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Five-day Resynch Programs in Dairy Cows Including Controlled Internal Drug Release at Two Stages Post-Artificial Insemination

S. L. Pulley, S. L. Hill, and J. S. Stevenson

Summary

Two experiments were conducted to assess pregnancy outcomes after a 5-day Ovsynch-56 Resynch (**RES**; gonadotropin-releasing hormone injection 5 days before [**GnRH-1**; d 0] and 56 hours (**GnRH-2**) after PGF_{2α} [**PG**] injections on day 5 and 6, timed artificial insemination [**TAI**] on day 8) with and without a progesterone-releasing intravaginal controlled internal drug release (**CIDR**) 5-day insert. In Exp. 1, nonpregnant cows were enrolled on day 34 post-AI: day 34 **RES-CON** (n = 528) or day 34 **RES-CIDR** (n = 503). Blood was collected for progesterone assay. Pregnancy per AI (**P/AI**) was diagnosed by uterine palpation per rectum at 34 and 62 days post-TAI. Only 76% of 1,031 cows had high progesterone (≥ 1 ng/mL) on day 34 at the nonpregnant diagnosis. No differences in P/AI were detected between treatments. The day-34 **RES-CIDR** cows with low (< 1 ng/mL) progesterone, however, had greater ($P = 0.036$) P/AI than day-34 **RES-CON** cows (37.7 vs. 29.4%), whereas day-34 **RES-CIDR** cows with high progesterone had lesser P/AI than day-34 **RES-CON** (27.4 vs. 34.3%). In Exp. 2, cows were enrolled on day 31 post-AI after a nonpregnant diagnosis: (1) day 31 PG-3-G (n = 102): Pre-PG on day 31, Pre-GnRH on day 34, and RES on day 41 (n = 102); (2) day 41 **RES-CON** (n = 108) as Exp. 1, but on day 41; and (3) day 41 **RES-CIDR** (n = 101) as Exp. 2, but on day 41. Blood was collected for progesterone assay and ovarian structures were mapped by ultrasonography on days 31, 34, 41, 46, and 48. Pregnancy was diagnosed by ultrasonography on days 31 and 59 post-TAI. The proportion of cows with high progesterone on day 31 was 70.6%. More ($P < 0.001$) cows ovulated after Pre-GnRH on day 31 PG-3-G (60.4%) than for day 41 **RES-CON** (12.5%) or day 41 **RES-CIDR** (17.1%). More ($P < 0.001$) PG-3-G cows had luteolysis after Pre-PG on day 31 than other treatments (73.7 vs. $< 11\%$). The proportion of cows with high progesterone on day 41 at GnRH-1 tended ($P = 0.10$) to be greater for PG-3-G (75.6%) than for other treatments (65 to 70%). The P/AI was greater in cows starting RES on day 41 when progesterone was low (44%) than when it was high (33%), but no treatment differences were detected 31 days after TAI (PG-3-G = 33.3%; d 41 **RES-CON** = 38.9%; d 41 **RES-CIDR** = 35.6%). We concluded that improved P/AI for cows initiating the 5-day RES on day 34 without a corpus luteum is progesterone-dependent because addition of the CIDR insert to the RES treatment improved P/AI in cows with low progesterone (Exp. 1). Although day-31 PG-3-G increased luteolysis and produced greater ovulation rates before the onset of RES, no increase in P/AI was detected compared with RES started on day 41 with or without a CIDR insert.

Key words: CIDR, Resynch, pregnancy

Introduction

Lactating dairy cows generally have poorer pregnancy outcomes at second and subsequent services when timed artificial insemination (**TAI**) programs are applied. Currently, many well-managed dairies are achieving TAI pregnancies in the 40% range at first service after calving. Pregnancy outcomes for TAI repeat services are in the 30 to 35% range for the most part.

Poorer pregnancy outcomes of repeat or Resynch (**RES**) inseminations seem to be associated with poorer ovulatory responses to gonadotropin-releasing hormone (**GnRH**) and failure of the corpus luteum to regress in response to $\text{PGF}_{2\alpha}$ (**PG**). Studies in which open cows are pretreated with GnRH or human chorionic gonadotropin (hCG) 7 days before RES have improved pregnancy outcomes, but they also extend the inter-insemination interval for individual cows because of the delay between open diagnosis and re-insemination. Injecting PG 7 or 11 days before RES also delays reinsemination, but when coupled with detection of estrus, more cows are reinseminated before the follow-up RES is applied to those cows not inseminated.

Our objective was to determine the value of applying progesterone in the form of a CIDR insert to cows diagnosed open at day 34 after last insemination (Exp. 1). A second experiment was performed with the same objective. The RES program with or without the CIDR insert, however, was applied on day 41, and a third treatment included a presynchronization (PG + GnRH) treatment before RES. In both experiments, a 5-day RES-Ovsynch protocol was used.

Experimental Procedures

Lactating dairy cows from two herds in northeast Kansas were enrolled in the study. All cows were milked three times daily and fed diets consisting of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals.

The herd enrolled in Exp. 1 comprised cows ($n = 1,031$) diagnosed open 34 days (range of 34 to 40 days, with the majority of cows on day 34) post-AI by uterine palpation per rectum by a single veterinarian from September 2010 through October 2012. Non-pregnant cows were assigned randomly to a 5-d Ovsynch-56 Resynch (RES; GnRH injection 5 days before [GnRH-1; day 0] and 56 hours (GnRH-2) after $\text{PGF}_{2\alpha}$ [PG] injections on days 5 and 6, TAI on day 8) with (day-34 **RES-CON**, $n = 528$) or without a progesterone-releasing intravaginal controlled internal drug release (CIDR) 5-day insert (day-34 **RES-CIDR**, $n = 503$, Figure 1).

The herd enrolled in Exp. 2 comprised cows ($n = 209$) diagnosed open 31 days (range of 30 to 36 days, with the majority of cows on day 31) post-AI by transrectal ultrasonography from November 2010 through October 2012. Non-pregnant cows were assigned randomly to either day-31 **PG-3-G** (Pre-PG on day 31, Pre-GnRH on day 34, and RES on day 41; $n = 102$), day-41 **RES-CON** (same as in Exp. 1, but on day 41; $n = 108$) or day-41 **RES-CIDR** (as Exp. 2 but on day 41, $n = 101$, Figure 2). Ovarian structures were recorded and mapped by ultrasonography on days 31, 41, 46, and 48 to determine the incidence of luteolysis, ovulation, and double ovulation in all cows. Cows returning to estrus before TAI were inseminated and designated as early bred (**EB**).

Pregnancy per AI (**P/AI**) was diagnosed at 34 days and confirmed at 62 days post-TAI by uterine palpation per rectum by a single veterinarian (Exp. 1) or at 31 days and confirmed 59 days post-TAI by ultrasonography (Exp. 2).

Blood was sampled from all cows by puncture of the coccygeal vein or artery into evacuated tubes at nonpregnant diagnosis. Blood samples were collected to determine progesterone concentrations at either day 34 (Exp. 1) or at days 31, 34, 46, and 48 (Exp. 2). Samples were immediately cooled and stored at 5°C for 16 hours. Blood tubes were centrifuged at 1,000 x g for 15 minutes in a refrigerated centrifuge at 5°C for serum separation and harvest. Serum samples were frozen and stored at -20°C until assayed for progesterone by radioimmunoassay.

Results and Discussion

Experiment 1

Only 76% of 1,031 cows had high (≥ 1 ng/mL) progesterone concentrations at day-34 non-pregnant diagnosis. Cows with low (< 1 ng/mL) progesterone concentrations at GnRH-1 and assigned to the day-34 RES-CIDR treatment had greater ($P = 0.028$) P/AI than day-34 RES-CON cows (39.1 vs. 31.3%; Figure 3). In contrast, day-34 RES-CIDR-treated cows with high progesterone concentrations had reduced P/AI compared with cows that received the day-34 RES-CON treatment (27.4 vs. 34.3%).

It seems clear from this experiment that no benefit to pregnancy outcome is accrued from using CIDR in a Resynch application for cows with elevated progesterone (functional corpus luteum; 76% of cows treated) at the onset of a 5-day Resynch program for cows that are between days 34 and 40 since last insemination. Increased P/AI was observed in cows treated with the CIDR insert only when they had low progesterone (no corpus luteum; 24% of cows treated) at the onset of RES.

Experiment 2

The proportion of cows with high progesterone on day 31 was 70.6%. More ($P < 0.001$) cows ovulated after Pre-GnRH of day-31 PG-3-G than for day-41 RES-CON or day-41 RES-CIDR (Table 1). More ($P < 0.001$) PG-3-G cows had luteolysis after Pre-PG on day 31 than other treatments (Table 1). The proportion of cows with high progesterone on day 41 at GnRH-1 tended ($P = 0.10$) to be greater for PG-3-G than for other treatments (Table 1). The P/AI was greater in cows starting RES on day 41 when progesterone was low (44%) than when it was high (33%), but no overall treatment differences in P/AI were detected 32 days after TAI (PG-3-G = 33.3%; day-41 RES-CON = 38.9%; day-41 RES-CIDR = 35.6%; Figure 4).

Some have reported improved P/AI in RES cows in which the RES protocol was delayed after open diagnosis and cows were pretreated with PG before initiating the RES protocol. Most of the advantage of using PG is accrued by inseminating those cows that are detected in estrus and enrolling only those not yet inseminated in the follow-up RES protocol. Therefore, although no P/AI advantage occurred after pretreating open cows with PG, the major benefit accrued when detection of estrus was included as part of the program before applying the RES protocol to cows not inseminated.

We concluded that improved P/AI occurred only when applying the CIDR insert to cows with low progesterone when initiating the 5-day RES on d 34 (Exp. 1). Although d 31 PG-3-G increased rates of luteolysis and ovulation before RES, no increase in P/AI was detected compared with the 5-day RES started on d 41 with or without a CIDR insert (Exp. 2).

Table 1. Ovarian characteristics during Experiment 2 treatment protocols

Item	Treatment			<i>P</i> -values
	Day-31 PG-3-G	Day-41 RES-CON	Day-41 RES-CIDR	
Pre-PG/GnRH				
Progesterone ≥ 1 ng/mL, %	74.8	69.3	67.6	0.42
Luteolysis ¹ , %	73.8 ^a	10.4 ^b	8.7 ^b	0.001
Ovulation after Pre-GnRH ² , %	60.4 ^a	12.5 ^b	17.1 ^b	0.001
GnRH-1				
Progesterone ≥ 1 ng/mL, %	76.6 ^a	65.5 ^b	70.2 ^{a,b}	0.05
CL present, %	78.5	73.4	67.0	0.13
Ovulation after GnRH ² , %	42.1 ^a	36.3 ^{a,b}	28.3 ^b	0.05
Double ovulation after GnRH ³ , %	8.4	4.4	5.7	0.46
GnRH-2				
Luteolysis ⁴ , %	96.6	93.5	95.7	0.63
Ovulation after GnRH ² , %	88.6	85.0	84.9	0.67
Double ovulation after GnRH ³ , %	19.1	28.3	24.5	0.18

^{a,b} Proportions within each row with different superscripts differ ($P < 0.05$).

¹ Luteolysis was determined by changes in progesterone concentration between Pre-PG (≥ 1 ng/mL) and Pre-GnRH injections 72 hours later (< 1 ng/mL).

² Ovulation of one follicle after GnRH administration.

³ Ovulation of more than one follicle after GnRH administration.

⁴ Luteolysis was determined by changes in progesterone concentration between PG-2 (≥ 1 ng/mL) and GnRH-2 injections 72 hours later (< 1 ng/mL).

REPRODUCTION

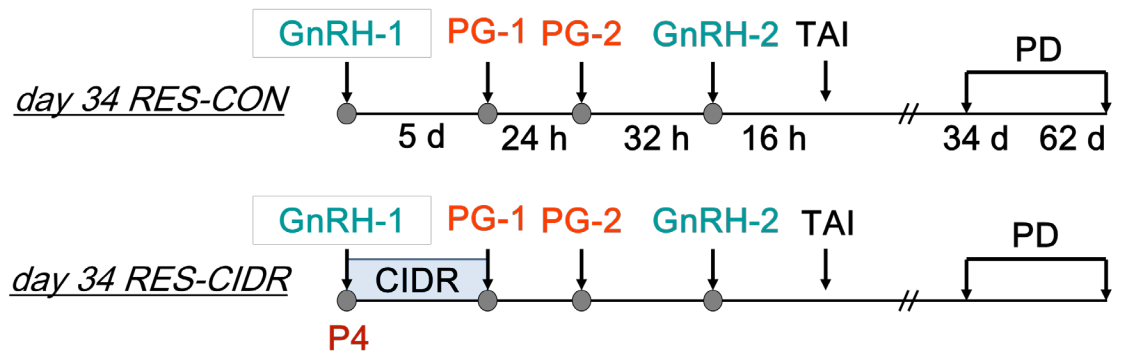


Figure 1. Experimental design for Experiment 1. PG = PGF_{2a}, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination, CIDR = controlled internal drug release insert (progesterone impregnated), P4 = progesterone, PD = pregnancy diagnosis.

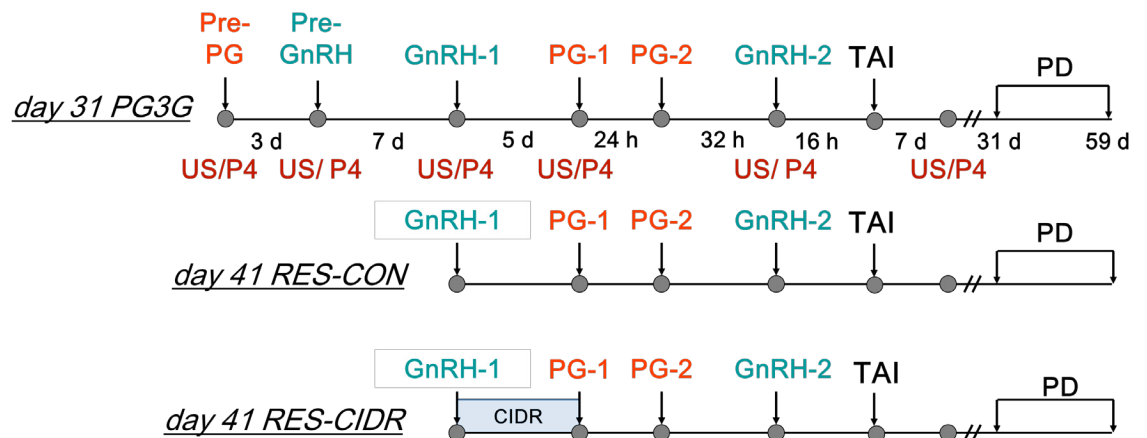


Figure 2. Experimental design for Experiment 2. PG = PGF_{2a}, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination, CIDR = controlled internal drug release insert (progesterone impregnated), P4 = progesterone, PD = pregnancy diagnosis, US = ovarian ultrasound examination.

REPRODUCTION

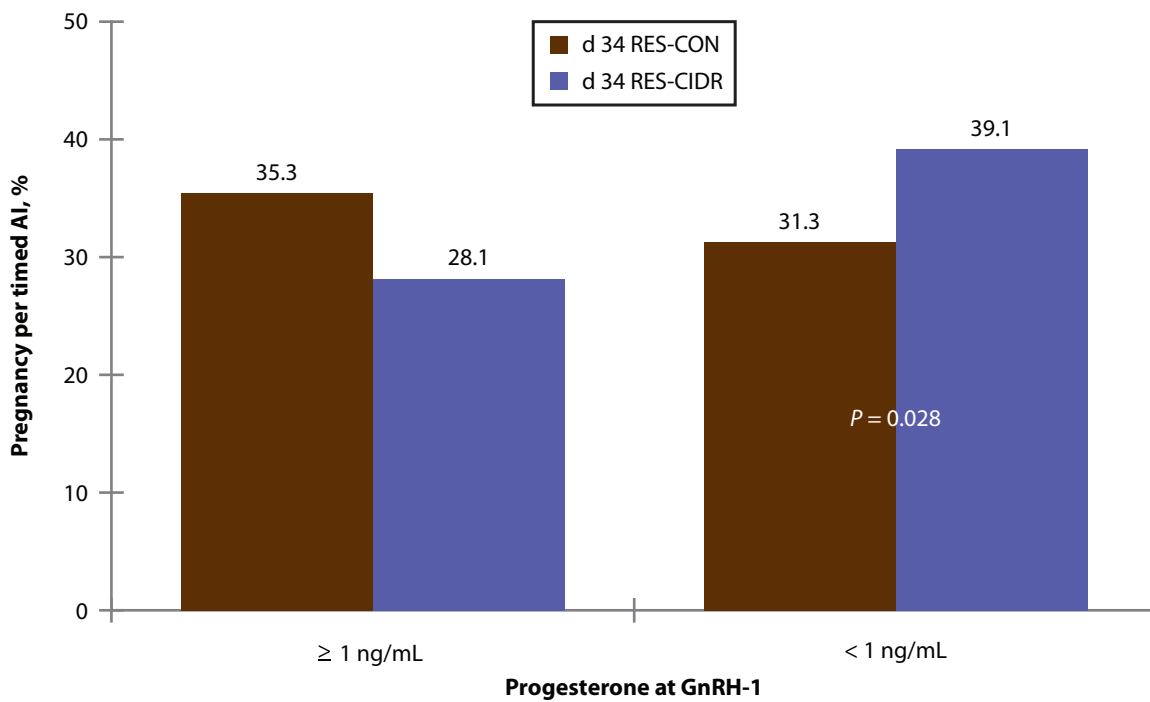


Figure 3. Pregnancy per timed AI determined 34 days post-insemination (Exp. 1) based on treatment and progesterone concentration at the onset of treatment.

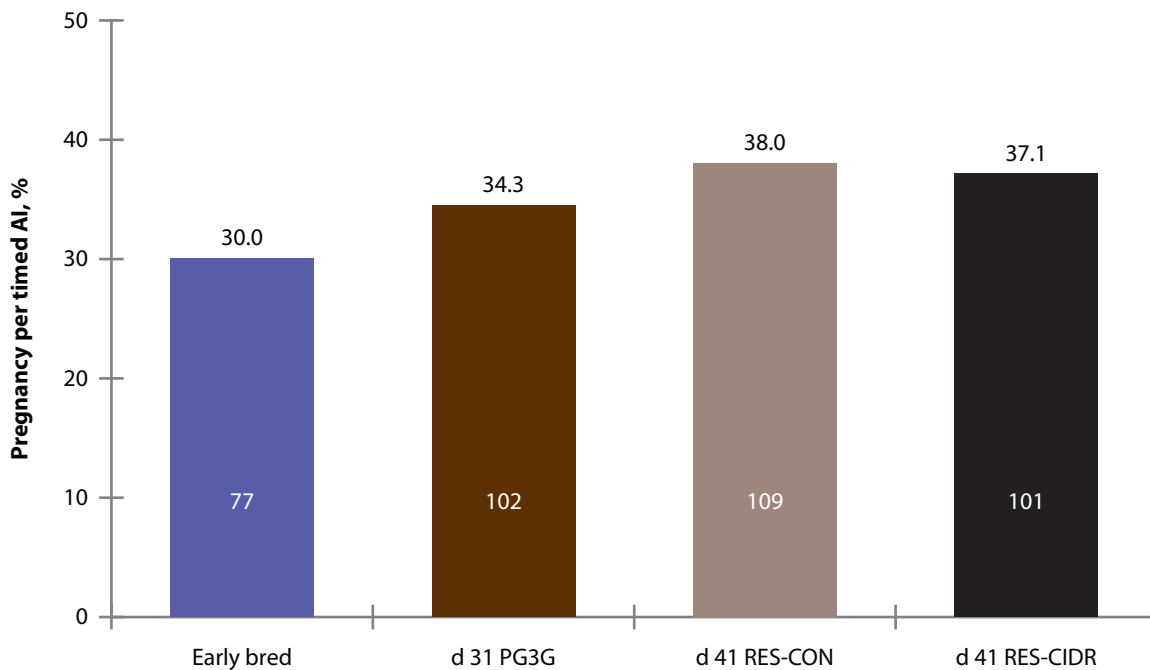


Figure 4. Pregnancy per timed AI at 32 days post-insemination for Experiment 2.