Results of Feeding Different Fatty Acids on the Cow's Transition and Reproductive Cycle

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Abstract

Recent studies indicate that supplemental fats differing in fatty acid profile impacts metabolism of different tissues in lactating dairy cows. Four experiments evaluated the effects of altering the fatty acid profile of the supplemental fat source fed to high producing dairy cows on lactational and reproductive performance. When cows were fed calcium salts of fish and palm oil, responses were dependent upon season of calving. Lactational performance was improved for cows receiving calcium salts when calving during the thermo neutral season, but cows fed tallow had higher yields of milk and milk components during heat stress. Reduction in performance of cows during heat stress was partially attributed to a higher content of free fatty acids in the calcium salt. When the experiment was replicated in the subsequent year, lactational performance was similar for cows under heat stress. Reproductive performance was not affected by source of fatty acids when cows were fed either tallow or calcium salts of fish and palm oil in two experiments. When cows in late gestation and early lactation were fed calcium salts of palm oil or calcium salts rich in linoleic and monoenoic *trans* fatty acids, yields of milk were not altered, but because of lower milk fat content for cows fed calcium salts rich in linoleic and monoenoic *trans* fatty acids, production of 3.5% fat corrected milk was suppressed. Changes in concentrations of milk fat and milk fatty acid profile were observed early in lactation, at 3 and 2 weeks postpartum, respectively. Feeding calcium salts rich in linoleic and monoenoic *trans* fatty acids improved reproductive performance of dairy cows, and the increased conception rates were partially attributed to higher fertilization rates and improved embryo quality.

Introduction

Feeding fat is a common method to increase the energy density of the diet. However, supplemental fat sources not only provide calories, but also impact tissue metabolism by altering genetic expression (polyunsaturated fatty acids; Sumida et al., 1993) or by supplying precursors (acetyl Co-A) for synthesis of other fatty acids or steroids (Staples et al., 1998), or competing with other cell components (Thatcher et al. 2004).

There are different commercial sources of rumen inert fats including hydrogenated fatty acids and calcium salts of fatty acids. These fat sources were originally designed to increase the caloric intake of dairy cows with minimal impact on rumen microbial activity. More recently, there has been an increased interest in designing fat sources rich in the polyunsaturated fatty acids of the ω-6 (linoleic acid, *cis*-9 *cis*-12 C18:2_{n6}) and ω-3 (linolenic acid, C18:3_{n3}; eicosapentaenoic - EPA, C20:5_{n3}; docosahexaenoic - DHA, C22:6_{n3}) for delivery to the lower gut for absorption. Because microbial activity in the rumen resulting in lipolysis and biohydrogenation alters the make up of fat sources rich in polyunsaturated fatty acids, methods to protect fatty acids from microbial activity in the rumen have been developed. Staples et al. (1998) reviewed the literature on the effects of fat feeding on reproduction in dairy cows and indicated that increasing dietary fat in the ration usually resulted in improvements in reproductive performance of cows. The authors indicated that the beneficial effects of supplemental fat were observed in spite of provision of calories (Staples et al., 1998).

The development of calcium salt technology has led to the ability to deliver polyunsaturated fatty acids to the small intestine of cattle. However, little is known about the degree of protection of these fatty acids and the estimated amounts reaching the duodenum. The new CPM model (CPM v. 3.0.4a) has a fat sub-model that predicts the amounts of specific fatty acids reaching the small intestine of dairy cattle, which has been described in detail by Moate et al. (2004). Strategies that integrate nutritional and reproductive management are expected to improve overall reproductive performance in dairy herds.

We have utilized Ca salt technology to design fat sources rich in specific fatty acids in an attempt to study their effects on reproductive functions in dairy cattle (Cerri et al., 2004; Juchem et al., 2004a, Juchem et al., 2004b, Juchem et al., 2004c, Juchem et al., 2004d).

Effect of Calcium Salts of Fish and Palm Oil on Lactational and Reproductive Performance of Dairy Cows

Seven-hundred and thirty eight multiparous Holstein cows from a commercial dairy farm were assigned to one of the two different diets in a randomized complete block design, from September 2001 to November 2002. Cows were blocked according to parity and previous lactation milk yield and, within each block, randomly assigned to one

of the two treatment diets. Both groups received the same diet except for the supplemental fat source, which was fed either as tallow (1.65 % diet DM) or as calcium salt of fish oil and palm oil (CaSFO, 1.8 % diet DM) to provide equal amounts of fatty acids (Table 1). All cows received a pre-treatment diet during the first 25 d in lactation that consisted of a blend of each fat supplement (0.8 % of Tallow + 0.9 % of CaSFO) and remained in the treatment diets until 145 d in lactation. The supplemental fat sources were designed to supply no ω-3 fatty acids as eicosapentaenoic (EPA, $C20:5_{n3}$) and docosahexaenoic (DHA, $C22:6_{n3}$) when tallow was fed or approximately 10 g of EPA and 10 g of DHA when calcium salts were fed. Based on previous studies (Thatcher et al., 2004), we hypothesized that feeding a calcium salt of ω-3 fatty acids would modulate uterine prostaglandin synthesis and benefit embryo survival in lactating dairy cows.

Because of an interaction between treatment and season of calving, production and reproduction outcomes were stratified by season: thermo neutral (TN) and heat stress (HS). Yields of 3.5% fat-corrected milk and milk fat were higher for cows fed CaSFO during the TN season, however, cows fed tallow produced more milk and 3.5% fat corrected milk during HS. Yields of milk fat and true protein were also higher for cows fed tallow during HS. Milk fatty acid profile was altered by feeding CaSFO, with increases in Linoleic, EPA, DHA, and *trans*-9 *cis*-11 CLA. Body condition score was lower for cows fed CaSFO at 70 d postpartum during TN, and at 70, 98 and 123 d postpartum during HS. Group dry matter intake was similar for both treatments, and estimated individual dry matter intake using alkanes at 52 and 100 d postpartum was also similar (P = 0.50) for CaSFO and tallow and it averaged 26 kg/d. Digestibility of dry matter in the total tract was higher for cows fed CaSFO, but efficiency of feed utilization (3.5% fat corrected milk/ dry matter intake) was similar for both treatments. No treatment effect was observed on plasma concentrations of glucose and NEFA.

Reproductive performance of dairy cows was generally not affected by treatment (Table 2), but interactions between treatment and season of calving were observed. Pregnancy rates were higher for cows fed CaSFO during the TN season, but lower during HS. Likewise, pregnancy loss during the first 63 d of gestation were similar for both treatments, but cows fed CaSFO tended to experience less embryonic mortality during the TN season, but higher during HS than cows fed tallow.

After analyzing Ca salts samples, we determined that the amount of free fat decreased as the storage time increased (Figure 1). In the first few days after manufacturing, the free fat content was approximately 12%, and it decreased to less than 6% after 60 days. Because at the beginning of the study, during the TN season, Ca salts had been stored for several weeks prior to feeding to the cows, and during the HS season it was utilized a few weeks after manufacturing, we suspect that some of the negative effects observed during the HS season are consequent to the higher availability of free polyunsaturated fatty acids in the rumen.

Effect of Calcium Salts of Fish and Palm on Lactational and Reproductive Performance of Dairy Cows under Heat Stress

Because of the negative effects observed in cows receiving CaSFO under HS in the previous experiment and the higher free fat content of the calcium salts fed during the HS period, we replicate the same experiment in 2003 during the summer.

Three-hundred and thirty-one multiparous Holstein cows were assigned to treatments based on lactation number and previous lactation 305-d milk yield. Cows calved from May 1st to August 31st, and the study was conducted from May to December of 2003. Both groups received the same diet except for the supplemental fat source, which was fed either as tallow (1.65 % diet DM) or as calcium salt of fish oil and palm oil (CaSFO, 1.8 % diet DM) to provide equal amounts of fatty acids. All cows received a pre-treatment diet during the first 25 d in lactation that consisted of a blend of each fat supplement (0.8 % of Tallow + 0.9 % of CaSFO) and remained in the treatment diets until 145 d in lactation. The supplemental fat sources were the same described previously (Table 1).

Group dry matter intake was similar for both groups and it averaged 25.7 kg/d. Yields of milk, 3.5% fat corrected milk, and milk fat were similar for both treatments, but cows fed CaSFO produced milk with lower concentrations of true protein, resulting in lower yields of milk protein. Similarly, concentrations of lactose and solids nonfat were also lower for cows fed CaSFO. Reproductive performance as determined by conception rates, pregnancy loss, days open, and proportion of cows pregnant at the end of the study were similar for both treatments.

Effect of Feeding Ca Salts Rich in ω-6 and trans Fatty Acids During Transition on Lactation, Health, and Reproduction of Dairy Cows

Four-hundred and twenty-three prepartum Holstein dairy cows from a commercial dairy farm were assigned to one of the two treatment diets that differed in the supplemental fat source. Cows were blocked according to parity, body condition score at dry off, and previous lactation milk yield (multiparous) and, within each block, randomly assigned to one of the two treatment diets. The study started 25 before the expected calving date, and cows were fed the supplemental fat sources until 70 to 75 d postpartum. The supplemental fat sources were added at equal amounts for both treatments during the pre- (1.9% of the diet DM; ~250 g/cow/d) and postpartum periods (1.5% of the diet DM; ~250 g/cow/d) and they consisted of calcium salts of palm oil (PO) or calcium salts of linoleic and a blend of monoenoic trans fatty acids (LTFA). The calcium salts were the only supplemental fat source fed to cows throughout the entire study.

Yields of milk and milk components were measured weekly during the first 11 weeks postpartum. Blood samples collected for measurements of plasma concentrations of β-OH-butyrate, nonesterified fatty acids, and glucose throughout the pre- and postpartum periods. Blood samples were also collected from 80 cows 4 x weekly during the first 21 DIM for measurements of plasma PGFM. Liver biopsies were collected from 30 cows (15/trt) at 5 and 28 DIM for measurements of hepatic glycogen and triacylglycerol. Cows had were examined by ultrasonography weekly, starting at 10 d

postpartum, for measurements of uterine horn diameter as an indicator of uterine involution, and for determination of ovarian structures to evaluate the interval from calving to first postpartum ovulation. Uterine cytology was performed in all cows at approximately 40 d postpartum to determine the incidence of subclinical endometritis. Cows were pre-synchronized with an injection of GnRH at approximately 50 d postpartum and 7 days later enrolled in a timed AI protocol. Pregnancy was diagnosed by ultrasonography at 28 d of gestation and pregnant cows were palpated per rectum 2 weeks later for pregnancy reconfirmation.

Yields of milk were similar for both treatments throughout the 70 d experiment. However, because milk fat content decreased beginning at 3 weeks postpartum (Figure 2), 3.5% fat corrected milk production was lower for cows fed LTFA. Yields of milk protein were similar between treatments, but milk from cows fed LTFA had higher milk true protein content (2.78 vs 2.74; $P < 0.01$). Feeding LTFA altered the fatty acid profile of milk fat as early as 2 weeks postpartum. Cow fed LTFA had milk fat with greater concentration of linoleic acid, *cis*-9 *cis*-11 CLA, and trans fatty acids. Conception rates at first postpartum insemination was higher for cows fed LTFA than those fed PO (38.9 vs 25.9%; $P = 0.02$).

Effect of Fat Sources Differing in Fatty Acid Profile on Fertilization Rate and Embryo Quality in Lactating Dairy Cows

One hundred and fifty-four lactating Holstein dairy cows were assigned to one of the two treatment diets that differed in the supplemental fat source. Cows were blocked according to parity, body condition score at dry off, and previous lactation milk yield (multiparous) and, within each block, randomly assigned to one of the two treatment diets. The study started 25 before the expected calving date, and cows were fed the supplemental fat sources until embryo collection at approximately 55 d postpartum. The supplemental fat sources were added at equal amounts for both treatments during the pre- (1.9% of the diet DM; ~250 g/cow/d) and postpartum periods (1.5% of the diet DM; ~250 g/cow/d) and they consisted of calcium salts of palm oil (PO; n = 75) or calcium salts of linoleic and a blend of monoenoic trans fatty acids (LTFA; n =79). The calcium salts were the only supplemental fat source fed to cows throughout the entire study. At approximately 33 d postpartum, all cows were pre-synchronized with the following protocol: d 30, 100 μg GnRH + CIDR insert; d 37, 25 mg of $PGF_{2\alpha}$ + CIDR removal. The Ovsynch protocol (GnRH, 7d $PGF_{2\alpha}$, 2 d GnRH, TAI 12 to 16 h later) started 48 h after the CIDR removal and all cows were fixed-time inseminated 12 h after the final injection of GnRH of the Ovsynch by the same technician with semen from two ejaculates from a single proven sire with expected relative conception rate of +3 (Figure). Throughout the synchronization protocol, the ovaries of cows were examined by ultrasonography to determine ovulatory responses to treatment and synchronization rates. Blood was sampled at each hormonal treatment for measurements of plasma concentrations of progesterone. Cows flushed 5 d after timed AI and structures were evaluated for fertilization rate, number of accessory sperm attached to the zona pellucida of embryos and oocytes, embryo quality, and number of total and dead blastomeres.

A total of 161 ovulations were detected in 154 cows, and 14 (18.7%) and 12 (15.2%) cows fed PO and LTFA, respectively, experienced double ovulation when inseminated. The number of structures recovered were 45 and 41 for PO and LTFA, respectively, and the recovery rate (number of structures/number of corpora lutea) were similar for both treatment and it averaged (53.4%). Fertilization rate tended to be higher (P = 0.11) for cows fed LTA than those fed PO (87.2 vs 73.3%). Similarly, the number of accessory sperm per structure collected was higher for cows fed LTA than PO (34.3 vs 21.5; $P < 0.001$), which might partially explain the higher fertilization rate. Cows fed calcium salts of LTFA throughout the transition period and early lactation had a great proportion of embryos classified as high quality compared to cows fed calcium salts of PO (73.5 vs 51.5; $P = 0.06$). Furthermore, the number of total cells (19.4 vs 14.0; $P = 0.13$) and the proportion of live cells (94.2 vs 85.3%; $P = 0.09$) tended to be higher for cows fed LTA than those fed PO. Results from this experiment indicate that improvements in fertility of dairy cows at first postpartum AI observed in the previous study might be attributed to improvements in fertilization rate and embryo quality when cows are fed fat sources containing linoleic and monoenoic *trans* fatty acids during the transition period.

Conclusions

Growing evidence indicates that the delivery of supplemental unsaturated fatty acids to the lower gut for absorption may target reproductive tissues to alter reproductive function and fertility. We presented results from 4 experiments indicating that reproductive performance of dairy cows is influenced by dietary fatty acids when the energy concentration in the ration is maintained. Feeding fat sources rich in the ω-3 fatty acids EPA and DHA inhibit prostanoid synthesis *in vitro* and *in vivo*. The results from these experiments indicate that feeding calcium salts of fish and palm oil was influenced by the higher availability of PUFA in the rumen. Furthermore, heat stress effects on reproduction overwhelmed the dietary intervention to prevent early embryonic loss. Feeding dairy cows calcium salts of linoleic and monoenoic *trans* fatty acids during late gestation and early lactation did not affect milk production, increased concentrations of protein in milk, but reduced concentrations of milk fat starting at 3 weeks postpartum. The reduction in milk fat content reduced yields of 3.5% fat corrected milk. Milk fatty acid profile was altered as early as 2 weeks postpartum. Cows fed calcium salts of LTFA had increased conception and pregnancy rates at first postpartum AI, and the enhanced reproductive performance was partially attributed to improvements in fertilization rate and embryo quality.

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Table 1. Nutrient composition of experimental diets in experiments 1 and 2.

	Treatment	
Nutrient	CaSFO	Tallow
NEL, Mcal/kg DM	1.62	1.61
CP, %	18.1	18.1
RUP, % CP	40.6	40.6
NDF, %	30.4	30.5
Fat, %	6.1	6.1
NFC, %	39.0	39.2

Fatty acid profile of supplemental fat sources

Fatty acids	- - - - - - - - - - - - - - - g/100g of fatty acids - - - - - - - - - - - - -	
C14:0	2.4 ± 0.09	2.8 ± 0.13
C16:0	40.9 ± 0.40	26.1 ± 0.56
C16:1	2.0 ± 0.14	3.2 ± 0.21
C18:0	4.2 ± 0.34	19.8 ± 0.48
C18:1 *cis* 9 + *cis* 10	30.2 ± 0.36	36.3 ± 0.50
C18:2 *cis* 9, *cis* 12, n-6	7.5 ± 0.23	5.6 ± 0.32
C20:5, n-3 (EPA)	2.3 ± 0.11	nd

C22:6, n-3 (DHA)[4]	2.5 ± 0.10	nd
Unsaturated fatty acids	53.5 ± 0.47	49.1 ± 0.67

Table 2. Effect of fat sources differing in fatty acid profile on reproductive performance in multiparous lactating Holstein cows.

			Season[1]						
	Treatment[2]		T N		HS		***P*** <		
Item	CaSFO	Tallow	CaSFO	Tallow	CaSFO	Tallow	Fat	Season	Fat x Season
Cycling (%)	82.2	83.3	83.8	81.3	80.9	85.2	0.08	0.54	0.11
Pregnancy Rate									
Day 28	35.9	40.7	41.6	37.6	31.2	43.7	0.45	0.37	0.02
Day 39	31.4	35.7	39.2	33.5	25.0	37.7	0.69	0.32	0.04
Day 63	26.3	32.0	32.4	28.5	21.4	35.4	0.98	0.23	0.03
Second AI	19.9	23.0	23.4	26.0	17.2	19.2	0.03	0.10	0.50
Pregnancy Loss									
Day 28 to 39	11.7	12.4	4.8	10.8	19.3	13.7	0.65	0.12	0.21
Day 39 to 63	12.9	8.9	12.7	14.0	13.0	4.5	0.26	0.35	0.31
Day 28 to 63	23.5	20.4	17.2	23.4	29.8	17.9	0.66	0.71	0.22

[1] TN = thermo neutral season; HS = heat stress season. [2] CaSFO = calcium salts of fish and palm oil.

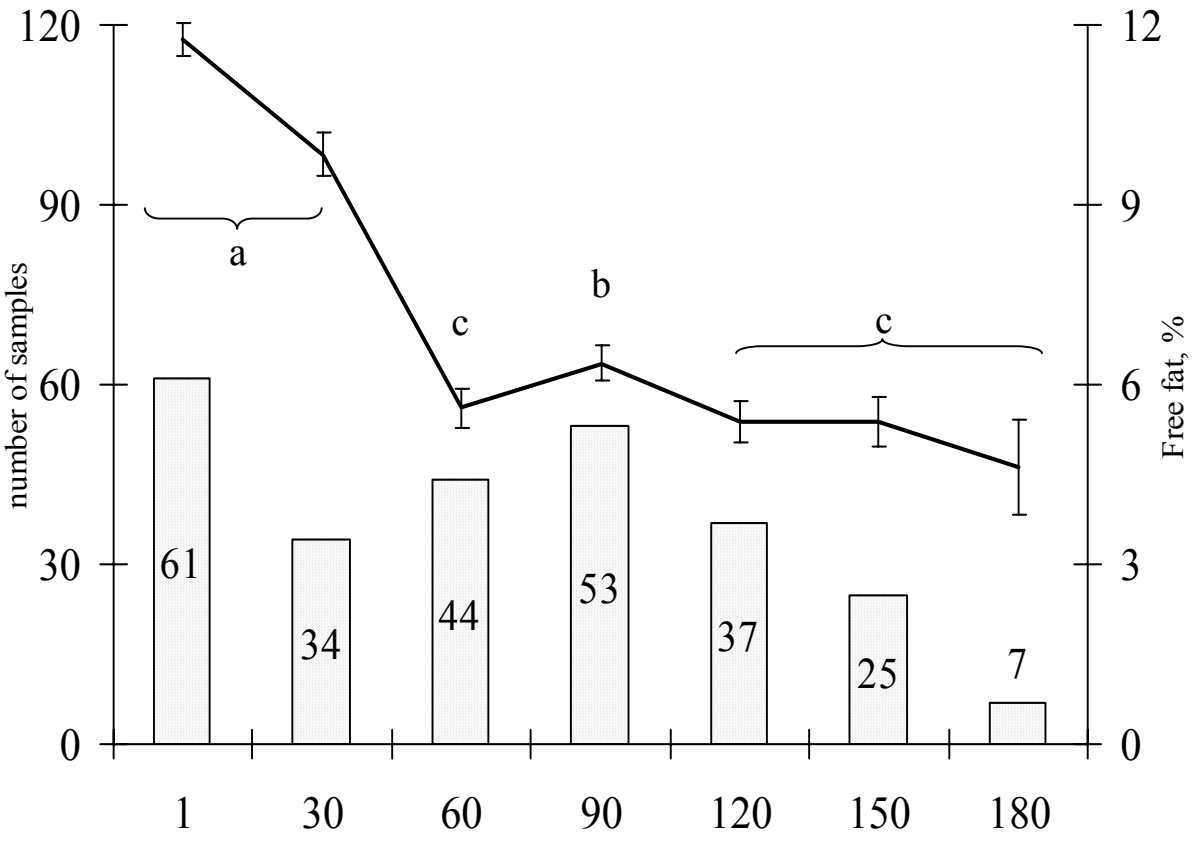

Figure 1. Free fat (% of total weight; solid line) in Ca salts of fish and palm oil over time after manufacturing, and number of samples (bars) analyzed in each time point. Time points with different superscripts differ ($P < 0.01$). Day 1 represents the day of manufacturing. Pooled SEM = 0.40.

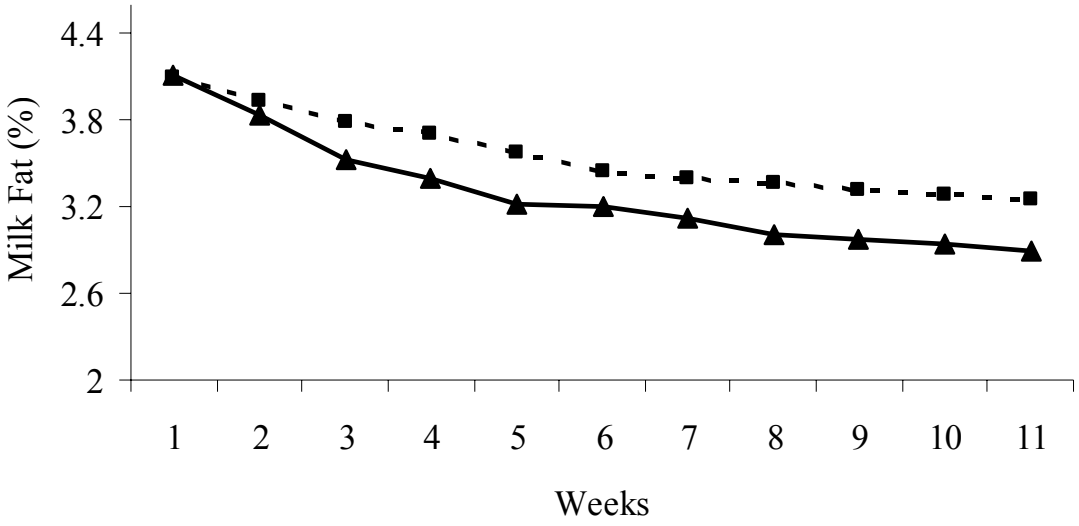

Figure 2. Milk fat % in cows fed calcium salts of palm oil (--■--) or calcium salts of linoleic and monoenoic *trans* fatty acids (—▲—). Pooled SEM = 0.02. Effect of treatment ($P < 0.01$) and interaction between treatment and week postpartum ($P < 0.02$).

Study Design

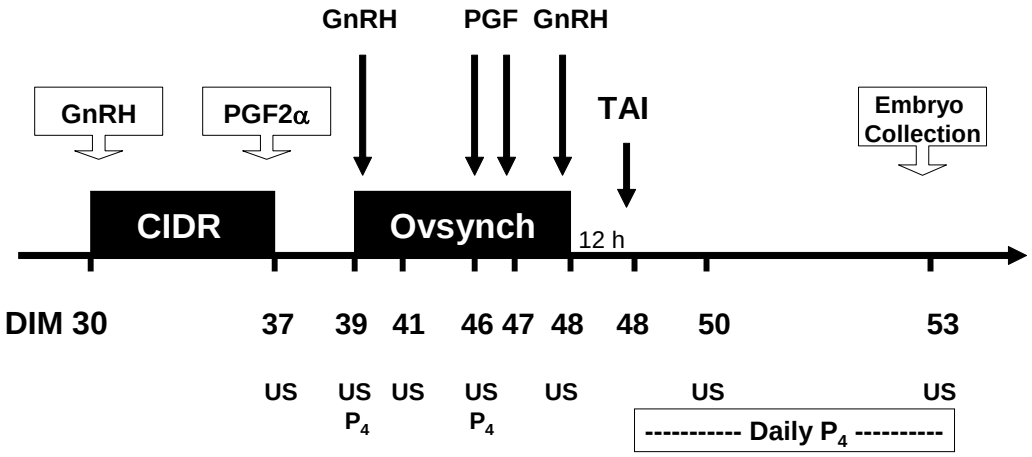

Figure 3. Diagram of activities during the experiment. CIDR = controlled intravaginal drug releasing containing 1.38 g of progesterone; DIM = days in milk; GnRH = gonadotropin releasing hormone; PGF = prostaglandin; P4 = progesterone; TAI = timed artificial insemination; US = ultrasonography.