Factors affecting first service conception rate in Holstein cows synchronized with double-ovsynch

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Abstract

Implementing a fertility protocol such as Double-Ovsynch (DO) in commercial dairy farms represents an opportunity to improve reproductive performance. However, no studies have tested this protocol nor identified factors that impact the first service conception rate (1SCR) in technified farms in the highlands of western Mexico. The objective was to identify factors that impact 1SCR in cows synchronized with the DO protocol. Records from 4759 cows synchronized for their first service with the DO protocol were used. To identify the factors that impact 1SCR, multiple logistic regression analyses were performed with the main effects: days in milk to first service, milk production at the peak of lactation, parity, calving assistance, service season of the year, sex of the calf, retained fetal membranes, metritis, and stillborn calf. The factors identified for 1SCR were the linear effect of days in milk to first service, service season, parity, retained fetal membranes, and metritis (P<0.05). Cows artificially inseminated during the summer had lower 1SCR compared to cows bred at other seasons of the year (P<0.05). The service season with the highest 1SCR was winter, with 42.5% (P<0.05). Multiparous cows showed a lower 1SCR compared to primiparous cows (29.9 and 47.3%, respectively; P<0.001). Cows with retained fetal membranes or cows with metritis had a lower 1SCR than those without these pathologies (P<0.05). In conclusion, multiparous cows, retained fetal membranes, metritis, and the summer service season are factors that negatively impact the 1SCR in cows synchronized with the DO protocol.

Keywords: Synchronization protocols; Holstein; Reproduction; Ovsynch; Conception rate

Introduction

Reproductive performance is a fundamental factor in dairy farm operations because it is directly associated with profitability (Ricci *et al*., 2020; Ríos-Mohar *et al*., 2022). Some of the most important reproductive indicators are conception rate, estrus detection efficiency, and pregnancy rate (Ríos-Mohar *et al*., 2022). With the advent of protocols to synchronize ovulation, such as Ovsynch (Pursley *et al*., 1995), the service rate was increased, which directly impacted the pregnancy rate (Pursley *et al*., 1997a; Pursley *et al*., 1997b). The Ovsynch protocol consists of first applying the gonadotropin-releasing hormone (GnRH), then Prostaglandin F2a (PG) seven days later, followed by a second dose of GnRH 56 hours later. This induces synchronized ovulation. Finally, 15-16 hours later, artificial insemination is performed at a fixed time (FTAI) (Moreira *et al*., 2001). The simplicity of this protocol, even with a conception rate lower than that obtained with visually detected estrus, contributed to its adoption by dairy farms worldwide (Wiltbank and Pursley, 2014).

On the other hand, with the advent of new protocols, such as Double-Ovsynch (DO), an improvement in the conception rate was also observed (Souza *et al*., 2008). The increase in the rates of conception and service contributed to the increase in the pregnancy rate, reproductive performance in general, and profitability in milk production farms (Wiltbank and Pursley, 2014; Ricci *et al*., 2020). The DO protocol consists of applying an Ovsynch to pre-synchronize the cows to start the second Ovsynch at the moment of the estrous cycle, where better fertility is obtained (Vasconcelos *et al*., 1999; Keith *et al*., 2005). Seven days after the first protocol, a second Ovsynch is started to synchronize ovulation, and artificial insemination service is provided at a fixed time. (Souza *et al*., 2008). It has been reported that the improved conception rate with DO is because the first Ovsynch is a therapeutic treatment for anestrous cows (Souza *et al*., 2008; Herlihy *et al*., 2012; Ayres *et al*., 2013). Also, the first Ovsynch synchronizes a surge of follicular development that allows the dominant follicle to ovulate with the first dose of GnRH from the breeding Ovsynch. This increases the amount of circulating progesterone during the development of the follicular wave in the second Ovsynch due to the formation of an accessory corpus luteum (Herlihy *et al*., 2012). It has been reported that the development of the ovulatory follicle under higher levels of progesterone improves the quality of the oocyte and increases the probability of establishing a pregnancy (Bisinotto *et al*., 2010; Stevenson, 2016; Madureira *et al*., 2021). All these factors contribute to improving the conception rate with the DO protocol.

Parity, calving assistance, and some reproductive puerperal diseases, such as metritis and subclinical endometritis, are factors that impact the conception rate to first service (1SCR) in cows synchronized with the DO protocol (Herlihy *et al*., 2012; Astiz and Fargas, 2013; Lima *et al*., 2013). Dairy farming is important occupation of livelihood in highlands of Mexico (Vieyra-Alberto *et al.,* 2018; Marin-Santana *et al.,* 2021). However, the effect of factors, such as days in milk to first service and the effect of milk production at the peak of lactation, have not yet been evaluated. Implementing the DO protocol in commercial farms represents an opportunity to improve reproductive performance and would allow less dependence on visual estrus detection and its auxiliary methods. Therefore, the objective of the present study was to determine the 1SCR in cows synchronized with the DO protocol in commercial dairy farms in the highlands of western Mexico and to identify factors that impact this indicator of reproductive performance.

Materials and methods

Location and weather

The study was conducted in two commercial farms located in the highlands of western Mexico, in the Los Altos region, in the state of Jalisco (21°24'N, 1880 masl). The climate is temperate and semi-dry, with an average temperature of 19°C and minimums and maximums of 5 and 31°C, respectively. The rainy season is from June to October, with an average annual rainfall of 850 mm.

Animals and management in the barn

Records of 4759 cows were obtained from two farms. The average number of cows per farm and milk production were 2395 cows and 36 kg/day for farm A and 3422 cows and 34 kg/day for farm B. The cows were housed in free stall barns with a cement floor and free access to dirt-floor sunbathing areas. The diet consisted of a total mixed ration with a 50:50 forage/concentrate ratio formulated to provide nutrients for maximum milk production of 650 kg cows with 17% crude protein in farm A and 730 kg cows and 16% diet of crude protein in farm B. Milking was performed three times a day in farm A and twice a day in farm B animals. Access to water was *ad libitum,* and the cows were fed twice a day on both farms. Food consumption was repeatedly stimulated during the day by bringing food closer to the bench-type feeders.

The veterinarians of each farm were responsible for checking the fresh cows to treat retained fetal membranes and metritis. The treatments consisted of local and systemic broad-spectrum antibiotics according to the protocols established in each production unit. Treatment for farm A included three consecutive transcervical infusions with 40 mL of Oxytetracycline/Saline (50:50), Ceftiofur 2 mg/kg sc, and Flunixin meglumine if systemic signs were present.

Farm B included Ceftiofur 2 mg/kg sc, the application of Ceftiofur by IM route only for cases with systemic symptoms, and two doses of PG analog, D-Cloprostenol 150 µg (InducelActive, Virbac, Mexico), and Flunixin meglumine.

Regarding the use of sexed semen, in farm A, the heifers received the first and second services with sexed semen. In contrast, for cows, the sexed semen was only used in the first and second services when the estrus was detected visually and only during the months of January to March. In farm B, sexed semen was used only in heifers for their first and second service. The voluntary waiting period in both farms was 60 days. Once they were approved from the reproductive postpartum check-ups, the cows began the synchronization protocol for the first service after 35 days in milk, and fixed-time artificial insemination was performed on average 71.52 ± 0.10 days postpartum. The pregnancy diagnosis was evaluated 40 days after service by transrectal palpation by the veterinarians of each farm.
Figure 1 outlines the DO protocol used in the farms. Doses of GnRH analogs were administered; farm A, Gonadorelin acetate, 100 µg (Ovosure, Lapisa, Mexico); farm B, Buserelin acetate, 10 µg (LiberActive, Virbac, Mexico). The following PG analogs were used: in farm A, Cloprostenol sodium 500 µg (Celosil, MSD, Mexico) and in farm B, D-Cloprostenol 150 µg (InducelActive, Virbac, Mexico). The cows were vaccinated against abortive infectious agents, pneumonia, diarrhea, and mastitis according to the protocols of each farm.

Fig 1. Scheme of the Double-Ovsynch protocol used to provide the first service postpartum in two commercial dairy farms. GnRH, Gonadotropin-releasing hormone; PG, Prostaglandin F2α; FTAI, fixed-time artificial insemination; d, days; h, hours.

Data management

The study consisted of a retrospective analysis of the first services performed during 14 months (corresponding to calvings between April 1, 2020 - May 31, 2021). The information was recorded by technical personnel of the dairy herds in the Dairy Comp 305® program. Subsequently, the information was exported to an Excel document with the following variables: date of calving, lactation number, type of calving (eutocic, mild and moderate help, or dystocia), sex of the calf, date of artificial insemination, the result of pregnancy diagnosis, and date/treatment of reproductive diseases. Retained fetal membranes were considered when the placenta was not eliminated for at least 24 hours after calving. The metritis diagnosis was performed by visual assessment based on the characteristics of the vaginal mucus (Sheldon *et al*., 2009). Calvings recorded with mild and moderate help were classified together with dystocia events as assisted calving. Milk measurements were carried out monthly, and only cows with a first weighing recorded within the first 60 days in milk were included in the study. From these records, milk production at peak production was obtained. For the service season categories, the following calendar dates were considered: Spring, March 20th-June 20th; Summer, June 21st-September 22nd; Fall, September 23rd-December 20th and finally, Winter, December 21st-March 19th.

Statistical analysis

All analyses were performed with the SAS program version 9.3 (SAS Institute Inc. Cary, NC, USA). The result of the pregnancy diagnosis was considered as the response variable (pregnant/non-pregnant). From this variable, the 1SCR (pregnant cows divided by the number of cows inseminated) was obtained. A series of logistic regression analyses were carried out using the LOGISTIC procedure to identify the factors that impact 1SCR and to calculate the odds ratio (OR). First, simple logistic regression analyses were performed between each potential factor and the result of the pregnancy diagnosis: days in milk to first service, milk production at the peak of lactation, farm, parity, service season, assisted calving, stillborn, sex of the calf, retention of fetal membranes, and metritis. The top and bottom 1% in the distribution of days to first service were removed from the analysis to represent outliers or capture errors (final range 60-100). A quadratic polynomial model was used for the continuous variables, days in milk to first service, and milk production at the peak of lactation. Variables with a significance of $P<0.20$ were retained for the second part of the analysis. In a preliminary multiple logistic regression analysis, barn was not significant as a main or interaction effect and was removed in both final models. Multiple logistic regression model 1 consisted of the following retained variables: days in milk to first service (linear and quadratic effects), milk production at the peak of lactation (linear and quadratic effects), parity (multiparous vs. primiparous), the season of service (summer vs. spring, autumn, and winter), sex of the calf (male vs. female), retention of fetal membranes (no RFM vs. RFM) and metritis (no metritis vs. metritis). Multiple logistic regression model 2 included all the main factors in model 1 and the corresponding second-order interactions between categorical variables. The backward option was used to comply with the principles of multiple models' parsimony. Finally, to retain the variables or interactions in the final model, a significance value of $P<0.05$ was defined.

Results

The overall 1SCR was 38.0%. Table 1 shows descriptive statistics for days in milk to first service and kilograms of milk at the peak of lactation. The polynomial logistic regression analysis results indicated a significant quadratic effect for days in milk to first service and milk production at the peak of lactation (Figure2, $P<0.05$). Table 2 shows descriptive statistics for factors with a potential impact on 1SCR. The variables that showed statistical significance ($P<0.05$) were days in milk at first service, service season, parity, retention of fetal membranes, and metritis. Table 3 shows the odds ratio and estimators for factors impacting the 1SCR of model 1 (for main effects). Days in milk showed a significant linear effect ($P<0.001$). Cows serviced during the summer had lower 1SCR than cows serviced at other times of the year ($P<0.05$). The season with the highest 1SCR was winter, with 42.5% ($P<0.05$). Multiparous cows presented a lower 1SCR compared to primiparous cows ($P<0.001$). Cows with retained fetal membranes or cows with metritis had a lower 1SCR than cows without these pathologies ($P<0.05$). For model 2, the only significant interaction was between parity and retention of fetal membranes (Figure 3; $P<0.01$). Multiparous cows had a lower 1SCR, and within each parity category, the presence of retained fetal membranes decreased the percentage of pregnant cows at first service.

Discussion

The polynomial logistic regression analyses indicated a quadratic effect of days in milk at first service and milk production at the peak of lactation. The decrease in 1SCR once the maximum is reached, due to the effect of milk production at peak lactation, is explained by multiparous cows with higher milk production levels at the peak of lactation (Atashi and Asaadi, 2019; Mellado *et al*., 2021). This is also the group of animals that showed lower fertility. These conditions, i.e., multiparous cows with higher peak yields with lower fertility, probably contributed to the significance of the parity effect in the multiple models but not the milk yield effect at the peak of lactation. On the other hand, even though the quadratic effect of days in milk at first service was also significant, the decrease in 1SCR, once the maximum was reached, is less evident. This also probably contributed to the fact that only the linear effect of days in milk was significant in the multiple model analyses. As the days in milk increase, the depth of the negative energy balance decreases, body condition score and uterine health improves, and the percentage of cows in anestrus decreases (Stangaferro *et al*., 2018; Karakaya-Bilen *et al*., 2019). These together augment the probability that the cows become pregnant at the first service. A practical application of this type of analysis is that it can contribute additional information to define the voluntary waiting periods in the farms, helping to achieve the conception rate at first service goals (Stangaferro *et al*., 2018; Niozas *et al*., 2019).

The cows bred during the summer had a lower 1SCR, while the season with the best fertility was the winter. A possible explanation for these results relies on the negative environmental impact of heat stress and the presence of higher humidity in the environment on fertility, which has been previously reported (Astiz and Fargas, 2013; Schüller *et al*., 2016). Heat stress reduces oocyte quality, compromises the uterine environment, and alters ovarian function, which is detrimental to embryonic development (Wolfenson and Roth, 2019; Amaral *et al*., 2021). Additionally, humidity in the environment deteriorates the general health of the cows, increasing their risk of suffering metritis and endometritis and thus compromising fertility (Adnane *et al*., 2017).

Primiparous cows showed higher 1SCR compared to multiparous cows. The results reported in other studies do not show a common agreement regarding the effect of parity on 1SCR in cows synchronized with DO (Giordano *et al*., 2012; Carvalho *et al*., 2015; Fuenzalida *et al*., 2015). However, most studies agree that DO-synchronized first-lactation cows have better 1SCR (Borchardt *et al*., 2017). A possible explanation that has been given to this finding relies on the therapeutic effect of DO to treat cows in anestrus and with ovarian cysts (Yaniz *et al*., 2004), some of the leading causes of infertility in primiparous cows (Herlihy *et al*., 2012; Astiz and Fargas, 2013). Therefore, the higher 1SCR observed in this group could be due to a direct therapeutic effect of the protocol on primiparous cows in anestrus. Another possible explanation is that a higher proportion of multiparous cows suffer incomplete luteolysis during the second Ovsynch of the DO protocol, decreasing their fertility (Carvalho *et al*., 2015; Wiltbank *et al*., 2015). In the present study, the difference in 1SCR is more than 15 percentage points in favor of primiparous cows, which would suggest using this protocol specifically for first-lactation cows (Borchardt *et al*., 2017).

This study observed a negative impact of RFM and metritis on 1SCR. The factor with the highest impact on fertility was the RFM, reducing the conception rate at first service by almost 14 percentage points. It has previously been reported that RFM is a risk factor for developing endometritis, and both negatively impact 1SCR (Potter *et al*., 2010; Montiel-Olguin *et al*., 2019; Kelly *et al*., 2020). The significant interaction of parity and RFM highlights the importance of this pathology in multiparous cows that present lower 1SCR. This effect is exacerbated when RFM is also present. It is unclear why multiparous cows are at higher risk of RFM, although it has been linked to a higher metabolic challenge from milk production and a lower ability to maintain redox balance (Kankofer *et al*., 2010; Dai *et al*., 2014; Endler *et al*., 2016).

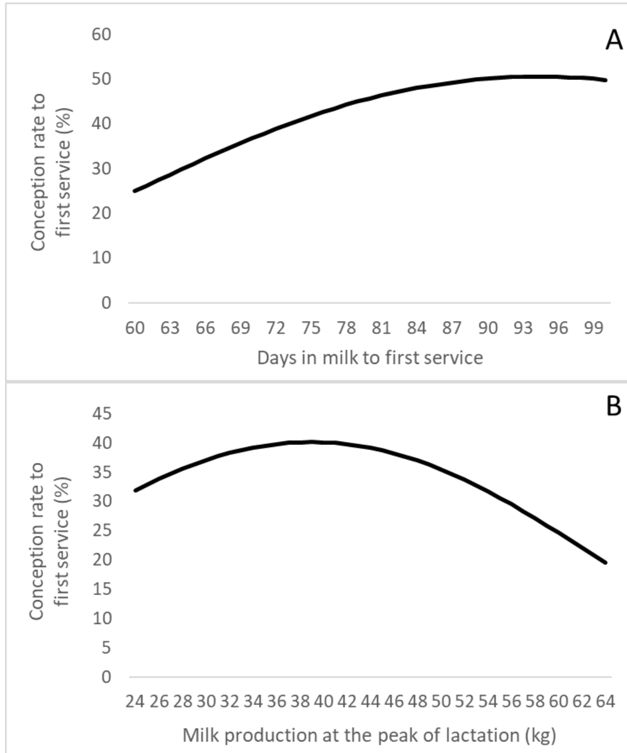

Fig 2. Effect of days in milk to first service (A) and milk production at the peak of lactation (B) on conception rate to first service (P<0.05).

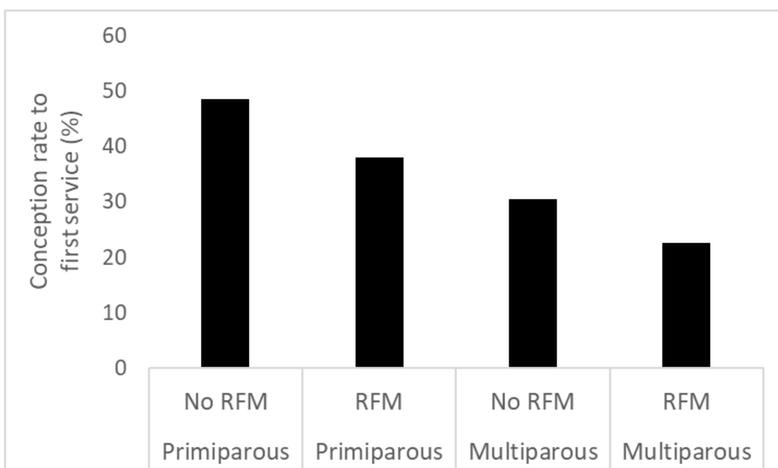

Fig 3. Conception rate to first service by the effect of the interaction between parity and retained fetal membranes (RFM) in model 2 (P<0.01).

Table 1. Descriptive statistics for the continuous variables days in milk to first service and milk production at the peak of lactation

Variable	N	Mean ± SE	Min	Q1	Median	Q3	Max
DIM1S (d)	4767	71.52±0.10	60	65	73	76	100
MPPL (kg)	3044	45.25±0.13	24	40	45	50	63

N, number of observations; SE, standard error; Min, minimum; Q1, first quartile; Q3, third quartile; Max, maximum; DIM1S, days in milk to first service; MPPL, milk production at the peak of lactation; d, days; kg, kilograms.

Table 2. Descriptive statistics for factors possibly impacting the first service conception rate (1SCR) in cows synchronized with a Double-Ovsynch protocol

Variable	Category	Pregnant cows	Total of cows	1SCR (%)
Farm	A	685	1715	39.9
	B	1125	3044	37.0
Parity	Multiparous	755	2528	29.9
	Primiparous	1055	2231	47.3
Assisted calving	Yes	56	145	38.6
	No	1754	4614	38.0
Service season	Spring	429	1091	39.3
	Summer	472	1375	34.3
	Fall	367	1017	36.1
	Winter	542	1276	42.5
Calf sex	Female	1163	2918	39.9
	Male	647	1841	35.1
Retained fetal membranes	Yes	98	383	25.6
	No	1712	4376	39.1
Metritis	Yes	137	436	31.4
	No	1673	4323	38.7
Stillbirth	Dead	50	124	40.3
	Alive	1760	4635	38.0

RFM, retained fetal membranes; %, percentage.

Table 3. Odds ratio and estimators for significant variables in model 1 of multiple logistic regression

Parameter	Nivel	OR	IC 95%	Estimate	Standard error	P
Intercept	N/A	N/A	N/A	-2.2233	0.599	<0.001
DIM1S	Linear effect	1.019	1.00-1.04	0.0189	0.00814	0.020
Service season	Summer	Ref.	NC	NC	NC	NC
	Fall	1.027	0.82-1.28	-0.2199	0.0714	0.002
	Winter	1.773	1.44-2.18	0.3261	0.0667	<0.001
	Spring	1.472	1.19-1.83	0.1402	0.0696	0.044
Parity	Multiparous	Ref.	NC	NC	NC	NC
	Primiparous	1.942	1.48-2.55	0.3319	0.069	<0.001
RFM	Yes	Ref.	NC	NC	NC	NC
	No	1.541	1.17-2.02	0.2161	0.0695	0.002
Metritis	Yes	Ref.	NC	NC	NC	NC
	No	1.590	1.05-2.40	0.232	0.1056	0.028

DIM1S, days in milk to first service; Ref., reference; OR, odds ratio, IC 95%, 95% confidence interval; P, p-value; N/A, not applicable; NC, not calculated for being the reference; RFM, retained fetal membranes.

Conclusion

In conclusion, multiparous cows, retention of fetal membranes, metritis, and summer are factors that negatively impact the conception rate at first service in cows synchronized with the DO protocol.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

The procedures applied to the animals during the development of this experiment were in accordance with the protocols indicated in the Animal Health Federal Law DOF 07-25-2007 and the Official Mexican Standard NOM-051-ZOO-1995.

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