms management software (DairyComp 305; Valley Agricultural Software, Tulare, CA, USA) and were considered as covariates in all the regression models.

2.4. Post-partum disease, ovarian cyclicity and uterine health monitoring

All cows were examined by veterinarians from the Scottish Centre for Production, Animal, Health and Food Safety, University of Glasgow between one and 7 DIM for health disorders. Clinical hypocalcemia, retained placenta (**RP**), metritis (Sheldon et al., 2006)and displaced abomasum (**DA**). Subclinical ketosis was diagnosed using a commercial urine-dip test strip (KetoStix®, Bayer Diagnostics Europe Ltd., Dublin, Ireland) (Carrier et al., 2004). Diagnoses of lameness (mobility score ≥2) (Whay et al., 2003), clinical mastitis, and bovine respiratory disease (**BRD**) were also recorded until first AI. If any health disorder was detected, cows were monitored weekly until they were considered healthy after veterinary clinical examination. Ovarian cyclicity and uterine health were monitored using transrectal ultrasonography (**US**) with a portable device equipped with a 7.5-MHz linear transducer (Easy-Scan II, BCF Technology Ltd., Livingston, UK) at 43 ± 3 DIM and 50 ± 3 DIM. The presence and size (using the Easy-Scan wrist screen grid) of follicles and CL were recorded for both ovaries at each examination. Cows were categorized into cyclic if a CL was observed on at least one of the two US evaluations, or categorized into anovular if no CL was observed at both US evaluations (Gümen and Wiltbank, 2005). Presence and size of follicles and number of CLs were also recorded in synchronized cows at US examination at G1 and P1, ovulatory size follicle (OSF) was defined as a follicle with size > 10 mm (Sartori et al., 2001). Uterine infection was defined as presence of echogenic intrauterine fluid at US (Kasimanickam et al., 2004).

2.5. Pregnancy diagnosis

Pregnancy diagnosis was performed between 29 and 35 d post-AI using transrectal US using the same device as for ovarian cyclicity and uterine health monitoring. Pregnant cows had a second US examination between 60 and 66 d post-AI to confirm pregnancy; non-pregnant cows at this point were recorded as pregnancy loss (**PL**).

2.6. Statistical analysis

Individual cow data were obtained from DairyComp 305, and exported to Microsoft Excel (Version 2011, Microsoft Corporation). Statistical analysis was performed in R (R Core Team, 2020). For the three treatment groups, with the 'pwr' package in R it was estimated that a sample of 330 cows per group would detect a 3.6–10.9% difference in P/AI between any two of these groups, with 95% confidence and 80% power, when using a two-tailed z-test (Cohen, 1988). Multivariable logistic regression models, as used in the final analysis, will have increased the precision further and allowed detection of even smaller differences between the groups. An additional 30% more cows were enrolled to account for cow losses during the observation period. Fisher's exact test was used to compare distributions of cows for categorical risk factors of reproductive strategy, farm and parity. Binary outcome variables— P/AI at 32 ± 3 d after first AI; P/AI at 63 ± 3 d after first AI; and PL—were modeled using logistic regression. Farm and reproductive strategy were included as fixed effects in all models, regardless of their effect sizes or statistical significance. The following covariates considered in the analysis were dichotomized (presence = 1; absence = 0): calving issues (including abortion, twins and stillbirth); health disorders before first postpartum AI (including hypocalcemia, subclinical ketosis, metritis, RP, DA, clinical mastitis, lameness and BRD), and ovarian cyclicity at 43 ± 3 and 50 ± 3 DIM. Parity was categorized into primiparous and multiparous cows. Body condition score at calving, at 43 ± 3 DIM and at 70 ± 3 DIM were dichotomized as BCS ≤ 2.5 and BCS > 2.5; BCS loss between calving and 43 ± 3 DIM (≥ 0.5 BCS lost; <0.5 lost, maintained or gained BCS). Other factors were also categorized including season (Spring: Mar-May; Summer: Jun-Aug; Autumn: Sep-Nov; Winter: Dec-Feb) and sire type (Beef; Holstein). At G1 and P1 US evaluations of the ovarian structures were classified by ovulatory size follicle (>10 mm) (presence = 1; absence = 0) and number of CLs (no CL = 0; 1 CL = 1; and 2 or more CLs = 2). Fourth week milk yield and MK30 were extracted from the monthly milk recordings. ANOVA was used to compare quantitative outcomes such as W4MK and DIM at first AI for the farm and protocol groupings. For all analyses, all variables were tested using univariable logistic regression and included in the multivariable analysis if P < 0.3. Multivariable logistic regression models were constructed using stepwise variable selection with Akaike's Information Criterion (AIC) to optimize predictive ability and model fit. All biologically plausible interactions were tested, including reproductive strategy × farm, reproductive strategy × parity, parity × farm and sire x parity. Covariates and interactions between reproductive strategies and covariates were retained only if they were statistically significant ($P<0.05$) in the final models. Diagnostic checks of the final models were done with the *DHARMa* package (Hartig, 2022). In particular, Kolmogorov-Smirnov tests were used to compare the distributions of the regression model residuals with simulated residuals; *DHARMa*'s built-in nonparametric dispersion tests were used to compare the variances of the model residuals and the simulated residuals; residual outlier tests were based on the binomial distribution; and visual checks of the residual distributions were plotted against each of the covariates included in the regression models.

3. Results

3.1. Descriptive statistics

From a total of 1491 dairy cows that were enrolled in this trial, data from 1037 cows were available for analysis. Reasons for exclusion for 454 cows (ED-Ov, n = 152; ED-PR, n = 145; DO, n = 157) were sold (51), DNB (78), UI (100), other reasons (53), missing data (74), non-compliance with protocols (71) and cows that died before AI (27).

3.1.1. Diseases before AI, milk yield, BCS and ovarian cyclicity

The proportion of primiparous and multiparous cows did not differ (*P*=0.85) among ED-Ov (39.9% *vs* 60.1%), ED-PR (39.2% *vs* 60.8%) and DO (37.8% *vs* 62.2%) reproductive strategies; however, they did differ (*P*<0.01) between farm 1 (primiparous = 43.3% *vs* multiparous = 56.7%) and farm 2 (primiparous = 32.4% *vs* multiparous = 67.6%).

Descriptive statistics are presented in Table 1. Combined calving issues were 9.6% (n = 32/333), 4.7% (n = 17/360) and 6.6% (n = 23/344) for ED-Ov, ED-PR and DO, respectively. There was a greater proportion (*P*=0.02) of cows with calving issues in ED-Ov compared to ED-PR; however, the proportion of cows with calving issues did not differ between ED-PR and DO (*P*=0.33), or ED-Ov and DO (*P*=0.21). There was no significant difference among reproductive strategies in combined diseases before AI (*P*= 0.14). However, farm 1 (21.7%; n = 135/623) had significantly lower number of cows suffering a disease before AI when compared to farm 2 (28%; n = 116/414). In addition, multiparous cows were more likely to suffer a disease before AI when compared to primiparous cows (30.3% *vs* 14.6%; *P*<0.01). Milk production between farms and protocols were comparable. Mean W4MK yields for farm 1 did not differ (*P*=0.99) among reproductive strategies and were (standard deviations and number of cows in brackets): OD-Ov, 42.1 liters (± 11.4; n = 192); ED-PR, 42.2 liters (± 11.8; n = 210); DO, 42.2 liters (± 11.5; n = 201). Mean W4MK yields for farm 2 did not differ (*P*=0.42) among reproductive strategies and were: OD-Ov, 36.0 liters (± 10.7; n = 93); OD-PR, 34.2 liters (± 9.5; n = 107); DO, 35.4 liters (± 9.6; n = 93). Cows on farm 1 had higher W4MK than on farm 2 (*P*< 0.01), with 42.2 liters (± 11.6; n = 603) and 35.1 liters (± 9.9; n = 293), respectively. In addition, mean yield for primiparous cows, 31.4 liters (± 7; n = 93), was significantly lower than for multiparous cows, 45.8 liters (± 10.3; n = 93) (*P*< 0.01). Week four milk yield and MK30 were highly correlated (r = 0.94), and therefore, only one of these variables was included in the regression models to avoid multicollinearity. Milk production was not significantly linked to pregnancy outcome in any of the regression models.

Overall, 92.9% (n = 853/918) of the cows calved with BCS > 2.5, 88.6% (919/1037) had BCS > 2.5 at 43 ± 3 DIM and 89.4% (n = 563/630) had BCS > 2.5 at 70 ± 3 DIM. A greater proportion of primiparous cows had BCS > 2.5 than multiparous cows at calving (*P*<0.01): 96.3% (334/347) *vs* 90.9% (519/571), respectively; at 43 ± 3 DIM 92.3% (373/404) *vs.* 86.3% (546/633), respectively; and at 70 ± 3 DIM 94.6% (210/222) *vs* 86.5% (353/408), respectively. Cows at farm 1 were more likely than cows at farm 2 to have BCS > 2.5 at calving: 99.1% (558/563) *vs* 83.1% (295/355), respectively; at 43 ± 3 DIM 95.3% (594/623) *vs* 78.5% (325/414), respectively (*P*<0.01); and at 70 ± 3 DIM 96.2% (358/372) *vs* 79.5% (205/258), respectively (*P*<0.01). However, there was no difference in BCS at calving (*P*=1.0); at 43 ± 3 DIM (*P*=0.85); and at 70 ± 3 DIM (*P*=0.3) between reproductive strategies.

Table 1

Descriptive statistics for 1037 lactating dairy cows enrolled in three reproductive strategies compared in the current study: combination of estrus detection and Ovsynch (ED-Ov), combination of estrus detection and PRIDsynch (ED-PR) and Double Ovsynch (DO) between November 2018 and February 2020.

Variable	Reproductive strategy				Farm			Parity		
	ED-Ov n = 333	ED-PR n = 360	DO n = 344	*P*-value	1 n = 623	2 n = 414	*P*-value	Primiparous n = 404	Multiparous n = 633	*P*-value
	(%/n)	(%/n)	(%/n)		(%/n)	(%/n)		(%/n)	(%/n)	
Calving issues[a]	9.6 (32)	4.7 (17)	6.7 (23)	0.04*	6.7 (42)	7.2 (30)	0.8	6.9 (28)	7.0 (44)	1.0
Diseases before 1st AI[b]	22.8 (76)	27.8 (100)	21.8 (75)	0.14	21.7 (135)	28.0 (116)	0.02	14.6 (59)	30.3 (192)	< 0.01
Ovarian cyclicity[c]				0.89			< 0.01			1.0
Cyclic	92.5 (308)	92.5 (333)	91.6 (315)		94.2 (587)	89.1 (369)		92.3 (373)	92.1 (583)	
Anovular	7.5 (25)	7.5 (27)	8.4 (29)		5.8 (36)	10.9 (45)		7.7 (31)	7.9 (50)	
BCS 43 ± 3 DIM				0.85			< 0.01			< 0.01
> 2.5	89.2 (297)	88.9 (320)	87.8 (302)		95.3 (594)	78.5 (325)		92.3 (373)	86.3 (546)	
≤ 2.5	10.8 (36)	11.1 (40)	12.2 (42)		4.7 (29)	21.5 (89)		7.7 (31)	13.7 (87)	
AI by sire				0.67			0.17			< 0.01
Holstein	82.9 (276)	80.6 (290)	80.5 (277)		82.7 (515)	79.2 (328)		85.9 (347)	78.4 (496)	
Beef	17.1 (57)	19.4 (70)	19.5 (67)		17.3 (108)	20.8 (86)		14.1 (57)	21.6 (137)	

[*] here was a significant difference between ED-Ov and ED-PR (*P*=0.02), but ED-OV and ED-PR do not differ from DO (*P*=0.21 and *P*=0.33, respectively)

[a] Calving issues recorded in the current lactation (abortion, stillbirth and twins)

[b] Diseases before AI in cows enrolled in the study (subclinical ketosis, retained placenta, metritis, displaced abomasum, lameness, clinical mastitis and bovine respiratory disease)

[c] Cows were categorized into cyclic or anovular, based on the presence or absence of a CL respectively at US evaluations performed at 43 ± 3 DIM and 50 ± 3 DIM

The proportion of anovular cows differed between farms (*P*<0.01), with 5.8% (n = 36/623) and 10.9% (n = 45/414) for farm 1 and 2, respectively. However, these differences were not associated with either parity (*P*= 1.0) or reproductive strategy (*P*= 0.89). A greater proportion of cows with BCS ≤ 2.5 (18.5%; n = 15/81) were anovular compared with cows with BCS > 2.5 at 43 ± 3 DIM (10.8%; n = 103/956) (*P*=0.04). Also, anovulation was higher in the cohort of cows that lost ≥ 0.5 points of BCS between calving and 43 ± 3 DIM (13%; n = 24/184) than cows that lost 0.25 points, gained, or maintained BCS (6.5%; n = 48/733) (*P*<0.01). Cyclic cows had higher W4MK than anovular cows (n = 947; *P*= 0.05). A greater proportion of multiparous cows (25.2%; n = 144/571) lost ≥ 0.5 BCS between calving and 43 ± 3 DIM when compared to primiparous cows (11.6%; n = 40/346) (*P*<0.01).

There was no difference in the proportion of cows bearing an OSF at G1 (*P*=0.29) and P1 (*P*=0.56) US evaluations between ED-Ov, ED-PR and DO. A greater proportion of cows (*P*= 0.01) in the DO (89.7%; n = 305/340) group had a CL than cows enrolled in the ED-Ov (81.0%; n = 115/142) and ED-PR (80.4%; n = 135/168) groups at US examination at G1. The proportion of cows bearing a CL at US examination at P1 was greater (*P*= 0.03) in DO (92.6%; n = 313/338) when compared to ED-Ov (86.5%; n = 122/141) and ED-PR (85.7%; n = 138/161) reproductive strategies. There was a greater proportion (*P*<0.01) of cows with 2 CLs at P1 in cows synchronized with DO (47.6%; n = 161/338) compared with cows synchronized with Ovsynch (22.0%; n = 31/141) and PRIDsynch (30.4%; n = 49/161) protocols. A greater (*P*<0.01) proportion of cows treated with DO (82.9%; n = 282/340) had an OSF and a CL at G1 than cows synchronized with Ovsynch (69.7%; n = 99/142) and PRIDsynch (72.6%; n = 122/168). At P1 US evaluation, 78.7%, 77.8% and 82.5% of the cows synchronized with Ovsynch, PRIDsynch and DO had an OSF and a CL, respectively (*P*=0.36). Farm 1 and farm 2 had similar proportions of cows with CL at G1 (84.7% *vs* 86.5%; *P*=0.73) and P1 (89.9% *vs* 89.0%, *P*=0.79); and there was no difference between primiparous and multiparous cows in the presence of CL at US examination at G1 (86.6% *vs* 84.7%; *P*=0.57) and P1 (91.6% *vs* 88.4%; *P*=0.28).

A greater proportion of multiparous cows (21.6%; n = 137/633) received AI from a beef sire compared to primiparous cows (14.1%; n = 57/404) (*P*<0.01). However, there was no difference in the proportion of cows receiving AI from Beef or Holstein sires between farms (*P*=0.17) or reproductive strategies (*P*=0.67).

3.1.2. Insemination risk for cows available for AI at detected estrus

Overall, the IR for cows after ED was 54.9%. There was no statistical difference (*P*=0.4) in the proportion of cows that received AI after ED between 50 ± 3 and 70 ± 3 DIM in the ED-Ov (56.8%; n = 189/333) and ED-PR (53.3%; n = 192/360) reproductive strategies. In addition, there was no observed difference in the proportion (*P*=0.7) of cows that received AI after ED between farms (farm 1 = 54.3%; farm 2 = 56%). A lower proportion (*P*<0.01) of primiparous cows (44.6%; n = 170/381) received AI after ED compared to multiparous cows (55.4%; n = 211/381) in ED-Ov and ED-PR reproductive strategies. Mean DIM for first postpartum AI was 68.3 (SD: ± 11.4), 68.9 (SD: ± 11.3) and 79.7 (SD: ± 1.9), for ED-Ov, ED-PR and DO, respectively. As expected, based on experimental design, no difference was found in DIM for first postpartum AI between ED-Ov and ED-PR (*P*=0.41); however, it differed between ED-Ov and DO (*P*<0.01) and between ED-PR and DO (*P*<0.01).

3.2. Pregnancy per AI after first insemination

In our initial data exploration, we found no statistically significant difference (*P*= 0.39) in P/AI for first AI in cows receiving AI at estrus detection (44.6%; n = 170/381), Ovsynch (37.5%; n = 54/144), PRIDsynch (41.7%; n = 70/168) and DO (45.3%; 156/344). There was no statistically significant difference (*P*= 0.26) in P/AI between subgroups of cows that were inseminated at detected estrus in ED-Ov (47.6%; 90/189) and ED-PR (41.7%; 80/192). Although P/AI was numerically higher in cyclic cows compared to anovular

Table 2
Regression coefficients for the final logistic regression models for P/AI 32 ± 3 d after first AI, 60 ± 3 d after first AI for 1037 lactating dairy cows enrolled in three reproductive strategies compared in the current study: combination of estrus detection and Ovsynch (ED-Ov), combination of estrus detection and PRIDsynch (ED-PR) and Double Ovsynch (DO). Odd ratios (OR) are given for each coefficient, with 95% CI.

Variable	n	Pregnancy 32 ± 3 d (%)	Odds ratio (95% CI)	*P*-value	Pregnancy 63 ± 3 d (%)	Odds ratio (95% CI)	*P*-value
Reproductive strategy							
ED-Ov	333	43.2	Referent		39.0	Referent	
ED-PR	360	41.7	0.95 (0.70–1.28)	0.72	40.0	1.06 (0.78–1.45)	0.71
DO	344	45.3	1.13 (0.83–1.53)	0.45	42.2	1.19 (0.87–1.63)	0.28
Farm							
1	623	42.1	Referent		39.2	Referent	
2	414	45.4	1.34 (1.03–1.75)	0.03	42.3	1.38 (1.05–1.80)	0.02
Parity							
Primiparous	404	53.7	Referent		50.0	Referent	
Multiparous	633	36.8	0.49 (0.38–0.64)	< 0.01	34.3	0.53 (0.41–0.68)	< 0.01
BCS 43 DIM							
≤ 2.5	118	34.7	Referent		29.7	Referent	
> 2.5	919	44.5	1.55 (1.02–2.38)	0.04	41.8	1.77 (1.16–2.77)	0.01
Sire AI							
Beef	194	38.1			32.5	Referent	
Holstein	843	44.6		NS[a]	42.2	1.45 (1.04–2.04)	0.03

[a] NS: not significant. Covariates and interactions between reproductive strategies and covariates were retained only if they were statistically significant (P < 0.05) in the final models.

cows (44% *vs* 35.8%), the difference was not significant ($P = 0.16$). Pregnancies per AI for first postpartum AI differed between cows found with no CL (19.4%; n = 13/67), 1 CL (41.3%; n = 137/332) and 2 CLs (50.2%; n = 121/241) at P1 US evaluation. The presence of both CL and OSF at G1 did not have significant effect on P/AI ($P = 0.93$); however, cows bearing an OSF and a CL at P1 had greater P/AI compared to cows either bearing a CL or an OSF only (44.6% *vs* 33.6%; $P = 0.03$).

The final logistic regression model results for P/AI 32 ± 3 d or P/AI 63 ± 3 d after first AI did not differ among ED-Ov, ED-PR and DO reproductive strategies (Table 2). Multiparous cows had lower P/AI than primiparous cows in all reproductive strategies 32 ± 3 d (OR = 0.49, 95% CI = 0.38–0.64; $P < 0.01$) (Fig. 2) and 63 ± 3 d after first AI (OR = 0.53, 95% CI = 0.41–0.68; $P < 0.01$) (Table 2). Cows on farm 2 were more likely to become pregnant than cows on farm 1 32 ± 3 d (OR: 1.34; 95% CI = 1.03–1.75; $P = 0.03$) and 63 ± 3 d after first AI (OR: 1.38; 95% CI = 1.05–1.80; $P = 0.02$) (Fig. 2). Moreover, cows with BCS > 2.5 at 43 ± 3 DIM on both farms had greater odds of becoming pregnant to first insemination compared with cows with BCS ≤ 2.5 (P/AI 32 ± 3 d after first AI = OR: 1.55; 95% CI=1.02–2.38; $P = 0.04$. P/AI 63 ± 3 d after first AI = OR: 1.77; 95% CI=1.16–2.77; $P = 0.01$) (Table 2). Cows receiving AI from Holstein sires had greater odds of being pregnant P/AI 63 ± 3 d after first AI than cows receiving AI from Beef sires (OR: 1.45; 95% CI=1.04–2.04; $P = 0.03$). There was no significant interaction between reproductive strategies and farm ($P = 0.86$), reproductive strategies and parity ($P = 0.63$), farm and parity ($P = 0.98$) and sire and parity ($P = 0.80$).

3.3. Pregnancy loss

An exploratory analysis showed that amongst the cows receiving first postpartum AI after a synchronization protocol (Ov, PR and DO), PL loss was lower ($P<0.01$) for cows with 2 CLs (1.7%; n = 2/120) compared with cows with no CL (23.1%; n = 3/10) and 1 (10.1%; n = 14/138) CL at P1 US evaluation.

The final logistic regression model results for PL did not show a significant difference between ED-Ov and DO ($P=0.20$), or ED-PR and DO ($P=0.52$). However, the cows in ED-Ov tended to have greater odds of PL than cows in ED-PR (OR = 2.46; CI = 0.91 – 6.7; $P=0.08$). Cows with BCS > 2.5 at 43 ± 3 DIM had reduced odds of PL than cows with BCS ≤ 2.5 (OR = 0.32; 95% CI = 0.11 – 0.92; $P = 0.04$). In addition, cows pregnant from a beef sire had greater odds of PL compared to cows pregnant from a Holstein sire (3.14; 95% CI = 1.4 – 7.05; $P<0.01$). There was no effect of farm and parity on PL after first AI ($P=0.41$ and $P=0.77$, respectively).

4. Discussion

This study compared reproductive performance between management strategies combining ED and Ovsynch, ED and PRIDsynch, and DO for FTAI. Our hypothesis was that DO would result in greater P/AI at first postpartum AI; however, P/AI at first AI did not differ between the three reproductive strategies. Whilst these results agree with comparable studies reporting no significant difference

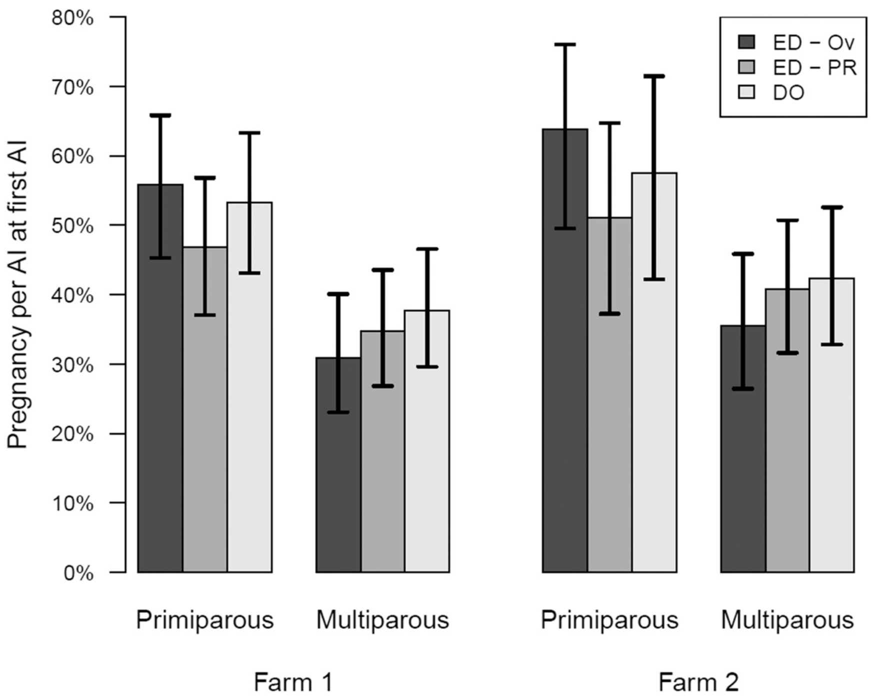

Fig. 2. Pregnancy at first AI in 1037 cows 32 ± 3 days after first postpartum AI, stratified by parity and farm, with error bars showing 95% Wilson type confidence intervals. The differences in P/AI for first AI between ED-Ov, ED-PR and DO were not statistically significant. We observed a difference in P/AI at first AI in parity ($P<0.01$) and farms ($P=0.03$).

(declared at $P<0.05$) in P/AI, comparing ED and FTAI with FTAI only (Denis-Robichaud et al., 2017; Neves et al., 2012; Stangaferro et al., 2018), they are in contrast to the results obtained in other work reporting greater P/AI at first postpartum AI in DO when compared with reproductive strategies combining ED and FTAI (Rial et al., 2022).

The lack of difference between the reproductive strategies might be partly explained by proportion of cows receiving AI after ED between 50 and 70 DIM in ED-Ov and ED-PR (ED-Ov = 56.8%; ED-PR = 53.3%), the resulting ED P/AI (ED-Ov = 47.6%; ED-PR = 41.7%) and the similar P/AI for TAI services after completion of Ovsynch (37.5%), PRIDsynch (41.7%) and Double-Ovsynch (45.4%). Whether ED increases or decreases P/AI in combined strategies depends on ED accuracy(Chebel and Santos, 2010). About 45% of the cows in our data set were not detected in estrus between 50 and 70 DIM in ED-Ov and ED-PR reproductive strategies. Although the farmers participating in this study performed visual ED three times/day for 30 min (Van Eerdenburg et al., 1996), it was hypothesized that they may have failed to detect cows with reduced intensity of estrus signs and estrus length, due to high milk yield (Lopez et al., 2004), the environment (Palmer et al., 2010), lameness (Somers et al., 2015) or anovulation (Wiltbank et al., 2002). Thus, reproductive strategies optimizing ovarian status such as DO may be an option to increase IRs and produce similar P/AI at first AI to reproductive strategies combining ED and FTAI (Denis-Robichaud et al., 2017; Stangaferro et al., 2018), especially on many UK dairy farms where ED is poorly implemented (median IR is 42%) (Hanks and Kossaibati, 2021).

We expected that an extended VWP in DO would have yielded greater P/AI, compared with ED-Ov and ED-PR. However, the low proportion of anovular cows before first postpartum AI in this trial may have countered the beneficial effects of DO and PR in this subgroup. Possibly a large proportion of cows were cyclic early after parturition, improving reproductive performance in cows receiving AI after ED (Galvão et al., 2010). Anovulation before first postpartum AI has a detrimental effect on fertility in cows receiving AI either after ED or FTAI (Gümen et al., 2003; Walsh et al., 2007a, 2007b). While others reported approximately 20% anovular lactating dairy cows before first postpartum AI (Bamber et al., 2009; Opsomer et al., 2000; Walsh et al., 2007a, 2007b), we observed a relatively low prevalence of 7.8% anovular cows (evaluated using US at 43 ± 3 and 50 ± 3 DIM). Cow health (Monteiro et al., 2020; Ribeiro et al., 2013), parity, energy balance (Lopez et al., 2004) and farm characteristics (Santos et al., 2009) have all been linked to anovulation. Monteiro et al. (2020) reported anovulatory cow prevalence by 49 DIM of 28.5%, with 33.4% of these cows suffering one disease event, and 30.4% suffering two or more disease events. In our study, 24% of cows had one disease event, and only 5% of cows had two or more disease events, which could partly explain the low anovulation prevalence. Our findings underscore previous evidence about the importance of developing management plans to minimize anovulation-related factors. In addition, our exclusion of cows diagnosed with UI during the VWP may have mitigated the negative effect of this subfertile group of cows in the fertility results (Sheldon et al., 2009b). After calving, uterine inflammation gradually decreases (LeBlanc, 2014; Sheldon et al., 2009a), thus, reproductive strategies with shorter VWP (ED-Ov; ED-PR) may have benefited from the exclusion of these cows than DO.

Our study was unable to detect statistically significant differences in P/AI between cows synchronized with Ovsynch, PRIDsynch, or DO. Similar P/AI after FTAI in the DO group, compared to the Ovsynch and PRIDsynch groups, was likely due to a high proportion of cows starting the synchronization protocols with a CL, similar BCS, and similar milk production at 70 DIM. However, the numerically greater P/AI in DO, compared to Ovsynch and PRIDsynch, could be attributed in part to the presynchronization effect of DO. The purpose of presynchronization with DO was to optimize P/AI by enhancing the effectiveness of hormonal treatments in the breeding Ovsynch protocol, and by improving synchronization and hormonal environment during follicular growth (Ayres et al., 2013). In this study, cows in DO were more likely to have a CL at G1 and P1, and 2 CLs at P1, compared to cows in the Ovsynch and PRIDsynch protocols. The presence of a 6-day-old CL and a 13-day-old CL at $PGF_{2\alpha}$ injection was previously associated with an increased probability of complete luteal regression at P1, resulting in lower P4 at AI and increased fertility in these cows, compared to those with only a single CL of about 6 days old (Carvalho et al., 2018).

In this study, 82.9% of the cows in the DO group had an OSF and a CL at G1; however, only 47% had two CLs at P1, suggesting a negative effect of P4 in ovulatory response to G1 due to inhibition of the GnRH-induced LH surge, and therefore, a lower ovulatory risk (Giordano et al., 2012a; Pulley et al., 2015; Stevenson and Pulley, 2016). On the other hand, it has been reported that follicular wave emergence is not usually affected by P4 circulating levels (Martínez et al., 2003), which may partly explain the high proportion of cows having an OSF at P1 and the P/AI obtained after FTAI in DO. The proportion of cows with two CLs at P1 in our study was considerably lower than in other trials (Kim et al., 2020), although literature reporting the occurrence of multiple CLs at P1 is limited.

The effect of parity (Herlihy et al., 2012; Moreira et al., 2001; Rial et al., 2022), uterine disease (Dubuc et al., 2010; Sheldon et al., 2009a) and BCS (Carvalho et al., 2014; Souza et al., 2008, 2007) in P/AI after first postpartum AI have been extensively documented. Similar to previous studies, we did not observe an interaction between parity and DO (Denis-Robichaud et al., 2017; Stangaferro et al., 2018). Treatment with two doses of $PGF_{2\alpha}$ in DO may have increased CL regression in multiparous cows, lowering P4 concentrations near AI and increasing P/AI (Borchardt et al., 2018; Wiltbank et al., 2015). Although we are unable to provide a formal explanation for the impact of the farm on fertility, we speculate that farm-specific characteristics (*i.e.*, stocking density, nutrition, genetic merit for fertility, *etc.*) could have potentially influenced the outcomes we obtained.

In our study, cows in ED-PR tended to have lower odds of PL than cows in ED-Ov. The effect of P4 supplementation during Ovsynch on embryo quality and survival has been extensively documented (Bisinotto et al., 2015; Cerri et al., 2009; Rivera et al., 2011). Interestingly, there was no difference in PL between ED-PR and DO. As mentioned earlier, the greater number of cows in DO bearing one or two CLs at P1 may have had increased circulating P4 levels (Cunha et al., 2022), which could have improved oocyte and embryo quality and reduced the probability of pregnancy loss.

Incidentally we found that cows that received a beef sire AI had greater pregnancy loss than cows receiving Holstein sire AI. These findings should, however, be interpreted cautiously as farm management decisions may have influenced these results (*i.e.*, cow selection for terminal beef sires). Further research is needed to elucidate the effect of terminal beef sires on pregnancy loss in lactating dairy cows.

5. Conclusions

We did not find significant differences in P/AI for first AI when comparing reproductive strategies combining ED and FTAI after Ovsynch and PRIDsynch to Double Ovsynch. High P/AI in cows receiving AI at ED, low postpartum disease incidence, and low proportion of cows with prolonged postpartum anovulation in our study may have contributed to the similar performance of DO, ED-Ov and ED-PR. Overall fertility was affected by cow factors such as BCS and parity, and unmeasured farm-specific characteristics. Presynchronization in the DO reproductive strategy increased the proportion of cows with one CL at initiation of the breeding Ovsynch, and the proportion of cows with one or two CLs at the moment of the $PGF_{2\alpha}$ administration, suggesting presynchronization success. In addition, reproductive strategies combining AI at ED and PRIDsynch for FTAI may reduce the risk of pregnancy loss in the first 60 d of gestation. Veterinarians should base their advice to dairy producers on a working knowledge of farm management practices and economics, and tailor hormone strategy recommendations to reduce disease incidence and promote early resumption of cyclicity.

CRediT authorship contribution statement

R. Vazquez Belandria: Conceptualization, Methodology, Project administration, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **K. Denholm:** Conceptualization, Writing – review & editing, Methodology, Formal analysis, Visualization. **P. T. Pepler:** Formal analysis, Data curation, Validation, Supervision, Writing – review & editing, Resources. **J. G. Cook:** Writing – review & editing, Supervision. **P. Pinho:** Writing – review & editing, Resources. **F. Randi:** Conceptualization, Methodology, Supervision, Writing – review & editing, Resources. **L. Viora:** Conceptualization, Writing – review & editing, Methodology, Resources, Project administration, Supervision, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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