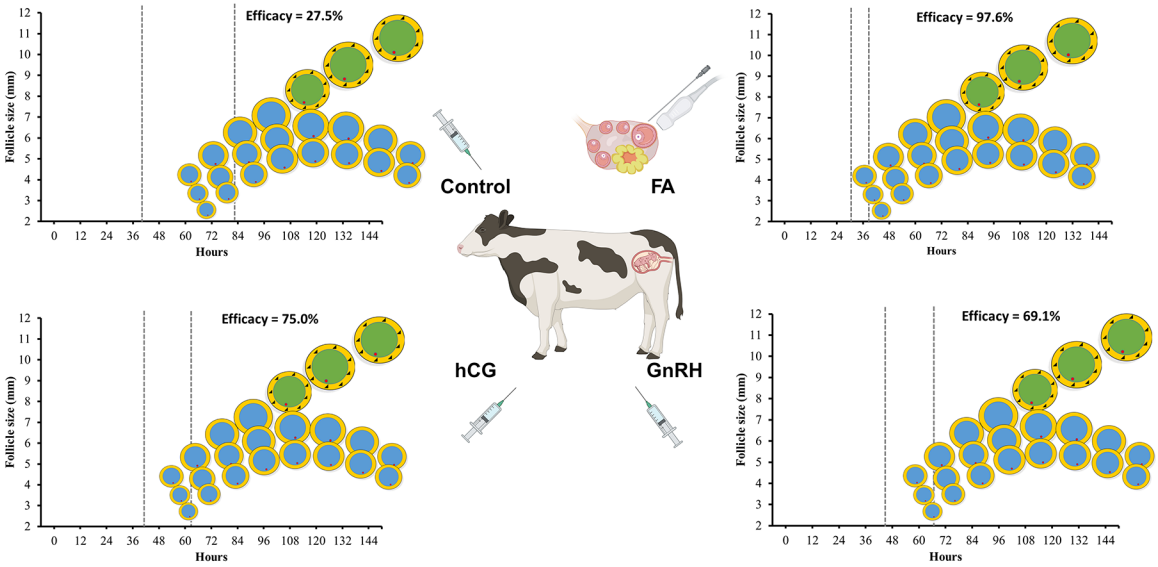
JDS Communications®
2024; 6:154–159

https://doi.org/10.3168/jdsc.2024-0629
Short Communication
Physiology

Efficacy of methods to synchronize follicular wave emergence in pregnant heifers

Cameron B. Hayden,[1] Jessica C. L. Motta,[1] Rodrigo V. Sala,[2] Nora M. Bello,[3] Marco A. Coutinho da Silva,[4] and Alvaro García-Guerra[1]*

Graphical Abstract



Summary

Synchronization of follicular wave emergence (FWE) and initiation of ovarian superstimulatory treatments at the time of FWE improves in vitro embryo production in pregnant cattle. Follicle ablation (FA) is often used to synchronize FWE; however, this method is technically complex and, thus, difficult to implement in field conditions. Induction of ovulation is a practical method to synchronize FWE; however, the follicular and endocrine characteristics of pregnancy pose a challenge for utilizing this approach. Thus, the present study investigated the efficacy of FA, gonadotropin-releasing hormone (GnRH), and human chorionic gonadotropin (hCG) for synchronization of FWE in pregnant heifers. Ovulatory response was greater for hCG- (81%) than GnRH-treated (50%) heifers. There was no evidence for a difference in FWE synchronization efficacy among hCG, GnRH, and FA. Nevertheless, FA resulted in a shorter and less variable interval from treatment to FWE, thus providing a more precise control of follicle development.

Highlights

- Synchronization of FWE improves in vitro embryo production.
- Methods for synchronization of FWE in pregnant cattle have not been evaluated.
- Follicle ablation, hCG, and GnRH are efficacious for synchronization of FWE.
- Follicle ablation results in a shorter and less variable interval to FWE.

[1]Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, [2]STgenetics, The Ohio Heifer Center, South Charleston, OH 45368, [3]USDA, Agricultural Research Service, Beltsville, MD 20705, [4]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH 43210.
*Corresponding author: garciaguerra.1@osu.edu. Received July 01, 2024. Accepted August 30, 2024.

The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-24. Nonstandard abbreviations are available in the Notes.

JDS Communications®
2024; 6:154–159

https://doi.org/10.3168/jdsc.2024-0629
Short Communication
Physiology

Efficacy of methods to synchronize follicular wave emergence in pregnant heifers

Cameron B. Hayden,[1] Jessica C. L. Motta,[1] Rodrigo V. Sala,[2] Nora M. Bello,[3] Marco A. Coutinho da Silva,[4] and Alvaro García-Guerra[1]*

Abstract: The objective of the present study was to evaluate the efficacy of various methods for synchronization of follicular wave emergence (FWE) in pregnant heifers. Pregnant (60 d of gestation) Holstein heifers (n = 86) arranged in cohorts were randomly assigned to be administered 172 µg of gonadorelin acetate (GnRH), 3,300 IU of human chorionic gonadotropin (hCG), follicular ablation of follicles >5 mm (FA), or saline (control). Ultrasonography was performed to determine ovulation and emergence of a new follicular wave. Data were analyzed using generalized linear mixed models with treatment as a fixed effect and cohort as a random effect. Ovulatory response was greater for hCG (81.0%; 95% CI: 58.0–92.9) than GnRH-treated (50.0%; 95% CI: 28.8–71.2) heifers, whereas ovulation was not observed for heifers in the FA or control groups. Heifers in the FA group had a shorter (34.8 ± 1.7 h) interval from treatment to FWE compared with heifers in the hCG (51.8 ± 5.3 h), GnRH (56.8 ± 5.3 h), and control (61.4 ± 9.8 h) groups. Furthermore, treatments differed in variability of time to FWE, whereby FA-treated heifers had less variable, more consistent responses than hCG and GnRH heifers. These groups were, in turn, less variable in time to FWE than heifers in the control group. Synchronization of FWE efficacy was greater in FA (97.6%; 95% CI: 69.8%–99.9%) and hCG-treated (75.0%; 95% CI: 52.8%–89.0%) heifers than control (27.5%; 95% CI: 12.2%–50.9%) heifers, with marginal evidence for a difference between GnRH (69.1%; 95% CI: 46.4%–85.2%) and control heifers. Overall, we found no evidence for differences in FWE synchronization efficacy between hCG, GnRH, and FA. Nevertheless, FA resulted in a shorter and less variable interval from treatment to FWE, thus providing a more precise control of follicular development.

The use of embryo transfer allows for rapid dissemination of desirable genetics by increasing the reproductive potential of genetically superior cattle. Ovum pick-up (**OPU**) and in vitro embryo production (**IVEP**) has increased greatly during the last decade, whereby ~80% of bovine embryos produced worldwide are done through IVEP (Viana, 2023). A distinct and unique advantage of IVEP is the ability for it to be used in pregnant females, which otherwise could not be used for embryo production with conventional methods. Furthermore, oocyte developmental competence and therefore IVEP efficacy appears to be greater in pregnant than nonpregnant heifers (Baruselli et al., 2016). Nevertheless, the unique reproductive physiology of pregnancy poses a challenge for the manipulation of ovarian function often required for IVEP.

Developmental competence of oocytes recovered after OPU is one of the major limitations for the successful implementation of IVEP (Baruselli et al., 2016). Factors associated with oocyte developmental competence include follicular size and developmental stage (Hagemann, 1999; Machatkova et al., 2004), underscoring the need for precise control of follicular development. Synchronization of follicular wave emergence (**FWE**) allows for the control of follicular development and has been reportedly shown to increase oocyte viability, blastocyst percentage and embryo yield in nonstimulated cows (Ongaratto et al., 2015; Cavalieri et al., 2018). Furthermore, ovarian superstimulation using FSH leads to greater oocyte developmental competence and embryo production (Hayden et al., 2023) and is commonly used to improve IVEP efficacy. Moreover, synchronization of FWE and initiation of ovarian superstimulation at the time of FWE improved superstimulatory response, oocyte developmental competence and embryo production (Hayden et al., 2022). The control of ovarian follicular development by synchronizing FWE therefore appears as an important step for the optimization of IVEP in cattle.

Synchronization of FWE is achieved by removal of the dominant follicle, either hormonally or through physical ablation, to eliminate its suppressive effects on the remaining follicles of the wave (Bó et al., 1995; García-Guerra et al., 2018c). Follicular ablation (**FA**) is an effective method for synchronization of FWE (Bergfelt et al., 1994); however, it is difficult to implement in field conditions. The induction of ovulation, using GnRH or human chorionic gonadotropin (**hCG**), is commonly used as a practical method to synchronize FWE in synchronization protocols for timed AI (Wiltbank and Pursley, 2014); however, there is a paucity of research on its use in pregnant females. Ovulatory response following administration of GnRH or hCG is associated with follicular size, such that greater follicle size increases the likelihood of ovulation (Sartori et al., 2001; García-Guerra et al., 2018a). Furthermore, ovulatory response after GnRH administration is associated with circulating progesterone ($\mathbf{P_4}$) and estradiol (**E2**) concentrations, as these hormones modulate the magnitude of LH release (Motta et al., 2020). Pregnancy represents a challenge due to elevated P_4 concentrations and relatively smaller dominant follicle size (Ginther et al., 1989; Ginther et al., 1996), which can decrease the likelihood of ovulation after GnRH or hCG administration. Owing to the limitations of FA, it is common for practitioners to forgo synchronization of

[1]Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, [2]STgenetics, The Ohio Heifer Center, South Charleston, OH 45368, [3]USDA, Agricultural Research Service, Beltsville, MD 20705, [4]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH 43210.
*Corresponding author: garciaguerra.1@osu.edu. Received July 01, 2024. Accepted August 30, 2024.

The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-24. Nonstandard abbreviations are available in the Notes.

FWE when conducting OPU/IVEP in pregnant females emphasizing the need for more practical approaches. The objective of the present study, therefore, was to evaluate the efficacy of GnRH, hCG, and FA for synchronization of FWE in pregnant heifers. It was hypothesized that FA would result in a shorter and less variable time of FWE than either hCG or GnRH, and thus have greater efficacy for synchronizing FWE.

Animal procedures were approved by the Institutional Animal Care and Use Committee at The Ohio State University (#2020A00000056). Pregnant Holstein heifers (n = 86) at 60 d of gestation (study d 0) with a mean (± SD) age of 17.8 ± 2.2 mo and 3.2 ± 0.3 body condition score (scale 1 to 5; 1 = emaciated 5 = obese) were blocked by cohort and randomly assigned into one of 4 treatment groups on d 0: GnRH (172 µg of gonadorelin acetate i.m.; 4 mL; Fertagyl, Merck, Kenilworth, NJ); hCG (3,300 IU of hCG i.m.; 3.3 mL; Chorulon, Merck, Kenilworth, NJ); FA (ultrasound-guided aspiration of all follicles >5 mm on both ovaries); or control (4 mL of 0.9% sodium chloride i.m.; Vet One Sterile Saline, Nova-Tec, Grand Island, NE). Follicular ablation was performed as previously described (Hayden et al., 2022) using a 9 MHz microconvex array transducer attached to a portable scanner (Ibex Evo II; E. I. Medical Imaging, Loveland, CO). The dosages of hCG and GnRH were selected to optimize ovulatory response, given the smaller dominant follicle size typically observed during pregnancy (Ginther et al., 1989) and the greater LH stimulation required to induce ovulation of small dominant follicles (Sartori et al., 2001). The GnRH dosage used was based on the reported increase in ovulatory response when twice the label-recommended dosage was administered (Giordano et al., 2013). A 3,300 IU hCG dosage was selected because this dosage was among those that maximized ovulatory response when administered on d 7 after ovulation (Cabrera et al., 2021).

Ovarian structures were evaluated using transrectal B-mode ultrasonography (7.5 MHz linear array probe, Ibex EVO, E.I. Medical Imaging) every 12 h from study d 0 through d 3, and every 24 h thereafter until d 7. At each examination, a cine-loop of each ovary was recorded and later analyzed by a single operator using image processing software (ImageJ version 1.54d, National Institutes of Health). A sketch was made of each ovary, and follicle and corpus luteum (**CL**) number, size, and relative location were recorded. Follicle diameter and CL volume were determined as previously described (García-Guerra et al., 2018b).

Blood samples were collected on d 0 and d 7 via coccygeal venipuncture into 10-mL evacuated collection tubes. Samples were centrifuged at 1,300 × *g* and 4°C for 13 min and serum was transferred into vials, frozen, and stored at −20°C until assayed. Serum P_4 concentration was determined using a solid-phase radioimmunoassay as previously described (García-Guerra et al., 2020). Samples were evaluated in a single assay, and assay sensitivity and intra-assay CV were 0.02 ng/mL and 0.3%, respectively.

Ovulation was defined as disappearance of a follicle ≥8.5 mm within 72 h of treatment followed by the presence of a new CL in the same location on d 7. Follicular wave emergence was determined retrospectively (Ginther et al., 1989) and was defined as the time point of first detection of a ~4 mm follicle subsequently identified as dominant (>8.5 mm). Efficacy of synchronization of FWE was defined as emergence of a new follicular wave within a predefined treatment specific 24-h period. The treatment specific periods for each treatment group were as follows: 36 h (range 24–48 h) for FA (Bergfelt et al., 1994); 48 h (range 36–60 h) for GnRH (Pursley et al., 1995); 48 h (range 36–60 h) for hCG (Cabrera et al., 2021); and 48 h (range 36–60 h) for control. The selection of the specified period for the control group was performed arbitrarily considering heifers in this group were expected to initiate a new follicular wave randomly.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.). Six heifers were removed from the study due to incomplete data; thus, data from 80 heifers were used for analysis. For continuous outcomes, general linear mixed models were fitted, assuming a normal distribution and identity link function. The linear predictor included the fixed effect of treatment and random effect of cohort. Heterogeneous residual variances were specified, when needed, as determined using fit statistics and likelihood ratio tests. For binary outcomes, generalized linear mixed models were fitted assuming a Bernoulli distribution and using Laplace approximation. Overdispersion was evaluated using the fit statistic Pearson chi-square over df. Extreme category problems were apparent for efficacy of FWE synchronization, for which a logistic regression model was fitted using Firth's correction. Pairwise comparisons between treatment groups were conducted using Tukey–Kramer adjustments, and data are presented as LSM and SEM (continuous outcomes) or 95% CI (binary outcomes). In all cases, evidence of a treatment effect was considered at an experiment-wise type I error of 5%.

As expected, on d 0 of the study, we found no evidence for differences between treatment groups on largest follicle diameter, luteal tissue volume, or P_4 concentration ($P \geq 0.3$; Table 1). Ovulation was not observed in any control or FA heifers; thus, ovulatory response was evaluated only for hCG and GnRH heifers. There was evidence for a differential effect of treatment on ovulatory response ($P = 0.05$), where hCG heifers had a greater (81.0%; 95% CI: 58.0%–92.9%) probability of ovulation than GnRH heifers (50.0%; 95% CI: 28.8%–71.2%). The greater ovulatory response of hCG compared with GnRH is consistent with previous reports in dairy cows treated during the postovulatory period or pregnancy (Stevenson et al., 2008; Cabrera et al., 2021). For example, Stevenson et al. (2008) reported that ovulatory response to GnRH and hCG administration at 26 to 71 d of gestation in lactating dairy cows was ~25% and ~50%, respectively (Stevenson et al., 2008). Ovulatory response in the present study, however, seemed larger in magnitude to that reported by Stevenson et al. (2008), likely due to the greater dosages used. However, the results presented herein are consistent with those reported for lactating cows treated with similar dosages (Giordano et al., 2013; Cabrera et al., 2021). Ovulatory response differs between GnRH and hCG likely due to the different mechanisms by which these induce ovulation. For instance, ovulation following GnRH treatment is dependent on both follicular size and steroid hormone concentrations. Specifically, smaller follicle size and greater P_4 concentration often lead to reduced ovulatory response, the latter due to a reduction in the magnitude of the GnRH-induced LH release (Giordano et al., 2013; García-Guerra et al., 2018a). Conversely, hCG has LH-like activity and binds directly to the LH/choriogonadotropin receptor (**LHCGR**) of the dominant follicle. Therefore, the ovulatory response to hCG is not affected by circulating P_4, but still is associated with follicular size (Price and Webb, 1989). As gestation advances, maximum dominant follicle diameter decreases (Ginther et al., 1989; Ginther et al., 1996). Thus, although the smaller follicle size would reduce

Table 1. Least squares means (± SEM) for ovarian characteristics and circulating progesterone for pregnant heifers treated with follicular ablation (FA), GnRH, human chorionic gonadotropin (hCG), or saline (control)

	Treatment				
Item	Control	FA	GnRH	hCG	*P*-value
N	19	20	20	21	
Study d 0					
Largest follicle (mm)	10.4 ± 0.3	10.8 ± 0.3	10.8 ± 0.3	10.8 ± 0.3	0.84
Total luteal tissue (cm^3)	6.3 ± 0.6	6.0 ± 0.5	5.5 ± 0.4	5.2 ± 0.5	0.30
Progesterone (ng/mL)	12.4 ± 1.2	12.5 ± 1.2	11.3 ± 1.2	10.4 ± 1.2	0.30
Study d 7					
Total luteal tissue (cm^3)	6.2 ± 0.4^{b}	6.3 ± 0.4^{b}	6.6 ± 0.4^{b}	9.5 ± 0.4^{a}	<0.0001
Progesterone (ng/mL)	15.4 ± 1.7^{ab}	13.3 ± 1.3^{b}	12.1 ± 1.3^{b}	19.8 ± 1.9^{a}	0.0008

^{a,b}Different letters indicate treatment differences within a row ($P < 0.05$).

ovulatory response to both GnRH and hCG administration, the greater P_4 concentration likely reduces the ovulatory response to GnRH but not hCG, explaining the observed differences. Although hCG appears to be more effective for the induction of ovulation in pregnant heifers, it is important to note that hCG can induce a humoral response when used repeatedly (Giordano et al., 2012), albeit the effect of hCG-induced antibodies on ovulatory response remains to be determined.

The first hypothesis was that the interval between treatment and FWE would be less for FA than for hCG and GnRH-treated heifers. Overall, evidence indicated an effect of treatment on the interval from treatment to FWE ($P < 0.0001$; Figure 1A). Heifers treated with FA had a shorter interval from treatment to FWE compared with hCG, GnRH, and control pregnant heifers ($P \leq 0.05$), thereby supporting our hypothesis. However, we found no evidence for differences ($P > 0.83$) in the interval from treatment to FWE among control, hCG, and GnRH pregnant heifers. Results from previous research in nonpregnant females indicate that FWE occurs ~36 h after FA (Bergfelt et al., 1994) and ~48 h after the induction of ovulation with GnRH or hCG (Pursley et al., 1995). These reports are in agreement with the intervals observed in the present study for pregnant heifers. Follicular wave emergence for GnRH- and hCG-treated heifers was delayed, as hypothesized, likely because the mechanism by which these induce synchronization is not direct and requires ovulation (Pursley et al., 1995). Furthermore, FA heifers had a shorter interval from treatment to FWE even when compared with heifers that ovulated after hCG or GnRH ($P \leq 0.002$; Figure 1B).

Ovarian asynchrony is a limiting factor for the implementation of reproductive technologies (Bó et al., 1995), therefore, consistency in the time of FWE is a critical feature of practical interest of any method used for synchronization of FWE. Analysis of the data from the present study revealed evidence for treatment differences in the variability of the interval from treatment to FWE ($P < 0.001$). Variance for the time to FWE was less for FA (59 ± 19 h^2) than for hCG or GnRH (560 ± 132 h^2) heifers, which in turn was less than that for control (1,637 ± 579 h^2) heifers. The lesser variability in time of FWE for FA is consistent with a previous report in cyclic heifers (Martinez et al., 2000), and supports the first hypothesis. Increased variability in time to FWE following GnRH and hCG maybe explained by the lack of ovulation induction in all treated animals. Accordingly, we found no evidence ($P > 0.50$) for a difference in the variance among FA heifers (59 ± 19 h^2) and heifers that ovulated in response to GnRH (83 ± 40 h^2) or hCG (99 ± 35 h^2). Thus, the use of FA resulted in a more precise synchronization of FWE, which could prove beneficial for ovarian stimulation.

Follicular ablation can be considered the gold standard for synchronization of FWE due to the ability to visually verify the success of the procedure. In addition, efficacy of this method does not appear to be affected by hormone concentrations or follicular size. Alternative methods like GnRH or hCG administration are more practical for widespread utilization but are only effective if ovulation occurs. As a result, it was hypothesized that FA would be more efficacious in synchronizing FWE than both hCG and GnRH. Our findings indicate evidence for an effect of treatment on the efficacy to synchronize FWE ($P = 0.002$; Figure 2). Efficacy was greater ($P < 0.03$) in FA and hCG than in control heifers, with marginal evidence ($P = 0.06$) for a difference in efficacy between GnRH and control heifers, leading to rejection of our hypothesis. Interestingly, the efficacy of hCG and GnRH was not significantly different even though ovulatory response was greater for hCG than GnRH heifers. Nevertheless, these results should be taken with caution given the relatively small sample size and the admittedly limited information available in binary data, both of which are known to impair statistical power (Stroup et al., 2024). Furthermore, it is important to highlight that even in the absence of ovulation, a relatively small percentage of heifers would be expected to have spontaneous FWE within a specific period, as observed in the heifers of the control group, due to the periodic nature of follicular waves (Ginther et al., 1989, 1996).

Although FA is admittedly efficacious for synchronization of FWE, this technique is not without limitations. Residual follicles (i.e., follicles that become fluid-filled and continue to grow after FA), may still be hormonally active and thus have the potential to alter follicular dynamics and the efficacy of the procedure (Viana et al., 2013). Residual follicles were reported to occur in ~73% of aspirated follicles, and most (~65%) displayed estradiol-active phenotypes (Viana et al., 2013). Estradiol-active residual follicles would be most detrimental to synchronization of FWE, due to the inhibitory action of E_2 on FSH secretion (García-Guerra et al., 2018c). Residual follicles in the present study were observed in 9 (45%) heifers of the FA group. However, we found no evidence ($P = 0.19$) for a difference in time to FWE for heifers with (37.3 ± 2.4 h) or without (32.7 ± 2.3 h) residual follicles. These results suggest that even though residual follicles occur after FA, there is no apparent effect on synchronization of FWE.

Ovulation in response to hCG or GnRH administration during the luteal phase or pregnancy leads to accessory CL, increased

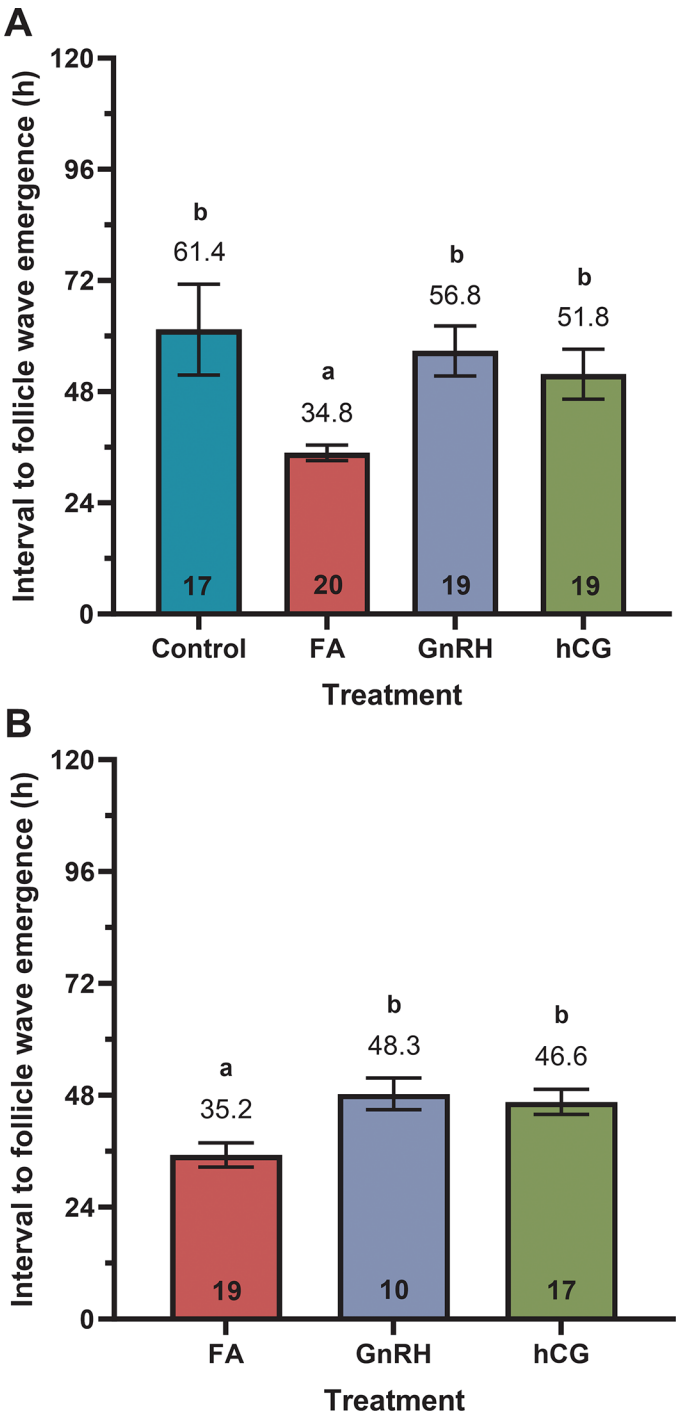


Figure 1. Least squares means (± SEM) for interval to follicular wave emergence in pregnant heifers treated with follicular ablation (FA), GnRH, human chorionic gonadotropin (hCG), or saline (control). (A) All treated heifers; (B) FA-treated heifers and GnRH- or hCG-treated heifers with a detected ovulation. Different letters (a,b) indicate treatment differences ($P \leq 0.05$).

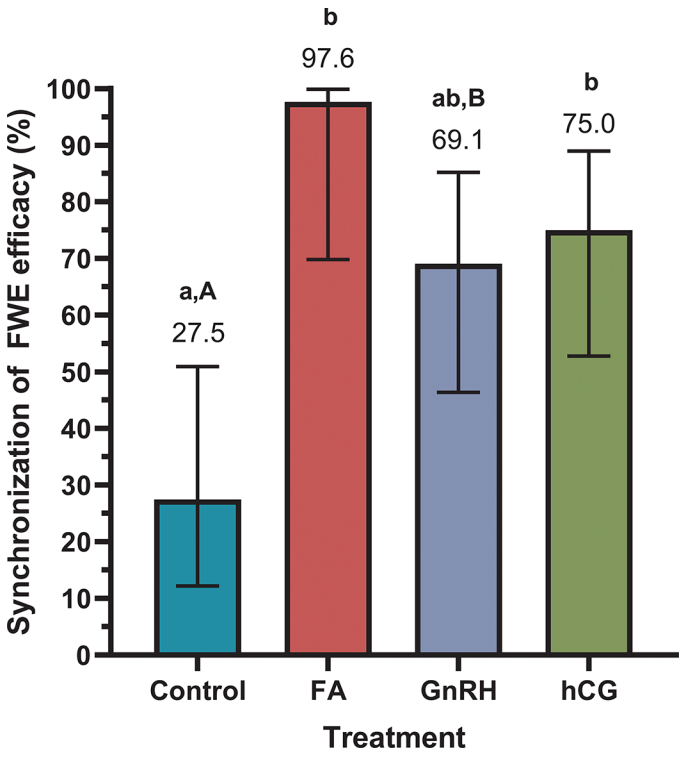


Figure 2. Efficacy of synchronization of follicular wave emergence (FWE; probability of successful FWE following treatment and 95% CI) in pregnant heifers treated with follicular ablation (FA), GnRH, human chorionic gonadotropin (hCG), or saline (control). Lowercase (a,b; $P \leq 0.05$) and uppercase (A,B; $P \leq 0.10$) letters indicate treatment differences.

luteal tissue volume and typically greater P_4 concentration (García-Guerra et al., 2020; Cabrera et al., 2021). In the present study, we observed an effect of treatment on total luteal tissue volume and circulating P_4 concentration on d 7 ($P < 0.001$; Table 1). Heifers treated with hCG had greater ($P < 0.003$) luteal tissue volume than control, FA, and GnRH-treated heifers, though no evidence ($P \geq 0.87$) for a difference was apparent between the latter. In addition, hCG-treated heifers had greater circulating P_4 concentration on d 7 than FA and GnRH-treated heifers ($P < 0.01$), whereas those of the control group were intermediate ($P > 0.10$). The larger luteal tissue volume and greater circulating P_4 concentration observed in hCG-treated heifers is consistent with previous reports (Schmitt et al., 1996; Cabrera et al., 2021) and likely explained by the greater ovulatory response following hCG administration. Furthermore, hCG has a long half-life, binds to LHCGR in small luteal cells stimulating P_4 production, and increases the number of large luteal cells of the existing CL (Farin et al., 1988; Schmitt et al., 1996). Conversely, the lack of evidence for any increase in luteal tissue volume and circulating P_4 in GnRH-treated pregnant heifers was unexpected (Stevenson et al., 2008; García-Guerra et al., 2020). Nevertheless, this is likely attributable to the poor ovulatory response after GnRH administration in the present study. It has been suggested that presence of luteal tissue at the time of OPU can hinder oocyte recovery efficiency (Ongaratto et al., 2015); therefore, before hCG can be used to synchronize FWE for OPU/IVEP, the effect of increased total luteal tissue volume on oocyte yield warrants further investigation.

In conclusion, results from the present study indicate that FA, hCG, and GnRH are efficacious for synchronization of FWE in pregnant heifers. Although FA is time-consuming, requires spe-

cialized equipment, and necessitates advanced technical skills, it results in a shorter and more consistent interval from treatment to FWE, which may be advantageous for ovarian stimulation before OPU/IVEP. The administration of hCG or GnRH is simpler and less costly, particularly in the case of GnRH, making it a practical alternative for FWE synchronization. However, the greater variability in the timing of FWE associated with hCG and GnRH could negatively affect the efficacy of ovarian stimulation. Additionally, induction of ovulation with hCG or GnRH is expected to increase luteal tissue volume, particularly with repeated use, and the effects of this on OPU remain unclear. Therefore, further research is needed to evaluate the impact of increased variability in FWE timing and greater luteal tissue volume on IVEP outcomes before hCG or GnRH can be recommended for use in ovarian superstimulation and OPU/IVEP protocols in pregnant heifers.

References

Baruselli, P., E. Batista, L. Vieira, R. Ferreira, B. Guerreiro, B. Bayeux, J. Sales, A. Souza, and L. Gimenes. 2016. Factors that interfere with oocyte quality for in vitro production of cattle embryos: Effects of different developmental & reproductive stages. Anim. Reprod. 13:264–272. https://doi.org/10.21451/1984-3143-AR861.

Bergfelt, D. R., K. C. Lightfoot, and G. P. Adams. 1994. Ovarian synchronization following ultrasound-guided transvaginal follicle ablation in heifers. Theriogenology 42:895–907. https://doi.org/10.1016/0093-691X(94)90113-W.

Bó, G. A., G. P. Adams, R. A. Pierson, and R. J. Mapletoft. 1995. Exogenous control of follicular wave emergence in cattle. Theriogenology 43:31–40. https://doi.org/10.1016/0093-691X(94)00010-R.

Cabrera, E. M., M. R. Lauber, E. M. Peralta, T. R. Bilby, and P. M. Fricke. 2021. Human chorionic gonadotropin dose response for induction of ovulation 7 days after a synchronized ovulation in lactating Holstein cows. JDS Commun. 2:35–40. https://doi.org/10.3168/jdsc.2020-0024.

Cavalieri, F. L. B., F. Morotti, M. M. Seneda, A. H. B. Colombo, M. A. Andreazzi, I. P. Emanuelli, and L. P. Rigolon. 2018. Improvement of bovine in vitro embryo production by ovarian follicular wave synchronization prior to ovum pick-up. Theriogenology 117:57–60. https://doi.org/10.1016/j.theriogenology.2017.11.026.

Farin, C. E., C. L. Moeller, H. Mayan, F. Gamboni, H. R. Sawyer, and G. D. Niswender. 1988. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. Biol. Reprod. 38:413–421. https://doi.org/10.1095/biolreprod38.2.413.

García-Guerra, A., A. M. O. Canavessi, P. L. J. Monteiro Jr., M. A. Mezera, R. Sartori, B. W. Kirkpatrick, and M. C. Wiltbank. 2018a. Trio, a novel bovine high fecundity allele: III. Acquisition of dominance and ovulatory capacity at a smaller follicle size. Biol. Reprod. 98:350–365. https://doi.org/10.1093/biolre/iox157.

García-Guerra, A., M. H. Kamalludin, B. W. Kirkpatrick, and M. C. Wiltbank. 2018b. Trio, a novel bovine high fecundity allele: II. Hormonal profile and follicular dynamics underlying the high ovulation rate. Biol. Reprod. 98:335–349. https://doi.org/10.1093/biolre/iox156.

García-Guerra, A., R. V. Sala, L. Carrenho-Sala, G. M. Baez, J. L. Motta, M. Fosado, J. F. Moreno, and M. C. Wiltbank. 2020. Postovulatory treatment with GnRH on day 5 reduces pregnancy loss in recipients receiving an in vitro produced expanded blastocyst. Theriogenology 141:202–210. https://doi.org/10.1016/j.theriogenology.2019.05.010.

García-Guerra, A., M. Wiltbank, S. E. Battista, B. W. Kirkpatrick, and R. Sartori. 2018c. Mechanisms regulating follicle selection in ruminants: Lessons learned from multiple ovulation models. Anim. Reprod. 15(Suppl. 1):660–679. https://doi.org/10.21451/1984-3143-AR2018-0027.

Ginther, O. J., L. Knopf, and J. P. Kastelic. 1989. Ovarian follicular dynamics in heifers during early pregnancy. Biol. Reprod. 41:247–254. https://doi.org/10.1095/biolreprod41.2.247.

Ginther, O. J., K. Kot, L. J. Kulick, S. Martin, and M. C. Wiltbank. 1996. Relationships between FSH and ovarian follicular waves during the last six months of pregnancy in cattle. Reproduction 108:271–279. https://doi.org/10.1530/jrf.0.1080271.

Giordano, J. O., M. C. Wiltbank, and P. M. Fricke. 2012. Humoral immune response in lactating dairy cows after repeated exposure to human chorionic gonadotropin. Theriogenology 78:218–224. https://doi.org/10.1016/j.theriogenology.2012.02.003.

Giordano, J. O., M. C. Wiltbank, P. M. Fricke, S. Bas, R. Pawlisch, J. N. Guenther, and A. B. Nascimento. 2013. Effect of increasing GnRH and PGF2α dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology 80:773–783. https://doi.org/10.1016/j.theriogenology.2013.07.003.

Hagemann, L. J. 1999. Influence of the dominant follicle on oocytes from subordinate follicles. Theriogenology 51:449–459. https://doi.org/10.1016/S0093-691X(98)00239-8.

Hayden, C. B., R. V. Sala, V. A. Absalón-Medina, J. C. L. Motta, D. Pereira, J. F. Moreno, and A. García-Guerra. 2022. Synchronization of follicle wave emergence before ovarian superstimulation with FSH and ovum pick-up improves in vitro embryo production in pregnant heifers. Theriogenology 188:71–78. https://doi.org/10.1016/j.theriogenology.2022.05.017.

Hayden, C. B., R. V. Sala, D. C. Pereira, J. F. Moreno, and A. Garcia-Guerra. 2023. Effect of use and dosage of p-follicule-stimulating for ovarian superstimulation before ovum pick-up and in vitro embryo production in pregnant Holstein heifers. J. Dairy Sci. 106:8110–8121. https://doi.org/10.3168/jds.2023-23576.

Machatkova, M., K. Krausova, E. Jokesova, and M. Tomanek. 2004. Developmental competence of bovine oocytes: Effects of follicle size and the phase of follicular wave on in vitro embryo production. Theriogenology 61:329–335. https://doi.org/10.1016/S0093-691X(03)00216-4.

Martinez, M. F., G. P. Adams, J. P. Kastelic, D. R. Bergfelt, and R. J. Mapletoft. 2000. Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. Theriogenology 54:757–769. https://doi.org/10.1016/S0093-691X(00)00388-5.

Motta, J. C. L., G. Madureira, L. O. Silva, R. L. O. R. Alves, M. Silvestri, J. N. Drum, C. E. C. Consentini, A. B. Prata, K. G. Pohler, M. C. Wiltbank, and R. Sartori. 2020. Interactions of circulating estradiol and progesterone on changes in endometrial area and pituitary responsiveness to GnRH. Biol. Reprod. 103:643–653. https://doi.org/10.1093/biolre/ioaa065.

Ongaratto, F., P. Rodriguez Villamil, A. Tribulo, and G. A. Bo. 2015. Effect of follicle wave synchronization and gonadotropin treatments on the number and quality of cumulus-oocyte complex obtained by ultrasound-guided ovum pick-up in beef cattle. Anim. Reprod. 12:876–883.

Price, C. A., and R. Webb. 1989. Ovarian response to hCG treatment during the oestrous cycle in heifers. J. Reprod. Fertil. 86:303–308. https://doi.org/10.1530/jrf.0.0860303.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915–923. https://doi.org/10.1016/0093-691X(95)00279-H.

Sartori, R., P. M. Fricke, J. C. P. Ferreira, O. J. Ginther, and M. C. Wiltbank. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biol. Reprod. 65:1403–1409. https://doi.org/10.1095/biolreprod65.5.1403.

Schmitt, É. J. P., T. Diaz, C. M. Barros, R. L. de la Sota, M. Drost, E. W. Fredriksson, C. R. Staples, R. Thorner, and W. W. Thatcher. 1996. Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. J. Anim. Sci. 74:1074–1083. https://doi.org/10.2527/1996.7451074x.

Stevenson, J. S., S. M. Tiffany, and E. K. Inskeep. 2008. Maintenance of pregnancy in dairy cattle after treatment with human chorionic gonadotropin or gonadotropin-releasing hormone. J. Dairy Sci. 91:3092–3101. https://doi.org/10.3168/jds.2008-1027.

Stroup, W. W., M. Ptukhina, and J. Garai. 2024. Generalized Linear Mixed Models: Modern Concepts, Methods and Applications. 2nd ed. Chapman and Hall/CRC, New York, NY.

Viana, J. H. M. 2023. 2022 Statistics of embryo production and transfer in domestic farm animals. Embryo Technol. Newsl. 41:20–38.

Viana, J. H. M., M. D. Dorea, L. G. B. Siqueira, E. K. N. Arashiro, L. S. A. Camargo, C. A. C. Fernandes, and M. P. Palhão. 2013. Occurrence and characteristics of residual follicles formed after transvaginal ultrasound-guided follicle aspiration in cattle. Theriogenology 79:267–273. https://doi.org/10.1016/j.theriogenology.2012.08.015.

Wiltbank, M. C., and J. R. Pursley. 2014. The cow as an induced ovulator: Timed AI after synchronization of ovulation. Theriogenology 81:170–185. https://doi.org/10.1016/j.theriogenology.2013.09.017.

Notes

Partial funding was provided by the USDA National Institute of Food and Agriculture (Washington, DC), Multistate Hatch project 7004144 (OHO01556-MRF).

We extend our gratitude to the personnel at the Ohio Heifer Center (South Charleston, OH) for assistance during field work and to Merck Animal Health (Kenilworth, NJ) for their donation of GnRH and hCG.

Animal procedures were approved by the Institutional Animal Care and Use Committee at The Ohio State University (#2020A00000056).

The authors have not stated any other conflicts of interest.

Nonstandard abbreviations used: CL = corpus luteum; E2 = estradiol; FA = follicular ablation; FWE = follicular wave emergence; hCG = human chorionic gonadotropin; IVEP = in vitro embryo production; LHCGR = LH/choriogonadotropin receptor; OPU = ovum pick-up; P_4 = progesterone.