EFFICIENCY OF INSEMINATION WITH SEXED SEMEN AT SPONTANEOUS ESTRUS AND SYNCHRONIZATION OF OVULATION IN LACTATING HOLSTEIN COWS

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Abstract. The objective was to determine the efficiency of insemination with sexed semen at spontaneous estrus and synchronisation of ovulation, and the effects of days in milk, insemination number, parity, and daily milk yield on pregnancy rates in lactating Holstein cows. A total 1,000 first to fourth parity cows, selected for their normal clinical and reproductive status, approaching a 50-day voluntary waiting period or non-pregnant subsequent to one to three inseminations were randomly assigned to insemination at spontaneous estrus or to Ovsynch protocol. At spontaneous estrus, 244 cows were inseminated with sexed semen 12 h after detection of estrus and 162 cows with unsexed semen according to the a.m.–p.m. rule. Using the Ovsynch protocol (GnRH-7d-$PGF_2\alpha$-2d-GnRH), 336 cows were inseminated with sexed and 258 with unsexed semen 18-20 h after the final GnRH treatment. The overall pregnancy rate for sexed semen at spontaneous estrus and the Ovsynch use was 9.6% lower of unsexed semen (38.6% vs 48.2%, P = 0.02). Pregnancy rates did not differ between protocols using sexed (37.2% vs 40.1%, P = 0.63) or unsexed (49.4% vs 47.1%, P = 0.77) semen. The effects of days in milk (50-100, 101-150, and > 150), insemination number (1 to 4), and parity (1 to 4) were not significant for both types of semen and protocols. Across studied cows median daily milk yield was 32.7 kg. Pregnancy rate with sexed semen at estrus appeared to increase in cows with above-mean milk yield, compared with those with below-mean yield (40.9 kg, 41.1% vs 25.7 kg, 35.3%, P = 0.41). A decrease of pregnancies was observed at the higher milk yield for sexed semen using the Ovsynch (38.1 kg, 35.2% vs 27.1 kg, 40.8%, P = 0.27). It is suggested that the Ovsynch protocol can be used for fixed-timed insemination with sexed semen of lactating dairy cows as an acceptable alternative to insemination upon detection of spontaneous estrus.

Keywords: sexed semen; lactating cows; estrus; Ovsynch; pregnancy rate; factors; effects

Introduction. The sex-sorted by flow-cytometry bovine semen separated into fractions containing predominantly X-chromosome bearing sperm has gained increased use in the dairy industry worldwide. Due to reduced fertilising potential attributed to impaired viability and quality of sperm by the sorting procedure (DeJarnette et al. 2008; Schenk et al. 2009) sexed semen is used mainly for heifers, as fertility is higher compared to lactating cows. Along with the use of sexed semen in heifers, the economic benefit and the rate of genetic gain would be greater through obtaining additionally high-value replacement heifers from genetically superior cows (Hohenboken 1999; Seidel 2003; De Vries et al. 2008).

With the commercial introduction of sexed semen, its use has been recommended for insemination at observed estrus, avoiding the use of a fixed-time insemination due to inherent variation in success of timed programmes (DeJarnette et al. 2010). However, a delay in the resumption of cycling in cows after calving, weak estrous expression and the low detection rates of estrus are primary factors limiting reproductive performance on dairy farms. For timed insemination of lactating cows, Pursley et al. (1995) elaborated the programme, providing the control of follicular growth, luteolysis and ovulation through a GnRH and $PGF_2\alpha$ combination (Ovsynch protocol). Induced release of LH by the first treatment with GnRH induces turnover of the follicle and the emergence of a new follicular wave. After induction of luteolysis by $PGF_2\alpha$ on Day 7 and the last GnRH treatment two days later, ovulation occurs between 24 and 32 h in up to 90% of cows, allowing insemination without the detection of estrus 16-18 h later from the final GnRH treatment. After the introduction of the Ovsynch, numerous modifications of the protocol were elaborated, providing a similar or higher pregnancy rate than at natural estrus (Rabiee et al. 2005; Wiltbank and Pursley 2014). Nevertheless, there are very few studies on sexed semen use in timed programmes in comparison with insemination upon detection of estrus in lactating dairy cows (Schenk et al. 2009; Sá Filho et al. 2013; Lucena et al. 2014). We suggest that the use of the Ovsynch protocol with sexed semen at defined conditions such as normal clinical and reproductive status of lactating cows might result in a pregnancy rate comparable to that achievable at insemination upon detection of spontaneous estrus.

Numerous fertility-related factors, other than methods of synchronisation, including stage of lactation, insemination number, parity, and milk production, may exert effect on success of insemination in lactating cows. The impact of the factors on pregnancy rate in the use of sexed semen is debatable (Bodmer et al. 2005; Schenk et al. 2009; DeJarnette et al. 2010; Karakaya et al. 2014). Thus, the information on the effect of the fertility-related

factors on the efficiency of sexed semen use at spontaneous estrus and synchronisation of ovulation is of interest in the perspective of the facilitation of the insemination of lactating cows.

The objective of the study was to determine the efficiency of insemination with sexed semen at spontaneous estrus and synchronisation of ovulation, and the effects of days in milk, insemination number, parity, and daily milk yield on pregnancy rates in lactating Holstein cows.

Material and methods

Study cows, conditions and selection. This study was conducted on three commercial dairy farms. The farms had herds of 600 to 1,200 Holstein breed cows housed year-round indoor in free-stall facilities with annual milk production from 9,430 to 11,076 kg per cow. The reproductive management on the farms involved insemination of cows after 50 days of a voluntary waiting period after calving at visually detected estrus according to the a.m-p.m. rule, and limited use of synchronisation of estrus. The cows were milked twice a day, fed a total mixed ration (grass silage supplemented with concentrate and minerals) based on milk yield, and had free access to fresh water. The first-insemination pregnancy rate of the herds ranged from 39.3 to 43.3%. A total 1,000 first to fourth parity cows with normal uterine and ovarian status, absence of lameness, mastitis and any clinical signs of metabolic disorders, approaching the 50-day voluntary waiting period or non-pregnant subsequent to one to three inseminations were used as experimental units.

Semen and insemination. For insemination of cows, commercial frozen-thawed sexed and unsexed semen doses of eight Holstein bulls (Cogent Ltd., Chester, UK and Select Sires Inc., Plain City, OH, USA), containing in 0.25 ml straws 2.1 x 10^6 X-chromosome-bearing sperm or 15 x 10^6 unsorted sperm were used. Cows were randomly assigned to the insemination with sexed or unsexed semen at spontaneous estrus or to the Ovsynch protocol. Unsexed semen was used for comparison, and to detect possible differences in the effect of the studied factors between two types of semen and insemination protocols.

A total 406 cows were inseminated at spontaneous estrus, visually detected by on-farm personnel. Single insemination was carried out with sexed semen on 244 cows not less than 12 h after the estrus was detected (Schenk et al. 2009) and with unsexed semen on 162 cows according to the a.m.-p.m. rule. At insemination with sexed or unsexed semen the cows had means (± SD) of 2.0 ± 0.85 and 2.2 ± 0.94 lactations (P = 0.04, t-test), 114.5 ± 57.1 and 114.8 ± 48.8 days in milk (P = 0.98), daily milk yield of 34.5 ± 9.2 and 34.7 ± 8.3 kg (P = 0.84) and 0.53 ± 0.82 and 0.50 ± 0.77 inseminations (P = 0.72), respectively.

The Ovsynch protocol was applied on 594 cows. The cows received at a random stage of the estrous cycle (Day 0) i.m.10 μg of buserelin (Receptal®, Intervet International B. V., EU), on Day 7 i.m. 25 mg of $PGF_{2}\alpha$ (Dinolytic®, Pharmacia N.V./S.A., Puurs, Belgium) and on Day 9 the second injection of buserelin. Single insemination was carried out on 336 cows with sexed and on 258 cows with unsexed semen 18 to 20 h after the final GnRH treatment. At insemination with sexed or unsexed semen the cows had means of 1.9 ± 0.88 and 1.9 ± 0.92 lactations (P = 0.92), 202.3 ± 68.1 and 221.4 ± 59.8 days in milk (P < 0.001), daily milk yield of 32.2 ± 6.8 and 32.0 ± 7.1 kg (P = 0.83) and 1.35 ± 0.94 and 1.65 ± 0.90 inseminations (P < 0.001), respectively.

The information on the parity, inseminations and days in milk were collected from on-farm records. Daily milk yield was recorded to compare pregnancy rates in cows with above and below mean yields. Across studied cows median daily milk yield was 32.7 kg. All inseminations with either type of semen carried out by on-farm technicians irrespective of estrous intensity for both insemination protocols to avoid using sexed semen at clearly defined estrous signs as a source of bias. The deposition site for both types of semen and protocols was the uterine body. Pregnancy status of cows was diagnosed 45-60 days after insemination by rectal palpation of the uterus.

Statistical analysis. Pregnancy rates from insemination at spontaneous estrus or synchronisation of ovulation were evaluated and compared between insemination numbers (1 vs 2 vs 3-4), parities (1 vs 2-4) and daily milk yield (above vs below mean), fitting a logistic model. In the model, fixed effects of type of semen, insemination protocol and interactions between the type of semen and insemination protocols were considered. Insemination number, parity and milk yield, as potential confounding factors, and interactions by type of semen and insemination protocol were included. Farms and bulls were included in the model as random effects. Higher order interactions were not considered to avoid unnecessary complexity of the model, and due to non-significant (P > 0.6) effects. Adjusted pregnancy rates were estimated and the pre-planned comparisons were carried out, using properly defined contrasts. A similar model was fitted to determine the effects of days in milk (50-100 vs 101-150 vs > 150 days). Due to a strong positive correlation between insemination number and days in milk (r = 0.82), these factors were analysed separately, replacing in the initial model insemination number with days in milk. Modelling was performed using the GLIMMIX procedure of the SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2013). Differences were considered significant at P < 0.05.

Results

After insemination with sexed semen of cows in spontaneous estrus or subjected to the Ovsynch (Table 1), the overall pregnancy rate was 9.6% lower (P = 0.02) or 80.1% of the rate obtained by using unsexed semen. Pregnancy rates did not differ between the two insemination protocols using sexed semen (P = 0.63), that was also observed in the use of unsexed semen (P = 0.77). Logistic regression revealed no effect on pregnancy rates of days in milk (P = 0.77), insemination number (P = 0.17) and parity (P = 0.34), and interaction of these factors by type of semen (P = 0.88, P = 0.93, P = 0.87) and insemination protocol (P = 0.82, P = 0.58, P = 0.42).

Table 1. **Adjusted pregnancy rates (aPR ± SEM) and odds ratios (aOR) estimated using logistic model considering fixed effects of type of semen and insemination protocols, interactions and potential confounding factors as insemination number, parity and milk yield, including farm and bull as random effects**

Insemination protocol	Unsexed semen		Sexed semen			
	n	aPR	n	aPR	aOR	P-value
Spontaneous estrus	162	49.4%	244	37.2%	1.65	0.05
Ovsynch	258	47.2%	336	40.1%	1.33	0.21
aOR		1.09		0.88		
P-value		0.77		0.63		
Total	420	48.2%	580	38.6%	1.48	0.02

Insemination with sexed semen from 50 to 100, 101 to 150 and > 150 days in milk resulted in pregnancy rates at spontaneous estrus 32.3%, 36.0% and 34.1% (P > 0.60) and using the Ovsynch 41.5%, 37.4% and 41.5% (P > 0.60). Unsexed semen pregnancy rates were 42.8%, 47.8% and 49.8% (P = 0.45) and 43.9%, 40.6% and 48.9% (P = 0.45), respectively.

Pregnancy rates with sexed semen were 32.8% at the first, 35.6% at second, and 43.4% at third and fourth inseminations at spontaneous estrus and 31.2%, 43.8% and 45.8%, respectively, using the Ovsynch. The increase of pregnancies from the second to fourth insemination at spontaneous estrus was not significant (P = 0.34) and tended to be in the Ovsynch group (P = 0.09). Unsexed semen pregnancy rates were 46.2%, 48.2% and 53.7% (P = 0.56) and 39.2%, 51.5% and 50.9% (P = 0.24), respectively. At insemination of cows in spontaneous estrus or using the Ovsynch pregnancy rate appeared to decrease in multiparous cows (Table 2). However, no statistical significance was found in both protocols neither for sexed nor unsexed semen.

Table 2. **Adjusted pregnancy rates[1] (aPR ± SEM) and odds ratios (aOR) for type of semen and insemination protocol by parity of cows**

	Sexed semen			Unsexed semen		
Parity	n	aPR		n	aPR	
Spontaneous estrus						
1	68	43.5%		38	59.3%	
≥ 2	176	35.6%		124	50.4%	
Ovsynch						
1	135	39.6%		108	59.5%	
≥ 2	201	37.2%		150	49.1%	
Estrus vs. Ovsynch		aOR	P-value		aOR	P-value
1		1.17	0.61		1.32	0.44
≥ 2		0.93	0.80		1.05	0.87

[1]Estimated considering fixed effects of type of semen and insemination protocol, including interactions of parity, insemination number and milk yield by type of semen and insemination protocol, and random effects of farms and bulls.

Table 3. **Adjusted pregnancy rates[1] (aPR ± SEM) and odds ratios (aOR) for type of semen and insemination protocol in cows producing above or below mean daily milk yield (median daily milk yield across studied cows 32.7 kg)**

	Sexed semen			Unsexed semen		
Milk yield	Mean (kg)	n	aPR	Mean (kg)	n	aPR
Spontaneous estrus						
Lower median	25.7 ± 3.9	103	35.3%	26.7 ± 3.8	70	47.3%
Higher median	40.9 ± 6.1	141	41.1%	40.7 ± 4.9	92	59.4%
Ovsynch						
Lower median	27.1 ± 3.4	181	40.8%	27.2 ± 3.9	143	50.2%
Higher median	38.1 ± 4.7	155	35.2%	38.1 ± 5.5	115	50.3%
Estrus vs. Ovsynch		aOR	P-value		aOR	P-value
Lower median		0.79	0.41		0.89	0.71
Higher median		1.29	0.38		1.45	0.26

[1]Estimated considering fixed effects of type of semen and insemination protocol, including interactions of parity, insemination number and milk yield by type of semen and insemination protocol, and farms and bulls as random effects.

At insemination with sexed semen at spontaneous estrus multiparous cows had greater milk yield than primiparous (37.3 ± 8.8 vs 27.5 ± 5.2 kg, P < 0.001) and relatively similar days in milk (111.4 ± 50.4 vs 122.3 ± 71.5 days, P = 0.25), as using unsexed semen (36.7 ± 8.1 vs 28.0 ± 4.7 kg, P < 0.001 and 114.7 ± 46.5 vs 114.0 ± 56.5 days, P = 0.94, respectively). In the inseminated with sexed semen Ovsynch groups, the milk yield in primi- and multiparous cows (31.6 ± 7.1 vs 32.6 ± 6.6 kg, P = 0.21) and days in milk (201.2 ± 71.0 vs 203.0 ± 66.3 days, P = 0.82) were not different, as using unsexed semen (31.0 ± 6.9 vs 32.8 ± 7.3 kg, P = 0.05 and 221.3 ± 58.2 vs 221.5 ± 61.0 days, P = 0.99, respectively).

There was no significant interaction between daily milk yield and type of semen (P = 0.39) or insemination protocol (P = 0.12). From insemination at spontaneous estrus the pregnancy rate appeared to increase in cows with an above-mean milk yield, compared to those with a below-mean yield using sexed or unsexed semen (Table 3). However, no statistical significance for observed differences was found. A decrease of pregnancies at an above-mean milk yield was observed using the Ovsynch with sexed semen, whereas unsexed semen pregnancy rates were similar.

Discussion. In the present work we have studied the efficiency of insemination with sexed semen at spontaneous estrus and synchronisation of ovulation, and the effects of several fertility-related factors on pregnancy rate in lactating Holstein cows. Previous studies on insemination of lactating dairy cows with sexed semen at observed estrus showed pregnancy rates of 23.8% (Bodmer 2005), 21% (Andersson et al. 2006) and 30% (DeJarnette et al. 2008), lower than achieved in this study (37.2%). Applying the Ovsynch protocol, the pregnancy rate with sexed semen was a mean of 40.1%, whereas others reported 31% (Schenk et al. 2009) and 25.7% (Karakaya et al. 2014). No significant difference in pregnancy rates was found between the two protocols neither using sexed nor unsexed semen. This is contrary to studies suggesting that the synchronisation of ovulation reduced pregnancy rates in the use of unsexed (Stevenson et al. 1999; Jobst et al. 2000) or sexed (Schenk et al. 2009; Abdel-Azim 2010) semen. Others reported similar conception rates with unsexed semen at observed estrus and Ovsynch use (Chebel et al. 2004) or a higher pregnancy rate in Ovsynch-treated cows (Marthold et al. 2016). Lucena at al. (2014) found no effect of the Ovsynch on pregnancy rate using sexed semen in lactating Jersey cows. These variations in results may stem from differences in experimental conditions, the fertility of the herds, timing of insemination, the fertility of bulls, and in an incidence of pregnancy loss.

An increase in pregnancy rate in the late stages of lactation was found at insemination with unsexed semen at detected estrus (Royal et al. 2000) or using the Ovsynch (Tenhagen et al. 2003). Yusuf et al. (2011) observed an increase of pregnancies at insemination of cows in spontaneous estrus from 40 to 100 days, with a subsequent decline. Corroborating our findings, in respective studies days in milk did not associate with pregnancy rate when the Ovsynch was applied on reproductively sound cows with sexed (Schenk et al. 2009; Karakaya et al. 2014) or unsexed (Keskin et al. 2010; Karakaya et al. 2014) semen. An increase of pregnancies using sexed semen was observed after 100 days in cows of unknown reproductive status (Schenk et al. 2009).

Data from studies on unsexed semen indicate that with increase in insemination number conception rates in Ovsynch-treated cows did not differ (Tenhagen et al. 2003; Keskin et al. 2010) or decreased (Chebel et al. 2004). Norman et al. (2010) documented a reduction in conception rates with sexed semen as service number increased. DeJarnette et al. (2010) reported a decrease of conception rates at advanced service numbers with unsexed semen at detected estrus, wherein a slight increase was observed for sexed semen, suggesting that, in lactating cows, service number has little influence on the conception rate of sexed semen. The non-significant effect of insemination number in the present study could be related to normal reproductive status of cows and restriction by fourth insemination. Higher insemination numbers might have a negative effect due to the inclusion of more of the less fertile cows.

The effect of parity on pregnancy rate was not significant in both protocols for either type of semen, corroborating data from insemination of lactating dairy cows with sexed semen at estrus (Bodmer et al. 2005; DeJarnette et al. 2008) and using the Ovsynch with sexed (Schenk et al. 2009) or unsexed (Keskin et al. 2010) semen. Others reported a greater pregnancy rate in primiparous cows inseminated using the Ovsynch with unsexed (Peters and Pursley 2002) or sexed (Karakaya et al. 2014) semen.

Differences in fertility between primiparous and multiparous cows may depend on differences in milk yield, energy balance, and metabolic changes. Greater energy demands for milk synthesis may increase the risk of fertility influencing problems due to the higher susceptibility of high-producing cows to metabolic and endocrine disorders (Chebel et al. 2004). In the present study, at insemination of cows in spontaneous estrus milk yield was greater in multiparous than that in primiparous cows. However, pregnancy rates did not differ significantly between multi- and primiparous cows in the use of sexed or unsexed semen. Similar lactation periods and normal clinical and reproductive status of cows may diminish the effect of the factors linked to greater milk yield. In the Ovsynch groups, inseminated with sexed or unsexed semen, primi- and multiparous cows had similar milk yields, and days in milk.

Several studies reported higher conception rates with unsexed semen, irrespective of parity in cows with above-mean milk yield than in those with below-mean yield using the Ovsynch (Peters and Pursley 2002) or GnRH treatment at the onset of estrus (Kaim at al. 2003). Others found no effect of milk yield inseminating Ovsynch-treated cows with unsexed (Tenhagen et al. 2003) or sexed (Karakaya et al. 2014) semen. We found that at insemination with sexed or unsexed semen at spontaneous

estrus, pregnancy rate appeared to increase in cows with above-mean milk yield. A decrease of pregnancies at a higher milk yield was observed in Ovsynch groups for sexed semen, wherein unsexed semen resulted similar pregnancy rates. This may have been caused by differences in the viability of sorted and unsorted sperm and in their sensitivity to the uterine environment. Treatment with GnRH and $PGF_{2}\alpha$ may alleviate or remove several of the factors decreasing fertility of high-producing cows (Tenhagen et al. 2003). It may be speculated that due to reduced viability sorted sperm are more sensitive to deviations in physiological state and uterine environment in cows with greater milk than unsexed sperm, despite possible positive hormonal and endometrial changes upon treatments.

Conclusions

The fixed-timed insemination with sexed semen of lactating Holstein cows with normal clinical and reproductive status applying the Ovsynch protocol resulted in the pregnancy rate similar to that obtained in those cows inseminated at spontaneous estrus. No significant difference in pregnancy rates was found between the two protocols using unsexed semen. In the both insemination protocols, the effect of days in milk, inseminations number and parity on pregnancy rate was not significant neither using sexed nor unsexed semen. At insemination with sexed or unsexed semen at spontaneous estrus, pregnancy rate appeared to increase in cows with above-mean milk yield. A non-significant decrease of pregnancies at a higher milk yield was observed in Ovsynch groups for sexed semen, wherein unsexed semen resulted similar pregnancy rates. The Ovsynch protocol can be used for fixed-timed insemination with sexed semen of lactating dairy cows as an acceptable alternative to insemination upon detection of spontaneous estrus.

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References

1. Abdel-Azim G. Effect of synchronization and semen sorting on artificial insemination bull fertility. J Dairy Sci 2010, 93, 420–425.

2. Andersson M., Taponen J., Kommeri M., Dahlbom M. Pregnancy rates in lactating Holstein-Friesian cows after artificial insemination with sexed sperm. Reprod Dom Anim 2006, 41, 95–97.

3. Bodmer M., Jannet F., Hässig M., Den Daas N., Reichert P., Thun R. Fertility in heifers and cows after low dose insemination with sex-sorted and non-sorted sperm under field conditions. Theriogenology 2005, 64, 1647–1655.

4. Chebel R.C., Santos J.E.P., Reynolds J.P., Cerri R.L.A., Juchem S.O., Overton M. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim Reprod Sci 2004, 84, 239–255.

5. DeJarnette J.M., Nebel R.L., Marshall C.E., Moreno J.F., McCleary C.R., Lenz R.W. Effect of sex-sorted sperm dosage on conception rate in Holstein heifers and lactating cows. J Dairy Sci 2008, 91, 1778–1789.

6. DeJarnette J.M., Nebel R.L., Marshall C.E. Use of flow cytometerically sex-sorted semen in single and superovulating cows and heifers. In: Proceedings of 26th Annual Scientific Meeting of A.E.T.E., Kuopio, Finland, September 2010, 79–95.

7. De Vires A., Overton M., Fetrow J., Leslie K., Eicker S., Rogers G. Exploring the impact of sexed semen on the structure of the dairy industry. J Dairy Sci 2008, 91, 847–856.

8. Hohenboken W.D. Application of sexed semen in cattle production. Theriogenology 1999, 52, 1421–1433.

9. Jobst S.M., Nebel R.L., McGilliard M.L., Peizer K.D. Evaluation of reproductive performance in lactating dairy cows with prostaglandin $F_{2}\alpha$, gonadotropin-releasing hormone, and timed artificial insemination. J Dairy Sci 2000, 83, 2366–2372.

10. Kaim M., Bloch A., Wolfenson D., Braw-Tal R., Rosenberg M., Voet H., Folman Y. Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conceptions. J Dairy Sci 2003, 86, 2012–2021.

11. Karakaya E., Yilmazbas-Mecitoglu G., Keskin A., Alkan A., Tasdemir U., Santos J.E.P., Gumen A. Fertility in dairy cows after artificial insemination using sex-sorted sperm or conventional semen. Reprod Dom Anim 2014, 49, 333–337.

12. Keskin A., Yilmazbas-Mecitoglu G., Gumen A., Karakaya E., Darici R., Okut H. Effect of hCG vs. GnRH at the beginning of the Ovsynch on first ovulation and conception rates in cyclic lactating dairy cows. Theriogenology 2010, 74, 602–607.

13. Lucena J.A., Kenyon A.G., Reynolds J.P., Moreno J.F., Lenz E.W., Carroll D., Lehenbauer T.W., Champagne J.D., Aly S.S. Comparison between low-dose, high-sort and high-dose, low-sort semen on conception and calf sex ratio in Jersey heifers and cows. J Dairy Sci 2014, 97, 1–8.

14. Marthold D., Detterer J., Koenig von Borstel U., Holtz W. Fixed-time insemination in pasture-based medium-sized dairy operations of Northern Germany and an attempt to replace GnRH by hCG. Reprod Dom Anim 2016, 51, 85–90.

15. Norman H.D., Hutchison J.L., Miller R.H. Use of sexed semen and its effect on conception rate, calf sex,

dystocia, and stillbirth of Holsteins in the United States. J Dairy Sci 2010, 93, 3880–3890.

16. Peters M.W., Pursley J.R. Fertility of lactating dairy cows treated with Ovsynch after presynchronization injections of $PGF_2\alpha$ and GnRH. J Dairy Sci 2002, 85, 2403–2406.

17. Pursley J.R., Mee M.O., Wiltbank M.C. Synchronization of ovulation in dairy cows using $PGF_2\alpha$ and GnRH. Theriogenology 1995, 44, 915–923.

18. Rabiee A.R., Lean I.J., Stevenson M.A. Efficacy of Ovsynch program on reproductive performance in dairy cattle: a meta-analysis. J Dairy Sci 2005, 88, 2754–2770.

19. Royal M.D., Darwash A.O., Flint A.P.F., Webb R., Woolliams J.A., Lamming G.E. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 2000, 70, 487–501.

20. Sá Filho M.F., Mendanha M.F., Sala R.V., Carvalho F.J., Guimarães L.H.C., Baruselli P.S. Use of sex-sorted sperm in lactating dairy cows upon estrus detection or following timed artificial insemination. Anim Reprod Sci 2013, 143, 19–23.

21. Schenk J.L., Cran D.G., Everett R.W., Seidel G.E. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: effects of sperm number per inseminate, sorting pressure and sperm storage before sorting. Theriogenology 2009, 71, 717–728.

22. Seidel G.E. Economics of selecting for sex: the most important genetic trait. Theriogenology 2003, 59, 585–598.

23. Stevenson J.S., Kobajashi Y., Thompson K.E. Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin F2α. J Dairy Sci 1999, 82, 506–515.

24. Tenhagen B-A., Vogel C., Drillich M., Thiele G., Heuwieser W. Influence of stage of lactation and milk production on conception rates after timed artificial insemination following Ovsynch. Theriogenology 2003, 60, 1527–1537.

25. Wiltbank M.C., Pursley J.R. The cow as an induced ovulator: Timed AI after synchronization of ovulation. Theriogenology 2010, 81, 170–185.

26. Yusuf M., Nakao T., Yoshida C., Long S.T., Gautam G., Ranasinghe R.M.S. B.K., Koike K., Hayashi A. Days in milk at first AI in dairy cows; its effect on subsequent reproductive performance and some factors influencing it. J Reprod Dev 2011, 57, 643–649.

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