

METHODOLOGY

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Comparison of the effect of a CIDR-Select Synch versus a long-term CIDR based AI protocol on reproductive performance in multiparous dairy cows in Swiss dairy farms

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Abstract

Background: Synchronization programs have become standard in the dairy industry in many countries. In Switzerland, these programs are not routinely used for groups of cows, but predominantly as a therapy for individual problem cows. The objective of this study was to compare the effect of a CIDR-Select Synch and a 12-d CIDR protocol on the pregnancy rate in healthy, multiparous dairy cows in Swiss dairy farms.

Methods: Cows (N = 508) were randomly assigned to CIDR-Select Synch (N = 262) or 12-d CIDR (N = 246) protocols. Cows in the CIDR-Select Synch group received a CIDR and 2.5 ml of buserelin i.m. on d 0. On d 7, the CIDR insert was removed and 5 ml of dinoprost was administered i.m.. Cows in the 12-d CIDR group received the CIDR on d 0 and it was removed on d 12 (the routine CIDR protocol in Swiss dairies). On d 0 a milk sample for progesterone analysis was taken. Cows were inseminated upon observed estrus. Pregnancy was determined at or more than 35 days after artificial insemination. As a first step, the two groups were compared as to indication for treatment, breed, stud book, stall, pasture, and farmer's business using chi square tests or Fisher's exact test. Furthermore, groups were compared as to age, DIM, number of AI's, number of cows per farm, and yearly milk yield per cow using nonparametric ANOVA. A multiple logistic model was used to relate the success of the protocols to all of the available factors; in particular treatment (CIDR-Select Synch/12-d CIDR), milk progesterone value, age, DIM, previous treatment of the uterus, previous gynecological treatment, and number of preceding inseminations.

Results: The pregnancy rate was higher in cows following the CIDR-Select Synch compared to the 12-d CIDR protocol (50.4% vs. 22.4%; $P < 0.0001$).

Conclusion: The CIDR-Select Synch protocol may be highly recommended for multiparous dairy cows. The reduced time span of the progesterone insert decreased the number of days open, improved the pregnancy rate compared to the 12-d CIDR protocol and the cows did not to have to be handled more often.

Background

The average size of a Swiss dairy farm herd is approximately 18 cows [1], compared with herds in the USA, for example, which had an average size of 120 in 2006 [2]. Hence, the use of synchronization programs and fixed-time AI (artificial insemination) is not common.

Busato et al., (1995) retrospectively analyzed the health data of 3581 cows from 80 Swiss dairy farms and identified endometritis (19.4%), silent heat (26.4%), non functional ovaries (7.6%) and cystic ovarian disease (5.5%) as the four main fertility disorders [3]. In Swiss dairy farms, the predominant therapy for cows with fertility disorders consists of a CIDR insert for 12 days without administering additional hormones (as described in the Eazy breedTM CIDR[®] B instruction leaflet), the cows being then inseminated upon observed estrus.

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Preparations of Gonadotropine Releasing Hormone (GnRH), human chorionic gonadotropin (hCG), Luteinizing Hormone (LH), and progesterone, either alone or in combination are frequently used to treat anestrus and/or COD [4-8]. Progesterone released from a CIDR insert inhibits the release of GnRH from the hypothalamus and therefore causes GnRH to accumulate in the hypothalamus; removal of the CIDR insert after 12 d causes a large amount of GnRH to be released and this results in ovulation [9-11]. However, the oocyte quality in 12-d CIDR protocols is poor [12]. Different studies showed an acceptable pregnancy rate after the use of a 7-d CIDR protocol in combination with GnRH and/or prostaglandin $F_{2\alpha}$ (CIDR-Select Synch) [13-15]. The objective of this study was to compare the effect of a CIDR-Select Synch and a 12-d CIDR protocol on the pregnancy rate in multiparous dairy cows in Swiss dairy farms. Based on a power analysis assuming a 10% increased pregnancy rate (from 35% to 45%) in the CIDR-Select Synch group, we needed a sample size of 380 cows per group to achieve 80% power for a two-sided hypothesis ($\alpha = 5\%$).

Methods

Animal selection criteria

Multiparous dairy cows at least 42 DIM (days in milk) of the breeds Holstein Friesian, Red Holstein, Brown Swiss and their crossbreds were included. The veterinarians performed a gynecological examination and cows diagnosed with anestrus, repeat breeders or cows diagnosed as not pregnant after a pregnancy check were included, if the uterus and uterine discharge were normal. Exclusion criteria were a preceding cesarean section, uterine torsion, uterine prolapse or birth-associated injuries of the genital tract. Also, cows with a history of lameness, acute mastitis or any systemic illness within 14 days prior to the CIDR insert were excluded. Any treatment within these 14 days resulted in exclusion. From CIDR removal to the point of insemination no therapy was allowed. Insemination had to be performed within 120 h after CIDR removal. Heifers and cows in first parity were excluded. Cows from farms using bulls were also excluded.

Treatment and insemination

Cows were randomly assigned to CIDR-Select Synch (N = 262) or 12-d CIDR (N = 246) protocols based on the cows' odd or even ear-tag numbers, respectively. Cows in CIDR-Select Synch group received a CIDR insert (Eazi-breed™ CIDR® B containing 1.9 g progesterone, Pfizer Animal Health, Zurich, Switzerland) and 2.5 mL of buserelin i.m. (Receptal® 4 µg/mL, Veterinaria AG, Zurich, Switzerland) on d 0. On d 7, the CIDR insert was removed and 5 mL of dinoprost i.m. (PGF_{2α};

Dinolytic®, 5 mg/mL, Pfizer Animal Health, Zurich, Switzerland) was administered. Cows in the 12-d CIDR group received a CIDR insert on d 0 and it was removed on d 12 (Figure 1). They were inseminated at observed estrus according to the AM-PM rule. The cows in both groups were observed for estrus three times daily for a minimum of 30 min. If no estrus was observed within 72 h after CIDR removal, each cow was examined by a veterinarian to exclude silent heat. Silent heat was defined by the presence of a mature follicle (≥ 13 mm in size), a regressing or no CL on one of the ovaries and a strong uterine tone. Cows found to be in silent heat were also inseminated. Cows were observed for estrus and inseminated up to 120 h from CIDR removal.

Milk progesterone test

A milk sample was collected from each cow on d 0 of treatment. About 5 mL was collected in a plastic tube containing 30 mg of sodium azide (Sigma-Aldrich, Bern, Switzerland) and was frozen until further analysis. The samples were centrifuged for 15 min at $1700 \times g$ and the fat layer was removed. This step was repeated, and skimmed milk samples were further tested. The progesterone concentrations were determined in skimmed milk by enzyme immunoassay as described by Meyer et al. (1986) [16]. The sensitivity of the test was 0.1 ng/mL. Intra- and inter-assay coefficients of variation were 8 and 12%, respectively. Cows with a progesterone level > 1 ng/mL were considered as having luteal activity. A progesterone level < 0.5 ng/mL was considered as

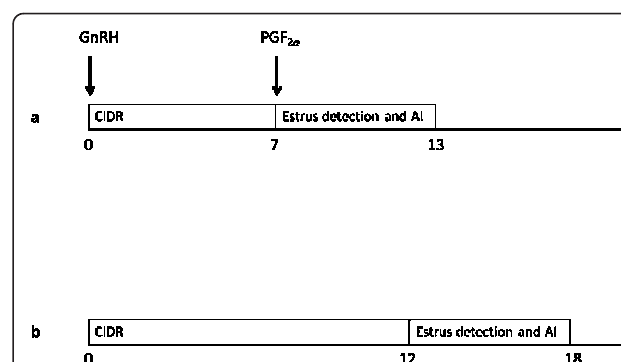


Figure 1 Schematic representation of the 2 synchronization protocols.

Cows in the CIDR-Select Synch group received a CIDR insert (CIDR; Eazi-Breed CIDR® cattle insert, Pfizer Animal Health, Zurich, Switzerland) and 10 µg of buserelin i.m. (GnRH; Receptal®, Veterinaria AG, Zurich, Switzerland) on Day 0. On Day 7, the CIDR insert was removed and 25 mg of dinoprost i.m. (PGF_{2α}; Dinolytic®, Pfizer Animal Health, Zurich, Switzerland) was administered. The cows were inseminated at observed estrus according to AM-PM rule up to 120 h from CIDR removal (a). Cows in the 12-d CIDR group received a Controlled Internal Drug Release insert (on Day 0) and it was removed on Day 12. The cows were inseminated at observed estrus using AM-PM rule up to 6 d from CIDR removal (b).

representing no luteal activity and the values in between belonged to the third group (indistinct luteal activity).

Pregnancy determination and definition of pregnancy rate

Pregnancy was determined by rectal palpation and/or transrectal ultrasound at or later than 35 days after artificial insemination. Pregnancy rates per group were determined by calculating the number of cows diagnosed as pregnant following the AI (after the protocol) divided by the total number of cows in the corresponding group. Those cows diagnosed as pregnant that were inseminated more than 6 d after CIDR removal were retrospectively excluded from the analysis.

Statistical analysis

Data were analyzed with a statistical software program (SAS Version 9.12). The primary end point was 'pregnancy after treatment'. As a first step, the baseline of the two groups were compared as to indication for treatment (non cyclic/negative pregnancy check/signs of estrus), breed (Brown Swiss, Holstein Friesian, Red Holstein and crossbreds), stud book (yes/no), type of stall (freestall/tiestall), pasture (yes/no), and farmer's business (regular/sideline) using chi square tests or Fisher's exact tests. The groups were also compared based on age, DIM, number of AI's, number of cows per farm, and yearly milk yield per cow using nonparametric ANOVA. The second step was to use a multiple logistic model to relate the success of the protocols to all of the available factors; in particular treatment (CIDR-Select Synch/12-d CIDR), milk progesterone value (< 0.5 /between 0.5 to 1/ > 1 ng/mL), age (< 3.5 yrs/between 3.5 to 5.5 yrs/ > 5.5 yrs), DIM (> 100 d; ≤ 100 d) previous treatment of the uterus (yes/no), previous gynecological treatment (yes/no), and insemination (farmer's observation/checked by veterinarian). We used a forward selection procedure. A test result was considered significant if the resulting p-value was < 0.05 .

Results

Sixteen Swiss practices participated in this study. Multiparous dairy cows ($N = 552$) were included from April 2009 to August 2010 following routine reproductive checks. The study was brought to an end because of the expiry date of the pharmaceuticals and as the time slot of data acquisition had elapsed. Forty-four cows were retrospectively excluded from the study due to the following reasons: lost CIDR ($n = 6$), wrong group assignment ($n = 10$), developing lameness ($n = 5$), developing vaginitis ($n = 6$), developing mastitis ($n = 5$), mounted by bull ($n = 5$), culling ($n = 7$). The only significant effect ($p < 0.0001$) was observed for the CIDR-Select synch treatment (132 cows pregnant of 262) versus

CIDR 12-d (55 cows pregnant of 246) with 28% more cows becoming pregnant following the CIDR-Select Synch protocol [with 95% confidence interval (0.199, 0.358)] (Figure 2).

No significant differences ($p > 0.1$) were found between groups based on: indication for treatment, breed, stud book, type of stall, pasture, farmer's business, age of the cows, DIM, number of AI's before treatment, number of cows per farm, yearly milk yield per cow, milk progesterone value, previous treatment of the uterus, previous gynecological treatment, and characteristic of insemination (Tables 1 and 2). When the cows were grouped according to age, previous gynecological or uterine treatment, DIM, and milk amount, no significant difference was found as to the pregnancy outcome (tested for every variable, without splitting the groups CIDR-Select Synch and 12-d CIDR). There were 78.7% (196/249) of cows in CIDR-Select Synch and 77.3% (157/203) in 12-d CIDR group observed in estrus ($P < 0.0001$; estrus observed vs. silent heat in both groups). Using the multiple logistic model with the forward selection method, none of the factors exceeded the significant level except for the factor CIDR.

Discussion

In Swiss dairy farms, synchronization protocols are more often used to treat individual animals than to synchronize herds. Fertility treatments have to be economical and practical to implement and acceptable to farmers. The CIDR-Select Synch is a viable alternative to the 12-d CIDR protocol because the cows do not need to be handled more frequently. The costs of the additional drugs were expected to be compensated for by the reduction in days open (extending the voluntary

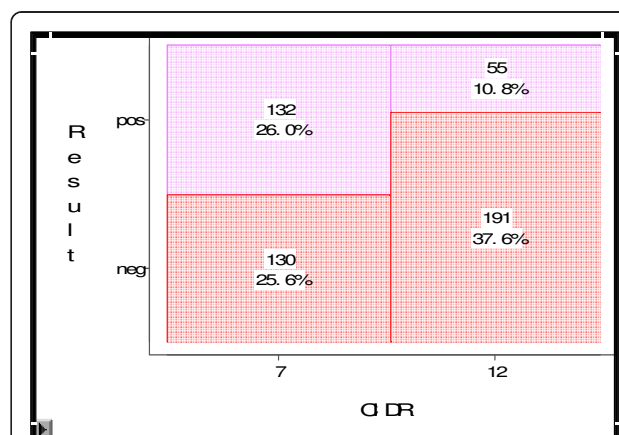


Figure 2 Mosaic plot of the primary endpoint "confirmed pregnancy". Graphic presentation of the percentage of cows in the groups CIDR-Select Synch and 12-d-CIDR with a negative/positive pregnancy test.

Table 1 Baseline comparison of groups at farm level

	CIDR-Select Synch	CIDR12	p- value
Number of cows	262	246	0.224
Age of cows (years)	5 (4/7)	5 (4/6.5)	0.627
DIM	98.5 (71/142)	100 (75/133)	0.709
Number of AI's	0 (0/1)	0 (0/1)	0.942
Number of cows/farm	24 (18/32)	25 (19/35)	0.224
Yearly milk yield/cow (kg)	7500 (6800/8050)	7300 (6800/ 8300)	0.554

There were no significant differences found between the two treatments for the parameters listed (median values (25%/75%), skewed distribution).

waiting period costs 10-20 Swiss francs/cow/day (personal communication Berger & Lauener)). Our study revealed a pregnancy rate for the cows in the CIDR-Select Synch group of 50.4% versus 22.4% in the 12-d CIDR group. Chebel et al., (2010) demonstrated that the use of a CIDR insert for 7 days during a TAI protocol increased the proportion of functional CL in anestrus cows after AI and pregnancy/AI compared to protocols without CIDR [17]. Lamb et al. described a better pregnancy rate in a Cosynch-CIDR protocol compared to Cosynch alone, but only in acyclic cows or cows with a low progesterone level when PGF was administered [18]. The use of exogenous progesterone over a longer period (as described in the Eazy breed™ CIDR® B instruction leaflet) might lead to the ovulatory follicles becoming persistent and to the ovulation of an excessively aged oocyte with a concurrent drop in the pregnancy rate [19]. As a consequence, Swiss practitioners using the 12-d CIDR protocol often skip the CIDR-provoked heat and inseminate cows in the consecutive estrus. The production of remaining large dominant follicles is inhibited by adding GnRH at the beginning of a CIDR protocol, the largest follicles being eliminated by ovulation or atresia [20]. GnRH induces a new follicular wave to emerge within 3-4 days after treatment [20]. Administering GnRH at or after insemination, administering hCG after insemination and supplementing progesterone after insemination have improved reproductive performance in normal, anoestrus and repeat breeder cows [21-25]. In the present study, no hormonal treatment was allowed around insemination. Perry & Perry (2009) demonstrated that treatment with GnRH at AI following the detection of standing estrus in cattle did not influence conception rates [26].

Milk progesterone was used as a covariable in this study and it was measured at d 0 of treatment. The pregnancy rate was not significantly different among the 3 milk progesterone groups (low/intermediate/high) at the beginning of the protocols. Ryan and coworkers (1995) already described this fact in a study using three

Table 2 Baseline comparison of groups at cow level

	CIDR-Select Synch	CIDR12	p-value
Indication for CIDR			0.677
Acyclic	71.4%	72%	
Neg. preg. check	22.9%	24%	
No AI	5.7%	4%	
Breed			0.364
Brown Swiss	40.9%	38.2%	
Holstein Friesian	24%	20.7%	
Red Holstein & crossbreds	35.1%	41%	
Studbook			0.341
Yes	79.5%	82.8%	
No	20.5%	17.2%	
Type of stall			0.542
Freestall	39.5%	36.9%	
Tiastall	60.5%	63.1%	
Pasture			0.285
Yes	91.9%	94.3%	
No	8.1%	5.7%	
Farmer's business			0.736
Regular	95.7%	95.1%	
Sideline	4.3%	4.9%	
Uterine treatment			0.275
Yes	13.4%	10.2%	
No	86.6%	89.8%	
Gynecological treatment			0.174
Yes	10.3%	6.9%	
No	89.7%	93.1%	
Insemination			0.733
Blind	78.7%	77.3%	
Checked	21.3%	22.7%	
Progesterone value			0.223
< 0.5 ng/ml	44.3%	39.4%	
0.5-1 ng/ml	14.9%	12.2%	
> 1 ng/ml	40.8%	48.4%	

No significant differences were found between the two treatments for the parameters listed (percentual distribution).

different CIDR protocols [27]. Progesterone levels were, however, only measured at the beginning of our study.

Ryan et al. (1999) describe an increased estrus detection rate and a decreased CIDR loss rate when the progesterone insertion period was decreased from 12 to 8 days [28]. In both our groups, nearly 80% of cows were inseminated at observed estrus. The estrus detection rate might be better in small herds with moderate milk production as is mostly observed in Switzerland. Lucy described the difficulties of heat detection, identification and insemination in large herds and assumed poor estrus expression as being a major problem in dairy farms with high production levels [29]. Conception rates tended to be higher when AI occurred after detected estrus as compared with fixed-time AI. Pregnancy rates

on the other hand, were higher after fixed-time AI when compared with insemination after detected estrus [30].

Conclusions

The CIDR-Select Synch protocol is a valuable and practical alternative to the 12-d CIDR protocol. Reducing the time span of the progesterone insert also means fewer CIDR inserts lost and fewer days open. We were able to demonstrate that in small herds with intensive observation periods to detect estrus, the CIDR-Select Synch protocol produced a pregnancy rate of 50.4% in multiparous dairy cows. The milk progesterone value at the beginning of the protocol did not significantly influence the outcome.

Abbreviations

CIDR: Controlled intravaginal drug release; AI: artificial insemination; COD: cystic ovarian disease; GnRH: Gonadotropine Releasing Hormone; hCG: human chorionic gonadotropin; LH: Luteinizing Hormone; PGF: prostaglandin F; DIM: days in milk; TAI: timed artificial insemination.

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Authors' contributions

This study represents the doctoral thesis of JR. JR had the basic idea of performing this study. RB helped with milk progesterone analysis. RK sensitized JR to synchronizing studies and revised the manuscript critically. AS and RK revised the manuscript critically and gave final approval of the version to be published. MK participated in the study design. JH performed the statistical design and analysis including calculating the sample size. GH made substantial contributions to the conception and the design of the study as well as the overall crosslinking. All authors read and approved the final manuscript.

Competing interests

The study was financially supported by Pfizer Animal Health, Zurich, Switzerland and Veterinaria AG, Zurich, Switzerland. Before the beginning of the study, publication of data was bound by contract whatever the results would prove.

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