RESYNCHRONIZING ESTRUS AND OVULATION IN OPEN COWS AND HEIFERS

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Summary

We compared outcomes of two protocols used to resynchronize estrus and ovulation in dairy females after found open at pregnancy checks. Replacement heifers and lactating cows in which AI occurred 41 ± 1 day earlier were presented every 2 to 3 weeks for a pregnancy check by ultrasonography. Ovaries were scanned, follicles were mapped and sized, presence of corpus luteum was noted, and GnRH was injected (day 0) . Females received $PGF_{2\alpha}$ 7 days later (day 7) and then were assigned randomly to either receive estradiol cypionate (ECP) 24 hours after $PGF_{2\alpha}$ (day 8; Heatsynch; n = 230) or a second GnRH injection after $PGF_{2\alpha}$ (day 9; Ovsynch; n = 224). Those detected in estrus were inseminated, whereas the rest received a timed AI (TAI) between 65 and 74 hours after $PGF_{2\alpha}$. Few females (5.1%) were inseminated between open diagnosis and day 8. On day 10, more ECP- than GnRH-treated females were inseminated after detected estrus (24 vs. 6%). Overall, more Ovsynch than Heatsynch females received a TAI (82 vs. 62%). Conception rates tended to be greater for females inseminated after estrus (37%) than after TAI (29%), and the tendency was more pronounced for those treated with Heatsynch (41 vs. 27%) than for those treated with Ovsynch (33 vs. 31%). Conception rates for females having elevated progesterone 7 days after the not-pregnant diagnosis were greater than conception rates of those having low progesterone in Heatsynch (42%; n = 133 vs. 25%; n = 55) and Ovsynch protocols (33%; n = 142 vs. 15%; n = 45). Conception rates were greater in heifers than in lactating cows (43 vs. 28%), regardless of protocol employed. Although overall pregnancy outcomes were similar in response to either the Ovsynch or Heatsynch protocol, inseminations performed after detected estrus before the scheduled TAI reduced days to eventual conception and tended to increase conception rates, particularly after Heatsynch.

(Key Words: Synchronized Estrus, Ovulation, Cysts.)

Introduction

Earlier identification of nonpregnant females is one way to reduce prolonged interinsemination intervals that occur because of poor efficiency in detecting post-insemination estrus. Treating nonpregnant cows with $PGF_{2\alpha}$ between 27 and 29 days after a previous AI induced regression of the corpus luteum (CL) before subsequent insemination after detected estrus or timed AI (TAI), and reduced days to re-insemination and to conception. Other options may include applying various TAI protocols to females that are diagnosed open. Applying the Ovsynch protocol or substituting estradiol cypionate (ECP) for GnRH in an Ovsynch-like protocol (known as Heatsynch) are viable options. Administering ECP to females in proestrus has been found to induce estrus, preovulatory LH surge, ovulation, and normal corpus luteum (CL) development in dairy heifers and dairy cows. Two previous studies have found that conception rates of Heatsynch-treated heifers are not different from those in heifers inseminated after detected estrus, and those in lactating cows after Heatsynch are similar to those after Ovsynch.

The objective of the present study was to determine fertility after applying the Ovsynch or Heatsynch protocols to dairy females that were diagnosed open by transrectal ultrasonography. An ancillary objective was to determine whether ovarian status at the initiation of the two protocols influenced subsequent pregnancy outcomes for either protocol.

Experimental Procedures

Lactating Holstein cows (n = 414) were housed in either tie stalls or free stalls at the Kansas State University Dairy Teaching and Research Center. Replacement Holstein heifers (n = 40) were housed in free stalls with an adjacent dirt lot and concrete feed apron behind the feed bunk. Females were presented for a pregnancy check every 2 to 3 weeks between August 2000 and November 2002, consisting of 46 separate groups. Days since AI at pregnancy diagnosis averaged 41 ± 1 day (range: 27 to 200). Slightly more than 91% of females were between days 27 and 53 since last AI. Those few females having prolonged intervals since AI had been pregnant, but aborted and had returned to estrus.

Pregnancy status was determined (presence of uterine fluid plus a CL, detection of embryo, or both) by using transrectal ultrasonography (real time, B-Mode, linear array, diagnostic, ultrasound scanner equipped with a 5-MHz transducer, Aloka 500V, Wallingford, CT). In open females, ovaries were scanned, follicles were mapped and sized by using electronic calipers (average of vertical and horizontal measures), and the presence of CL was noted. Ovarian characteristics quantified were number of CL, number of follicles ≥10 mm on each ovary and their total per female, diameter of the largest and second-largest follicle (excludes the largest cystic structure[s] in those females bearing cysts), and diameter of all cystic structures (defined later).

After the not-pregnant diagnosis and ovarian examination, all females received i.m. 100 μg of GnRH (d 0; Cystorelin, Merial, Iselin, NJ). Females were then blocked by lactation number (1 vs. 2+), and replacement heifers were balanced by body weight and age before random assignment to one of two treatments: 1) 25 mg of $PGF_{2\alpha}$ (day 7; Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) 7 days after not-pregnant diagnosis, plus 1 mg of ECP (day 8; ECP, Pharmacia Animal Health, Kalamazoo, MI) 24 hours after $PGF_{2\alpha}$ (Heatsynch; n = 230); or 2) 25 mg of $PGF_{2\alpha}$ 7 days after not-pregnant diagnosis, plus a second GnRH injection 48 hours after $PGF_{2\alpha}$ (day 9; Ovsynch; n = 224).

To maximize pregnancy outcomes in both treatments, any female detected in estrus after the initial GnRH injection, but at least 24 hours before the scheduled TAI, was inseminated 8 to 16 hours after first detected estrus (a.m. - p.m. rule). Females were observed for estrus at least twice daily (morning and late afternoon), in addition to other casual observations during the work day (7:30 a.m. to 5:00 p.m) and while various groups of cows were moved to the milking parlor (5:30 to 10:00 a.m and 5:30 to10:00 p.m.). In the absence of previous insemination, all remaining females received a TAI at 65 to 74 hours after $PGF_{2\alpha}$ (16 to 20 hours after GnRH or 46 to 50 hours after ECP). After treatment inseminations, pregnancy outcome was determined as described previously by using transrectal ultrasonography between 33 and 40 days after AI.

Blood samples were collected from females in 39 of 46 groups (83.2% of females) before the GnRH injection (day 0), before $PGF_{2\alpha}$ (day 7), and 24 hours after $PGF_{2\alpha}$ (day 8) for later radioimmunoassay analysis of blood concentrations of progesterone (P4) in serum.

Ovarian scans and blood collected before injections for P4 analysis were used to classify females into one of four ovarian status groups:

anestrus, follicular cysts, luteal cysts, and cycling. Females were classified to be anestrus (n = 20) when serum concentration of P4 was < 1 ng/mL on days 0, 7, and 8 and few follicles > 10 mm were detected on day 0. Females having follicular cysts (n = 12) had multiple follicles, including at least one follicle > 20 mm in diameter, and had concentrations of P4 < 1 ng/mL on day 0. Females having luteal cysts (n = 12) had multiple follicles, including at least one follicle > 20 mm in diameter, and had serum concentration of P4 ≥ 1 ng/mL on day 0. Cycling females (n = 344) had normal ovarian structures and various concentrations of P4 in blood serum, but none had serum concentrations of P4 < 1 ng/mL at all three sampling times.

Results and Discussion

Cumulative percentages of all inseminations conducted after detected estrus during 10 days after not-pregnant diagnosis are illustrated in Figure 1. By day 8 (day of ECP injection), 5.2% of females pre-assigned to Heatsynch and 4.9% pre-assigned to Ovsynch had been inseminated. On day 9 (day of GnRH injection), another 9.6% were inseminated in the Heatsynch treatment and another 6.7% were inseminated in the Ovsynch treatment. Of those inseminated on day 10, more ($P < 0.05$) females were inseminated after detected estrus in the Heatsynch (23.5%; n = 54) than in the Ovsynch (6.3%; n = 14) protocol. Not shown in Figure 1 are all remaining females that received a TAI on day 10. More ($P<0.01$) Ovsynch (82.1%; n = 184) than Heatsynch (61.7%; n = 142) females received the TAI.

No differences in conception rates were detected between treatments, when considering all inseminations (Table 1). Replacement heifers had greater ($P<0.05$) conception rates than lactating cows (Table 1). Conception rates also tended ($P<0.10$) to be greater for females inseminated after detected estrus than for those receiving one TAI (37.1%; n = 128 vs. 28.9%; n = 326) in both protocols (Table 1). Conception rates for the 76 females inseminated during 48 hours after ECP were 38.2%, compared with 31% of the 29 Ovsynch females inseminated during the same period, including those that received GnRH midway during the same 48-hour period. For females inseminated after detected estrus, average days from treatment AI until when conception finally occurred were fewer ($P<0.01$) than for those receiving the TAI (45 ± 10 [n = 81] vs. 76 ± 7 [n = 174]).

For females having concentrations of P4 ≥ 1 ng/mL seven days after treatment initiation, regardless of subsequent treatment (Table 1), conception rates were greater ($P<0.001$) than those having < 1 ng/mL P4 (37.5%; n = 275 vs. 20%; n = 100). Concentrations of P4 in females of different ovarian and cycling status are illustrated in Figure 2. Cycling females and those having luteal cysts had greater ($P<0.05$) concentrations of P4 on day 0 (day of GnRH) than did anestrous females and those bearing follicular cysts. These average differences were maintained on day 7, except in those having follicular cysts, in which serum P4 increased to concentrations similar to those having luteal tissue. In all 12 females having follicular cysts on day 0 (serum P4 was < 1 ng/mL), only 2 failed to have an increase in serum P4 ≥ 1 ng/mL by 7 days after GnRH injection. By day 8, 24 hours after $PGF_{2\alpha}$, only anestrous females had less ($P<0.05$) serum P4 than all others.

Conception rates for these females of different ovarian and cycling status are summarized in Table 1. Because of limited numbers of observations in all but the cycling group, potential differences in conception rates were not statistically detectable, but data are presented for informational purposes.

Eight of the 24 cystic females (only 1 heifer, which had a follicular cyst; otherwise, 4 follicular and 4 luteal cysts) expressed estrus after not-pregnant diagnosis on the day of TAI, except for 1 female having a luteal cyst that expressed estrus 1 day before TAI. On the basis of our initial classifications of the cystic structure before serum P4 concentrations were assessed, we identified 3 cows with a luteal cyst and 21 females with follicular cysts. In retrospect, on the basis of serum P4, we had classified incorrectly 9 of the 21 females having follicular cysts, because each of the 9 had elevated serum P4 on day 0 and, therefore, were luteal, rather than follicular, cysts. Concentrations of P4 on the day of not-pregnant diagnosis for females having follicular cysts were less ($P<0.001$) than those in females bearing luteal cysts (0.5 ± 0.5 vs. 3.3 ± 0.5 ng/mL).

We have demonstrated that early-pregnancy diagnosis of dairy females via transrectal ultrasonography is a practical means of identifying nonpregnant females for prompt reinsemination. That process was facilitated by applying either the Ovsynch or Heatsynch protocol after transrectal ultrasonographic diagnosis of no pregnancy. Although overall pregnancy outcomes were similar between protocols, females that expressed estrus and were re-inseminated before the scheduled TAI tended to have greater conception rates and did eventually conceive sooner than those receiving the TAI.

It is unfortunate that the estrogen (ECP) used in the Heatsynch protocol is no longer available in the U.S. market. The concept, however, of applying an early open diagnosis, whether it be by transrectal ultrasonography or palpation, and promptly re-inseminating, is valid by any means, including using the Ovsynch protocol. When early diagnosis of pregnancy by ultrasonography is not possible, more frequent detection by palpation (e.g., weekly vs. biweekly) on a herd basis reduces the inter-insemination interval and increases AI submission rate. When AI submission rate is increased, increases in total pregnancy rate should result because submission rate is one of two factors that determines overall pregnancy rate (i.e., product of AI submission rate × actual conception rate).

Table 1. Conception Rates in Dairy Females after Applying the Ovsynch or Heatsynch Protocol

Item	Protocol[1]	
	Heatsynch	Ovsynch
	--- (% [no.])---	
Overall[2]	34.1 (230)	31.8 (224)
Lactation number[3]		
0	46.2 (20)	40.1 (20)
1	29.5 (108)	22.4 (92)
2+	26.8 (102)	32.8 (112)
AI after detected estrus[4]	41.2 (88)	33.0 (40)
Timed AI	27.1 (142)	30.6 (184)
Serum P4 7 days after not pregnant diagnosis[5]		
High (≥ 1 ng/mL)	42.2 (133)	32.9 (142)
Low (< 1 ng/mL)	25.3 (55)	14.6 (45)
Cycling	34.8 (172)	33.8 (174)
Anestrus	15.6 (13)	16.2 (7)
Follicular cyst	25.8 (6)	38.4 (6)
Luteal cyst	53.1 (6)	28.2 (6)
Unknown	37.4 (33)	24.7 (31)

[1]Heatsynch = injection of GnRH 7 days before an injection of $PGF_{2\alpha}$, followed in 24 hour by 1 mg of ECP and one fixed-time AI (TAI) 42 to 50 hours after ECP. Ovsynch = injection of GnRH 7 days before and 48 hours after an injection of $PGF_{2\alpha}$, with one fixed TAI at 16 to 22 hours after the second GnRH injection. Adjusted least-squares percentages are illustrated.
[2]Includes all inseminations since treatment initiation on day 0.
[3]Replacement heifers differed ($P<0.05$) from lactating cows.
[4]Pregnancy rate for AI after detected estrus (37.1%; n = 128) tended ($P<0.10$) to be greater than that after TAI (28.9%; n = 326).
[5]Females with concentration of P4 ≥ 1 ng/mL 7 days after treatment initiation had greater ($P<0.001$) pregnancy rates than those with P4 < 1 ng/mL(37.5%; n = 275 vs. 20%; n = 100).

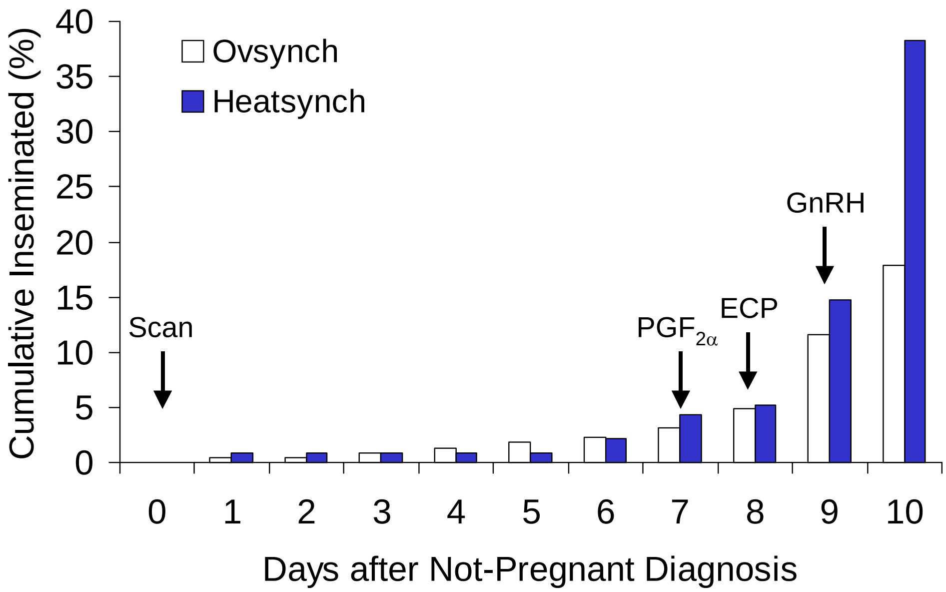

Figure 1. Cumulative Percentages of Females Inseminated after Detected Estrus During 10 Days after the Not-pregnant Diagnosis and Initiation of Treatments, as a Proportion of All Females Inseminated. More (P<0.05) females were inseminated after detected estrus on day 10 in the Heatsynch (23.5%; n = 54) than Ovsynch (6.3%; n = 14) protocols. Not shown on day 10 are all remaining females that received a TAI in the Ovsynch (82.1%; n = 184) and Heatsynch (61.7%; n =142) protocols.

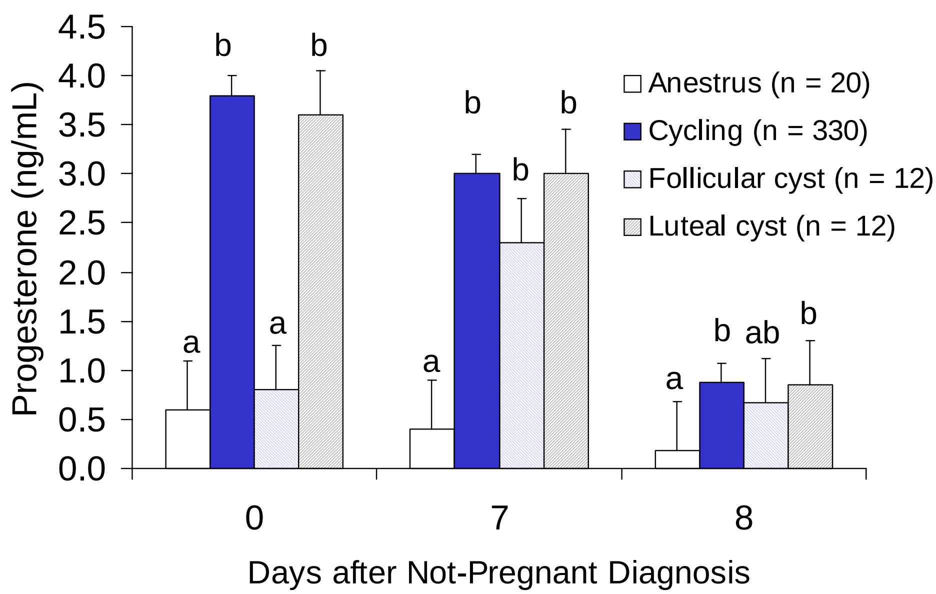

Figure 2. Serum Concentrations of Progesterone in Dairy Females Classified According to Ovarian Structures and Progesterone Status After the Not-pregnant Diagnosis and Initiation of Treatments. Injections of GnRH were administered on day 0, $PGF_{2\alpha}$ on day 7, and ECP on day 8 (those in Heatsynch protocol) or GnRH on day 9 (those in the Ovsynch protocol). Bars within day having different superscript letters differ ($P<0.05$).