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ORIGINAL ARTICLE

Effect of oestrous expression prior to timed artificial insemination with sexed semen on pregnancy rate in dairy cows

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Abstract

The objectives of the study were to determine (1) oestrous expression rate and (2) the effect of oestrous expression prior to progesterone-based Ovsynch protocol on pregnancy rate in Holstein cows. All cows ($n = 917$) were subjected to 7-day progesterone-based Ovsynch protocol. In this protocol, cows that expressed oestrus before (HEAT1) the scheduled second GnRH were inseminated 20 h later after the onset of oestrus without GnRH administration. Cows that expressed oestrus after the second GnRH administration (HEAT2) or did not express oestrus (NOHEAT) received fixed-timed AI. Oestrous expression was determined by using activity-rumination monitoring system and all cows were inseminated with sexed semen. Oestrous expression rate prior to FTAI was 40.5% and the majority ($p < .01$) of oestrous expression were in HEAT2 compared with HEAT1 in both primiparous (71.8 vs. 28.1%) and multiparous cows (69.5 vs. 30.5%). The mean interval from intravaginal device removal to the onset of oestrus was 47.4 ±0.9 h and 62.9 ±0.5 in HEAT1 and HEAT2, respectively. Primiparous cows (47.7%) had a higher ($p < .01$) expression rate compared with multiparous cows (37.2%). Overall pregnancy rate was 37.4% and there was two-way significant interaction between parity and oestrous expression on pregnancy rate ($p < .01$). Both primiparous (48.1 vs. 35.8%) and multiparous cows (47.4 vs. 28.4%) that expressed oestrus had greater ($p < .01$) pregnancy rate compared with cows that did not express oestrus. There was no difference in pregnancy rates of HEAT1 and HEAT2 in both primiparous (44.7 vs. 49.5%) and multiparous cows (47.2 vs. 47.6%). Pregnancy rate was not influenced ($p = .21$) by milk production (high or low) in both primiparous (47.6 vs. 48.6%) and multiparous (54.9 vs. 42.1%) cows that expressed oestrus, respectively. In conclusion, cows showing oestrus before or after second GnRH of the Ovsynch protocol had greater pregnancy rate than cows not showing oestrus.

KEYWORDS
dairy cow, oestrous expression, Ovsynch, pregnancy rate, sexed semen

1 | INTRODUCTION

Poor or inadequate oestrous detection is the major reason for low insemination risk and reproductive inefficiency in dairy herds (Souza et al., 2007). Oestrous detection rate is estimated at less than 50% in many commercial dairy farms (Ferguson & Skidmore, 2013; Rabiee et al., 2005). Increased herd size and different management conditions affect the oestrous detection rate in dairy herds. Higher milk production decreases the oestrous detection rate and probability of insemination due to elevated blood flow and metabolism of steroid hormones in high-yielding dairy cows (Lopez et al., 2004; Sangsritavong et al., 2002; Walsh et al., 2011). Timed artificial insemination (TAI) protocols are widely used without oestrus detection to increase the probability of insemination and pregnancy rate in commercial farms (Baruselli et al., 2012). The Ovsynch protocol is the most commonly used TAI protocol because resulting pregnancy rates are similar to those obtained when cows are bred at detected oestrus (Gümen et al., 2003; Rabiee et al., 2005; Wiltbank & Pursley, 2014). However, oestrous expression prior to TAI is critical for gamete transport, uterine environment may have resulted with better pregnancy rates (Bishop et al., 2017; Pereira et al., 2016; Richardson et al., 2016). In dairy cows, the previous studies demonstrated that oestrous expression prior to TAI increased (Pereira et al., 2016; Perry et al., 2005) or did not change (Kasimanickam et al., 2005; Souza et al., 2007) the pregnancy rate.

It is well known that sexed semen has been globally used to control the gender of the calf in recent years in many countries (Vishwanath & Moreno, 2018). Using sexed semen in cows expressing oestrus is recommended to maximize the pregnancy rate in cows (Kurykin et al., 2017). Recent studies demonstrated that oestrous expression near the time of TAI was critical to obtaining a higher pregnancy rate with sexed semen in beef heifers (Colazo et al., 2018) and beef cows (Crites et al., 2018). Although sexed semen is primarily preferred in dairy cattle (Holden & Butler, 2018), there has been a limited report investigating the effect of oestrous expression on pregnancy rate with sexed semen in dairy cows that received Ovsynch protocol (Crego et al., 2015). The objectives of this study were two-fold: firstly, to determine the proportion of dairy cows that exhibit oestrous prior to the initiation of fixed-time AI and, secondly, to determine whether their pregnancy rates are different from their contemporaries that do not exhibit oestrous when both groups are bred with sexed semen following induction of ovulations with Ovsynch protocols.

2 | MATERIALS AND METHODS

2.1 | Animals

A total of 917 Holstein-Friesian cows (primiparous cows; $n = 283$ and multiparous cows; $n = 634$) were enrolled in the study from January 2019 to May 2020. A commercial dairy farm (had an average of 1100 lactating dairy cows) was located in the north-western province of Turkey. Following National Research Council recommendations (NRC, 2001) cows were grouped in free-stall barns and fed a total mixed ration according to their milk yield. Average herd milk production per cow was 40–43 kg per day during the lactation period. During the study, the months between June and August were determined as the hot season and the months between September and May as the cold season. Body condition score (BCS) was assessed using a scale from 1 to 5, with 1 = very thin and 5 = obese (Ferguson et al., 1994). The milk production for 100 and 305 days and also the average milk yield at insemination week (±3 days) were recorded via a herd management program (Dataflow II™, Allflex, Israel). All animal procedures were approved (2019/2) by the Balıkesir University of Animal Care and Use Committee.

2.2 | Experiment

Primiparous and multiparous cows received progesterone-based Ovsynch protocol at 80 ± 5 and 60 ± 5 days in milk (DIM), respectively. In this TAI protocol, the cows received GnRH (100 μg gonadorelin acetate, im, Gestavet GnRH®, Hipra, Girona, Spain) and a progesterone-releasing intravaginal device (1.55 gr progesterone, PRID-DELTA®, Ceva-Sante Animale, Libourne, France) on Day 0. Progesterone releasing device was removed on Day 7 and the cows received $PGF_{2\alpha}$ (150 μg d-cloprostenol, im, Gestavet Prost®, Hipra, Girona, Spain) treatments 24 h apart on Days 7 and 8. The second administration of 100 μg GnRH was performed 56 h later after the first $PGF_{2\alpha}$ administration. The fixed-TAI was applied to all cows 16 h later after the second GnRH administration.

All cows were equipped with a neck collar comprised of an electronic identification tag (Dataflow II™, Allflex, Israel). The activity monitoring system used a wireless receiver box to record the average activity at 2-h intervals and computes and activity index value between 0 and 100. Activity indices above 35 are associated with cows that display oestrous activity (Guner et al., 2020). The oestrous expression of each cow was checked via activity monitoring from the day of intravaginal progesterone device removal (Day 7) to the day of TAI (Day 10). The cows displaying oestrus before the second GnRH administration (HEAT1) were inseminated 16–20 h after the onset of oestrus. The cows displaying oestrus after the second GnRH administration (HEAT2) and not detected oestrus (NOHEAT) were inseminated 16 h after the second GnRH administration. In all cows, sexed semen (0.25 ml per straw) was used for insemination. Frozen straws were thawed at 37 °C for 30 s and deposited into the uterine body by the insemination technicians of the farm.

The pregnancy status was determined by the presence of a viable embryo by trans-rectal ultrasonographic examination (Ibex Pro®, E.I. Medical Imaging, Loveland, CO) on Day 31 after AI. The cows were re-examined to confirm continuation (the presence of the fetus with heart beat) or to determine loss of pregnancy on Day 62.

TABLE 1 Pregnancy per artificial insemination (P/AI) according to the presence of oestrous expression in primiparous and multiparous cows received progesterone-based Ovsynch protocol

	Primiparous ($n = 283$)		Multiparous ($n = 634$)		
Oestrus expression	**YES***	**NO†**	**YES***	**NO†**	***p* value**
Number of animals, % (*n*/*n*)	47.7%[a] (135/283)	52.3% (148/283)	37.2%[b] (236/634)	63.9% (405/634)	<.01
P/AI at d 32, % (*n*/*n*)	48.1%[a] (65/135)	35.8%[b] (53/148)	47.4%[a] (112/236)	28.4%[b] (113/398)	<.01
P/AI at d 60, % (*n*/*n*)	43.0%[a] (58/135)	31.1%[b] (46/148)	44.1%[a] (104/236)	26.6%[b] (106/398)	<.01
Pregnancy loss, % (*n*/*n*)	10.8% (7/65)	13.2% (7/53)	7.1% (8/112)	6.2% (7/113)	.40

Note: [a,b]: Percentages with different superscripts within a row are different ($p < .01$). *p*-values refer to the differences in cows that exhibited oestrous (YES) and those that did not (NO).

*Cows displayed oestrus prior to TAI after progesterone device removal.

†Cows did not display oestrus prior to TAI after progesterone device removal.

2.3 | Statistical analysis

The data related to pregnancy rate at Day 31, 62 and pregnancy loss were analysed using PROC GLIMMIX procedure of SAS 9.4 (SAS Institute Inc.; Cary, NC, USA). The final model included oestrous expression (HEAT and NOHEAT), parity (primiparous vs multiparous), level of milk yield (low vs high producers), season (warm vs cold) and their two-way interactions as fixed effects. Cow within oestrous expression was considered as a random effect in the model just to take the individual differences into account. Statistical differences were considered significant for $p < .05$.

3 | RESULTS

The total oestrous expression rate was 40.5% (371/917) prior to the scheduled FTAI. Primiparous cows (47.7%, 135/283) had a greater ($p < .01$) oestrous expression rate than multiparous cows (37.2%, 236/634). The average body condition score (mean ± SEM) was 3.49 ± 0.4 and there was no difference ($p = .17$) in both primiparous and multiparous cows with (3.53 ± 0.4; 3.45 ± 0.5, respectively) or without (3.50 ± 0.3; 3.50 ± 0.5, respectively) oestrous expression. The distribution of cows was 12.0% (110/917), 28.5% (261/917), and 59.5% (546/917) in HEAT1, HEAT2 and NOHEAT groups, respectively. The majority of both primiparous (71.8 vs. 28.1%) and multiparous cows (69.5 vs. 30.5) displaying oestrus were in HEAT2 compared with HEAT1 ($p < .01$). Irrespective of parity, the mean interval from intravaginal device removal to the onset of oestrus was 58.3 ± 0.5 h (47.4 ± 0.9 h for HEAT1 and 62.9 ± 0.5 h for HEAT2) in cows that expressed oestrus.

Total pregnancy rate was found 37.4% on Day 31 and 34.2% on Day 62 after insemination with sexed semen in the current study. Pregnancy loss was 8.4% in all cows. Pregnancy rate was higher ($p < .01$) in primiparous cows than in multiparous cows at days 31 (41.7 vs. 35.5%) and at Days 62 (36.7 vs. 33.1%). Pregnancy loss was not different ($p = .10$) between primiparous (11.9%) and multiparous cows (6.7%). Similar to parity, oestrous expression was significant factor ($p < .01$) to obtain a higher pregnancy rate. The pregnancy rate was greater ($p < .01$) in cows that expressed oestrus (47.7%, 177/371) compared with cows that did not express oestrus (30.4%, 166/546), irrespective of parity (Table 1). There was two-way significant interaction between parity and oestrous expression on pregnancy rate ($p < .01$). Both primiparous (48.1 vs. 35.8%) and multiparous cows (47.4 vs. 28.4%) that expressed oestrus had greater ($p < .01$) pregnancy rate compared with cows that did not express oestrus (Table 1). Pregnancy rates for HEAT1 and HEAT2 were not different in both primiparous (44.7 vs. 49.5%) and multiparous cows (47.2 vs. 47.6%), respectively. There was no seasonal effect on oestrous expression and was 36.8% (46/125) and 41.0% (325/792) for the hot and cold seasons, respectively ($p = .37$). Additionally, there was no significant two-way interaction ($p = .62$) between parity and season on the pregnancy rate. Pregnancy rate was not statistically different ($p = .19$) between the cold season (38.3%, 303/792) and the hot season (32.0%, 40/125).

Average milk production (mean ± S.E.M) was 3394.6 ± 56.7 kg, 10,593.1 ± 127.1 kg and 4902.6 ± 38.7 kg, 13,121.9 ± 87.1 kg for primiparous and multiparous cows at 100 and 305 days, respectively. The average milk yield on the day of insemination for primiparous and multiparous cows was 39.6 kg/day and 53.4 kg/day, respectively. The average milk yield on the day of insemination was classified as low or high producers for primiparous (<39.6 kg/day, ≥39.6 kg/day) and multiparous cows (<53.4 kg/day, ≥53.4 kg/day). According to milk production classification, the average milk production was 44.4 ± 0.6 kg and 34.7 ± 0.6 kg for primiparous cows and 60.1 ± 0.5 and 46.1 ± 0.4 kg for multiparous cows, respectively. The overall pregnancy rate was not significantly affected ($p = .17$) by milk production in both primiparous and multiparous cows. Oestrous expression rate and pregnancy rate were not different ($p > .05$) between high producer primiparous and multiparous cows. However, oestrous expression rate and pregnancy rate were higher ($p < .01$) in low producer primiparous cows compared with low producer multiparous cows. Oestrous expression rates and pregnancy rates according to parity and milk classification are presented in Table 2.

4 | DISCUSSION

The aims of this study were to assess the proportion of dairy cows that expressed oestrus and the effect of oestrous expression

TABLE 2 Percentage of oestrous expression and pregnancy per artificial insemination (P/AI) for low- and high-producing cows that synchronized with progesterone-based Ovsynch protocol

	Primiparous ($n = 283$)†		Multiparous ($n = 634$)‡		
Parameters	**Low producers**	**High producers**	**Low producers**	**High producers**	***p*-value**
Average milk yield* (kg/day)	34.7 ± 0.6^{a}	44.4 ± 0.6^{b}	46.1 ± 0.4^{c}	60.1 ± 0.5^{d}	<.01
Oestrous expression %, (*n*/*n*)	50.7 (72/142)[a]	44.7 (63/141)[ab]	37.5 (114/304)[b]	36.9 (122/330)[ab]	<.01
P/AI in oestrus %, (*n*/*n*)	48.6 (35/72)	47.6 (30/63)	40.3 (46/114)	54.1 (66/122)	.21
P/AI not in oestrus %, (*n*/*n*)	45.7 (32/70)[a]	27.0 (21/78)[b]	26.8 (51/190)[b]	29.8 (62/208)[b]	<.05
Overall P/AI %, (*n*/*n*)	47.2 (67/142)[a]	36.2 (51/141)[ab]	31.9 (97/304)[b]	38.8 (128/330)[ab]	<.01

Note: [a,b,c,d]Percentages with different superscripts within a row are different. *p*-values refer to the differences in cows that exhibited oestrous (YES) and those that did not (NO).

*The average milk yield (kg/day) at insemination week (±3 days).

†Primiparous cows classified as lower (<39.6 kg/day) or higher (≥39.6 kg/day) producers according to average milk yield.

‡Multiparous cows classified as lower (<53.4 kg/day) or higher (≥53.4 kg/day) producers according to average milk yield.

on pregnancy rate after insemination with sexed semen in dairy cows synchronized with progesterone-based Ovsynch protocol. Ovsynch is the most preferred protocol to optimize the first insemination and provides a similar pregnancy rate compared to natural oestrus in dairy herds (Gümen et al., 2003; Karakaya-Bilen et al., 2019; Rabiee et al., 2005). Our finding of 40.5% of cows expressing oestrus was higher than in some previous studies (Jobst et al., 2000; Kasimanickam et al., 2005; Perry et al., 2005) and similar to other studies (Brusveen et al., 2009; Souza et al., 2007). Kasimanickam et al. (2005) found that the oestrous expression rate was 11.7% in Ovsynch protocol in dairy cows. However, several studies demonstrated that the oestrous expression rate was approximately 20% in cows synchronized with Ovsynch in previous studies (Jobst et al., 2000; Perry et al., 2005). Contrary to previous studies, Brusveen et al. (2009) and Souza et al. (2007) reported that the oestrous expression rate in cows synchronized with the Ovsynch protocol was 37.2% and 44.4%, respectively. The higher oestrous expression rate probably resulted from difference in the administration of the second GnRH at 56 h (Brusveen et al., 2009; Souza et al., 2007) instead of 48 h (Jobst et al., 2000; Kasimanickam et al., 2005; Perry et al., 2005) after $PGF_{2\alpha}$ in the Ovsynch protocol. Early administration of the second GnRH (at 48 h instead of 56 h) could inhibit oestrous expression due to an induced early preovulatory gonadotropin surge and maturity of dominant follicles (Perry et al., 2005). Additionally, progesterone supplementation for 7 days and second administration of $PGF_{2\alpha}$ during Ovsynch protocol could have improved oestrous expression by decreasing progesterone concentration after $PGF_{2\alpha}$ injection and increasing the magnitude of the LH surge (Gümen & Wiltbank, 2005; Wiltbank et al., 2015). One of the main factors also affecting oestrous expression or explaining differences between studies may be nutrition, negative energy balance and body condition score.

Pregnancy rate after insemination ranged from 24.7% to 45% in cows that received Ovsynch (Brusveen et al., 2009; Kasimanickam et al., 2005; Souza et al., 2007). Sexed semen resulted in a lower pregnancy rate (75 to 90% of conventional semen) due to physical and biochemical damage (Seidel, 2014). It is difficult to directly compare fertility when sex-sorted and conventional semen was not used from the same sires (Cerchiaro et al., 2007; Guner et al., 2021). However, it was revealed that modification of the sorting process by decreasing the cellular damage of sperm partially compensated the reduced fertility with sexed semen (González-Marín et al., 2018; Maicas et al., 2020; Vishwanath & Moreno, 2018). Similar to our results (37.4%), Karakaya et al. (2014) found that pregnancy rate was 36.4% after insemination with sexed semen in Ovsynch protocol in dairy cows. In addition, Kurykin et al. (2017) reported that pregnancy rate was 47.2% after the first insemination with sexed semen in Ovsynch protocol. A similar result has been reported by Drake et al. (2020) which was 49.0% pregnancy rate after insemination with sexed semen following the Ovsynch protocol.

It is challenging to compare fertility between cows expressing oestrus or not since cows are not allowed to show oestrus due to the administration of GnRH 56 h after prostaglandin. However, similar to our results, previous studies demonstrated higher pregnancy rate in cows that expressed oestrus compared with cows that did not (Crego et al., 2015; Jobst et al., 2000; Pereira et al., 2016; Perry et al., 2005). The pregnancy rate by using sexed semen was impressively higher in cows that expressed oestrus compared to cows that did not display oestrus (47.7–30.4 = 17.3% absolute difference; 17.3÷30.4 = 56.9% relative difference) in this study. Therefore, the importance of oestrous expression was highlighted for pregnancy success with sexed semen in TAI protocols in the present study. In terms of sexed semen, there have been just a few studies in the literature that investigate the effect of oestrous expression in TAI protocol on pregnancy rate using sexed semen in dairy cows (Crego et al., 2015). Similar to our findings, Crego et al. (2015) found that cows expressing oestrus (54%) had higher pregnancy rates than cows that did not express oestrus (34%) in the Ovsynch protocol (Crego et al., 2015).

It was reported that insemination after oestrous expression significantly increased the pregnancy rate by 13.4% (from 25.5% to 38.9%) compared with cows that expressed oestrus (Pereira et al., 2016). Oestrous expression increased the pregnancy rate

by approximately 2-fold (Jobst et al., 2000) or 3-fold (Perry et al., 2005) compared with cows that did not display oestrus in the Ovsynch protocol. Consistent with the previous studies (Crego et al., 2015; Pereira et al., 2016; Richardson et al., 2016), a higher pregnancy rate could be explained by increased follicle diameter and preovulatory oestrogen concentration in cows that expressed oestrus during TAI in this study (Perry et al., 2007). In addition to previous beneficial effects associated with oestrous expression near the time of TAI, enhanced conceptus size (Davoodi et al., 2016) and greater mRNA expression of interferon tau (Cooke et al., 2019) could result in a higher pregnancy rate in cows that displayed oestrus.

Contrary to our results, Kasimanickam et al. (2005) reported that the pregnancy rate did not change with (31%) or without (23.9%) oestrous expression in dairy cows that received Ovsynch protocol (Kasimanickam et al., 2005). Similarly, Souza et al. (2007) also determined that oestrous expression did not change (45.1% vs 40.5%) the pregnancy rate in dairy cows treated with Ovsynch protocol. It was reported that the lack of complete regression of the corpus luteum (10 to 25%) resulted in a low pregnancy rate due to increased progesterone concentration near the time of AI in cows subjected to Ovsynch protocol (Wiltbank et al., 2015). The second administration of PGF2α during Ovsynch protocol could have reduced progesterone concentration near the time of AI in this study. Low-progesterone concentration and elevated oestrogen concentration near the time of AI properly regulate the uterine environment for subsequent sperm transport and gamete survival (Brusveen et al., 2009; Richardson et al., 2016). Furthermore, failure to detect the onset of oestrus may not have resulted in an expected increase in pregnancy rate due to suboptimal insemination time with sexed semen in cows that expressed oestrus (Kasimanickam et al., 2005; Souza et al., 2007). In this study, 12% of cows (HEAT1) displayed oestrus before the administration of the second GnRH and the mean interval from intravaginal device removal to the onset of oestrus was 47.4 ± 0.9 h. Delaying the insemination time for long time (interval between onset of oestrus and FTAI more than 24 h) could result in low pregnancy rate in HEAT1 cows. In primiparous cows, delaying insemination time from 16 to 24 h reduced pregnancy rate (50 to 44%) in Ovsynch protocol (Lauber et al., 2020). In Jersey heifers, delaying insemination time more than 24 h after onset of oestrus resulted in reduced pregnancy rate (45.5%) compared with insemination at 16–20 h (Sá Filho et al., 2010). Therefore, combination of activity monitoring technologies or tools with TAI protocols have been recommended to achieve a higher pregnancy rate with sexed semen in dairy herds (Denis-Robichaud et al., 2018). Considering the limited use of sexed semen in TAI protocols, the selection of cows that expressed oestrus might be a critical strategy for increasing the pregnancy rate in Ovsynch protocol.

It is well known that increased milk production is inversely associated with oestrous expression and pregnancy rate (Lopez et al., 2004; Pereira et al., 2016; Peters & Pursley, 2002). The average milk production was higher in multiparous cows than in primiparous cows. Many previous studies revealed that primiparous cows had a higher pregnancy rate than multiparous cows (Karakaya et al., 2014; Kurykin et al., 2017; Peters & Pursley, 2002) in the Ovsynch protocol. Consistent with the previous studies (Pereira et al., 2016), primiparous cows had a higher oestrous expression rate and pregnancy rate in primiparous cows compared to multiparous cows in this study. Pereira et al. (2016) determined a similar reduction in pregnancy rate in primiparous (13.2%) and multiparous (13.4%) cows that did not express oestrus. Unlike in previous results, pregnancy rate was drastically lower in multiparous cows (21%) than in primiparous cows (12.3%) in the absence of oestrous expression in this study. Oestrous expression was more necessary to achieve greater pregnancy rate in high-producer cows than in low-producer cows. Unlike multiparous cows and high-producer primiparous cows, there was no difference in pregnancy rate regardless of oestrous expression in low producer primiparous cows. However, pregnancy was not influenced by parity and milk production in cows that expressed oestrus. Additionally, there was no difference in pregnancy between high-producer primiparous and multiparous cows. Producing high milk yield could demonstrate fewer periparturient disorders in multiparous cows and high-producer multiparous cows are more likely to have double ovulation. Multiple ovulations may lead to a greater pregnancy rate compared to high-producer primiparous cows (Peters & Pursley, 2002).

5 | CONCLUSION

The pregnancy rate was greater in cows that expressed oestrus following synchronization with progesterone-based Ovsynch than cows that did not express oestrus. Thus, the present study highlighted the importance of oestrous expression prior to FTAI to obtain a higher pregnancy rate.

AUTHOR CONTRIBUTIONS

Baris Guner contributed to conceptualization, methodology, investigation and writing—original draft. Melih Erturk contributed to resources, investigation and methodology. Mehmet Dursun contributed to investigation. Buse Ozturk contributed to writing—original draft. Gulnaz Yilmazbas-Mecitoglu, Abdulkadir Keskin and Ahmet Gumen contributed to conceptualization, methodology, review and editing. Serdal Dikmen contributed to statistical analysis, review and editing.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Baruselli, P. S., Sales, J. N. S., Sala, R. V., Vieira, L. M., & Filho, M. F. S. (2012). History, evolution and perspectives of timed artificial insemination programs in Brazil. *Animal Reproduction*, *9*(3), 139–152.

Bishop, B. E., Thomas, J. M., Abel, J. M., Poock, S. E., Ellersieck, M. R., Smith, M. F., & Patterson, D. J. (2017). Split-time artificial insemination in beef cattle: III. Comparing fixed-time artificial insemination to split-time artificial insemination with delayed administration of GnRH in postpartum cows. *Theriogenology*, *99*, 48–52. https://doi.org/10.1016/j.theriogenology.2017.04.046

Brusveen, D. J., Souza, A. H., & Wiltbank, M. C. (2009). Effects of additional prostaglandin F2α and estradiol-17β during Ovsynch in lactating dairy cows. *Journal of Dairy Science*, *92*(4), 1412–1422. https://doi.org/10.3168/jds.2008-1289

Cerchiaro, I., Cassandro, M., Dal Zotto, R., Carnier, P., & Gallo, L. (2007). A field study on fertility and purity of sex-sorted cattle sperm. *Journal of Dairy Science*, *90*(5), 2538–2542. https://doi.org/10.3168/jds.2006-694

Colazo, M. G., Whittaker, P., Macmillan, K., Bignell, D., Boender, G., de Carvalho Guimaraes, R., & Mapletoft, R. J. (2018). Evaluation of a modified GnRH-based timed-AI protocol associated with estrus detection in beef heifers inseminated with sex-selected or conventional semen. *Theriogenology*, *118*, 90–95. https://doi.org/10.1016/j.theriogenology.2018.05.037

Cooke, R. F., Pohler, K. G., Vasconcelos, J. L. M., & Cerri, R. L. A. (2019). Estrous expression during a fixed-time artificial insemination protocol enhances development and interferon-tau messenger RNA expression in conceptuses from Bos indicus beef cows. *Animal*, *13*(11), 2569–2575. https://doi.org/10.1017/S1751731119000636

Crego, S. E., Larimore, E., & Perry, G. (2015). Effect of timing of artificial insemination and estrus expression using sexed semen on pregnancy rates in Holstein dairy cows.

Crites, B. R., Vishwanath, R., Arnett, A. M., Bridges, P. J., Burris, W. R., Mcleod, K. R., & Anderson, L. H. (2018). Conception risk of beef cattle after fixed-time artificial insemination using either SexedUltra™ 4M sex-sorted semen or conventional semen. *Theriogenology*, *118*, 126–129. https://doi.org/10.1016/j.theriogenology.2018.05.003

Davoodi, S., Cooke, R. F., Fernandes, A. C. C., Cappellozza, B. I., Vasconcelos, J. L. M., & Cerri, R. L. A. (2016). Expression of estrus modifies the gene expression profile in reproductive tissues on day 19 of gestation in beef cows. *Theriogenology*, *85*(4), 645–655. https://doi.org/10.1016/j.theriogenology.2015.10.002

Denis-Robichaud, J., Cerri, R. L. A., Jones-Bitton, A., & LeBlanc, S. J. (2018). Performance of automated activity monitoring systems used in combination with timed artificial insemination compared to timed artificial insemination only in early lactation in dairy cows. *Journal of Dairy Science*, *101*(1), 624–636. https://doi.org/10.3168/jds.2016-12256

Drake, E., Holden, S. A., Aublet, V., Doyle, R. C., Millar, C., Moore, S. G., Maicas, C., Randi, F., Cromie, A. R., Lonergan, P., & Butler, S. T. (2020). Evaluation of delayed timing of artificial insemination with sex-sorted sperm on pregnancy per artificial insemination in seasonal-calving, pasture-based lactating dairy cows. *Journal of Dairy Science*, *103*(12), 12059–12068. https://doi.org/10.3168/jds.2020-18847

Ferguson, J. D., Galligan, D. T., & Thomsen, N. (1994). Prinicipal descriptiors of body condition score in Holstein cows. *Journal of Dairy Science*, *77*(9), 2695–2703. https://doi.org/10.3168/jds.S0022-0302(94)77212-X

Ferguson, J. D., & Skidmore, A. (2013). Reproductive performance in a select sample of dairy herds. *Journal of Dairy Science*, *96*(2), 1269–1289. https://doi.org/10.3168/jds.2012-5805

González-Marín, C., Góngora, C. E., Gilligan, T. B., Evans, K. M., Moreno, J. F., & Vishwanath, R. (2018). In vitro sperm quality and DNA integrity of SexedULTRA™sex-sorted sperm compared to non-sorted bovine sperm. *Theriogenology*, *114*, 40–45. https://doi.org/10.1016/j.theriogenology.2018.03.025

Gümen, A., Guenther, J. N., & Wiltbank, M. C. (2003). Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. *Journal of Dairy Science*, *86*(10), 3184–3194. https://doi.org/10.3168/jds.S0022-0302(03)73921-6

Gümen, A., & Wiltbank, M. C. (2005). Length of progesterone exposure needed to resolve large follicle anovular condition in dairy cows. *Theriogenology*, *63*(1), 202–218. https://doi.org/10.1016/j.theriogenology.2004.04.009

Guner, B., Erturk, M., Yilmazbas-Mecitoglu, G., Keskin, A., Karakaya-Bilen, E., Cakircali, R., Serim, E., Orman, A., & Gumen, A. (2020). The effect of delaying the time of insemination with sex-sorted semen on pregnancy rate in Holstein heifers. *Reproduction in Domestic Animals*, *1–7*, 1411–1417. https://doi.org/10.1111/rda.13789

Guner, B., Selcuk, G., Guclu, S., Sengul, S., Altun, I., Dikmen, S., & Gumen, A. (2021). Comparison of pregnancy per AI of heifers inseminated with sex-sorted or conventional semen after oestrus detection or timed artificial insemination. *Reproduction in Domestic Animals*, *56*(9), 1254–1260. https://doi.org/10.1111/rda.13984

Holden, S. A., & Butler, S. T. (2018). Review: Applications and benefits of sexed semen in dairy and beef herds. *Animal*, *12*(s1), s97–s103. https://doi.org/10.1017/S1751731118000721

Jobst, S. M., Nebel, R. L., McGilliard, M. L., & Pelzer, K. D. (2000). Evaluation of reproductive performance in lactating dairy cows with prostaglandin F2α, gonadotropin-releasing hormone, and timed artificial insemination. *Journal of Dairy Science*, *83*(10), 2366–2372. https://doi.org/10.3168/jds.S0022-0302(00)75125-3

Karakaya, E., Yilmazbas-Mecitoglu, G., Keskin, A., Alkan, A., Tasdemir, U., Santos, J., & Gumen, A. (2014). Fertility in dairy cows after artificial insemination using sex-sorted sperm or conventional semen. *Reproduction in Domestic Animals*, *49*(2), 333–337. https://doi.org/10.1111/rda.12280

Karakaya-Bilen, E., Yilmazbas-Mecitoglu, G., Keskin, A., Guner, B., Serim, E., Santos, J. E. P., & Gumen, A. (2019). Fertility of lactating dairy cows inseminated with sex - sorted or conventional semen after Ovsynch, Presynch – Ovsynch and Double - Ovsynch protocols. *Reproduction in Domestic Animals*, *54*, 309–316. https://doi.org/10.1111/rda.13363

Kasimanickam, R., Cornwell, J. M., & Nebel, R. L. (2005). Fertility following fixed-time AI or insemination at observed estrus in Ovsynch and Heatsynch programs in lactating dairy cows. *Theriogenology*, *63*(9), 2550–2559. https://doi.org/10.1016/j.theriogenology.2004.11.003

Kurykin, J., Jalakas, M., Kaart, T., & Jaakma, Ü. (2017). Efficiency of insemination with sexed semen at spontaneous estrus and synchronization of ovulation in lactating Holstein cows. *Veterinarija Ir Zootechnika*, *75*(97), 30–35.

Lauber, M., McMullen, B., Parrish, J., & Fricke, P. (2020). Short communication: Effect of timing of induction of ovulation relative to timed artificial insemination using sexed semen on pregnancy outcomes in primiparous Holstein cows. *Journal of Dairy Science*, *103*(11), 10856–10861. https://doi.org/10.3168/jds.2020-18836

Lopez, H., Satter, L. D., & Wiltbank, M. C. (2004). Relationship between level of milk production and estrous behavior of lactating dairy cows. *Animal Reproduction Science*, *81*(3–4), 209–223. https://doi.org/10.1016/j.anireprosci.2003.10.009

Maicas, C., Holden, S. A., Drake, E., Cromie, A. R., Lonergan, P., & Butler, S. T. (2020). Fertility of frozen sex-sorted sperm at 4 × 106 sperm per dose in lactating dairy cows in seasonal-calving pasture-based herds. *Journal of Dairy Science*, *103*(1), 929–939. https://doi.org/10.3168/jds.2019-17131

NRC. (2001). *Nutrient requirements of dairy cattle* (7th ed.). The National Academies Press.

Pereira, M. H. C., Wiltbank, M. C., & Vasconcelos, J. L. M. (2016). Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. *Journal of Dairy Science*, *99*(3), 2237–2247. https://doi.org/10.3168/jds.2015-9903

Perry, G. A., Smith, M. F., Lucy, M. C., Green, J. A., Parks, T. E., MacNeil, M. D., Roberts, A. J., & Geary, T. W. (2005). Relationship between follicle size at insemination and pregnancy success. *Proceedings of the National Academy of Sciences*, *102*(14), 5268–5273. https://doi.org/10.1073/pnas.0501700102

Perry, G. A., Smith, M. F., Roberts, A. J., Macneil, M. D., & Geary, T. W. (2007). Relationship between size of the ovulatory follicle and pregnancy success in beef heifers 1. *Journal of Animal Science*, *85*, 684–689. https://doi.org/10.2527/jas.2006-519

Peters, M. W., & Pursley, J. R. (2002). Fertility of lactating dairy cows treated with ovsynch after presynchronization injections of PGF2α and GnRH. *Journal of Dairy Science*, *85*(9), 2403–2406. https://doi.org/10.3168/jds.S0022-0302(02)74322-1

Rabiee, A. R., Lean, I. J., & Stevenson, M. A. (2005). Efficacy of Ovsynch program on reproductive performance in dairy cattle: A meta-analysis. *Journal of Dairy Science*, *88*(8), 2754–2770. https://doi.org/10.3168/jds.S0022-0302(05)72955-6

Richardson, B. N., Hill, S. L., Stevenson, J. S., Djira, G. D., & Perry, G. A. (2016). Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. *Animal Reproduction Science*, *166*, 133–140. https://doi.org/10.1016/j.anireprosci.2016.01.013

Sá Filho, M. F., Ayres, H., Ferreira, R. M., Nichi, M., Fosado, M., Campos Filho, E. P., & Baruselli, P. S. (2010). Strategies to improve pregnancy per insemination using sex-sorted semen in dairy heifers detected in estrus. *Theriogenology*, *74*(9), 1636–1642. https://doi.org/10.1016/j.theriogenology.2010.06.036

Sangsritavong, S., Combs, D. K., Sartori, R., Armentano, L. E., & Wiltbank, M. C. (2002). High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. *Journal of Dairy Science*, *85*(11), 2831–2842. https://doi.org/10.3168/jds.S0022-0302(02)74370-1

Seidel, G. E. (2014). Update on sexed semen technology in cattle. *Animal*, *8*(Suppl. 1), 160–164. https://doi.org/10.1017/S1751731114000202

Souza, A. H., Gümen, A., Silva, E. P. B., Cunha, A. P., Guenther, J. N., Peto, C. M., Caraviello, D. Z., & Wiltbank, M. C. (2007). Supplementation with estradiol-17β before the last gonadotropin-releasing hormone injection of the ovsynch protocol in lactating dairy cows. *Journal of Dairy Science*, *90*(10), 4623–4634. https://doi.org/10.3168/jds.2007-0172

Vishwanath, R., & Moreno, J. F. (2018). Review: Semen sexing - current state of the art with emphasis on bovine species. *Animal*, *12*(1), 85–96. https://doi.org/10.1017/S1751731118000496

Walsh, S. W., Williams, E. J., & Evans, A. C. O. (2011). A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science*, *123*(3–4), 127–138. https://doi.org/10.1016/j.anireprosci.2010.12.001

Wiltbank, M., Baez, G., Cochrane, F., Barletta, R., Trayford, C., & Joseph, R. (2015). Effect of a second treatment with prostaglandin F2α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. *Journal of Dairy Science*, *98*(12), 8644–8654. https://doi.org/10.3168/jds.2015-9353

Wiltbank, M. C., & Pursley, J. R. (2014). The cow as an induced ovulator: Timed AI after synchronization of ovulation. *Theriogenology*, *81*(1), 170–185. https://doi.org/10.1016/j.theriogenology.2013.09.017

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