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Proestrus, Follicle Dynamics, and Hormone Profiles in Jersey Cows during Synchronization and Re-synchronization after Preovulatory Follicle Aspiration

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Abstract

Reports of length of proestrus, follicle dynamics, circulating hormones, and intrafollicular hormones of estrual animals are scarce. Furthermore, no studies have investigated alterations in cow response to re-synchronization following preovulatory follicle aspiration. Therefore, objectives of this study were to determine length of proestrus, follicle dynamics, and circulating estradiol (E2) and progesterone (P4) concentrations in estrual animals; determine factors influential of proestrus; compare intrafollicular E2 and P4 at estrous onset, 11 h, and 18 h post first standing mount; and determine if re-synchronization beginning 9-10 days after preovulatory follicle aspiration was related to changes in preovulatory follicle dynamics, steroid hormone profiles, and estrual response. Non-lactating, Jersey cows (n=40) underwent pre-synchronization, synchronization, preovulatory follicle aspiration, and re-synchronization. Blood collection and ovarian mapping were performed during each synchronization. Analysis of variance was used to determine differences in intrafollicular hormones at estrous onset and 11 or 18 h post estrous onset, as well as differences in proestrus, follicle dynamics, and circulating hormones between synchronization and re-synchronization. Development of a preovulatory follicle and estrous expression were similar between synchronizations. Length of proestrus was 43.1 ± 1.35 h and 44.6 ± 1.37 h during synchronization and re-synchronization. Serum E2 at prostaglandin F2α (PGF) was negatively associated with length of proestrus. Serum E2 concentration at estrus, and P4 at and prior to PGF were higher in re-synchronization. Follicle diameter at PGF was smaller in re-synchronization. Follicular fluid E2:P4 ratio was 30.03 ± 3.92, 6.35 ± 4.68, and 2.65 ± 5.06, at estrous onset, 11 h, and 18 h after estrous onset, respectively. This study sheds light on preovulatory follicle development and hormone profiles during synchronization and re-synchronization in Jersey cows. Detailed account of length of proestrus, hormone profiles in estrual animals, and intrafollicular hormone profiles add to scarce, but essential, data surrounding the bovine follicular phase.

Keywords: Bovine; Follicle dynamics; Hormone profiles; Preovulatory follicular fluid; Synchronization

Introduction

Pregnancy rates to a single insemination (artificial or natural service) can range from 50 to 65% in beef and <30 to 50% in dairy cattle [1-4]. Although numerous factors contribute to reproductive efficiency, ovulation of a physiologically optimal preovulatory follicle is essential for pregnancy to occur [reviewed by [5]]. Numerous hormonal protocols have been developed to synchronize estrus or ovulation of a preovulatory follicle [6-11]. To that end, variations in preovulatory follicle development, length of proestrus, and fertility have been described among protocols and individual animal responses within a protocol [6-9, 11-22]. Follicle dynamics and circulating steroid hormone profiles are well described in cattle during critical timepoints of preovulatory follicle development from luteolysis to estrus and/or ovulation. This said, aside from two studies in 2013 [23,24], studies reporting follicle dynamics or hormone profiles during the follicular phase are ≥ 37 years old [25-28] or focus only on specific timepoints such as prostaglandin F2α (PGF) administration and fixed-time artificial insemination (FTAI) versus onset of estrus [12, 14, 17, 22].

Furthermore, limited studies have reported estradiol (E2) and progesterone (P4) hormone profiles within the preovulatory follicular fluid at specific timepoints from onset of estrus [24, 29-30] or an induced luteinizing hormone (LH) surge [24, 31-33] to ovulation. Intrafollicular E2 production reaches its peak near the time of estrous onset and the peak LH surge [23, 25-26] that follows approximately 4 h after [29]. Hormone production then shifts to favor P4 and oocyte maturation commences. Milestones of 11 or 18 h after first mount are of great relevance to oocyte maturation, as these timepoints correspond to the hours immediately before major events of oocyte nucleus and cumulus-oocyte gap junction breakdown at 9 to 12 h post peak LH surge and extrusion of the first polar body at 18 to 20 h post peak LH surge [34,35]. Documentation of intrafollicular balance of E2 and P4 from estrus to ovulation during these critical time periods is essential.

In addition to the important modifications in intrafollicular steroid hormones during the preovulatory period, research teams are beginning to explore differential composition (metabolome, proteome, etc.) of preovulatory follicles [31-33, 36-39]. Such studies

require transvaginal aspiration of the ovulatory follicle contents, thus removing a proportion of the follicular granulosa cells destined to become large luteal cells [40]. Aside from one study that determined a normal luteal phase following aspiration of an estrogen active preovulatory follicle [41], it is unknown if preovulatory follicle aspiration may impact follicle dynamics or hormone profiles in animals re-synchronized soon after preovulatory follicle aspiration.

Objectives of the current study were to (1) determine length of proestrus, follicle dynamics, and circulating E2 and P4 concentrations in estrual cows; (2) determine if hormone concentrations or follicle sizes at gonadotropin releasing hormone (GnRH) or PGF administration were influential of length of proestrus; (3) compare intrafollicular E2 and P4 at estrous onset, 11 h post first standing mount, and 18 h post first standing mount; and (4) determine if re-synchronization beginning 9-10 days after preovulatory follicle aspiration was related to changes in preovulatory follicle dynamics, steroid hormone profiles, and estrual response. We hypothesized that size of the follicle and the circulating concentrations of E2 and P4 at administration of PGF influence the length of proestrus, follicular fluid hormone profiles differ from estrous onset to timepoints 11 and 18 h thereafter, and that there would be no difference in follicle dynamics, circulating hormone concentrations, or length of proestrus between initial and re-synchronizations.

Materials and Methods

Synchronization of estrus and preovulatory follicle aspiration:

All protocols and procedures were approved by the Institutional Animal Care and Use Committee at the University of Tennessee-Knoxville (protocol approved on 12/20/2019). Estrus was synchronized twice in non-lactating, multiparous Jersey cows (n=40) maintained on fescue-based pasture (Figure 1). Before synchronization of estrus, estrous cycles of all animals were pre-synchronized by administration of GnRH (Cystorelin®; 100 ug; i.m, Boehringer Ingelheim; Ingelheim am Rhein, Germany) and insertion of a controlled internal drug release (CIDR intervaginal insert; 1.38 g P4; Eazi-Breed™ CIDR®; Zoetis Animal Health, Kalamazoo, MI, USA). Nine days later, the CIDR was removed, and PGF (12.5 mg of dinoprost tromethamine/mL; Lutalyse® HighCon; Zoetis Animal Health, Kalamazoo, MI, USA) was administered. Forty-eight h after PGF administration and CIDR removal, when animals were expected to be in the follicular phase of the estrous cycle, cows were administered GnRH and a CIDR was inserted to synchronize development of a first wave dominant follicle. Seven days later, the CIDR was removed, PGF was administered, and cows were visually monitored for estrus every 4 h until estrous behavior began. Monitoring then occurred continuously for 75 h post PGF administration. Onset of estrus was defined as the time of first recorded instance of an animal standing to be mounted. Fifteen, 9, and 11 animals underwent preovulatory follicle aspiration at 0 h, 11 h, and 18 h post estrous onset, respectively. Procedures for re-synchronization began 9-10 days after preovulatory follicle aspiration was performed. PGF was administered, and 68 h later cows underwent procedures described above for the insertion of a 7-day CIDR and GnRH injection, PGF injection, and estrous detection.

Blood collection, ovarian mapping, cow weight, and body condition

Blood was collected and ovarian status recorded during each synchronization at the time of GnRH administration and 7-day CIDR insertion, CIDR removal and PGF administration, and onset of estrus. Blood was collected via venipuncture in the coccygeal vein of the tail into BD Vacutainer® serum collection tubes (BD, Franklin Lakes, NJ, USA). Blood samples remained at room temperature for 1 h to allow for clotting to occur. Samples were then moved to a refrigerator to be stored at 4°C for 24 h, after which centrifugation was performed at 1200 x g for 25 min at 4°C. Serum was collected and stored in borosilicate glass tubes at -20°C for hormone analysis. Transrectal ultrasonography was performed using an IBEX EVO® II ultrasound and the eL7 linear probe (E.I Medical Imaging, Loveland, CO, USA). For both ovaries, follicles >7 mm were measured using the average of the largest diameter of the follicle and the diameter perpendicular to the largest diameter. Animal weight (kg) and body condition score (BCS, dairy body condition score scale of 1-5 [35]) were assessed at the onset of synchronization and records for age were acquired.

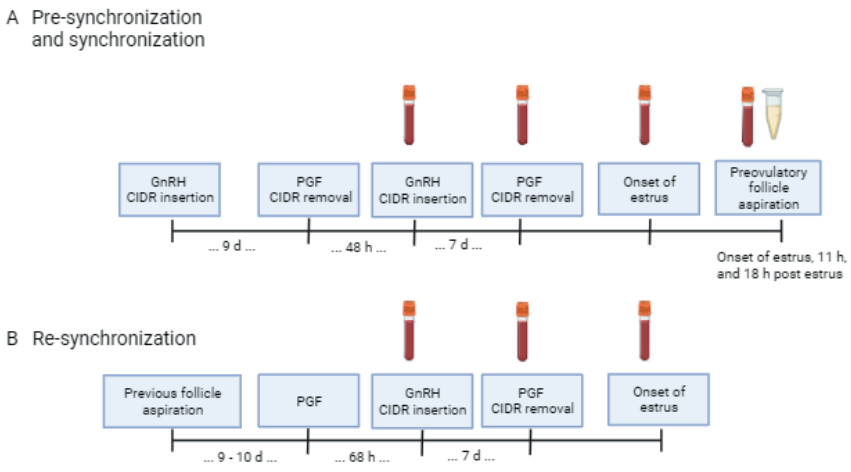

Figure 1: Timeline for synchronization (A) and re-synchronization (B) of estrus. GnRH: gonadotropin releasing hormone, PGF: prostaglandin F2α. Figure created with BioRender.com.

Preovulatory follicle aspiration and follicular fluid collection

Transvaginal aspiration of the preovulatory follicle occurred at either estrous onset, 11 h post estrous onset, or 18 h post estrous onset during the initial synchronization [39]. Cows were administered a spinal block using 5 mL of 2% lidocaine in the first intercoccygeal space of the tailhead prior to transvaginal aspiration. The perineal and vulvar area was thoroughly cleaned to remove potential contaminants from entering the vagina. An ultrasound guided transvaginal aspiration apparatus encompassing a CFA-9 convex ultrasound probe connected to a Samsung HM70A ultrasound was inserted into the anterior vagina. Using the ultrasound for visualization, the ovary was manipulated until the follicle of interest was in the best position for aspiration. The needle was advanced through the wall of the vagina and ovarian cortex until the tip was inserted in the follicular antrum. Follicular fluid was collected into a 5 mL syringe until the follicle was visualized to have fully collapsed. The transvaginal aspiration device was cleaned with chlorhexidine and rinsed with water, and all tubing was completely flushed with sterile water between each cow.

After aspiration, follicular fluid was distributed into one well of a 4-well plate. A trained technician searched the dish under a microscope for the cumulus oocyte complex (COC) which, if found, was removed and collected for future use in a different study. Follicular fluid was collected from the 4-well petri dish and distributed into 1.7 mL Eppendorf tubes which were centrifuged at 500 x g for 5 min at 4°C to pellet potential cellular debris within the sample. Supernatant was aliquoted into multiple tubes and stored at -80°C for future analyses. One hundred µL of follicular fluid was placed into a 2 mL microcentrifuge tube for follicular fluid E2 and P4 assays (Stellar Scientific, Baltimore, MD, USA).

Follicular fluid and serum hormone profiles

Follicular fluid samples underwent dilution from 1:2500 to 1:50000 before E2 and P4 assays were performed. Serum and follicular fluid P4 concentrations were evaluated using the ImmuChem progesterone double antibody radioimmunoassay kit (catalog number: 0717010-CF, MP Biomedicals, Costa Mesa, CA, USA) and following the manufacturer's specifications and procedures previously validated and described [42]. Assay sensitivity was 0.11 ng/mL. Serum P4 intra- and inter-assay coefficients of variation (CV)s were 8.00% and 4.18%, respectively. Follicular fluid P4 intra-and inter-assay CVs were 4.42% and 5.70%, respectively. Serum E2 concentration was determined using radioimmunoassay following methods previously validated and described [43]. Serum E2 intra- and inter-assay CVs were 3.55% and 6.70%, respectively, and assay sensitivity was 1.03 pg/mL. Follicular fluid free E2 concentration was quantified using the DetectX® Serum 17β-Estradiol ELISA Kit (catalog number: KB30-H, Arbor Assays, Ann Arbor, MI, USA) according to manufacturer's specifications and as previously described by [44]. Intra- and inter-assay CVs for follicular fluid E2 were 1.65% and 7.33%, respectively (assay sensitivity=2.21 pg/mL).

Statistical analyses

All statistical procedures were performed using R Studio version 4.2.3. Outliers were identified using interquartile range, and outlying data were removed in analyses related to follicular fluid hormones and factors related to time from PGF to estrous onset (first standing mount). Outlier data were descriptive of potential differences between synchronization and re-synchronization and were thus included in analyses. Potential differences between synchronization and re-synchronization in distribution of estrous response, follicle dynamics, and serum E2 and P4 concentrations at GnRH / 7-day CIDR insertion, PGF / 7-day CIDR removal, and estrous onset were assessed using the type III analysis of variance (ANOVA) function in a linear, mixed model blocked by cow. To determine factors influential of time from PGF to estrous onset (first observed standing mount) or serum E2 concentration at PGF, we performed mixed model regression (blocking on cow within synchronization) between time from PGF to estrous onset or serum E2 at PGF and cow phenotypes, follicle size, and circulating hormone concentrations. After all independent variables were assessed individually, a single best fit model was derived using step down procedures and Akaike information criterion values. The relationship between timepoint post estrous onset and follicular fluid E2 concentration, P4 concentration, and E2: P4 ratio following initial synchronization was assessed using ANOVA. If time post estrous onset was significant, we performed pairwise comparisons between timepoints and utilized Tukey's Honestly Significant Differences Post Hoc test. Significance in all analyses was inferred if $P \leq 0.05$. Plots were generated using ggplot2.

Results

Expression of estrus during synchronization and re-synchronization

Overall, 87.5 and 92.5% of cows displayed estrus within 75 h following synchronization and re-synchronization post preovulatory follicle aspiration, respectively. Interestingly, 2 of the 3 cows that failed to display estrus following re-synchronization also did not display estrus during initial synchronization. Time from PGF administration to first observed standing mount (i.e., length of proestrus) following re-synchronization was 43.1 ± 1.35 h and did not differ from synchronization (44.6 ± 1.37 h; P=0.45, Figure 2).

Factors influential of proestrus

Cow age, BCS, and weight did not influence interval from PGF administration to estrous onset (Table 1). Although neither serum E2 or P4 concentration at GnRH administration was related to interval from PGF to first standing mount, size of the largest follicle at GnRH administration was positively associated with length of proestrus (Table 1). At the time of PGF administration, both future preovulatory follicle diameter and serum E2 concentration were negatively associated with interval from PGF to first standing mount (Table 1).

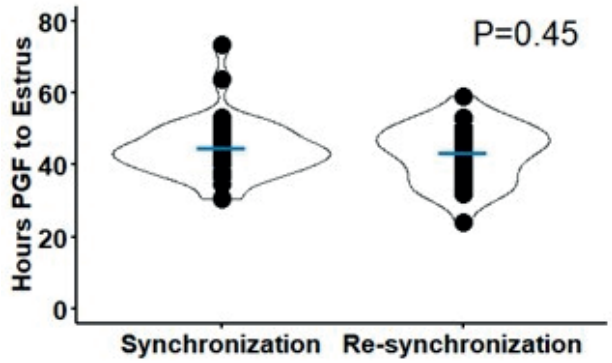

Figure 2: Scatterplot depicting hours from prostaglandin F2α (PGF) administration to onset of standing estrus following synchronization and re-synchronization.

Table 1: Parameters assessed for association with length of proestrus.

Parameter	Estimate	P
Cow age, days	-0.001	0.44
Cow body condition score	-2.518	0.74
Cow weight, kg	-0.025	0.19
Serum E2 at GnRH, pg/mL	0.002	0.99
Serum P4 at GnRH, ng/mL	1.335	0.84
Largest follicle diameter at GnRH, mm	0.44	0.02
Serum E2 at PGF, pg/mL	-2.336	<0.01
Serum P4 at PGF, ng/mL	3.456	0.29
Preovulatory follicle diameter at PGF, mm	-1.356	<0.01

E2, estradiol; GnRH, gonadotropin releasing hormone administration; P4, progesterone; PGF, prostaglandin F2α.

Alternatively, serum P4 concentration at PGF was not related to length of proestrus (Table 1).

When largest follicle diameter at GnRH administration, future preovulatory follicle diameter at PGF, and serum E2 concentration at PGF were analyzed to determine a single best fit model, serum E2 concentration was the only variable that maintained a significant relationship with interval from PGF to estrous onset/first standing mount (Figure 3A). As such, a secondary analysis was performed to determine factors influential of serum E2 concentration at this time. Cow age (P=0.29), BCS (P=0.79), weight (P=0.54), largest follicle at GnRH (P=0.17), serum E2 at GnRH (P=0.36), nor serum P4 at GnRH (P=0.11) were related to serum E2 concentration at PGF. Alternatively, at the time of PGF administration, future preovulatory follicle diameter was positively associated, and serum P4 concentration was negatively associated with serum E2 concentration (P<0.01). When both independently significant variables were fit into the same linear model, only serum P4 concentration at PGF maintained its relationship with serum E2 concentration at PGF (Figure 3B).

Follicle dynamics during synchronization and re-synchronization

During initial synchronization and re-synchronization, largest follicle diameter at GnRH was 14.4 ± 0.85 mm and 14.3 ± 0.79 mm, respectively (P=0.91, Table 2). Diameter of the largest follicle at PGF administration was 12.5 ± 0.64 mm and 14.1 ± 0.63 mm during synchronization and re-synchronization, respectively (P=0.08, Table 2). Largest follicle diameter at the time of first standing mount (at estrous onset) was 15.4 ± 0.50 mm during synchronization and 16.5 ± 0.47 mm during re-synchronization (P=0.14, Table 2). One cow during synchronization, and 11 cows during re-synchronization developed a follicular cyst that did not prohibit development of a separate preovulatory follicle. Therefore, the largest growing follicle's diameter was recorded as the future preovulatory follicle at PGF administration and the preovulatory follicle at estrus. Diameter of the future preovulatory follicle at PGF during synchronization (12.8 ± 0.32 mm) was larger than during re-synchronization (11.8 ± 0.30 mm; P=0.04, Table 2). Nevertheless, diameter of the preovulatory follicle at estrous onset was not different between synchronization and re-synchronization (15.3 ± 0.28 mm, 15.2 ± 0.27 mm, respectively; P=0.73, Table 2).

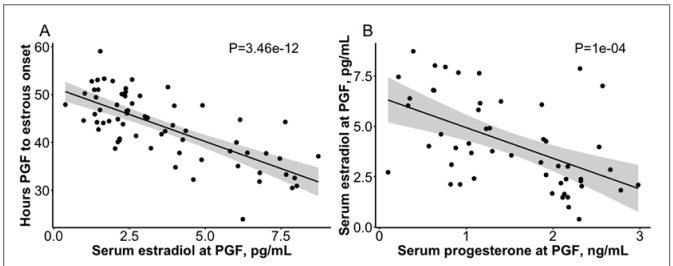

Figure 3: Scatterplots depicting relationships between serum estradiol at the time of prostaglandin F2α (PGF) administration and hours from PGF to estrous onset as defined by first standing mount (A) or serum progesterone concentration at PGF administration (B).

Table 2: Follicle dynamics during synchronization and re-synchronization.

Parameter	Synchronization	Re-synchronization	P
Largest follicle diameter at GnRH, mm	14.4 ± 0.85	14.3 ± 0.79	0.91
Largest follicle diameter at PGF, mm	12.5 ± 0.64	14.1 ± 0.63	0.08
Largest follicle diameter at estrus, mm	15.4 ± 0.50	16.5 ± 0.47	0.14
Preovulatory follicle diameter at PGF, mm	12.8 ± 0.32	11.8 ± 0.30	0.04
Preovulatory follicle diameter at estrus, mm	15.3 ± 0.28	15.2 ± 0.27	0.73

Data are presented as mean ± standard error of the mean.
GnRH, gonadotropin releasing hormone; PGF, prostaglandin F2α.

Circulating E2 and P4 during synchronization and re-synchronization

Serum E2 concentration at GnRH during synchronization was 5.53 ± 0.47 pg/mL, which did not differ from serum E2 concentration during re-synchronization (6.09 ± 0.47 pg/mL; P=0.41, Table 3). Serum E2 concentration at PGF was 3.53 ± 0.40 pg/mL and 3.73 ± 0.40 pg/mL, during synchronization and resynchronization respectively; P=0.69, Table 3). Serum E2 concentration at the time of first standing mount (estrous onset) was higher in cows during re-synchronization (12.6 ± 0.66 pg/mL) compared to synchronization (10.1 ± 0.67 pg/mL; P=0.01, Table 3).

Circulating P4 concentration was <1 ng/mL in all cows at GnRH administration during synchronization. Alternatively, 5 cows had serum P4 concentration >1 ng/mL at GnRH during re-synchronization. As such, serum P4 concentration at GnRH was higher during re-synchronization (0.41 ± 0.09 ng/mL) compared to synchronization (0.17 ± 0.09 ng/mL; P=0.05, Table 3). A higher P4 concentration was also observed in re-synchronized cows, compared to synchronization, at the time of PGF (2.50 ± 0.21 ng/mL *vs* 1.79 ± 0.21 ng/mL; P=0.02, Table 3), but by estrous onset there were no differences in circulating P4 concentration (0.16 ± 0.02 ng/mL in re-synchronization and 0.15 ± 0.02 ng/mL in synchronization; P=0.64; Table 3).

Relationship between time post estrous onset and follicular fluid hormones

To determine differences in intrafollicular steroid hormone

Table 3: Serum estradiol and progesterone concentration during synchronization and re-synchronization.

Parameter	Synchronization	Re-synchronization	P
Serum E2 at GnRH, pg/mL	5.53 ± 0.47	6.09 ± 0.47	0.41
Serum P4 at GnRH, ng/mL	0.17 ± 0.09	0.41 ± 0.09	0.05
Serum E2 at PGF, pg/mL	3.53 ± 0.40	3.73 ± 0.40	0.69
Serum P4 at PGF, ng/mL	1.79 ± 0.21	2.50 ± 0.21	0.02
Serum E2 at estrus, pg/mL	10.1 ± 0.67	12.6 ± 0.66	0.01
Serum P4 at estrus, ng/mL	0.15 ± 0.02	0.16 ± 0.02	0.64

Data are presented as mean ± standard error of the mean
E2, estradiol; GnRH, gonadotropin releasing hormone administration; P4, progesterone; PGF, prostaglandin F2α.

concentrations at onset of estrus, 11 h and 18 h post estrous onset, preovulatory follicular fluid was aspirated from cows at the onset of estrus, 11 h and 18 h post estrus during the initial synchronization. At onset of estrus, 11 h and 18 h post onset of estrus, intrafollicular free E2 concentration was (1137 ± 63.1 ng/mL, 522 ± 66.6 ng/mL, and 136 ± 75.5 ng/mL, respectively; P=0.0006, Figure 4A), P4 concentration was (33.3 ± 5.59 ng/mL, 57.5 ± 5.59 ng/mL, and 50.8 ± 5.98 ng/mL, respectively; P=0.018, Figure 4B) and E2 to P4 ratio was (30.03 ± 3.92, 6.35 ± 4.68, and 2.65 ± 5.06, respectively; P=0.0003, Figure 4C).

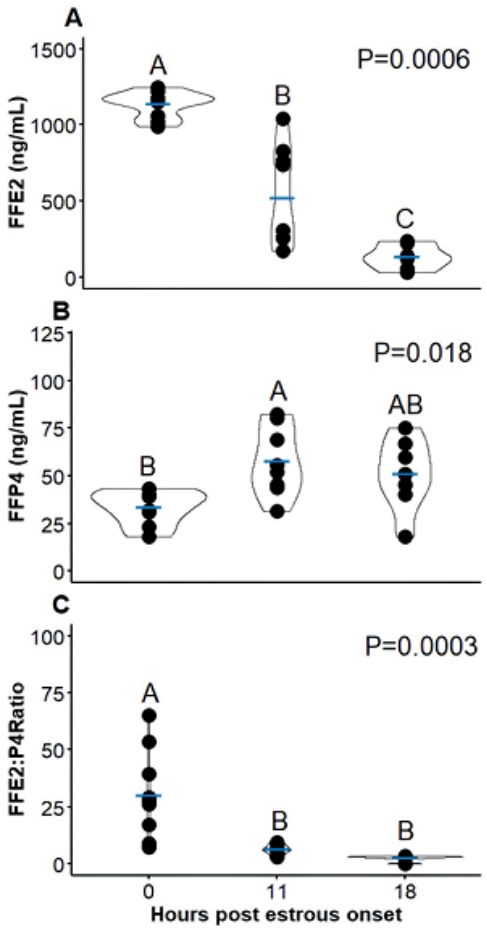

Figure 4: Scatterplots depicting follicular fluid estradiol (FFE2), follicular fluid progesterone (FFP4), and follicular fluid estradiol to progesterone ratio (FFE2:P4 ratio) at onset of estrus, 11 h and 18 h post estrous onset.

Discussion

Data described herein provide a detailed account of length of proestrus, follicle dynamics, and both circulating and intrafollicular steroid hormone profiles in non-lactating Jersey cows undergoing estrous synchronization. In the current study, 87.5% and 92.5% of cows exhibited estrus during synchronization and re-synchronization, respectively, which is higher than reported estrous expression ranges of 22% to 83% in the literature [7-9, 13, 15-17, 45-48]. Length of proestrus during synchronization and re-synchronization procedures did not differ and was shorter than observed in studies by others that employed a similar [13] or different [15-16,46] synchronization protocol.

The increased estrous response and shortened proestrus observed in both synchronizations was likely influenced by pre-synchronization procedures. In an attempt to synchronize development of a first wave dominant follicle, procedures were employed to increase the likelihood that animals were in the follicular phase of the estrous cycle at the onset of each synchronization. After pre-synchronization procedures placed animals in the luteal phase of the estrous cycle, PGF was administered 2-3 days prior to initiation of the 7-Day CIDR protocol utilized in both synchronization and re-synchronization. Such procedures led to circulating P4 concentration of 1 ng/mL or less in all cows during initial synchronization and 35/40 (87.5%) of cows during re-synchronization. One shortcoming of the current study was failure to collect blood samples at the time of pre-synchronization PGF administration to confirm luteolysis of a functional corpus luteum. Ability of a follicle to transform into a normal corpus luteum is questionable after preovulatory follicular aspiration since many of the follicular granulosa cells are disturbed and stripped from the follicular cavity. This said, a functional corpus luteum was reported to develop in 82% of lactating dairy cows that underwent ultrasound guided aspiration of a preovulatory follicle [41].

Reduced P4 in the follicular phase of the estrous cycle allows for increased gonadotropin production, increased growth of the follicle, and an increase in E2 production [49-51]. Although ovulatory response to GnRH was not measured, animals in the present study developed a large, preovulatory follicle following pre-synchronization and PGF administration. This likely led to an increased ovulatory response to GnRH administration (or the cow's endogenous LH surge) at the start of the 7-Day CIDR protocol during both synchronization and re-synchronization. Animals ovulating to GnRH administration at the time of CIDR placement have previously been reported to have a more advanced follicle at the time of PGF administration and/or a more advanced follicle at the time of FTAI [53-54]. A shorter proestrus can be attributed to maturity of the dominant follicle at the time of PGF administration [55-56]. Indeed, our findings demonstrate that increased future ovulatory follicle diameter and serum E2 concentration at PGF were related to shorter length of proestrus.

It is interesting, however, that animals in the current study experienced a longer proestrus with increasing follicle diameter at GnRH and CIDR insertion. This is contrary to the notion that a larger follicle at GnRH would lead to faster follicular turnover and a shorter proestrus due to increased development of the synchronized follicle at PGF administration. It is likely that presynchronization could have reduced the importance of follicle size on follicle turnover to GnRH since all follicles in the current study should have been capable of response to GnRH administration. This does not explain the positive relationship between follicle diameter at GnRH and length of proestrus, and this relationship should not be overinterpreted.

In addition to likely impacts of pre-synchronization to shorten length of proestrus, frequency of estrous observation also likely influenced recorded interval from PGF to first standing mount. Previous studies which describe the length of proestrus monitored animals 2-3 times a day [13,46] or report estrous expression in 12-hour intervals [15-16], while the current study employed visual checks every 4 h until the first animal displayed estrous activity and then proceeded with continuous monitoring thereafter. The beginning of standing estrus was likely more precisely determined in the current study due to more frequent monitoring, which allowed for animals to be recorded as estrual sooner.

Although follicle dynamics were as expected at PGF and onset of estrus during both synchronizations, interesting findings emerged that suggest variation in stage of estrous cycle in some cows at onset of re-synchronization. Further, analysis of largest follicle diameter during re-synchronization highlights a subset of cows the developed a large follicular cyst that did not influence preovulatory follicle development. One animal displayed such a cyst during synchronization, but it is interesting that 11 different animals presented with cysts during re-synchronization. Such findings suggest that re-synchronizing follicle development shortly after aspiration of preovulatory follicle contents could lead to non-influential cyst formation. We have found no accounts in the literature relating preovulatory follicle aspiration to cyst development, and cysts were equally present on the previously aspirated ovary (6/11) and non-aspirated ovary (5/11) in the current study. No influence of the cyst on preovulatory follicle growth or hormone production was inferred, as animals with a recorded cyst were within range of all other animals for preovulatory follicle sizes and circulating E2 and P4 concentration at all timepoints measured.

Intrafollicular hormone profiles described within add to the sparse, but essential, reports of modified intrafollicular E2 and P4 concentration after estrous expression in cattle [24,29,30]. Shifts in intrafollicular E2 and P4 following estrus are attributed to granulosa cells showing a time-dependent response to the LH surge. Leading up to estrus, the follicle increases its E2 production resulting in a peak of follicular E2 concentration around onset of estrus [23, 25-26]. A high concentration of E2 was detected in bovine preovulatory follicles obtained before the LH surge [29,30], similar to levels reported in the current study. However, at 6 h post estrous onset, intrafollicular hormone concentrations abruptly shift in favor of P4 production, leading to lower E2 and higher P4 concentrations at 11 h and 18 h post estrus. This shift occurs as theca and granulosa cells within the preovulatory follicle differentiate into large and small luteal cells, transitioning steroid hormone production towards P4. For instance, bovine follicular fluid collected 16-22 h after the second GnRH injection, was reported to have E2 concentration, P4 concentration, and E2 to P4 ratio in the largest pre-ovulatory follicles of 818.5 ng/mL, 46.63 ng/mL, and 19.6 respectively [24]. At 19 h after GnRH2 administration, follicular fluid E2 and P4 concentrations averaged 52 pg/mL and 68 ng/mL [31], similar to concentrations 18 h post estrus in our study. Data from follicular fluid collected from women and ewes follow the same trend in E2 and P4 profiles, with a follicular fluid E2:P4 ratio of approximately 0.16 reported in women 36 h after human chorionic gonadotropin administration to induce the LH surge [57]. In ewes, follicular fluid E2 concentrations declined from peak pre-LH surge levels by the time of the LH surge onset and further declined by 8 hr post LH surge detection, whereas P4 concentrations began to rise at 16 h post LH surge detection [58]. Due to similarities among species in follicular steroid hormone production following a natural or induced preovulatory LH surge, the thorough examination of intrafollicular hormone status at three timepoints relative to bovine estrous onset offers valuable insights into the complex interplay of ovarian hormones, enriching our understanding of the mechanisms governing follicular maturation.

Conclusions

In conclusion, this study reported precise measurements of length of proestrus, follicle dynamics, and circulating E2 and P4 concentrations in Jersey cows undergoing synchronization and re-synchronization following preovulatory follicle aspiration. Proestrus averaged 43.78 ± 0.95 h and was shorter than reported previously by others. This difference is likely due to pre-synchronization procedures and increased frequency of estrous observation in the current study. There were few differences in follicle dynamics and circulating hormone profiles between initial and re-synchronizations. This said, notable differences were that stage of the estrous cycle was less precisely controlled at onset of re-synchronization, circulating P4 was higher from CIDR placement to removal in re-synchronized cows, and 27.5% of re-synchronized cows developed a large follicular cyst that did not influence development of a separate preovulatory follicle. This study also reports follicular fluid E2, P4, and E2 to P4 ratio at estrous onset and 11 or 18 h after first standing mount during the initial synchronization. Follicular fluid E2 to P4 ratio dropped abruptly from 30.03 ± 3.92 at estrous onset to 6.35 ± 4.68 at 11h and 2.65 ± 5.06 at 18 h thereafter, respectively.

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Institutional Review Board Statement

The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of the University of Tennessee, Institutional Animal Care and Use Committee (12/20/2019).

Data Availability Statement

The data presented in the study are available on request from the corresponding author. The data are not publicly available due to their inclusion in a larger, not yet published experiment beyond the scope of this manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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