

Fate of cystic ovarian follicles, clinical responses, and pregnancy in dairy cows subjected to Ovsynch and timed artificial insemination, with or without an intravaginal progesterone device

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Abstract

Our objectives were to determine the fate of cystic ovarian follicles (COF), clinical responses, and pregnancy to timed artificial insemination (TAI) in lactating Holstein cows subjected to an Ovsynch protocol, with or without insertion of a progesterone releasing intravaginal device (PRID). Cows diagnosed with at least 1 COF (≥ 25 mm) were given 100 μ g of gonadorelin acetate (GnRH; day -10 and day -1), 500 μ g cloprostenol (prostaglandin $F_{2\alpha}$; day -3), and TAI (day 0). Cows randomly received PRID[®] (n = 49) for 7 days or NO-PRID (n = 62). Transrectal ultrasonography was conducted on days -10, -3, 0, 1, and 11 to monitor ovarian responses and on day 32 for pregnancy diagnosis. Cows were also categorized as low progesterone (LP; n = 50) or high progesterone (HP; n = 61) based on plasma progesterone concentrations ($<$ or ≥ 1.0 ng/ml) or luteal status (presence or absence of luteal tissue) on day -10. Mean ($\pm$ SEM) diameter of COF was 33 ± 0.6 mm. Although ovarian responses did not differ by PRID treatment, a higher ($p = 0.05$) proportion of COF in HP compared to LP cows underwent complete luteinization (21 versus 8%) after 1st GnRH treatment. In contrast, the proportion of cows ovulating an existing follicle after 1st GnRH treatment was higher ($p = 0.03$) in LP than in HP cows (72 versus 54%). No interactions existed between treatment (PRID or NO-PRID) and progesterone categories (LP, HP) in any of the ovarian or endocrine responses. Furthermore, 10% of COF ovulated and 62% of other preexisting follicles ovulated after 1st GnRH treatment. A new follicle was detected (day -3) in 95% of cases and 84% ovulated after 2nd GnRH. Pregnancy per AI did not differ ($p = 0.46$) between PRID and NO-PRID cows (37 versus 45%). Plasma progesterone at TAI was elevated ($p = 0.02$) in LP than HP cows (0.7 versus 0.2 ng/ml) and pregnancy tended to be lower ($p = 0.13$) in LP than in HP cows (34 versus 48%). In summary, cows with COF responded normally to Ovsynch protocol; although incorporating PRID into the protocol was not beneficial, cows with HP status at the start of the protocol tended to have higher pregnancy per AI.

Keywords: Cattle, ovary, cyst, luteinization, ovulation, treatment

Introduction

Cystic ovarian follicles (COF) are widely prevalent in lactating dairy cows, occurring usually during the early postpartum period, with incidence from $< 20\%$ ¹ up to 50% in individual herd situations.² Because of COF, dairy industry had substantial economic losses,³ mainly by increased calving interval^{3,4} and the likelihood of cows being culled.⁴ Although estimated losses of up to \$160 per cow per lactation was reported in the 1980's,⁵ a recent economic simulation study estimated the annual loss to the global dairy industry due to COF at \$4 billion.⁶

The conventional definition of COF is a large (≥ 25 mm diameter) anechoic follicle-like structure persisting for 10 days or longer in the absence of a corpus luteum (CL), although later definitions have included smaller follicular structures of 17-20 mm in diameter.⁷ The coexistence of CL with COF has been reported^{8,9} and it has been opined that COF coexisting with CL are benign persistent structures that exert no negative influence on ovarian follicular growth or pregnancy establishment.¹⁰ Although managing COF in cattle has been an active topic of research for nearly a century,^{11,12} treating cows diagnosed with COF continues to be a vexing problem for practitioners and dairy producers.

Exogenous gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), progesterone, and corticosteroids have been used with varying degrees of success to treat follicular cysts, whereas prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the preferred treatment for luteinized follicular cysts^{3,4,13,14} that often results in induced estrus within a few days after treatment.

For over 2 decades, the protocol used for synchronization of ovulation in cattle, widely known as Ovsynch,¹⁵ with timed artificial insemination (TAI) has been used in cows with COF, achieving reasonable pregnancy per artificial insemination (P/AI).^{10,16–20} Treatment with exogenous progesterone (progesterone-releasing intravaginal device; PRID) decreased mean LH and LH pulse frequency, regressed COF, and initiated new follicular growth.²¹ Moreover, PRID as a standalone treatment^{22,23} or progesterone given as controlled internal drug release (CIDR) device, with $PGF_{2\alpha}$ and GnRH,¹⁷ or incorporating CIDR into an Ovsynch protocol,¹⁹ have been effective in treating bovine COF. It has been emphasized that strategies for therapeutic management of COF should include induction of follicular turnover and an increase in progesterone concentrations that can be achieved by the Ovsynch protocol, when used in combination with an exogenous progesterone insert such as CIDR.¹⁷ Although the efficacy of Ovsynch/TAI treatment and that of CIDR, PRID, and other forms of progesterone treatments on cows diagnosed with COF have been tested adequately, only a few studies have concurrently compared the efficacy of Ovsynch/TAI and Ovsynch/TAI including a progesterone device in treating cows with COF. Furthermore, the fate of COF, including that of persistent cysts that linger in coexistence with a CL and their response to treatment, have not been adequately documented.

Our objectives, therefore, were to determine the fate of COF, clinical responses to treatments, and P/AI in dairy cows subjected to TAI following Ovsynch, with or without exogenous progesterone treatment in the form of an intravaginal progesterone device. Exogenous progesterone treatment can reduce LH pulse frequency resulting in regression of COF and initiation of new follicular growth.^{4,21} Moreover, an elevated progesterone environment at the initiation of Ovsynch improved P/AI in cyclic and acyclic dairy cows,^{24,25} and in cows with COF.¹⁹ Therefore, we hypothesized that incorporating a PRID during the initial 7 days of Ovsynch/TAI protocol improves P/AI in cows diagnosed with COF, particularly in cows with low progesterone status at diagnosis compared to those assigned to Ovsynch/TAI without PRID.

Materials and methods

Animals and housing

This randomized clinical trial conducted over 4 years included 111 lactating Holstein cows diagnosed with COF. Cows were of mixed parities (mean $\pm$ SD, 2.9 ± 1.5 ; range 1–7) from 9 dairy farms in Alberta, including 1 university farm, with a majority (75%) from 2 farms and the remainder from the other 7 farms (Figure 1). Mean ($\pm$ SD) days in milk at COF diagnosis was 103 ± 54 (range 54–265) and the mean 305 days milk yield was $10,144 \pm 1657$ kg (range 7,077–12,921 kg). Cows had a mean ($\pm$ SD) body condition score of 2.90 ± 0.30 using the common 1 (emaciated) to 5 (obese) scale, with scores assigned in 0.25 increments.²⁶ Animal use and treatment protocols were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP no. 052/10/09) and cows

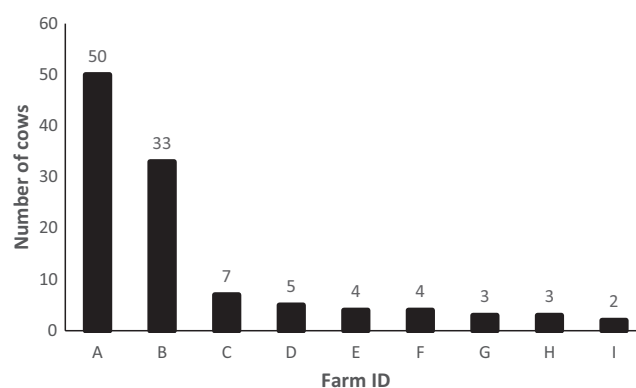


Figure 1. Distribution of cows with cystic ovarian follicles

were cared for in accordance with the requirements of Canadian Council on Animal Care. Housing types included both tie-stall and free-stall barns and herd sizes ranged 50–450 cows. Cows were fed total mixed rations formulated according to National Research Council guidelines²⁷ with primary ingredients of barley or alfalfa silage, grass or alfalfa hay, and concentrates. Cows had unrestricted access to potable water and were milked either twice (8 herds) or thrice daily (1 herd).

Of the 111 cases, 83 were from 2 farms (1 university, 1 commercial) often used in research and the other 28 cases were directed to the researchers by collaborating dairy practitioners. Ovaries were examined via transrectal ultrasonography on day –10, and cows that had at least 1 large (≥ 25 mm in diameter) anechoic structure, regardless of the presence or absence of other ovarian structures,²⁸ were considered to have a COF and randomly assigned to either NO-PRID or PRID treatment group. Although persistent cysts existing alongside a CL may not fit the conventional definition of COF, such cases were also included to fulfil our objective of determining their fate and response to treatment.

Treatments and transrectal ultrasonography

Cows received 2 treatments of intramuscular gonadorelin acetate (100 μ g; Fertiline® [GnRH]; Vetoquinol Inc. Lavaltrie, QC, Canada) 9 days apart (on day –10 and day –1), 1 treatment of intramuscular cloprostenol (500 μ g [$PGF_{2\alpha}$]; Estrumate®; Merck Animal Health, Kirkland, QC, Canada) on day –3, and were subjected to TAI on day 0, ~16–20 hours after 2nd GnRH treatment (Figure 2). Cows ($n = 49$) received PRID® (1.55 gram of progesterone; Vetoquinol Inc.) during the initial 7 days of Ovsynch and 62 cows did not receive PRID (NO-PRID).

Transrectal ultrasonography was performed on day –10 (at 1st GnRH), day –3 (at $PGF_{2\alpha}$), and day 0 (at TAI) of the Ovsynch protocol and on days 1, 11, and 32 after TAI. Experimental events (Figure 1) and the purpose of each event (Table 1) are provided. Pregnancy was diagnosed (visualization of a viable embryo) via transrectal ultrasonography on day 32 after TAI. Observations made at ultrasonography including size and location of major ovarian structures and ovarian responses to treatments were manually recorded on paper as ovarian maps and data were later entered into an Excel spreadsheet for analyses. If the thickness of the COF wall exceeded 3 mm, it was considered a luteinized follicular cyst. If the antrum of the COF filled completely with presumed luteal tissue of uneven echogenicity on day 7 after 1st GnRH treatment, and this CL-like

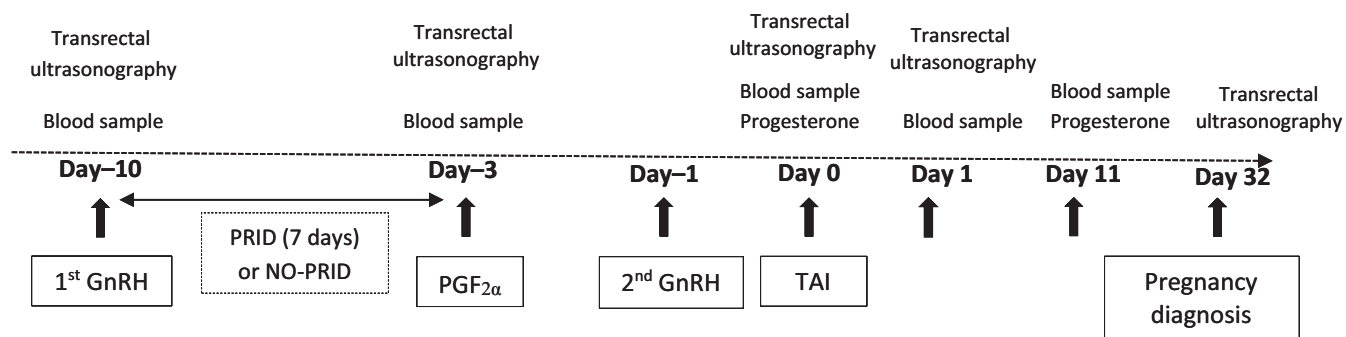


Figure 2. Timeline for treatments and procedures

Table 1. Schedule and description of experimental events

Day	Activity	Description or purpose
-10	Transrectal ultrasonography, blood sample, GnRH + PRID	Transrectal ultrasonography to document characteristics of COF and the presence of other major ovarian structures > 9 mm; blood sample for progesterone and estradiol; 1 st GnRH; PRID inserted or NO-PRID inserted.
-3	Transrectal ultrasonography, blood, PGF _{2α} -PRID	Transrectal ultrasonography to document ovarian response (luteinization of cyst or ovulation of COF or an existing follicle; presence of a new dominant follicle); document major ovarian structures; blood sample drawn to quantify progesterone and estradiol; PGF _{2α} to induce luteal regression; PRID removed.
-1	GnRH	2 nd GnRH to synchronize ovulation; no transrectal ultrasonography and no blood sample.
0	Transrectal ultrasonography, blood sample, TAI	Transrectal ultrasonography to measure preovulatory follicle diameter; document ovarian structures including COF; blood sample to quantify progesterone; TAI 16-20 hours after 2 nd GnRH.
1	Transrectal ultrasonography	Transrectal ultrasonography to confirm ovulation of new preovulatory follicle
11	Transrectal ultrasonography, blood sample	Transrectal ultrasonography to confirm presence of new CL; blood sample to quantify progesterone and determine luteal status.
32	Transrectal ultrasonography	Transrectal ultrasonography primarily to determine insemination outcome (pregnant or not pregnant) and secondarily to determine persistence of COF until pregnancy diagnosis.

structure lacked the characteristic homogenous appearance of a CL, the COF was considered to have undergone complete luteinization. Disappearance of a COF or another follicle that existed on day -10 in response to 1st GnRH treatment, and appearance of a distinct CL at the same location on day -3, confirmed 1st ovulation. Likewise, disappearance, on day 1, of a new follicle detected on days -3 and 0, and the presence of a new CL on day 11 at the same location, confirmed 2nd ovulation in response to 2nd GnRH treatment.

Blood sampling and quantification of plasma progesterone and estradiol concentrations

Blood samples were collected by coccygeal venipuncture into evacuated tubes containing sodium heparin (Vacutainer, Beckton Dickinson and Co. Franklin Lakes, NJ) in a subset of 77 cows on days -10, -3, 0, and 11 to determine plasma progesterone concentrations, and on days -10 and -3 for plasma estradiol concentrations. Samples were placed on ice after collection and centrifuged at 1,500 g for 20 minutes at 4°C, plasma was harvested and frozen at -20°C until assayed.

Plasma progesterone concentrations were quantified using a solid-phase radioimmunoassay (Coat-a-Count; Diagnostic Products Corp. Los Angeles, CA) with a minimum detection limit of 0.1 ng/ml, in 2 separate assays. Mean intra- and inter-assay coefficients of variations for low (1.9 ng/ml), medium (3.1 ng/ml) and high (15.0 ng/ml) progesterone reference samples were 14.8, 3.9, and 5.9% and 13.8, 6.5, and 6.2%, respectively. Estradiol was quantified using a commercial assay (ImmuChem Double Antibody 17β-estradiol ¹²⁵I RIA kit, MP Biomedicals, Santa Ana, CA) with 0.55 pg/ml lowest detection limit. Mean intra- and interassay coefficients of variations for low (3.6 pg/ml) and high (41.9 pg/ml) estradiol reference samples were 16.9 and 13.1%, and 24.8 and 14.0%, respectively.

Categorization of cows by progesterone status on day -10 and by type of cystic ovarian follicle

Cows were retrospectively categorized as having had COF under a low progesterone (LP; n = 50) or high progesterone (HP; n = 61) environment, based on either plasma progesterone concentrations (< 1.0 or ≥ 1.0 ng/ml)²⁹ or absence

(LP) or presence (HP) of a CL on day -10. Presence of a luteinized follicular cyst was evident by ultrasonography (follicular walls > 3 mm thickness)^{23,29} but if plasma progesterone data were unavailable, the cow was assigned to HP category. If luteinization was not evident (absence of CL) and progesterone concentration at day -10 was not available, the cow was assigned to LP category.

Additionally, cows with at least 1 large (≥ 25 mm) follicular structure and no detectable signs of luteinization or CL, and low progesterone concentrations (< 1 ng/ml) at enrolment (day -10) were categorized, retrospectively, as having had 'true follicular cyst'. Cows with at least 1 large follicular structure (≥ 25 mm) and visible signs of luteinization but no detectable CL or those with progesterone concentrations ≥ 1 ng/ml in the absence of a detectable CL were categorized as having had 'luteinized follicular cyst'. Cows with at least 1 large follicular structure (≥ 25 mm) and a distinct CL on either ovary, with progesterone ≥ 1 ng/ml or without progesterone data were categorized as having had 'persistent follicular cyst'. We chose the above categorization merely as an alternate approach to understand the clinical responses to Ovsynch/TAI treatment, based on structurally distinct types of cysts. Although the aforesaid categories were used retrospectively to describe the types of cysts and determine insemination outcomes (pregnant versus open) by cyst category, because the effect of PRID treatment was not significant, all 3 types of cysts (true follicular, luteinized follicular, and persistent follicular) were considered under the common category of COF during analyses to determine main effects of treatment (NO-PRID versus PRID) and progesterone category (LP versus HP). Terms clinical responses and ovarian responses are used synonymously to indicate ovarian changes in response to GnRH, PGF_{2a} or PRID treatment and endocrine responses indicate changes in hormone (progesterone and estradiol) concentrations.

Data analyses

Data were analysed using SAS version 9.4 (SAS Institute Inc., Cary, NC) or IBM SPSS Statistics (Version 29.0). UNIVARIATE procedure of SAS was used to determine the descriptive statistics such as mean ($\pm$ standard error of mean; SEM), minimum and maximum diameters of COF. FREQ procedure of SAS was used to determine the following outcomes: incidence of COF on right ovary, left ovary, and both ovaries, proportion of cows with > 1 COF, proportion of cows in which the COF grew (that is, became larger in diameter) after starting Ovsynch, proportion of cows that ovulated or highly luteinized the COF in response to 1st GnRH, and proportion of cows that developed a new COF during the Ovsynch-TAI protocol.

Differences in continuous dependent outcomes such as plasma progesterone and estradiol concentrations by PRID treatment (NO-PRID versus PRID), progesterone status at enrolment (LP versus HP) and their interactions were evaluated by ANOVA using MIXED procedure of SAS, with the effect of farm considered as random.

Differences in continuous dependent outcomes such as mean diameter of COF at 1st GnRH and preovulatory follicle at 2nd GnRH and binomial dependent outcomes such as the proportion of cows that ovulated or luteinized a COF after 1st GnRH; in which the COF grew in size between 1st GnRH and PGF_{2a}; ovulated an existing follicle after 1st GnRH; developed a

new dominant follicle at PGF_{2a}, ovulated a new follicle after 2nd GnRH, and ovulated > 1 follicle in response to 2nd GnRH of Ovsynch/TAI; were pregnant on day 32 after AI were determined by 2 x 2 factorial analyses between PRID treatment (NO-PRID versus PRID) and progesterone status at enrolment (LP versus HP) and their interactions using Generalized Linear Mixed Model in SPSS. In addition, differences in mean BCS, DIM, and 305 days MY were determined by 2 x 2 factorial analyses between PRID treatment (NO-PRID versus PRID) and progesterone status at enrolment (LP versus HP) and their interactions using Generalized Linear Mixed Model in SPSS. Differences were declared significant if $p \leq 0.05$ and considered trends if $p > 0.05$ and ≤ 0.15 .

Results

Mean ($\pm$ SEM), minimum, and maximum diameter of the COF were 33 (± 0.6), 25, and 49 mm; in 72% of the cows COF diameter ranged 25-36 mm, 19% ranged 37-42 mm, and 9% of the COF were > 43 mm diameter (Figure 3). Prevalence of COF was greatest on the right ovary (58%), followed by the left ovary (26%), and in 16% of the cases COF were present on both ovaries. More than 1 COF was detected in 25% of cases, present either on the same ovary or both ovaries.

None of the clinical and endocrine responses or P/AI differed between cows subjected to Ovsynch without (NO-PRID) or with PRID insertion during the 1st 7 days of the protocol (Table 2). Thus, when all cows were considered together, ignoring the PRID treatment, the COF either ovulated (11/111) or completely luteinized (17/111) in response to 1st GnRH in 25.2% of cases. Cystic ovarian follicles that completely luteinized presented a CL-like structure with nonhomogenous echogenicity at ultrasonography on day -3 at PGF_{2a} treatment. Ovulation of an existing follicle other than the COF occurred in 69 instances (62.2% of cases). In response to PGF_{2a} given on day -3, luteal regression occurred in 94.7% of cows.

Cystic ovarian follicle grew in 28 (25.2%) cases, determined by ultrasonography as an increase in its diameter between 1st GnRH (day -10) and PGF_{2a} (day -3) treatments. Development of a new COF (≥ 25 mm) was recorded in 9 (8.1%) cows of LP category; none of the HP category cows developed a new cyst during treatment period of 10 days.

In response to 1st GnRH treatment of the Ovsynch protocol, a new dominant follicle (mean diameter 17.4 mm) developed in

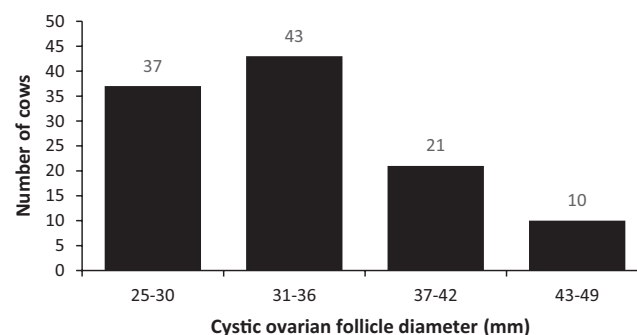


Figure 3. Distribution of cows ($n = 111$) based on the diameter (mm) of COF; mean $\pm$ SEM (33 ± 0.6), minimum (25) and maximum (49)

Table 2. Ovarian responses and pregnancy per AI in cows diagnosed with COF subjected to the Ovsynch/TAI protocol without (NO-PRID) or with (PRID) an intravaginal progesterone releasing device treatment (TRT) for 7 days and categorized as having either low (LP) or high progesterone (HP) at the initiation of the Ovsynch/TAI protocol

Observations, ovarian responses and outcomes	NO-PRID		PRID		TRT	Progesterone category	TRT x progesterone category interaction
	LP	HP	LP	HP	p	p	p
Number of cows	26	36	24	25			
Mean ($\pm$ SEM) diameter of cyst in mm	32.6 $\pm$ 1.1	33.9 $\pm$ 1.1	31.2 $\pm$ 1.1	35.2 $\pm$ 1.1	0.95	0.22	0.24
Ovulation or luteinization ¹ of cyst after 1 st GnRH, n (%)	8 (30.8)	9 (25.0)	5 (20.8)	6 (24.0)	0.53	0.92	0.32
Ovulation of cyst after 1st GnRH, n (%)	5 (19.2)	2 (5.5)	4 (16.7)	0 (0.0)	0.27	0.11	0.11
Luteinization ¹ of cyst after 1 st GnRH, n (%)	3 (11.5)	7 (19.5)	1 (4.2)	6 (24.0)	0.88	0.05	0.76
Ovulation of existing follicle after 1 st GnRH, n (%)	18 (69.2)	23 (63.9)	18 (75.0)	10 (40.0)	0.38	0.03	0.12
Cyst grew between 1st GnRH and PGF _{2α} , n (%)	10 (38.4)	6 (16.7)	6 (25.0)	6 (24.0)	0.82	0.18	0.22
New cyst developed during treatment, n (%)	5 (19.2)	0 (0.0)	4 (16.7)	0 (0.0)	1.00	ND	ND
Cows with new dominant follicle at PGF _{2α} , n (%)	24 (92.3)	34 (94.4)	22 (91.7)	25 (100)	0.99	0.99	0.99
Mean ($\pm$ SEM) diameter of new dominant follicle in mm	17.4 $\pm$ 0.6	16.9 $\pm$ 0.5	18.4 $\pm$ 0.6	16.9 $\pm$ 0.6	0.41	0.11	0.50
Ovulation of new follicle after 2 nd GnRH, n (%)	22 (84.6)	31 (86.1)	19 (79.1)	21 (84.0)	0.60	0.66	0.83
Double ovulation after 2 nd GnRH, n (%)	8 (36.6)	4 (12.9)	6 (31.5)	0 (0.0)	0.99	0.99	0.99
Cows pregnant on day 32 after AI (%)	10 (38.4)	18 (50.0)	7 (29.1)	11 (45.8)	0.46	0.13	0.76

ND: not determinable

¹cyst completely luteinized presenting a CL-like appearance

105 (94.6%; Table 2) cases and 93 (88.6%) of those follicles or 83.8% of all cases ovulated in response to 2nd GnRH treatment given on day -1. Incidence of ≥ 2 ovulations (defined as 'double ovulation') was recorded in 18 cases (16.2%; Table 2).

Mean P/AI determined by transrectal ultrasonography on day 32 after TAI was 41.4% (46/111 cows pregnant) when NO-PRID and PRID groups were considered together. Information on persistence of COF until pregnancy determination was available in 37 cows. Among those, the COF persisted for the entire 42 days (day 10 treatment + day 32 until pregnancy determination) in 14 cows (37.8%) of which 4 were pregnant. Thus, based on the subset of cows from whom data were available on persistence (or not) of COF until pregnancy diagnosis, 28.5% of the pregnant cows had a persistent follicular cyst on day 32 after TAI when pregnancy was diagnosed.

Proportions of cows that were categorized as having true follicular cyst, luteinized follicular cyst, and persistent follicular cyst

(either ipsilateral or contralateral) were 45 (50/111), 20 (22/111), and 35% (39/111), respectively, and percentages of cows determined pregnant from the above categories were 33.8, 58.5, and 41.3, respectively ($p = 0.16$). Ovulation of a cyst in response to 1st GnRH treatment occurred in 18 (9/50), 0 (0/22), and 5% (2/39); a new cyst developed during the treatment period in 18 (9/50), 0 (0/22) and 0% (0/39); and double ovulation after 2nd GnRH occurred in 28 (14/50), 14 (3/22) and 3% (1/39) of cows categorized as true follicular cyst, luteinized follicular cyst, and persistent follicular cyst, respectively.

Ovarian and endocrine responses, and P/AI of cows with COF categorized by treatment (NO-PRID or PRID), progesterone category (LP or HP, based on progesterone concentrations or presence of a CL on day -10) and their interactions are presented (Table 2). Treatment did not affect ovarian (clinical) responses and pregnancy per AI, whereas some ovarian responses differed or tended to differ by progesterone category. Mean diameter of COF on day -10 in LP

versus HP cows was 31.9 versus 34.6 mm ($p = 0.22$). Proportion of COF that ovulated from LP (18.0%) versus HP cows (3.3%) tended to be higher ($p = 0.11$), with a mean diameter of 28.7 ± 1.1 (min, max; 25, 34) mm. On the contrary, the proportion of COF that underwent complete luteinization was higher ($p = 0.05$) in HP than in LP category cows (21.3 versus 8.0%). The mean diameter of COF that completely luteinized was 34.0 ± 1.3 (minimum, maximum; 25, 42) mm. Moreover, ovulation of an existing follicle after 1st GnRH was higher (72 versus 54%; $p = 0.03$) in LP than in HP cows (Table 2). Although neither the proportions of cows that developed a new dominant follicle in response to 1st GnRH treatment nor their ovulation responses to 2nd GnRH treatment differed between LP and HP cows (Table 2), the putative preovulatory follicle tended ($p = 0.11$) to be larger by 1 mm in LP cows.

Despite the lack of differences ($p = 0.66$) in the proportions of LP and HP cows that ovulated a new follicle after 2nd GnRH of Ovsynch, cows that started the protocol in a LP environment tended ($p = 0.13$) to have lower P/AI on day 32 after AI than cows that started the protocol in a HP environment (34.0 versus 47.5%; Table 2).

Mean body condition score, days in milk, and 305 days milk yield did not differ by treatment, by progesterone category, or their interactions (Table 3).

Progesterone profiles (subset of 77 cows) of days -10, -3, 0, and 11, corresponding to 1st GnRH, PGF_{2α}, TAI, and 11 days after TAI, assigned to NO-PRID versus PRID as well as from cows classified as LP versus HP are presented (Figure 4). Progesterone concentrations did not differ ($p > 0.30$) at any of the days mentioned above between NO-PRID and PRID cows. However, mean progesterone concentrations (ng/ml) at 1st GnRH (0.4 versus 4.2) and at PGF_{2α} (3.4 versus 5.1) were lower ($p \leq 0.02$) in LP than in HP cows. On the contrary, plasma progesterone on day 0 (TAI) was higher ($p = 0.02$) in LP cows than in HP (0.7 versus 0.2 ng/ml).

Plasma estradiol concentrations (pg/ml) of day -10 (1st GnRH) and day -3 (PGF_{2α}) did not differ between NO-PRID and PRID cows. Although mean estradiol concentrations were higher in LP than in HP cows on day -10 (2.6 ± 0.3 versus 1.3 ± 0.3 ; $p = 0.01$) they did not differ ($p = 0.66$) on day -3 (1.2 ± 0.2 versus 1.3 ± 0.2).

Discussion

Cows diagnosed with COF responded adequately to Ovsynch treatment and irrespective of the type of COF (follicular, luteinized follicular or persistent follicular cyst) present at diagnosis, acceptable P/AI were obtained after TAI. Nevertheless, our hypothesis that incorporating PRID during the initial 7 days of Ovsynch/TAI improves P/AI in cows diagnosed with COF was not supported. In fact, the PRID treatment may have worsened P/AI, as it was numerically lower by 8.5-percentage points than NO-PRID treatment. Besides, none of the ovarian and endocrine responses measured was changed by including a PRID into the Ovsynch protocol.

Treating cows diagnosed with COF with GnRH triggered an LH release within minutes,³⁰ peaking at 2 hours, and led to a measurable increase in serum progesterone concentrations in < 20 minutes after GnRH treatment in some cows. Moreover, a sustained increase in serum progesterone concentrations occurred up to 14 days, presenting a progesterone profile akin to that of a normal estrous cycle.³⁰ Therefore, 1st GnRH treatment of the Ovsynch protocol in the present study would have induced an acute LH release that either induced ovulation of LH-receptive COF and dominant follicle(s) or caused luteinization of the COF. Although it is impossible to rule out that an endogenous LH surge may have occurred prior to 2nd GnRH treatment in cows that received no PRID, leading to the development of a spontaneous CL, it is unlikely. We anticipated COF to ovulate in response to 1st GnRH treatment only in rare instances; however, ovulation of the COF occurred in ~ 10% of the cases, and complete luteinization resulting in the development of a CL-like structure occurred in ~ 15% of cases, indicating the luteotropic effects of LH released in response to 1st GnRH treatment. Among cows that underwent complete luteinization of COF, 76% were categorized as HP and 24% as LP.

Differences in the quantity of luteal tissue present in COF and their LH receptivity may determine how they respond to GnRH treatment³⁰; therefore, variations in progesterone concentrations on day -3 (at PGF_{2α}) and differences in progesterone concentrations between LP and HP cows may be attributed to the above notion. Although reports of either complete or partial luteinization of the COF after GnRH treatment are not uncommon, ovulation of COF, either spontaneously or in response to GnRH treatment, is less commonly reported. In a previous study¹⁰ with only 18 cows, none of the COF ovulated in response to 1st GnRH although in 2 (~ 11%) cases the COF

Table 3. Differences in body condition score (BCS), days in milk (DIM), and 305 days milk yield (MY) in kg in a subset of 71 cows diagnosed with COF subjected to Ovsynch/TAI protocol without (NO-PRID) or with (PRID) an intravaginal progesterone releasing device treatment (TRT) for 7 days and categorized as having either LP or HP at Ovsynch/TAI protocol initiation

	NO-PRID		PRID		TRT	Progesterone category	TRT x progesterone category interaction
	LP	HP	LP	HP	p	p	p
Number of cows	17	27	14	13			
BCS (mean ± SEM)	3.0 ± 0.05	3.0 ± 0.05	3.0 ± 0.05	3.0 ± 0.05	0.84	0.41	0.84
DIM (mean ± SEM)	104 ± 14	109 ± 11	139 ± 15	102 ± 16	0.32	0.25	0.14
MY (mean ± SEM)	10,283 ± 485	10,579 ± 396	9,911 ± 551	11,136 ± 536	0.85	0.13	0.35

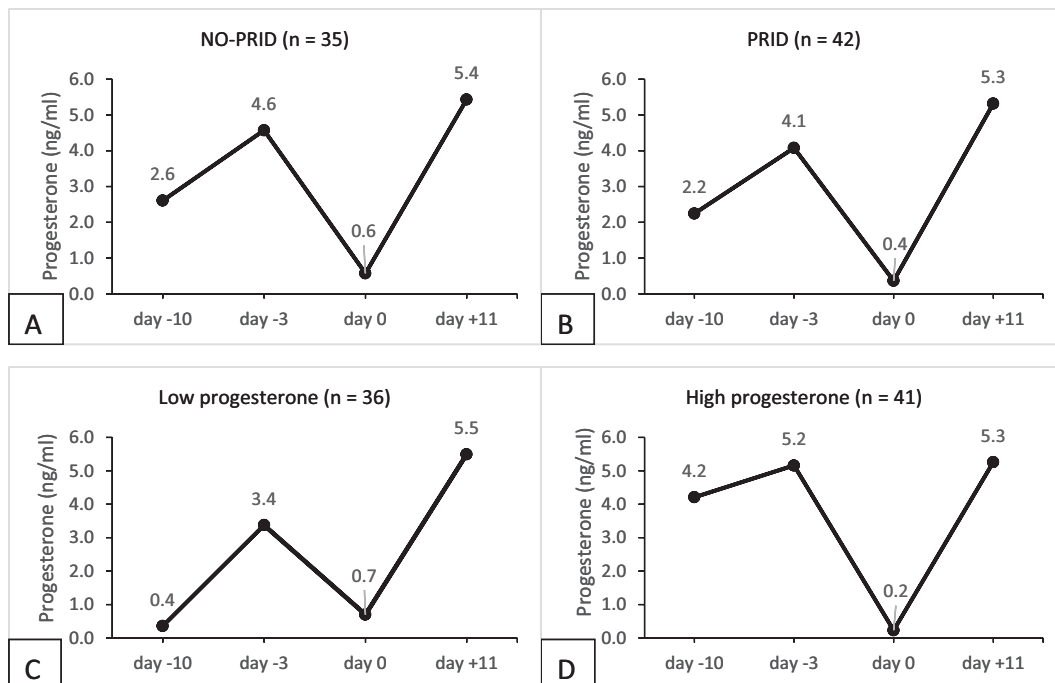


Figure 4: Plasma progesterone profiles at 1st GnRH treatment (day -10), at PGF_{2α} treatment (day -3), at TAI (day 0) and 11 days after TAI (day +11) in cows that received NO-PRID (A) versus PRID (B) or categorized as LP (C; < 1 ng/ml) versus HP (D; ≥ 1 ng/ml). Standard errors of the means (common to charts A, B, C, and D) on days -10, -3 and +11 were ± 0.3, 0.4, and 0.4, respectively. Standard errors of the means on day 0 for charts A, B, C, and D were 0.5, 0.4, and 0.1, 0.1, respectively.

underwent complete luteinization presenting CL-like structures 7 days after treatment. Instances of spontaneous ovulation of COF after persisting for 21 days,³¹ and induced ovulation of COF in response to GnRH treatment during an Ovsynch protocol¹⁹ have been reported in isolated cases. Ovulation of low-dose-progesterone-induced follicular cysts after persisting for more than 30 days was also reported in one study.³² Of the 11 cows that ovulated a COF in response to 1st GnRH treatment, 9 were in the LP category and 2 cows in which progesterone concentrations were not quantified on day -10, were assigned to the HP category based on luteal tissue detected during ultrasonography on day -10. On further examination of data, it was evident that the 9 LP cows that ovulated a COF also belonged to the 'true follicular cyst' category and the 2 HP cows belonged to the 'persistent follicular cyst' category. Although induced ovulation after GnRH treatment occurred more readily when circulating progesterone concentrations were lower than elevated,³³ it is likely that the 2 COF categorized as 'persistent follicular cyst' were responsive to the GnRH-induced LH surge. Furthermore, the mean diameter of the COF that ovulated in response to 1st GnRH was smaller (28.7 mm) compared to that of COF that underwent luteinization (34.0 mm) suggesting that the former may have been in an active growing phase presenting a milieu conducive to induced ovulation.

Three of the 9 cows (33.3%) that developed a new cyst were diagnosed pregnant at day 32, indicating that even cows that developed a new COF responded positively to the Ovsynch/TAI protocol resulting in an acceptable P/AI.

Overall incidence of double ovulation in the present study (16.2%) was higher than what was reported for cyclic cows of mixed parities (14.1%) and cows with COF (3.6%).¹⁶ That the incidence of double ovulation was higher 14/18 (78%) in LP

cows than in the HP category 4/18 (22%) was not surprising; because, as previously discussed, induced ovulation after GnRH treatment occurred more readily when circulating progesterone concentrations were lower³³ and COF is a known risk factor for double ovulation.³⁴

In addition to the 11 cows that ovulated a COF in response to 1st GnRH treatment, over 60% of cows with COF responded to that GnRH by ovulating an existing follicle and developed a CL on day -3, as determined by ovarian ultrasonography prior to PGF_{2α} treatment. More importantly, 95% of cases developed a new dominant follicle in response to 1st GnRH treatment and 89% of the aforesaid new follicles (or 84% of all cows) ovulated in response to 2nd GnRH treatment, providing evidence that the Ovsynch protocol works in cows with COF as well as it does in normal cyclic cows. New dominant follicle development in response to 1st GnRH treatment in 100% of COF cases has been reported^{10,19} and synchronized ovulation rates in response to 2nd GnRH treatment were 73,¹⁶ 83,¹⁰ and 100%.¹⁹

Although ovulation response to 2nd GnRH treatment did not differ between LP and HP categories, cows in which the Ovsynch protocol was initiated in LP status tended to have reduced P/AI compared to cows in HP status in the present study. It is known that starting the Ovsynch protocol during the luteal phase (HP status) results in higher P/AI in cows because adequate exposure to progesterone during ovulatory follicle development increased the fertility of dairy cows subjected to TAI programs^{24,35} and cows that start the protocol in HP status have higher odds of conceiving and sustaining the pregnancy to term through reduced embryo losses. Cows categorized as LP in the current study presented no evidence of luteal tissue at ultrasonography and/or plasma progesterone concentrations were < 1 ng/ml at the initiation of the Ovsynch

protocol. Thus, cows in the LP category (no CL, progesterone < 1 ng/ml) may be considered as having 'true COF' with little progesterone exposure and at a higher risk of not conceiving. Indeed, overall P/AI in LP cows, irrespective of PRID treatment, tended to be lower than in HP cows by 13.5-percentage points (Table 2). However, when LP cows that received NO-PRID treatment, hence expected to have the lowest P/AI, were compared against LP cows that received PRID, mean P/AI was numerically higher in LP cows by 9.3-percentage points (Table 2), suggesting that LP cows with NO-PRID appeared to have a slight advantage. However, no conclusions may be drawn based on these observations because we did not have an adequate sample size for these tests, a limitation of the study. Mean progesterone concentrations at 1st GnRH and at PGF_{2α} were lower in LP than in HP cows, attributable to the lack of prior progesterone exposure. In contrast, mean plasma progesterone concentrations on d 0 (TAI), albeit < 1 ng/ml, was higher in LP cows than in HP (0.7 versus 0.2 ng/ml). Suprabasal concentrations of progesterone on the day of TAI are detrimental to pregnancy establishment^{35,36} and this may be the main reason why LP cows tended to have reduced P/AI.

Although the use of Ovsynch/TAI in the reproductive management of cystic cows has been widely reported,^{10,16–18,20,37} P/AI has varied 3–41% in the cited studies. One study¹⁹ tested the effectiveness of Ovsynch/TAI + CIDR against a protocol involving AI at detected estrus and reported that P/AI in cows with COF subjected to Ovsynch/TAI + CIDR greatly improved (52% > 27%; *p* < 0.05) compared to control group. Nonetheless, few studies have directly compared treatment outcomes to Ovsynch and Ovsynch + progesterone within the same trial. One such study³⁸ compared P/AI after Ovsynch and Ovsynch + CIDR and reported that P/AI in the Ovsynch + CIDR group of cows with COF improved (37.5 > 16.2%; *p* < 0.05) whereas in another study³⁹ that made a direct comparison between Ovsynch and Ovsynch + CIDR in cows with true follicular cysts, the addition of a CIDR did not improve P/AI although both Ovsynch/TAI and Ovsynch/TAI + CIDR yielded above average P/AI (mean 44.7%).

Although past studies^{10,21,23} have demonstrated the positive impact of exogenous progesterone on managing ovarian cysts in dairy cattle, our findings and that of another³⁹ do not support the blanket use of a progesterone device in all COF cases. Some studies had significant improvements in P/AI when a progesterone device was incorporated into the Ovsynch protocol to manage either COF cows³⁸ or normal cyclic cows;⁴⁰ however, given the increased input cost associated with PRID or CIDR, selective use of a progesterone device only where its inclusion would be most beneficial could be justified. In this regard, a study⁴¹ that compared the economic benefits of TAI versus PRID as therapeutic options for managing COF in dairy cows, using a modelling approach, concluded that the overall benefit of treatment by TAI was \$11.39 higher than by PRID. Thus, based on our findings and because of a calculated cost advantage to using TAI over PRID for managing dairy cows with COF,⁴¹ addition of exogenous progesterone to the Ovsynch/TAI protocol cannot be recommended.

In summary, Ovsynch/TAI protocol was effective in dairy cows diagnosed with COF, regardless of the type of cyst present. First GnRH treatment induced ovulation of the COF in ~ 10% of cases and caused complete luteinization in ~ 15% of cases. Presence of COF did not hinder the ovulation of an existing follicle or the development of a new follicle. Moreover, COF did not interfere with conception or have any apparent harmful effects on early embryonic development at least until day

32 after TAI. Our findings reiterated previous reports that the application of Ovsynch/TAI is an effective approach to manage cows with COF to attain normal P/AI. Cows with COF that started on the Ovsynch program with HP status tended to attain higher pregnancy to TAI. Indiscriminate inclusion of a PRID in the Ovsynch/TAI protocol, in cows with all types of COF, was not beneficial.

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Conflict of interest

None to report.

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