Journal Pre-proof

Efficacy of ovulation synchronization with timed artificial insemination in treatment of follicular cysts in dairy cows

Hany Abdalla, Amanda M. de Mestre, Shebl E. Salem

PII: S0093-691X(20)30313-7

DOI: https://doi.org/10.1016/j.theriogenology.2020.05.029

Reference: THE 15535

To appear in: *Theriogenology*

Received Date: 19 October 2019

Revised Date: 18 May 2020

Accepted Date: 19 May 2020

Please cite this article as: Abdalla H, de Mestre AM, Salem SE, Efficacy of ovulation synchronization with timed artificial insemination in treatment of follicular cysts in dairy cows, *Theriogenology* (2020), doi: https://doi.org/10.1016/j.theriogenology.2020.05.029.

Hany Abdalla: Conceptualization, Methodology, Software, Validation, Investigation, Resources, Data Curation, Writing - Original Draft

Amanda M. de Mestre: Writing - Review & Editing

Shebl E. Salem: Methodology, Software, Formal analysis, Resources, Data Curation, Writing - Review & Editing

Revised

Efficacy of ovulation synchronization with timed artificial insemination in treatment of follicular cysts in dairy cows

Hany Abdalla [a*], *Amanda M. de Mestre* [b], *Shebl E. Salem* [c,d]

[a] Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig City 44511, Al Sharquiya Governorate, Egypt

[b] Department of Comparative Biomedical Sciences, The Royal Veterinary College, Hawkshead Lane, Hatfield AL9 7TE, UK

[c] Department of Surgery, Faculty of Veterinary Medicine, Zagazig University, Zagazig City 44511, Al Sharquiya Governorate, Egypt

[d] Veterinary Epidemiology, Economics and Public Health Group, Department of Pathobiology and Population Sciences, Royal Veterinary College, Hawkshead Lane, Hatfield AL9 7TE, UK

* **Correspondence author:** hlabdallah@zu.edu.eg; Lotfi_hany@yahoo.com

ABSTRACT

The efficacy of five ovulation-synchronization protocols with FTAI in treatment of cows diagnosed with follicular cysts was investigated in a nonrandomized study in a single herd. Cows identified with follicular structures >25 mm on two subsequent ultrasonographic (USG) examinations 7 to 10 days apart (n = 552) were assigned to one of the five treatment regimens on the day of the second USG examination. Treatment regimens were Ovsynch (GnRH-7d-PGF2α-56h-GnRH-16h-FTAI), New-CIDR (as Ovsynch with a new CIDR insert being fitted between days 0 and 7), Reused-CIDR (as New-CIDR but the CIDR insert was previously used for 7 days in another cow), G-New CIDR (Pre-GnRH on day 0 with the New-CIDR regimen being initiated 7 days later), and RG-Ovsynch (Pre-GnRH on day 0 and then every 7 days until detection of a luteal structure upon USG examination; at that point the Ovsynch was initiated). A subset of cows was subjected to ovarian USG examination at the time of $PGF_{2\alpha}$ administration, at insemination, and 8 to10 days post-insemination. Progesterone-releasing ability of new and reused CIDR inserts were evaluated in cows diagnosed with severe ovarian inactivity (n = 16). The data were analyzed using logistic regression with pregnancy per AI on days 30 (P/Al 30) and 70 (P/AI 70) post-insemination were included as outcome measures. Compared with Ovsynch, RG-Ovsynch improved the P/AI 30 (OR = 2.6, P = 0.03) and the P/AI 70 (OR = 2.5, P = 0.05). New-CIDR and G-New CIDR were associated with non-significant increase in P/AI 30 (OR = 2.1, P = 0.09 and OR = 2.3, P = 0.07, respectively) and P/AI 70 (OR = 2.01, P = 0.09 and OR = 2.2, P = 0.09, respectively). Reused-CIDR was not associated with improvement in P/AI (P = 0.93 and 0.79 for P/AI 30 and P/AI 70, respectively). RG-Ovsynch had a longer diagnosis-to-FTAI interval (median 24, IQR 17,31). The dominant ovarian structures and the presence of a cyst or a luteal structure at $PGF_{2\alpha}$ administration or at insemination were not associated with

P/AI. The new and re-used CIDR inserts produced comparable concentrations of serum progesterone 3 h, 3 days and 7 days post CIDR insertion. In conclusion, the RG-Ovsynch improved the P/AI whereas the New-CIDR and the G-New CIDR regimens tended to increase the P/AI compared with Ovsynch. Marginal differences in P/AI between RG-Ovsynch, New-CIDR, and G-New-CIDR together with prolonged diagnosis-to-FTAI in RG-Ovsynch should be considered if to evaluate the economic value of these regimens.

Keywords

Dairy cattle, follicular cyst, ovulation synchronization, reused CIDR

1. Introduction

Cystic ovarian disease has negative impacts on the reproductive performance of dairy cattle, and hence it may decrease the overall productivity of dairy enterprises [1]. In cystic ovarian disease, the mature follicle continues to grow to larger diameters without ovulation and persists for a prolonged period [2, 3]. These cows, however, have follicular waves that grow to different stages followed by regression without ovulation [2, 3]. The diameter at which an ovarian structure is defined as a follicular cyst varied among literatures from 17 to 25 mm [4-10], and the reported prevalence of cystic ovarian disease varied from 10 to 16 % with higher prevalence being reported around the end of the voluntary waiting period [11]. Cystic ovarian disease is commonly treated with LH-inducing hormones such as GnRH, LH-like medications such as human chorionic gonadotrophin (hCG) [9, 12-14], or with intravaginal progesterone-releasing inserts [15-18]. Cows receiving these treatments are monitored carefully for estrous sings and inseminated at the first post-treatment estrus. Such treatment regimens, however, have been associated with prolonged treatment-to-insemination intervals [5, 9, 12, 13, 19, 20] with difficulties to differentiate between recovery in response to treatment and spontaneous recovery [21].

Because cows with cystic ovarian disease develop follicular waves [2, 3, 17], ovulation-synchronization treatment regimens with fixed-time artificial insemination (FTAI) has been suggested as a therapeutic intervention for these cows [3, 22, 23]. Ovsynch (GnRH on day 0, $PGF_{2\alpha}$ on day 7, GnRH on day 9, and insemination on day 10) has been found to perform sub-optimally in anovular cows compared with cyclic cows [7, 24, 25], and therefore, several modifications to the Ovsynch such as starting the Ovsynch with $PGF_{2\alpha}$ plus GnRH followed by $PGF_{2\alpha}$ 14 days later [24, 26], replacing the second GnRH dose with hCG [27], or treatment of the cows with hCG 5 days post-insemination [28] have been investigated to improve the efficiency of Ovsynch in anovular cows. The suboptimal performance of the Ovsynch in anovular cows is thought to occur because the new synchronized follicular wave that emerges after the first GnRH treatment of the Ovsynch develops and matures under a low progesterone level [25, 29, 30]. To mitigate this, cows with ovarian cysts have been pre-treated with GnRH 6 to7 days (Pre-GnRH) before initiating the Ovsynch [31, 32]. Pre-GnRH treatment has been found to improve the pregnancy per artificial insemination (P/AI) in one study [32], however, another study reported reduced P/AI following treatment with Pre-GnRH [31]. This controversy could have resulted due to differences in the response to the pre-GnRH treatment between cows. Therefore, we hypothesised in the current study that the performance of the Ovsynch in cows with ovarian cystic disease could be improved if the response to the Pre-GnRH treatment is evaluated via ultrasonographic ovarian examination with the Pre-GnRH dosing being repeated until a luteal structure is identified before initiating the Ovsynch treatment regimen.

Another alternative to improve the efficiency of Ovsynch in anovular cows is to incorporate a controlled internal drug release (CIDR) between days 0 and 7 of the Ovsynch (CIDR-Ovsynch). Several studies have investigated the efficacy of CIDR-Ovsynch in reproductive management of dairy cows, but a limited number of studies have investigated its efficiency in cows with follicular cysts [10, 20]. Furthermore, most of these studies used a limited number of cows, and the results obtained were contradictory [4, 10, 33]. In the GnRH-$PGF_{2\alpha}$ based ovulation-synchronization regimen, the progesterone-releasing inserts are usually used for 7 days, however, the commercially available CIDR is designed to produce an elevated concentration of progesterone for 14 days [34]. Therefore, reusing the progesterone-releasing inserts has been investigated to reduce the cost of the reproductive management of dairy cows [35-38]. Previously used CIDR and Sincrogest inserts have been reported to induce a lower progesterone concentration than the new ones in high lactating dairy cows and anestrus buffaloes [35-37]. The follicular growth and the P/AI in seasonal anestrus buffaloes, however, were similar after incorporating new or previously used Sincrogest insets into an estradiol-progesterone based ovulation-synchronization regimen [37]. To the best of our knowledge, the efficiency of reused CIDR has not been evaluated in cows with follicular cysts. Therefore, the objectives of the current study were (i) to investigate the efficiency of some modifications to the Ovsynch (mainly repeated pretreatment with GnRH until detection of a luteal structure or CIDR insertion) in treatment of cows diagnosed with follicular cysts and (ii) to compare the progesterone-releasing ability and treatment efficacy of new and reused CIDR inserts.

2. Materials and methods

2.1. Herd and animals

A prospective study was conducted over a 2-year period in a commercial dairy herd of approximately 1,300 dairy cows that is located in the outskirts of Cairo, Egypt. The herd contained Holstein, Brown Swiss, and crossbred cows (Holstein × Brown Swiss and Holstein × Fleckvieh) that were housed in shaded free stalls fitted with fans and water sprinklers. The nutritional requirements of the cows were determined according to the National Research Council nutritional guidelines [39]. Cows were fed a total mixed ration consisted mostly of corn silage, citrus silage, corn, and soybean. Cows were milked three times daily and were vaccinated against bovine viral diarrhea, infectious bovine rhinotracheitis, foot and mouth disease, ephemeral fever, lumpy skin disease, rift valley fever, and clostridial diseases. Cows were equipped with a pedometer system to record their walking activity, and this was employed as the main estrus detection method. The routine reproductive management program of the herd included a weekly examination of all open cows beginning at 45 days in milk (DIM), with a voluntary waiting period set at 60 days. A commercial farm management software (AfiFarm version 4.1, Afimilk Ltd, Kibbutz Afikim,1514800, Israel) was used to record all reproductive data. The study was approved by the Institutional Animal Care and Use Committee, Zagazig University, Egypt (ZU-IACUC).

2.2. Case definition

Cows potentially suffering from follicular cysts were identified during ultrasonographic (USG) examination of open cows at 45 to 50 DIM or during pregnancy diagnosis on day 30 to 32 post-insemination. These were defined as cows that had a thin-walled follicular structure >25 mm without a luteal structure. Potentially affected cows were re-examined 7 to 10 days later, and the diagnosis of the follicular cyst was confirmed if a luteal structure could not be visualized and the cystic structure was still present [40]. All examinations were performed using an IMAGO ultrasound unit (IMV imaging UK, Ltd, Bellshill, Scotland, ML4 3NJ) supplied with a 7.5–10 MHz rectal probe.

2.3. Treatment regimens

On each visit to the farm, all cows diagnosed with follicular cysts were immediately treated using one of five ovulation-synchronization treatment regimens, namely Ovsych, New-CIDR, Reused-CIDR, G-Reused-CIDR and RG-Ovsynch. Cows in which the Ovsynch treatment regimen was used (n = 38) were treated with GnRH (100 µg gonadorelin acetate im; Ovurelin, Bayer, New Zealand) on day 0, $PGF_{2\alpha}$ (500 mg cloprostenol im; Ovuprost, Bayer, New Zealand) on day 7, a second dose of GnRH 56 hours after the $PGF_{2\alpha}$ treatment, and inseminated 16 h later. The New-CIDR treatment regimen (n = 261) was similar to the Ovsynch treatment regimen, but with a new intravaginal CIDR insert containing 1.38 g of progesterone (Eazi-Breed CIDR; Zoetis, NJ, USA) being inserted between days 0 and 7. Cows treated with the Reused-CIDR treatment regimen (n = 63) received the same treatments described in the Ovsynch treatment regimen, but with a reused CIDR insert being fitted between days 0 and 7. The reused-CIDR insert was previously used once for 7 days in a different cow and was cleaned by an antiseptic solution before reusing. The G-New CIDR treatment regimen (n = 161) involved treatment of cows with a single dose of Pre-GnRH 7 days before initiating the New-CIDR treatment regimen. The RG-Ovsynch treatment regimen (n = 141) involved pretreatment of cows with Pre-GnRH on day 0 followed by USG examination 7 days later. Identification of a luteal structure upon USG examination indicated a successful response to Pre-GnRH treatment, and the Ovsynch treatment regimen was initiated. If a luteal structure could not be identified, a second dose of Pre-GnRH treatment was administered, and the cows were examined again 7 days later. This procedure was repeated every 7 days until a luteal structure was identified before initiating the Ovsynch treatment regimen. The luteal structure identified was either a corpus luteum (CL) with regression of the cyst, a luteinized cyst, or a CL in the presence of the cyst. Assignment of cows to these treatments was nonrandomized. A graphical illustration of the treatment regimens is shown in Fig. 1.

Cows in all treatments received FTAI performed by a total of 12 inseminators using semen doses from 30 bulls. Cows that did not return to estrus underwent a per rectum USG pregnancy diagnosis 30 to 32 days post-insemination. Pregnant cows underwent a second USG examination approximately 70 days post-insemination and their pregnancy status was noted. The P/AI 30 and P/AI 70 were calculated as the proportion of cows out of all treated cows that were diagnosed as pregnant on day 30 or 70 post-insemination, respectively. Cows were defined as recovered from the cystic ovarian disease if they were returned into estrus 17 to 29 days post insemination, were diagnosed as pregnant 30 to 32 days post-insemination or were not identified with a follicular cyst on pregnancy diagnosis examination in the case of negative pregnancy scan.

2.4. Data collection

Additional information recorded included the date of insemination, breed, age, parity, DIM, number of inseminations and 305-day milk yield. The daily ambient temperature and humidity information were collected from a local weather station located at the Cairo airport; about 10 km to the west of the farm. The monthly mean temperature-humidity index (THI) was calculated from the temperature and humidity data as follows: $THI = (1.8 \times AT + 32) - ((0.55 - 0.0055 \times RH) x (1.8 \times AT - 26))$ [41]; where AT refers to the ambient temperature and RH is the relative humidity. THI was measured on the day of insemination and the monthly mean of THI was calculated. The mean monthly value was included in the analysis as a categorical variable where the calendar year was split according to the monthly THI into 4 periods; THI < 65, 65 ≤ THI < 70, 70 ≤ THI <75 and THI ≥ 75.

2.5. Ultrasonographic examinations

On day 0, cows in all treatment regimens had a follicular cyst that persisted for at least 7 to 10 days. To monitor the disappearance of the follicular cyst and the development of a luteal structure during and after the end of the treatment regimens, a subgroup of cows in the New-CIDR, Reused-CIDR, G-New CIDR and RG-Ovsynch treatment regimens were subjected to USG examinations on the day of $PGF_{2\alpha}$ administration, on the day of insemination, and 8 to10 days post-insemination. Because of limited time and resources, not all cows were examined on every examination time point. Table 1 provides a summary of findings that were considered on each examination time point. During each examination, signs of ovulation (development of a CL that was not present on the previous examination), and changes in the USG appearance of the follicular cyst such as the development of a luteinized thick wall around the follicular cyst (luteinization) or the disappearance of the follicular cyst were recorded. According to the ovarian structures identified on each examination, cows were classified as either having a follicular cyst (a thin-walled follicular cyst without a luteal tissue), a follicle (a follicular structure without a follicular cyst or a luteal structure), a CL (a CL without a follicular cyst), a CL plus a follicular cyst (both a thin-walled follicular cyst and a CL), or a luteal cyst (a cyst with >3 mm wall thickness). To investigate the association between the development of a luteal structure and P/AI, cows identified with a CL, a luteal cyst, or a CL plus a follicular cyst were grouped together and classified as having a luteal structure. Similarly, cows identified with a follicular cyst or a CL plus a follicular cyst were grouped and classified as having a follicular cyst.

2.6. Evaluation of the progesterone-releasing ability of new and reused CIDR

Sixteen Holstein cows that were diagnosed with severe ovarian inactivity were randomly fitted with new or reused CIDR inserts for 7 days. Severe ovarian inactivity was characterized by the absence of a CL and ovarian follicles >8 mm during 3 subsequent USG examinations at 7 days intervals before the experiment. These findings were also confirmed by USG examination on each of the blood sampling occasions and 7 days after removing the CIDR inserts. These cows were used to assure the absence of an ovarian source of progesterone. Venous blood samples were collected at the time of CIDR insertion, and 3 h, 3 days, and 7 days post CIDR insertion. Blood samples were transported in an icebox to the laboratory where they were centrifuged at 3000 g for 10 minutes, and sera were harvested and stored at –20 ^{o}C until testing. Progesterone concentration in serum samples was measured using an electrochemiluminescence immunoassay “ECLIA” kit on an Elecsys immunoassay analyzer (Elecsys, Roche Diagnostic Gmbh, Mannheim, Germany). The test has a measurement range of 0.05 to 60 ng/ml and functional sensitivity (limit of quantification) of 0.15 ng/ml. The coefficient of variation of reference samples at a concentration of 0.659 and 3.03 ng/ml were 5.2 and 3.0, respectively.

2.7. Statistical analyses

The P/AI 30 and P/AI 70 were the primary outcomes of interest in all analyses. The prevalence of a positive pregnancy within each level of categorical variables together with 95% confidence intervals were reported. The summary statistics (median and interquartile range [IQR]) of continuous variables in cows identified with a positive or a negative pregnancy were also calculated (Supplementary Tables 1 and 2). Univariable logistic regression was performed to investigate the association between explanatory variables including the treatment regimens, and each of the outcome variables. The linearity of the relationship between continuous explanatory variables and the log odds of a positive pregnancy scan was investigated using generalized additive models (GAMs) [42]. Continuous variables were then fitted as linear terms in logistic regression analyses or categorized based on the results of the GAMs. The cut-off points used were informed by plotting the GAMs results. The variable DIM was categorized into <200, 200–40, and >400 days for the P/AI 30 outcome but included as a linear fit in the model investigated risk factors for P/AI 70. Cows were classified as primiparous (first lactation) or multiparous (second or more lactations). The likelihood ratio test (LRT) P value was calculated for each of the explanatory variables by comparing the null logistic regression model and the model containing the variable.

Variables identified with an LRT P value <0.25 were used to build multivariable logistic regression models. The multivariable models were built using a forward selection approach in which variables were added in succession into the models. The improvement in model fit was justified if the added variable resulted in an LRT P value of <0.05 and/or a reduction in AIC by at least 2 when nested models were compared. Excluded variables were forced back into the model to make sure no confounding or important variables have been excluded. Interaction between variables retained in the final model were evaluated for statistical significance. The model fit was then evaluated using the Hosmer-Lemeshow goodness-of-fit test and influential observations were investigated by calculating and plotting delta betas. In final models, the random effects of cow, bull, and inseminator were evaluated by comparing the model with and without the random effect using the LRT. The amount of variability in the log odds of positive pregnancy attributed to each of the random effects was calculated using the latent-variable approach. The method assumes the binary outcome arises from an underlying continuous distribution and that the level one (individual pregnancies) variance on the logit scale is $\pi^2/3$

The relationships between the dominant ovarian structures identified on each of the examination occasions and the log odds of P/AI 30 and were examined using logistic regression models following adjustment for the treatment regimen and the THI. Furthermore, the proportion of ovarian structures identified during examination were compared between treatment regimens using a chi-square test. Fishers' exact test was used if one or more of the cells in the contingency table contained an expected frequency of <5 observations. The progesterone concentration was compared between cows treated with reused or new CIDR inserts using a random intercept linear mixed-effects model. The model takes into account the dependent nature of the data where progesterone concentration was measured within the same cows over time through including the time and the type of CIDR (both were categorical variables) as fixed effects variables and 'cow' as a random effect. Post-hoc comparisons of least square means were performed using the Tukey's post-hoc test. All analyses were performed using R software version 3.5.3 [43] and the critical probability was set at $P < 0.05$ for all analyses.

3. Results

3.1. *Factors affecting the odds of pregnancy*

Follicular cyst was diagnosed on 664 occasions in 522 cows during the study period. Of the cows that had repeated treatments (n = 116), 65 cows were treated in the same lactation and 51 cows were treated in different lactations. At the time of insemination, cows had a median age of 49.8 months (IQR 37.1, 65.8), a median parity of 2 (IQR 1, 3), a median DIM of 136.5 days

(IQR 86, 261), and a median insemination number of 2 (IQR 1,4). The cows had a median actual 305-day milk yield of 9905 kg (IQR 8741, 11119).

Cows expressed estrus sings 17 to 29 days post insemination, diagnosed as pregnant 30 to 32 days post-insemination, or diagnosed as non-pregnant, but with the absence of a follicular cyst were defined as recovered from the cystic ovarian disease. The overall recovery rate within 30 days of the end of the treatment regimens was 89.8%, and it did not differ significantly between treatment regimens (X^2 = 1.42, P = 0.84). The total percentage of P/AI 30 and P/AI 70 were 32.2% (n = 214) and 28.2% (n = 187), respectively. Pregnancy loss between gestational days 30 and 70 was 12.7% (n = 27). The proportion of cows with pregnancy loss did not differ between treatment regimens (P = 0.68). Of note that, the P/AI 30, P/AI 70, and pregnancy loss in cows that were inseminated in standing estrus with a normal inter-estrus interval (i.e. 17 to 25 days) in the same herd during the study period were 31.6% (797/2523), 27.9% (705/2523) and 11.5% (92/797), respectively.

Results of univariable analyses of variables associated with the log odds of P/AI 30 and P/AI 70 are presented in supplementary Tables 1 and 2, respectively. The results of the final multivariable models are presented in Table 2. Cows inseminated when the THI was >70 showed lower P/AI 30 and P/AI 70 than those inseminated when THI was <65. Cows treated with the RG-Ovsynch treatment regimen showed higher P/AI 30 (P = 0.03) and P/AI 70 (P = 0.05) compared with those treated with the Ovsynch treatment regimen. Cows treated with the New-CIDR and the G-New CIDR treatment regimens showed non-significant improvement in P/AI 30 (P = 0.09 and 0.07, respectively) and P/AI 70 (P = 0.09). The differences in the odds of pregnancy in cows treated with RG-Ovsynch, New-CIDR, and G-New CIDR treatment regimens were marginal and did not reach statistical significance. There was no interaction between the treatment regimens and the THI on the P/AI. The likelihood ratio test P values of the random effect of the cow in the final P/AI 30 and P/AI 70 multivariable models were 0.08 and 0.06, respectively. The intra-class correlation coefficients for the cow random effect were 26% and 32% for the two models, respectively. Neither the random effect of bull nor that of the inseminator explained any variation in the log odds of the P/AI 30, or the P/AI 70 in the final models.

3.2. *Dominant ovarian structures at the time $PGF_{2\alpha}$ administration, on day of insemination, and 8–10 days post-insemination and their relation to P/AI*

The intervals between the onset of the treatment regimen and FTAI varied among treatment regimens. This was 10 days in the Ovsynch, New-CIDR, and Re-used CIDR treatment regimens,17 days in the G-New CIDR treatment regimen, and 17–52 days (median 24, IQR 17,31) in the RG-Ovsynch treatment regimen. The number of weekly Pre-GnRH treatment administrations until detection of a luteal structure in cows treated with the RG-Ovsynch treatment regimen varied from 1 to 6 (median 2, IQR = 1, 3). Of the 141 cows treated with RG-Ovsynch, the proportion of cows that showed a luteal structure after 1, 2, 3, 4, 5, or 6 Pre-GnRH treatment administrations were 41.2, 25.5, 18.4, 11.4, 2.8, and 0.7 %, respectively. Luteal structures identified after GnRH pretreatments and at the beginning of the Ovsynch were either a CL in the presence of the cyst (34.7%, n = 49), a CL with disappearance of the follicular cyst (53.2%, n = 75), or luteinization of the cyst (12.1%, n = 17). Cows identified with a CL in the presence of a follicular cyst at the beginning of the Ovsynch in the RG-Ovsynch treatment regimen tended to have lesser P/AI 30 (P = 0.07) compared with those identified with a CL without a follicular cyst (Table 3).

At the time of $PGF_{2\alpha}$ administration, the proportion of cows identified with a luteal structure was higher in the RG-Ovsynch (97.3%, $P < 0.001$) compared with the New-CIDR (46.9%) and the Reused-CIDR (63.1%) treatment regimens. The proportions of cows that retained the follicular cyst until the time of $PGF_{2\alpha}$ administration did not vary among treatment regimens (Fig. 2A). The dominant ovarian structures, development of a luteal structure, or the presence of a follicular cyst at the time of $PGF_{2\alpha}$ administration were not associated with P/AI 30 (Table 3). The proportion of cows that retained the follicular cyst up to the time of insemination were similar among treatment regimens (Fig. 2B), and this was not associated with P/AI 30 (Table 3).

The proportion of cows ovulated at the end of the treatment regimens (i.e. had a CL or a CL plus a cyst) did not differ between treatment regimens. Similarly, the proportion of cows that retained a follicular cyst (i.e. had a follicular cyst or a CL plus a cyst) up to 8 to 10 days post-insemination did not differ among treatment regimens. Only 4.4 to 13.5% of the cows showed a follicular cyst without a luteal structure on day 8 to 10 post-insemination (Fig. 2C). The P/AI 30 was similar in cows that ovulated after the disappearance of the follicular cyst (i.e. had a CL with no follicular cyst on day 8 to10 post-insemination) and those ovulated in the presence of the follicular cyst (i.e. showed a CL plus a follicular cyst on day 8 to 10 post-insemination) (41.3 vs 30.4%, respectively; Table 3).

3.3. *Progesterone concentration in cows treated with new and reused CIDR inserts*

The linear mixed-effects model showed that the use of new or reused CIDR inserts was not associated with significant differences in blood progesterone concentrations at all sampling occasions ($P = 0.1$). The mean ± standard deviation baseline serum progesterone concentrations in cows received new or reused CIDR inserts were 0.60 ± 0.08 and 0.47 ± 0.19, respectively. By 3 h after CIDR insertion, the mean serum concentration of progesterone increased ($P < 0.001$) to

1.96 ± 0.15 and 1.49 ± 0.86 ng/ml in cows received new and reused CIDR inserts, respectively. The serum progesterone concentration 3 days post CIDR insertion was comparable to that measured 3 h after CIDR insertion in cows received new or reused CIDR inserts. On day 7 post CIDR insertion, the serum progesterone concentration significantly decreased compared with the concentration on day 3 but remained significantly higher than the baseline concentration in both groups (Fig. 3).

4. Discussion

The present study investigated the efficacy of some modifications to the Ovsynch in the treatment of cows diagnosed with follicular cysts. These included either inclusion of a new or a previously used CIDR inserts or pretreatment with GnRH until detection of a CL before initiating the Ovsynch. Repeated administration of Pre-GnRH treatments until the development of a luteal structure before initiating the Ovsynch (RG-Ovsynch) significantly improved the P/AI 30 and P/AI 70 compared with the Ovsynch treatment regimen. There was also a trend toward improved P/AI 30 and P/AI 70 in cows treated with New-CIDR and G-New CIDR treatment regimens.

The low P/AI 30 (21.1%) observed in the current study after imposing the Ovsynch treatment regimen agrees with previous reports [5-7, 27, 32]. Two previous studies [10, 44],however, reported high P/AI (41 to 46.5%) following treatment with Ovsynch. These two studies used a small number of cows with cystic ovarian disease (n = 15 to 17), and this high P/AI was likely to be a chance finding. In the current study, the use of RG-Ovsynch treatment regimen improved the P/AI compared with the Ovsynch regimen. Pretreatment with a single dose of Pre-GnRH before starting the Ovsynch improved the P/AI in cows with cystic ovarian disease in one study [32], but it was associated with a lesser P/AI in another study [31]. The previously mentioned studies did not differentiate between cows that responded or did not respond to the Pre-GnRH treatment. Noncyclic cows that responded to pretreatment with CIDR before the Ovsynch have been reported to have greater P/AI than nonresponding cows [45]. In the current study, only 41.2% of cows with a follicular cyst showed a luteal structure after a single Pre-GnRH treatment. This indicates that a single Pre-GnRH treatment may not be enough to induce the desired effect of the Pre-GnRH treatment (i.e. induction of ovulation and formation of a luteal structure before the initiation of the Ovsynch).

Inclusion of a New CIDR insert into the Ovsynch (i.e New-CIDR treatment regimen) tended to improve the P/AI 30 and P/AI 70 in the current study. Previous studies reported that the inclusion of CIDR inserts into the Ovsynch improved the P/AI in cows diagnosed with a persistent follicle but not in cows diagnosed with cystic ovarian disease [4, 10]. A previous study reported a P/AI percentage of 52% in cows with cystic ovarian disease treated with a CIDR-based ovulation-synchronization treatment [20], which is much higher than the P/AI percentage (34.86%) reported in the current study following the same treatment. The differences between studies could be attributed to differences in herd management plans and the cow's genetic between herds. The addition of a single Pre-GnRH treatment before imposing the New-CIDR protocol (i.e. G-New CIDR treatment regimen) did not achieve significant improvement in the odds of pregnancy compared with the New-CIDR treatment regimen.

Although the New-CIDR and the G-New CIDR treatment regimens achieved an important clinical improvement in the P/AI percentage compared with the Ovsynch treatment regimen, this effect did not reach statistical significance. This could have been occurred because of the smaller number of cows treated with the Ovsynch treatment regimen in the current study with subsequence reduction in the study power to detect significance differences between groups (e.g.

the number of cows treated with New-CIDR treatment regimen was 6 times as those treated with Ovsynch). Post-hoc sample size calculations for this given example of the Ovsynch and the New-CIDR treatment regimens identified that 183 cows per group are required to identify a difference in P/AI 30 of 13% at 5% significance level and 80% power. Of note that, the differences in the odds of pregnancy in cows treated with RG-Ovsynch, New-CIDR, and G-New CIDR treatment regimens were marginal, however, the diagnosis to insemination intervals in cows treated with the RG-Ovsynch treatment regimen (24.7 days) is about 14 and 10 days longer than that in cows treated with New-CIDR or G-New CIDR treatment regimens, respectively. Before deciding which treatment regimen could achieve better economic benefit, any additional improvement in P/AI associated with RG-Ovsynch treatment regimen should be weighed against the prolonged days open occurred in cows treated with this treatment regimen. Of note also that, the dose of Pre-GnRH treatment applied in the current study was that the same as that commonly used in ovulation-synchronization regimens, i.e. 100 μg. Using larger doses might minimize the number of required Pre-GnRH treatment doses and the duration from diagnosis till insemination in the RG-Ovsynch treatment regimen.

The Reused-CIDR and the New-CIDR treatment regimens showed similar ability to induce cyst regression and ovulation at the end of these treatment regimens, but the odds of pregnancy in cows treated with the New-CIDR treatment regimen was as twice as that in those treated with the Reused-CIDR treatment regimen. The CIDR insert as a source of exogenous progesterone provides a mimicked physiological hormonal milieu for the growth and maturation of the synchronized follicular wave [25, 46]. Furthermore, it reduces the pulse frequency and the mean concentration of LH to a normal concentration and restores the ability of the hypothalamo-pituitary axis to generate an LH surge in response to an increase in circulating estradiol [15-17].

The required number of progesterone-releasing inserts is controversial. The inclusion of two CIDR inserts had improved the conception rate in cows that had no CL at the start of the CIDR-Ovsynch treatment [46]. However, two other studies reported that using two or three progesterone-releasing inserts did not improve the P/AI compared with one treatment [47, 48]. In the current study, the new and the reused CIDR inserts induced similar serum progesterone concentration at 3 h, 3 days, and 7 days post insertion. It seems that the serum progesterone concentration induced by the reused CIDR insert was enough to restore the ability of the hypothalamo-pituitary axis to generate an LH surge and ovulation at the end of the treatment regimen. However, lower pregnancy probability in the Reused-CIDR treatment regimen raises a question of whether the very narrow non-significant difference in the serum progesterone concentration (about 0.27 ng/ml) observed after using of new and reused CIDR inserts could affect the fertilizing and the developmental ability of the oocytes in cows treated with Reused-CIDR treatment regimen. Further research is required to investigate the underlying mechanism.

A larger proportion of cows in the RG-Ovsynch and the G-New CIDR treatment regimens were found to have a CL or a luteal structure at the time of $PGF_{2\alpha}$ administration. This observation is logical as in RG-Ovsynch treatment regimen, the Ovsynch was not initiated until after detection of a luteal structure, and in the G-New CIDR treatment regimen the cows received 2 doses of GnRH before the time of $PGF_{2\alpha}$ administration. Cows developed or did not develop a luteal structure by the time of $PGF_{2\alpha}$ administration showed similar P/AI percentages. This agrees with a previous study that reported that the luteal status at the time of $PGF_{2\alpha}$ administration was not associated with the pregnancy probability in non-cyclic cows treated with a CIDR-based ovulation-synchronization treatment [33]. The presence of a CL at the time of $PGF_{2\alpha}$ administration indicated an ovulation and the initiation of a new follicular wave in response to the previous GnRH treatment/s. Cyclic cows that ovulate after the first GnRH treatment of the Ovsynch regimen have been reported to have higher P/AI than those that did not ovulate [49, 50]. The emergence of a synchronized follicular wave may occur without the development of a CL [44, 51]. In the current study, we did not monitor the emergence of new follicular waves after GnRH treatment, but the presence of a functional follicle at insemination and high ovulation rate (83 to 88%) at the end of the treatment regimens indicated that the emergence of a new follicular wave may have occurred without development of a CL in a large proportion of cows. This may explain why we could not detect differences in the pregnancy probability between cows that had or did not have a luteal structure at the time of $PGF_{2\alpha}$ administration.

We observed similar pregnancy probability in cows that retained or did not retain the follicular cyst up to the time of insemination. Our results are supported by a previous study that reported 70% ovulation and 36.7% conception rates in cows that had an ovarian cystic structure at the time of insemination [52]. In the current study, the pregnancy probability in cows ovulated with retention of the follicular cyst up to 8 to 10 days post-insemination tended to be lower than that in cows ovulated with regression of the follicular cyst (P = 0.1). However, the pregnancy probability in cows ovulated in the presence of follicular cyst (30.4%) was still reasonable.

5. Conclusions

In comparison with Ovsynch, the application of weekly GnRH doses until detection of a luteal structure before initiating the Ovsynch (RG-Ovsynch) significantly improved the P/AI 30 and 70 in cows diagnosed with follicular cysts. Furthermore, New-CIDR and G-New CIDR treatment regimens tended to improve the pregnancy probability on day 30 and 70 post-insemination. The differences in the odds of pregnancy in cows treated with RG-Ovsynch, New-

CIDR, and G-New CIDR treatment regimens were marginal and any economic benefits achieved from this marginal increase in the P/AI should be interpreted in light of prolonged diagnosis-to-insemination interval occurred in the RG-Ovsynch compared with the New-CIDR and G-New CIDR treatment regimens. Overall, these three treatment regimens could have a positive impact on the fertility of the dairy herd investigated here as the P/AI in cows diagnosed with follicular cysts following these treatment regimens was comparable to that of cows inseminated after spontaneous estrus during the same period. The dominant ovarian structures identified at the time of $PGF_{2\alpha}$ administration, or retention of the cyst up to the time of insemination, or 8 to10 days post-insemination were not associated with the P/AI.

Conflict of interest

Authors declare that no conflicts of interest could be perceived as prejudicing the impartiality of the research reported.

Acknowledgements

The authors would like to thank the owner of the AL-Kasem farm for consenting to use the farm data. Thanks to Dr Mohamed Hussein for the farm data managing and curation.

References

[1] Peter AT. An update on cystic ovarian degeneration in cattle. Reprod Doms Anim 2004: 39:1-7.

[2] Wiltbank MC, Gümen A, Sartori R. Physiological classification of anovulatory conditions in cattle. Theriogenology 2002;57:21-52.

[3] Peter AT, Vos PLAM, Ambrose DJ. Postpartum anestrus in dairy cattle. Theriogenology 2009;71:1333-42.

[4] López-Gatius F, Santolaria P, Yániz J, Rutlant J, López-Béjar M. Persistent ovarian follicles in dairy cows: a therapeutic approach. Theriogenology 2001;56:649-59.

[5] Crane MB, Bartolome J, Melendez P, de Vries A, Risco C, Archbald LF. Comparison of synchronization of ovulation with timed insemination and exogenous progesterone as therapeutic strategies for ovarian cysts in lactating dairy cows. Theriogenology 2006;65:1563-74.

[6] Crane MB, Melendez P, Bartolome J, de Vries A, Risco C, Archbald LF. Association between milk production and treatment response of ovarian cysts in lactating dairy cows using the Ovsynch protocol. Theriogenology 2006;66:1243-8.

[7] Bartolome JA, Archbald LF, Morresey P, Hernandez J, Tran T, Kelbert D, et al. Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies for bovine ovarian cysts in the dairy cow. Theriogenology 2000;53:815-25.

[8] Lopez-Gatius F, Mirzaei A, Santolaria P, Bech-Sabat G, Nogareda C, Garcia-Ispierto I, et al. Factors affecting the response to the specific treatment of several forms of clinical anestrus in high producing dairy cows. Theriogenology 2008;69:1095-103.

[9] Probo M, Comin A, Mollo A, Cairoli F, Stradaioli G, Veronesi MC. Reproductive performance of dairy cows with luteal or follicular ovarian cysts after treatment with buserelin. Anim Reprod sci 2011;127:135-9.

[10] Kawate N, Watanabe K, Uenaka K, Takahashi M, Inaba T, Tamada H. Comparison of plasma concentrations of estradiol-17 β and progesterone, and conception in dairy cows with cystic ovarian diseases between Ovsynch and Ovsynch plus CIDR Timed AI Protocols. J Reprod Develop 2011;57:267-72.

[11] Garverick HA. Ovarian follicular cysts in dairy cows. J Dairy Sci 1997: 80;995-1004.

[12] Nakao T, Tomita M, Kanbayashi H, Takagi H, Abe T, Takeuchi Y, et al. Comparisons of several dosages of a GnRH analog with the standard dose of hCG in the treatment of follicular cysts in dairy cows. Theriogenology 1992;38:137-45.

[13] Osawa T, Nakao T, Kimura M, Kaneko K, Takagi H, Moriyoshi M, et al. Fertirelin and buserelin compared by LH release, milk progesterone and subsequent reproductive performance in dairy cows treated for follicular cysts. Theriogenology 1995;44:835-47.

[14] Picard-Hagen N, Lhermie G, Florentin S, Merle D, Frein P, Gayrard V. Effect of gonadorelin, lecirelin, and buserelin on LH surge, ovulation, and progesterone in cattle. Theriogenology 2015;84:177-83.

[15] Calder MD, Salfen BE, Bao B, Youngquist RS, Garverick HA. Administration of progesterone to cows with ovarian follicular cysts results in a reduction in mean LH and LH pulse frequency and initiates ovulatory follicular growth. J Anim Sci 1999;77:3037-42.

[16] Todoroki J, Yamakuchi H, Mizoshita K, Kubota N, Tabara N, Noguchi J, et al. Restoring ovulation in beef donor cows with ovarian cysts by progesterone-releasing intravaginal silastic devices. Theriogenology 2001;55:1919-32.

[17] Todoroki J, Kaneko H. Formation of follicular cysts in cattle and therapeutic effects of controlled internal drug release. J Reprod Develop 2006;52:1-11.

[18] Zulu VC, Nakao T, Yamada K, Moriyoshi M, Nakada K, Sawamukai Y. Clinical response of ovarian cysts in dairy cows after PRID treatment. J Vet Med Sci 2003;65:57-62.

[19] Iwakuma A, Suzuki Y, Haneishi T, Kajisa M, Kamimura S. Efficacy of intravaginal progesterone administration combined with prostaglandin f(2alpha) for cystic ovarian disease in Japanese Black cows. J Vet Med Sci 2008;70:1077-83.

[20] Kim IH, Suh GH, Kim UH, Kang HG. A CIDR-based timed AI protocol can be effectively used for dairy cows with follicular cysts. Anim Reprod Sci 2006;95:206-13.

[21] Jou P, Buckrell BC, Liptrap RM, Summerlee AJS, Johnson WH. Evaluation of the effect of GnRH on follicular ovarian cysts in dairy cows using trans-rectal ultrasonography. Theriogenology 1999;52:923-37.

[22] Yániz JL, Murugavel K, López-Gatius F. Recent developments in oestrous synchronization of postpartum dairy cows with and without ovarian disorders. Reprod Domest Anim 2004;39:86-93.

[23] El-Zarkouny SZ, Shaaban MM, Stevenson JS. Blood metabolites and hormone-based programmed breeding treatments in anovular lactating dairy cows. J Dairy Sci 2011;94:6001-10.

[24] López-Gatius F, López-Béjar M. Reproductive performance of dairy cows with ovarian cysts after different GnRH and cloprostenol treatments. Theriogenology 2002;58:1337-48.

[25] Bisinotto RS, Chebel RC, Santos JE. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. J Dairy Sci 2010;93:3578-87.

[26] Gundling N, Drews S, Hoedemaker M. Comparison of two different programmes of ovulation synchronization in the treatment of ovarian cysts in dairy cows. Reprod Domest Anim 2015;50:893-900.

[27] De Rensis F, Bottarelli E, Battioni F, Capelli T, Techakumphu M, Garcia-Ispierto I, et al. Reproductive performance of dairy cows with ovarian cysts after synchronizing ovulation using GnRH or hCG during the warm or cool period of the year. Theriogenology 2008;69:481-4.

[28] Navanukraw C, Khanthusaeng V, Kraisoon A, Suwannarit D, Jarassaeng C, Aiumlamai S. Synchronization of ovulation with human chorionic gonadotropin in lactating dairy cows with ovarian cysts during heat stress. Trop Anim Health Prod 2015;47:945-51.

[29] Denicol AC, Lopes G, Jr., Mendonca LG, Rivera FA, Guagnini F, Perez RV, et al. Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. J Dairy Sci 2012;95:1794-806.

[30] Wiltbank MC, Souza AH, Carvalho PD, Cunha AP, Giordano JO, Fricke PM, et al. Physiological and practical effects of progesterone on reproduction in dairy cattle. Animal 2014;8 Suppl 1:70-81.

[31] Bartolome J, Hernandez J, Sheerin P, Luznar S, Kelbert D, Thatcher WW, et al. Effect of pretreatment with bovine somatotropin (bST) and/or gonadotropin-releasing hormone (GnRH) on conception rate of dairy cows with ovarian cysts subjected to synchronization of ovulation and timed insemination. Theriogenology 2003;59:1991-7.

[32] Bartolome JA, Sozzi A, McHale J, Melendez P, Arteche AC, Silvestre FT, et al. Resynchronization of ovulation and timed insemination in lactating dairy cows, II: assigning

protocols according to stages of the estrous cycle, or presence of ovarian cysts or anestrus. Theriogenology 2005;63:1628-42.

[33] Stevenson JS, Pursley JR, Garverick HA, Fricke PM, Kesler DJ, Ottobre JS, et al. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J Dairy Sci 2006;89:2567-78.

[34] Rathbone MJ, Bunt CR, Ogle CR, Burggraaf S, Macmillan KL, Burke CR, et al. Reengineering of a commercially available bovine intravaginal insert (CIDR insert) containing progesterone. J Control Release. 2002;85:105-15.

[35] Cerri RL, Rutigliano HM, Bruno RG, Santos JE. Progesterone concentration, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. Anim Reprod Sci 2009;110:56-70.

[36] Long ST, Yoshida C, Nakao T. Plasma progesterone profile in ovariectomized beef cows after intra-vaginal insertion of new, once-used or twice-used CIDR. Reprod Domest Anim 2009;44:80-2.

[37] Carvalho NA, Soares JG, Souza DC, Vannucci FS, Amaral R, Maio JR, et al. Different circulating progesterone concentrations during synchronization of ovulation protocol did not affect ovarian follicular and pregnancy responses in seasonal anestrous buffalo cows. Theriogenology 2014;81:490-5.

[38] de Graaff W, Grimard B. Progesterone-releasing devices for cattle estrus induction and synchronization: Device optimization to anticipate shorter treatment durations and new device developments. Theriogenology 2018;112:34-43.

[39] National Research Council. Nutrient Requirements of Dairy Cattle. Seventh Revised Edition. National Academy of Science, 2001.

[40] Gümen A, Guenther JN, Wiltbank MC. Follicular Size and Response to Ovsynch Versus Detection of Estrus in Anovular and Ovular Lactating Dairy Cows. J Dairy Sci 2003;86:3184-94.

[41] Kendall PE, Webster JR. Season and physiological status affects the circadian body temperature rhythm of dairy cows. Livest Sci 2009;125:155-60.

[42] Hastie T. gam: Generalized Additive Models. R package version 1.12. http://CRAN.R-project.org/package=gam. 2015.

[43] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014.

[44] Ambrose DJ, Schmitt EJP, Lopes FL, Mattos RC, Thatcher WW. Ovarian and endocrine responses associated with the treatment of cystic ovarian follicles in dairy cows with gonadotropin releasing hormone and prostaglandin F2alpha, with or without exogenous progesterone. Can Vet J 2004;45:931-7.

[45] Yilmazbas-Mecitoglu G, Karakaya E, Keskin A, Alkan A, Okut H, Gumen A. Effects of presynchronization with gonadotropin-releasing hormone-prostaglandin F2alpha or progesterone before Ovsynch in noncyclic dairy cows. J Dairy Sci 2012;95:7186-94.

[46] Bisinotto RS, Castro LO, Pansani MB, Narciso CD, Martinez N, Sinedino LD, et al. Progesterone supplementation to lactating dairy cows without a corpus luteum at initiation of the Ovsynch protocol. J Dairy Sci 2015;98:2515-28.

[47] Lima JR, Rivera FA, Narciso CD, Oliveira R, Chebel RC, Santos JE. Effect of increasing amounts of supplemental progesterone in a timed artificial insemination protocol on fertility of lactating dairy cows. J Dairy Sci 2009;92:5436-46.

[48] McDougall S, Compton CW, Anniss FM. Effect of exogenous progesterone and oestradiol on plasma progesterone concentrations and follicle wave dynamics in anovulatory anoestrous post-partum dairy cattle. Anim Reprod Sci 2004;84:303-14.

[49] Gumen A, Keskin A, Yilmazbas-Mecitoglu G, Karakaya E, Alkan A, Okut H, et al. Effect of presynchronization strategy before Ovsynch on fertility at first service in lactating dairy cows. Theriogenology 2012;78:1830-8.

[50] Bello NM, Steibel JP, Pursley JR. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of ovsynch in lactating dairy cows. J Dairy Sci 2006;89:3413-24.

[51] Sahu SK, Cockrem JF, Parkinson TJ, Laven RA. Effects of progesterone inclusion in a gonadotropin-prostaglandin-gonadotropin programme on follicular dynamics and ovulation synchronisation of pasture-based dairy cows with anoestrous. Research Vet Sci 2015;102:200-5.

[52] Kaneko K. Conception rate in Holstein dairy cows having both normal sized follicles and cystic follicles at estrus. Asian Pac J Reprod 2016;5:406-10.

Figure legend

Fig. 1. A Schematic illustration of ovulation synchronization treatments used in the present study. GnRH = 100µg gonadorelin; $PGF_{2\alpha}$= 500 µg cloprostenol; FTAI= Fixed time artificial insemination; New-CIDR = insertion of a new controlled internal drug-release containing 1.38 g of progesterone during the shaded period; Reused-CIDR= insertion of a reused CIDR insert that was previously used for 7 days in another cow.

Fig. 2. Results of ultrasonographic examination of follicular cystic cows treated with CIDR, G-New CIDR, RG-Ovsynch or Reused-CIDR treatments. (A) Dominant ovarian structure at the time of CIDR removal or PG administration. (B) proportion of cows with a follicular cyst at insemination (C) Dominant ovarian structures at days 8 to 10 post- insemination. The cows were classified as having either a follicular cyst [cows with a follicular cyst without a luteal tissue], a follicle [cows with a follicular structure without a follicular cyst or a luteal structure], a CL [cows with a CL without a follicular cyst], a CL plus follicular cyst [cows with both a follicular cyst and a CL], or luteal cyst [cows with a luteal cyst]. Luteal structures collectively refer to cows that have a CL, a luteal cyst or a CL plus a follicular cyst. Follicular cyst collectively refers to cows that have a follicular cyst or a CL plus a follicular cyst.

Fig. 3. Least square means of serum progesterone concentration at different time point in cows with severe ovarian inactivity that received a new or a reused CIDR inserts. Error bars indicate 95% confidence intervals of the least square means. Different superscripts indicate differences between new and reused CIDR inserts within the same examination time point as well as differences between time points within the same insert type (alpha = 0.05, Tukey-adjusted).

Table 1

Examination time points and findings to be recorded during ultrasongraphic examination of

	At the time of PGF2α administration	At the time of insemination	8-10 days post-insemination
Number of cows	238	179	256
Finding to be recorded	• Disappearance of cyst by this time • Appearance of luteal structure by this time (corpus luteum or cyst luteinization)	• Disappearance of cyst by this time	• Disappearance of cyst by this time • Ovulation at the end of the treatment protocol based on appearance of corpus luteum that was not present at the time of insemination

subgroup of cows submitted to RG-Ovsynch, New-CIDR and Reused-CIDR treatment protocols

Table 2

Multivariable logistic regression model for variables associated with P/AI 30 and P/AI 70 in follicular cystic cows.

Variables	**Level**	**P/AI n (%)**	**OR**	**95% confidence interval of OR**	P-value
P/AI 30					
	Less than 200	161/445 (36.2)	Ref		
DIM	200-400	39/179 (21.8)	0.56	0.37, 0.86	0.01
	> 400	14/40 (35.00)	0.85	0.41, 1.68	0.64
THI	<65	58/150 (38.7)	Ref		
	≥65 to <70	76/185 (41.1)	1.04	0.66, 1.65	0.85
	≥70 to <75	39/160 (24.4)	0.49	0.29, 0.81	0.01
	≥ 75	41/169 (24.3)	0.48	0.29 - 0.78	0.01
	Ovsynch	8/38 (21.1)	Ref		
	New-CIDR	91/261 (34.9)	2.09	0.94, 5.16	0.09
Treatments	G-New CIDR	51/161 (31.7)	2.26	0.98, 5.73	0.07
	RG-Ovsynch	49/141 (34.7)	2.63	1.13, 6.72	0.03
	Reused CIDR	15/63 (23.8)	1.05	0.39, 2.94	0.93
P/AI 70					
	<65	51/150 (34.0)	Ref		
THI	≥65 to <70	65/185 (35.1)	1.01	0.64, 1.61	0.73
	≥70 to <75	34/160 (21.3)	0.46	0.27, 0.78	0.002
	≥ 75	37/169 (21.9)	0.46	0.27, 0.77	0.002
	Ovsynch	7/38 (18.4)	Ref		
	New-CIDR	78/261 (29.9)	2.01	0.87, 5.17	0.09
Treatments	G-New CIDR	47/161 (29.2)	2.19	0.93, 5.84	0.09
	RG-Ovsynch	43/141 (30.5)	2.49	1.04, 6.66	0.05
	Reused CIDR	12/63 (19.1)	0.96	0.34, 2.86	0.79

OR = odds ratio; DIM = Days in milk; THI = Temperature humidity index; Ovsynch = GnRH injection at day 0 and 9, $PGF_{2\alpha}$ administration at day 7 and insemination at day 10; New-CIDR = the same as Ovsynch with insertion of a new CIDR between days 0 and 7; Reused-CIDR = the same as Ovsynch with insertion of reused CIDR between day 0 and 7, G-New CIDR = the same as New-CIDR with administration of Pre-GnRH 7 days before the start of the New-CIDR; RG-Ovsynch = repeated weekly doses of Pre-GnRH until a detection of a luteal structure upon ultrasonographic examination, at this point the Ovsynch was initiated.

Table 3

Dominant ovarian structure and cyst retention at each examination time points and its relation to pregnancy probability at day 30

Examination time (n)	Variable	Descriptive statistics			Relationship between dominant ovarian structure and the risk of P/AI 30*		
		Level	NO. (%)	P/AI 30 NO (%)	Odds ratio	P-value	95% confidence interval of OR
On the beginning of the Ovsynch (141) **	Dominant Ovarian structures	CL	75 (53.2)	31 (41.3)	Ref		
		Luteal cyst	17(12.1)	7 (41.3)	0.96	0.94	0.31, 2.87
		CL+ Follicular cyst	49(34.7)	11 (22.5)	0.46	0.07	0.19, 1.06
at the time of $PGF_{2\alpha}$ administratoin (238)	Dominant Ovarian structures	Follicle	21 (8.8)	6 (28.6)	Ref		
		CL	42 (17.7)	17 (40.5)	1.70	0.38	0.52, 5.96
		Luteal cyst	33 (13.9)	8 (24.2)	0.82	0.76	0.23, 3.05
		Follicular cyst	66 (27.7)	20 (30.3)	1.06	0.92	0.36, 3.39
		CL + Follicular cyst	76 (31.9)	25 (32.9)	1.286	0.67	0.42, 4.25
	Luteal structure#	No	87 (36.6)	26(29.9)	Ref		
		yes	151 (63.4)	50 (33.1)	1.19	0.59	0.64, 2.25
	Follicular cyst¶	No	96 (40.3)	31(32.3)	Ref		
		Yes	142 (59.7)	45 (31.7)	0.97	0.91	0.55, 1.75
At day of insemination (179)	Follicular cyst	No	83 (46.4)	24(28.9)	Ref		
		yes	96 (53.6)	25(26.1)	0.85	0.63	0.43, 1.67
At 8-10 days post-insemination (256)	Follicular cyst¶	No	141 (55.1)	55(39.0)	Ref		
		Yes	115 (44.9)	28(24.4)	0.50	0.01	0.28, 0.87
	Dominant ovarian structures***	CL	133 (52.0)	55 (41.4)	Ref		
		CL+ Follicular cyst	92 (36.0)	28 (30.4)	0.61	0.1	0.34, 1.09

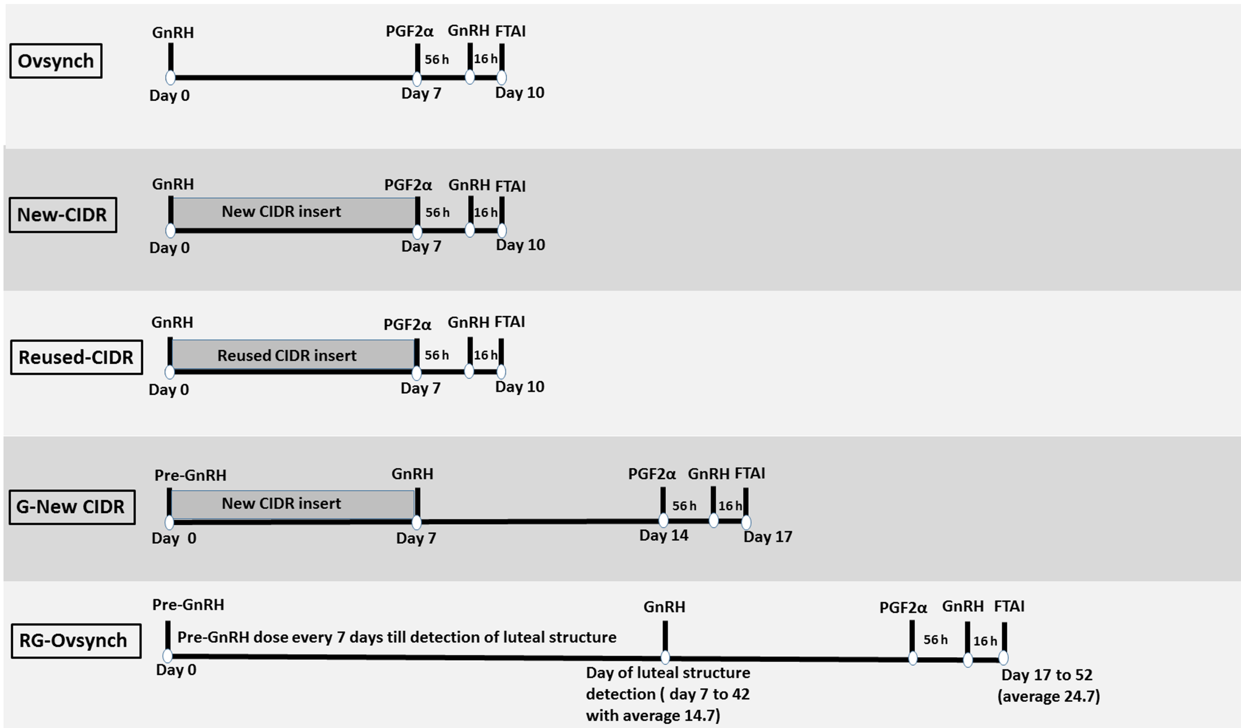
*Calculated after adjustment for THI and treatment protocols

**Applies only to the RG-Ovsynch

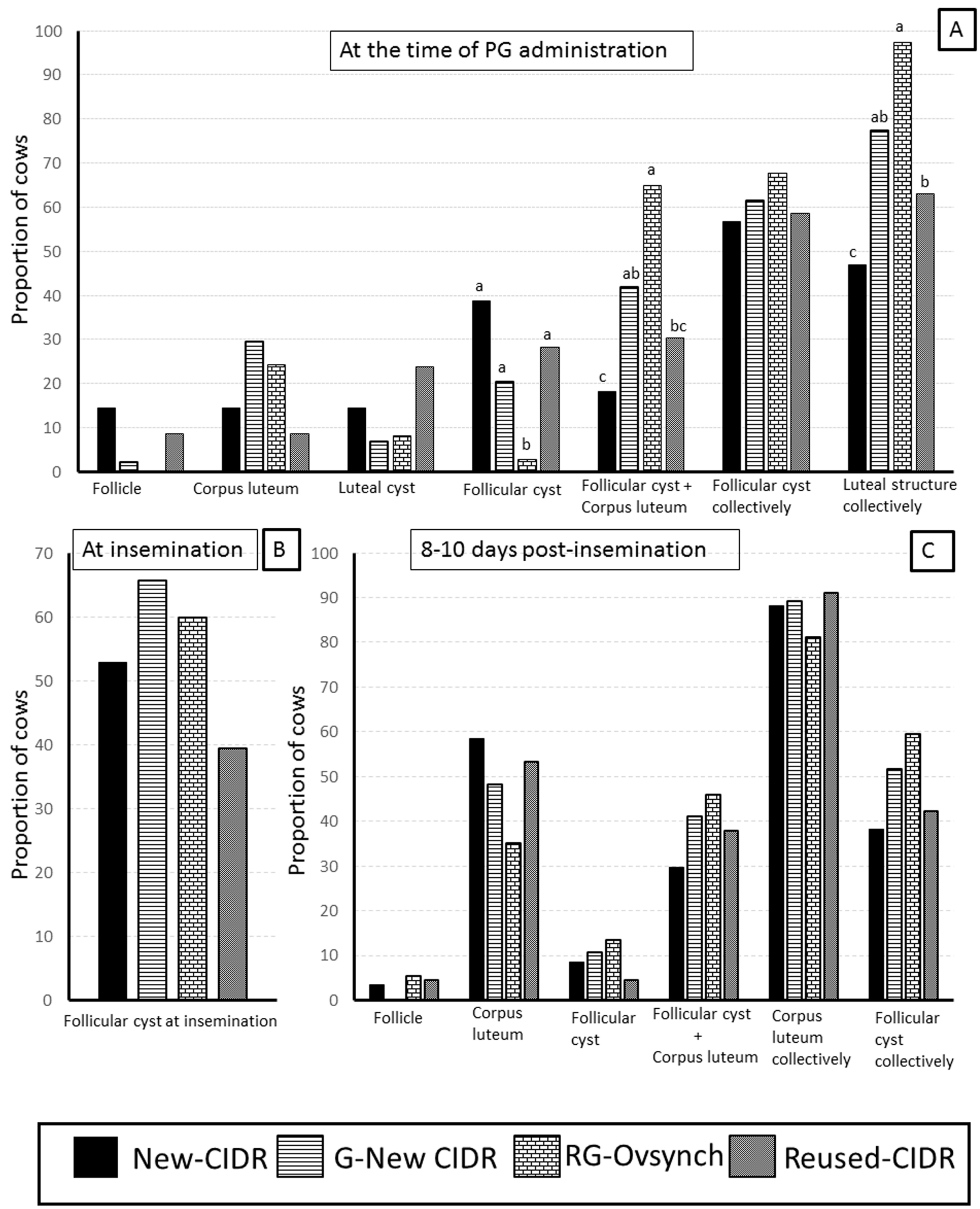
*** 8 cows that were identified with a follicle and 23 cows identified only with a follicular cyst were excluded from the analysis as these resulted in complete separation in the model

Cows classified as having a luteal structure were identified either with a CL, a Luteal cyst or a CL plus a follicular cyst. Cows without a luteal structure were identified with a follicle or a follicular cyst.

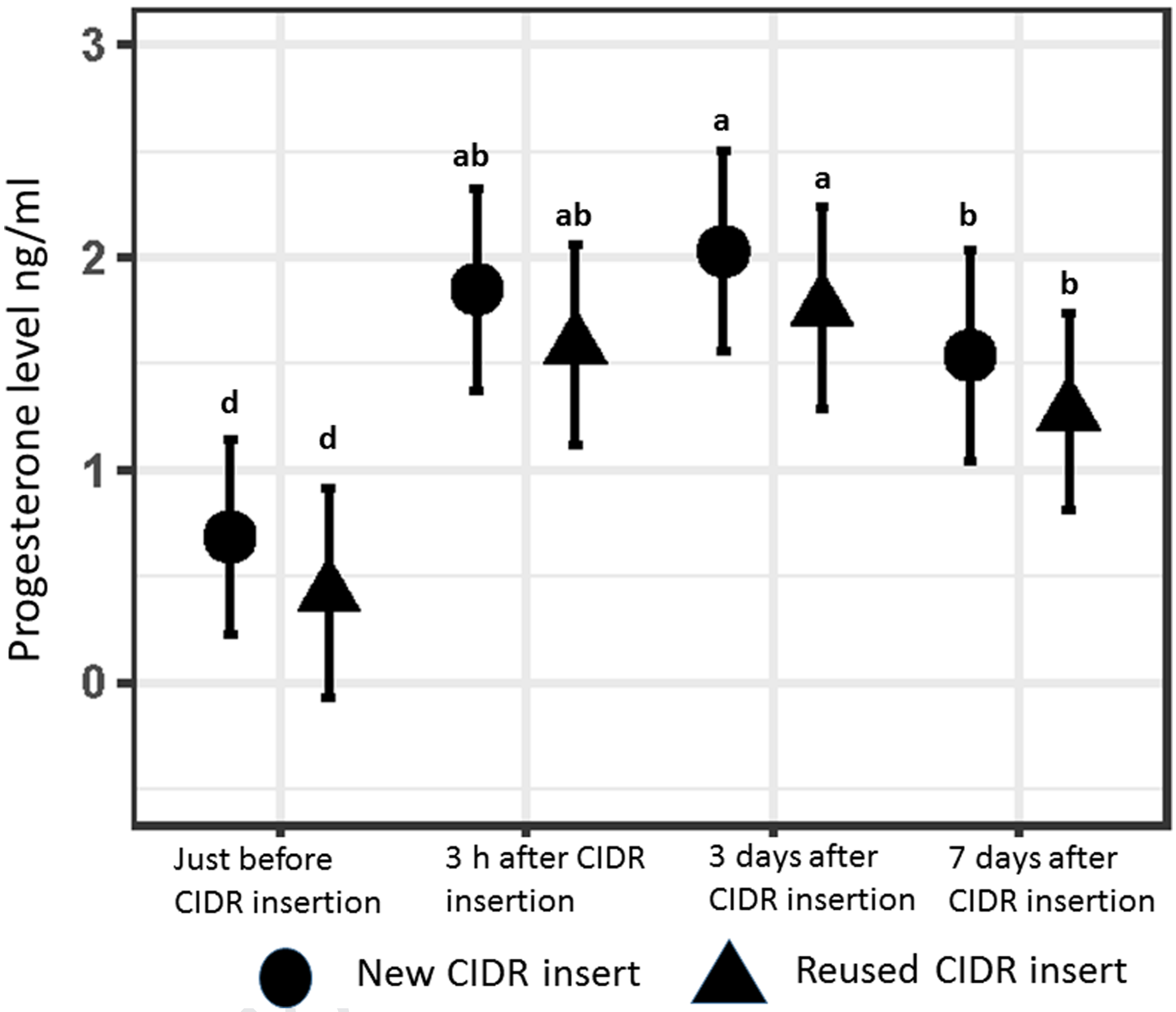
¶Cows with a follicular cyst included cows that had a follicular cyst or a CL plus a follicular cyst. Cows without a follicular cyst included cows that had a follicle or a CL as dominant ovarian structures.



Ovsynch
GnRH
PGF2α
56 h
GnRH
16 h
FTAI
Day 0
Day 7
Day 10
New-CIDR
GnRH
New CIDR insert
PGF2α
56 h
GnRH
16 h
FTAI
Day 0
Day 7
Day 10
Reused-CIDR
GnRH
Reused CIDR insert
PGF2α
56 h
GnRH
16 h
FTAI
Day 0
Day 7
Day 10
G-New CIDR
Pre-GnRH
New CIDR insert
GnRH
PGF2α
56 h
GnRH
16 h
FTAI
Day 0
Day 7
Day 14
Day 17
RG-Ovsynch
Pre-GnRH
Pre-GnRH dose every 7 days till detection of luteal structure
GnRH
PGF2α
56 h
GnRH
16 h
FTAI
Day 0
Day of luteal structure detection (day 7 to 42 with average 14.7)
Day 17 to 52 (average 24.7)



A
At the time of PG administration
Proportion of cows
100
90
80
70
60
50
40
30
20
10
0
a
ab
a
b
c
a
ab
bc
a
a
b
a
c
Follicle
Corpus luteum
Luteal cyst
Follicular cyst
Follicular cyst + Corpus luteum
Follicular cyst collectively
Luteal structure collectively
At insemination
B
70
60
50
40
30
20
10
0
Follicular cyst at insemination
8-10 days post-insemination
C
Follicle
Corpus luteum
Follicular cyst
Follicular cyst + Corpus luteum
Corpus luteum collectively
Follicular cyst collectively
New-CIDR
G-New CIDR
RG-Ovsynch
Reused-CIDR


Progesterone level ng/ml
3
2
1
0
ab
ab
a
a
b
b
d
d
Just before CIDR insertion
3 h after CIDR insertion
3 days after CIDR insertion
7 days after CIDR insertion
New CIDR insert
Reused CIDR insert

- In follicular cyst, weekly GnRH until detection of CL before Ovsynch improved P/AI
- New CIDR and G-New CIDR protocols tended improve the P/AI in follicular cystic cows
- Reused CIDR inserts on days 0 to 7 of Ovsynch did not improve the P/AI
- P4 concentration up to 7 days post-insertion of new and reused-CIDR were similar