REPRODUCTIVE BIOLOGY

ORIGINAL RESEARCH

Circulating concentrations of ovarian steroids and follicle-stimulating hormone (FSH) in ewes with 3 or 4 waves of antral follicle emergence per estrous cycle

Tanya E. Baby, Pawel M. Bartlewski[1]

Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

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SUMMARY

The mechanism governing the number of follicle-stimulating hormone (FSH) peaks and emerging follicular waves in ruminants remains unknown. The main purpose of the present study was to examine the relationships between progesterone (P_4) levels, circulating concentrations of FSH and antral follicular development throughout the interovulatory interval in sheep. We retrospectively analyzed and compared daily serum concentrations of P_4, FSH and estradiol (E_2) obtained in cyclic (November-December) Western White Face ewes (Columbia×Rambouillet) that had 3 (n=10) or 4 (n=19) follicular waves per estrous cycle. Follicular growth was monitored in all animals by daily transrectal ultrasonography. Mean P_4 concentrations

[1] Corresponding author: Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada; e-mail: pmbart@uoguelph.ca

were greater ($p<0.05$) in sheep with 4 waves per cycle compared to their counterparts with 3 waves of follicular growth. The ewes with 3 waves exceeded ($p<0.05$) animals with 4 follicular waves in mean serum FSH concentrations on days 0–2, 6, 7, 9–11, 14 and 15 post-ovulation. Animals with 4 follicular waves exceeded ($p<0.05$) the ewes with 3 waves in mean serum E_2 concentrations on days – 1, 2 and 10 of the estrous cycle studied (day 0=ovulation). The present results are supportive of the notion that luteal P_4 is an important endocrine signal, which controls the periodicity of FSH peaks and the number of emerging follicular waves in cyclic ewes. *Reproductive Biology 2011* ***11*** *1: 19–36.*

Key words: sheep, follicle-stimulating hormone, antral follicles, follicular waves, progesterone

INTRODUCTION

Ovarian antral follicles in ruminant species develop in an orderly fashion, producing sequential follicular waves [1, 3, 10]. This pattern of antral follicular growth is closely associated with periodic elevations in serum concentrations of follicle-stimulating hormone (FSH); peaks of transient increases in FSH concentrations occur just prior to follicle wave emergence. Several studies in cattle have described the numbers of follicular waves and their associated endocrine variables [1]. Approximately 95% of the bovine estrous cycles studied expressed either the 2- or 3-wave pattern [1]; however, the 3-wave pattern seems to be predominant [6]. Unlike in sheep, the number of emerging follicular waves in individual cows seems to be maintained from cycle to cycle [15]. It was also demonstrated that bovine estrous cycles with 2 waves (20.4±0.3 days in duration) were 2–3 days shorter as compared to those with 3 waves (22.8±0.6 days; [11]). It was suggested that this difference was due to an earlier regression of the corpus luteum (CL) in 2-wave cycles (around day 16) than in 3-wave cycles (day 19; [2]). In a more recent study, it was confirmed that cattle with the 2-wave pattern had a shorter estrous cycle compared to those exhibiting the 3-wave pattern (19.8±0.2 vs. 22.5±0.3 days, respectively

[15]). However, the determining factor governing the number of antral follicular waves during the estrous cycle was attributed mainly to the duration of follicular dominance in Wave 1 [15]. This suggests that factors influencing the development of individual waves and dominant follicles (i.e. gonadotropins) play an important role in regulating the duration of the interovulatory interval and the pattern of wave emergence in cows. However, the precise mechanism regulating the number of antral follicular waves in cattle still remains undefined and is not likely to shed any light on the possible mechanism in sheep.

The duration of the estrous cycle in sheep is relatively constant between different breeds and ages, with only a slight variation of 1–2 days [13]. Hence, sheep typically exhibiting 3 or 4 waves of follicular growth per interovulatory period do not extend the length of their cycle to incorporate an extra wave like cattle, but rather the intervals between consecutive waves are shortened [3]. In ewes exhibiting the 3-wave pattern, Wave 1 emerges around the day of ovulation (day 0), whereas Waves 2 and 3 emerge ~5.5 and 10 days post-ovulation, respectively [3]. In comparison, Wave 1 in ewes with the 4-wave pattern also emerges around day 0, while Waves 2 and 3 emerge on days 4 and 8, respectively, allowing Wave 4 to emerge on day 12 and to be incorporated within the confinements of the 17-day estrous cycle [19].

The first wave of the ewe's estrous cycle was found to display changes in follicular size that were accompanied by increases in serum concentrations of FSH, estradiol (E_2) and inhibin A [22]. However, when follicular Waves 2 and 3 were analyzed, the changes in follicular diameter were similar to those seen in Wave 1, but the hormone concentrations did not increase in the same fashion as during the lifespan of Wave 1. Therefore, it was concluded that increased serum inhibin A and E_2 concentrations controlled the FSH peak heralding the first follicular wave of the estrous cycle (i.e. during the periovulatory endocrine events [22]). Bartlewski *et al*. [3] did not observe any differences between the amplitude and duration of serum E_2 and FSH fluctuations throughout the entire estrous cycle of Western White Face (Columbia×Rambouillet) ewes. There were no temporal relationships between serum FSH and E_2 concentrations throughout diestrus in ewes [3].

The controlling mechanism(s) of FSH increases associated with Waves 2, 3 and 4 of the ovine estrous cycle remains to be elucidated.

To the best of our knowledge, only one study [19] has been performed to compare the endocrine profiles and antral follicular development in cyclic ewes with different numbers of waves. The first inter-wave interval and the interval from the emergence of the final wave to the day of ovulation, was found to be longer in ewes with 3-wave cycles as compared to ewes exhibiting the 4-wave cycles. Specifically, the inter-wave intervals were associated with a longer regression phase in the first wave along with an extended growth phase and lifespan of follicles in the ovulatory wave. All FSH peaks, except for the first, periovulatory peak, occurred earlier in the 4-wave than 3-wave cycles [19]. This divergence in the growth pattern of the largest follicles of waves between the first and subsequent waves may be due to alterations in serum concentrations of progesterone (P_4) across the ewe's estrous cycle. The prolonged follicle growth early in the luteal phase may be associated with the presence of developing or non-fully functional CL secreting low levels of P_4 [3, 9]. Therefore, the objective of the current study was to compare daily serum concentrations of P_4, FSH and E_2 obtained in normally cycling Western White Faced ewes that had 3 or 4 follicular waves per estrous cycle. We hypothesized that the shortened inter-peak intervals for peak serum FSH concentrations associated with the occurrence of 4 follicular waves per cycle would be the result of elevated P_4 concentrations during the luteal phase of the ewe's estrous cycle.

MATERIALS AND METHODS

This report is comprised of retrospective analyses of ultrasonographic recordings and hormone measurements obtained in several previous studies performed on separate, age-matched groups of cyclic Western White Face (WWF) ewes. The data from all experiments were pooled for analyses, as there were no differences in any of the follicular or endocrine variables analyzed. The hormonal and ovarian data were obtained from 29 cyclic WWF ewes with either 3 (n=10) or 4 follicular waves (n=19) per cycle in the middle portion of the breeding season (November-December). The techniques used in collecting the data can be referred to in the original publications in which they were described [3, 9]. In brief, blood sampling and transrectal ovarian ultrasonography were performed daily. Ultrasonography utilized a portable, Aloka 900-SSD echo camera (Aloka Co., Ltd., Tokyo, Japan) equipped with a rigid 7.5-MHz rectal transducer. To minimize error due to inappropriate classification of the 3- and 4-wave estrous cycles, the inclusion criteria for determining the number of follicular waves were as follows: sheep with "3 or 4" waves had to have "3 or 4" ultrasonographically detected waves of antral follicular growth and "3 or 4" associated FSH peaks (i.e., occurring within 24 hours from the day of wave emergence). In any case where discrepancies occurred between the numbers of waves and FSH peaks, animals were withdrawn from the analyses.

A cycle detector program (or the threshold adaptive technique) was used to analyze the FSH and E_2 data. It used the concentrations of the hormones and the corresponding assay coefficients of variation (CV's) to determine the peaks of successive FSH and E_2 fluctuations [7]. The additional variables analyzed for each peak are listed in Table 1. A follicular wave was defined as a group of follicles that emerged from 2–3 mm in diameter and grew to ≥5 mm before regression or ovulation [3]. Follicles that emerged within a 24-hour period were included in a wave. The number of waves per estrous cycle, days of wave emergence, and numbers of follicles in each wave were noted. In addition, the largest antral follicle in each wave was analyzed for its maximum diameter (mm), duration of the growth, static and regressing phase of the lifespan, and the growth rate [3]. Daily numbers of follicles in different size categories (2, 3, 4 and ≥5 mm in diameter) were also analyzed.

Single time point observations were compared between the two subsets of animals by Student *t*-test. Differences in serial hormonal and ovarian data were assessed by two-way repeated-measures analysis of variance (RM-ANOVA, General linear model procedures) and post-hoc ANOVA comparisons were performed using Fisher's protected least significant difference (LSD; SigmaStat®, version 3.0 for Windows®, 2003 Systat Software, Inc. Richmond, CA, USA). All results are given as mean±standard error of the mean (SEM). The statistical significance was regarded as $p<0.05$.

Table 1. Mean (±SEM) days of FSH peak detection, peak amplitude, concentration, duration of fluctuations (nadir-to – peak-to-nadir), and basal concentration (preceding nadir) determined by a cycle detection computer program in cyclic Western White Face ewes (retrospective analysis).

FSH	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**
Mean time of peak detection*	0.1±0.1 (-1 to 1) *0.2±0.2 (-1 to 1)*	4.5±0.2^{A} (3 to 6) *6.4±0.3^{B} (6 to 9)*	8.2±0.2^{A} (7 to 9) *11.0±0.3^{B} (10 to 13)*	12.2±0.3 (10 to 14) -
Mean peak amplitude (ng/ml)	ND ND	1.69±0.16^{a} *2.11±0.34^{a}*	1.07±0.13^{b} *1.39±0.23^{b}*	1.07±0.18^{b} -
Mean peak concentration (ng/ml)	2.61±0.22^{A} *3.80±0.42^{B}*	2.89±0.17^{A} *3.81±0.43^{B}*	2.57±0.16^{A} *3.48±0.40^{B}*	2.56±0.23 -
Peak duration (days)	ND ND	4.5±0.2^{a} *5.1±0.2*	3.5±0.2^{Ab} *5.1±0.4^{B}*	4.3±0.3^{a} -
Preceding nadir concentration (ng/ml)	ND ND	1.14±0.07^{Aa} *1.69±0.14^{Ba}*	1.55±0.09^{Ab} *2.10±0.27^{Bb}*	1.48±0.10^{b} -

*Day 0=ovulation; ND-not determined; regular font: 4 waves; italics: 3 waves; AB represents differences ($p<0.05$) between groups (3 waves vs. 4 waves); ab represents differences ($p<0.05$) within groups

RESULTS

General results

The mean ovulation rates and numbers of detected CL at the beginning and end of the inter-ovulatory period studied did not vary between WWF ewes with 4 waves (1.8±0.1 and 2.1±0.1, respectively) or 3 waves per cycle (1.7±0.3 and 1.7±0.2, respectively), in the 29 estrous cycles studied.

Hormone concentrations

Mean daily P_4 concentrations were significantly higher ($p<0.05$) in cyclic WWF ewes with 4 waves as compared to animals exhibiting 3 waves of antral follicular development per estrous cycle (fig. 1A); however, no significant difference was observed between the groups on any specific day. Animals with 4 follicular waves exceeded ($p<0.05$) the ewes with 3 waves in mean circulating E_2 concentrations on days – 1, 2 and 10 of the estrous cycle (day 0=ovulation; fig. 1B). Serum FSH concentrations were significantly lower ($p<0.05$) in ewes with 4 waves as compared to the ewes with 3 waves on days 0–2, 6, 7, 9–11, 14 and 15 (fig. 1C).

Characteristics of FSH and E_2 peaks

Successive peaks (Peaks 1–4) of circulating FSH concentrations were detected between days – 1 to 1, 6 to 9 and 10 to 13 in ewes exhibiting 3 waves and between days – 1 to 1, 3 to 6, 7 to 9 and 10 to 14 in ewes with 4 waves (fig. 2). The first peak of FSH occurred within the same time interval (day – 1 to 1) in all sheep exhibiting the 3- and 4-wave patterns. However, Peaks 2 and 3 occurred 2–3 days earlier ($p<0.05$) in ewes with 4 waves as compared to animals with 3 waves. Furthermore, the mean peak concentration and basal FSH levels (preceding nadir concentration) for Peaks 2 and 3 were lower ($p<0.05$) in ewes with 4 waves as compared to ewes with 3 waves (tab. 1). The mean duration of Peak 3 was not only significantly shorter in ewes with 4 waves as compared to the ewes with 3 waves, but

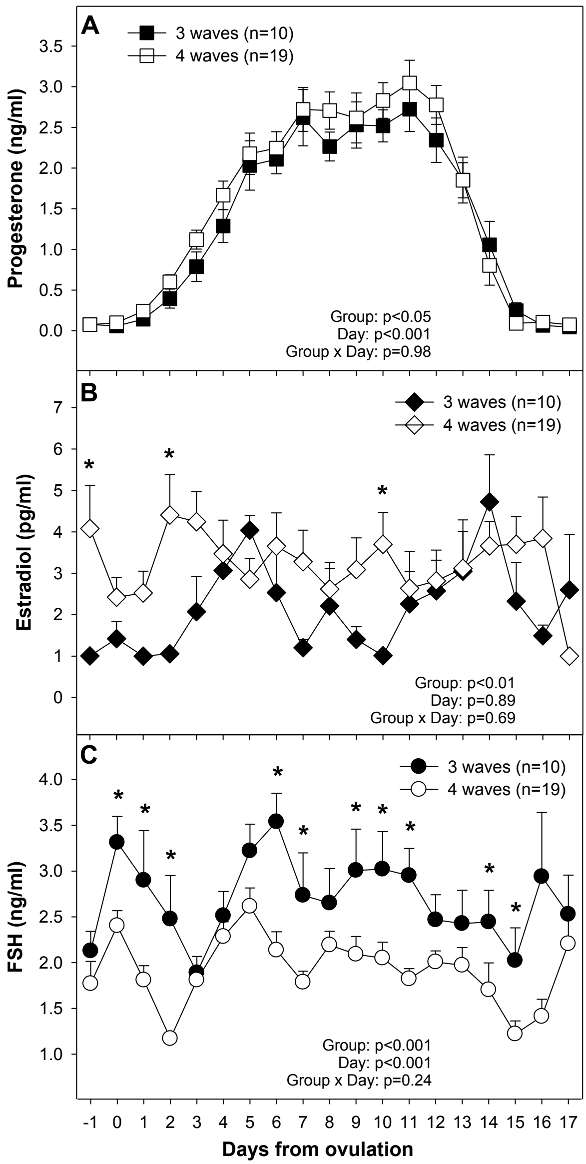

Figure 1. Mean (±SEM) serum concentrations of progesterone (A), estradiol (B), and FSH (C) in cyclic Western White Face ewes (retrospective analysis). Twenty-nine estrous cycles consisting of 3 (n=10; ■, ♦, ●) or 4 waves (n=19; □, ◊, ○) of antral follicular growth were monitored in their entirety in the middle portion of the breeding season. Statistically significant differences between the two groups are indicated by * ($p<0.05$).

it was also shorter than for Peaks 2 and 4 within ewes with 4 follicular waves per estrous cycle ($p<0.05$). Lastly, basal FSH concentrations for Peak 2 were significantly lower than those for Peaks 3 and 4 in ewes with 4 waves, and those in Peak 3 within ewes with 3 waves. Recurrent peaks (Peaks 1, 2, 3) for serum E_2 concentrations were detected between days 4 to 6, 8 to 12 and 13 to 16 in ewes exhibiting 3 waves, and between days 2 to 6, 6 to 11, 9 to 14 and 13 to 16 in ewes with 4 waves (fig. 2). Peaks 1, 2 and 3 were detected earlier ($p<0.05$) in ewes with the 4-wave pattern than in ewes with 3 waves in their estrous cycles (tab. 2). The amplitude of Peak 2 was lower and the duration of Peak 3 was shorter in ewes with 4 waves as compared to those in ewes with 3 waves; in addition, the 4-wave cycles had larger mean and basal E_2 concentrations for Peaks 1 and 2 in comparison with the 3-wave cycles ($p<0.05$). Furthermore, mean E_2 concentrations for Peak 1, in both the 3- and 4-wave cycles, were higher as compared to those observed for Peak 2 ($p<0.05$). Lastly, the duration of Peak 3 was greater ($p<0.05$) in ewes displaying the 3-wave pattern than in ewes exhibiting the 4-wave pattern.

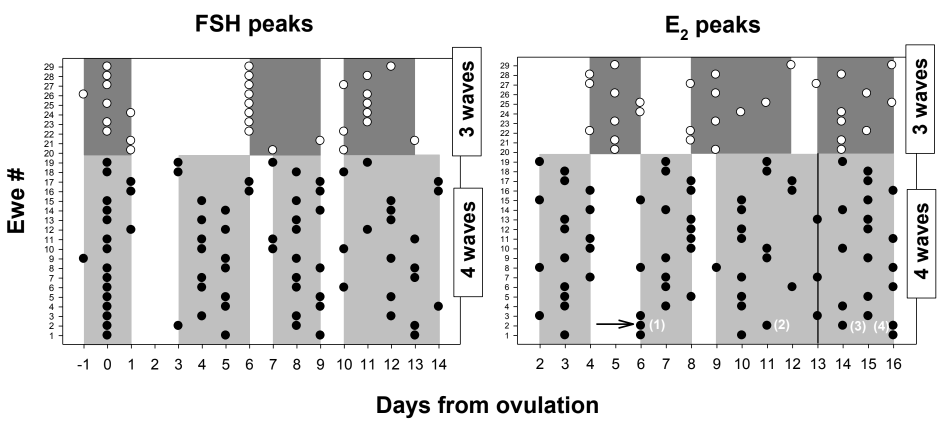

Figure 2. Distribution and grouping of FSH and estradiol (E_2) peaks in individual ewes based on the peak detection using a cycle detector computer program [7]. Numbers in parentheses (right panel, lower chart area) denote the sequence of E_2 peaks in only one ewe (#2) with 4 follicular waves per cycle that had the first peak in serum E_2 concentrations after day 4 post-ovulation (day 6).

Table 2. Mean (±SEM) days of estradiol peak detection, peak amplitude, concentration, duration of fluctuations (nadir-to – peak-to-nadir), and basal concentration (preceding nadir) determined by a cycle detection computer program in cyclic Western White Face ewes (retrospective analysis).

Estradiol	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**
Mean time of peak detection*	3.2±0.2^{A} (2 to 6) *3.9±0.2^{B} (4 to 6)*	7.4±0.3^{A} (6 to 11) *8.3±0.4^{B} (8 to 12)*	11.0±0.3^{A} (9 to 14) *13.5±0.3^{B} (13 to 16)*	15.0±0.2 (13 to 16) -
Mean peak amplitude (pg/ml)	2.8±0.6 *2.8±0.2*	1.7±0.3^{A} *3.0±0.5^{B}*	2.2±0.5 *2.7±0.6*	3.1±0.8 -
Mean peak concentration (pg/ml)	5.5±0.5^{Aa} *4.0±0.2^{Ba}*	3.9±0.4^{Ab} *2.6±0.4^{Bb}*	4.2±0.4^{ab} *4.4±0.8^{ab}*	4.8±0.5^{ab} -
Peak duration (days)	4.2±0.3 *5.0±0.3^{a}*	3.6±0.6 *3.3±0.2^{b}*	3.6±0.4^{A} *5.5±0.2^{Ba}*	3.8±0.5 -
Preceding nadir concentration (pg/ml)	2.1±0.4^{A} *1.0±0.0^{B}*	2.4±0.6^{A} *1.2±0.2^{B}*	2.3±0.7 *1.1±0.1*	2.0±0.4 -

*Day 0=ovulation; regular font: 4 waves; italics: 3 waves; AB represents differences ($p<0.05$) between groups (3 waves vs. 4 waves); ab represents differences ($p<0.05$) within groups.

Antral follicle numbers and wave characteristics

When antral follicle numbers were analyzed for the duration of the inter-ovulatory period, it was found that ewes with 4 waves had a greater number of 2-mm follicles on days – 1, 0, and 15, but on days 3 and 4 they had fewer 2-mm follicles compared with the ewes with 3 follicular waves per cycle ($p<0.05$; fig. 3A). Ewes with 4 waves had a larger number of 3-mm follicles as compared to ewes with 3 waves on days 0, 5–7, 9, 11–14 ($p<0.05$; fig. 3B). The number of 4-mm follicles was greater in ewes with 4 waves as compared to ewes with 3 waves on day 9 ($p<0.05$, fig. 3C). Lastly, the ewes

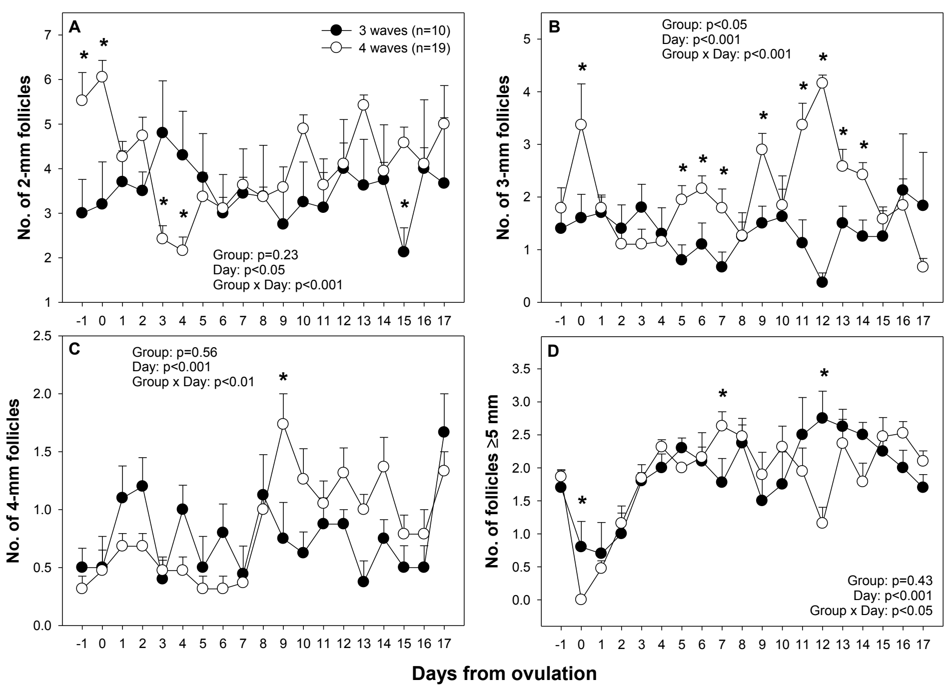

Figure 3. Mean (±SEM) numbers of 2-mm (A), 3-mm (B), 4-mm (C) and ≥5-mm (D) antral follicles in Western White Face ewes with 3 waves (n=10; ●) or 4 follicular waves per estrous cycle (n=19; ○), which underwent daily transrectal ovarian ultrasonography for one full inter-ovulatory interval during the middle portion of the breeding season (November-December).

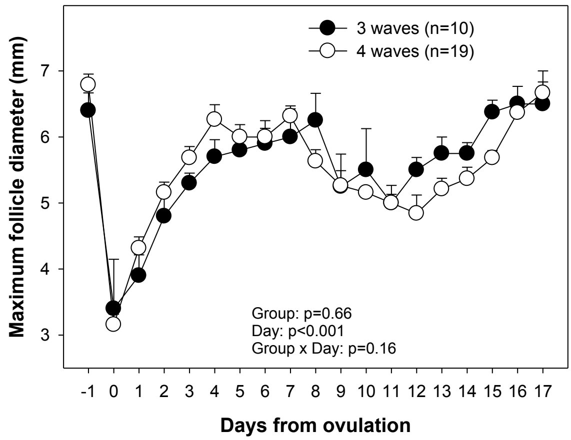

Figure 4. Maximum follicle diameter (mean±SEM) in Western White Face ewes with 3-wave cycles (n=10; ●) or 4-wave cycles (n=19; ○), which underwent daily transrectal ultrasonography during one full inter-ovulatory interval during the middle portion of the breeding season (November-December).

with the 3-wave cycles had a larger number of ≥5-mm follicles on days 0 and 12 but had fewer ovulatory-sized follicles compared to animals with the 4-wave cycles on day 7 ($p<0.05$, fig. 3D). No difference ($p>0.05$) was seen in the daily maximum follicle diameter between the two subsets of ewes (fig. 4). The characteristics of antral follicular waves monitored with transrectal ovarian ultrasonography are summarized in Table 3. Waves 2 and 3 emerged approximately 2 and 3 days earlier, respectively in ewes with 4 waves than in ewes with 3 follicular waves per cycle ($p<0.05$). Additionally, the inter-wave interval between Waves 1 and 2, and Waves 2 and 3 was ~2 and 1 day(s) shorter, successively in ewes with 4 waves as compared to ewes with 3 waves ($p<0.05$). No differences ($p>0.05$) were observed amongst the maximum diameter, growth phase and growth rate of the largest antral follicles of waves between ewes with 3 or 4 waves. However, the static phase was longer in Wave 1 compared to subsequent waves ($p<0.05$), and its duration declined ($p<0.05$) over the estrous cycle in ewes exhibiting both the 3- and 4-wave patterns. In addition, the regressing phase was shortest ($p<0.05$) in Wave 3 within the ewes with the 4-wave cycles.

Table 3. Characteristics of ovarian follicular waves (follicles attaining ≥5 mm in diameter; mean±SEM) in Western White Face ewes with the 3-wave (n=10) or 4-wave (n=19) cycles.

Variable/Group	Wave 1	Wave 2	Wave 3	Wave 4
Day of emergence*	*0.5±0.2* 0.1±0.1	*6.7±0.3*^{A} 4.8±0.4^{B}	*10.9±0.3*^{A} 7.8±0.4^{B}	- 11.7±0.3
No. of follicles/wave	*2.0±0.4* 1.4±0.3	*1.2±0.2* 1.6±0.4	*1.5±0.2* 1.2±0.2	- 1.9±0.5
Largest follicle maximum diameter (mm)	*6.4±0.2* 6.1±0.3	*5.8±0.3* 6.2±0.3	*6.2±0.3* 5.9±0.3	- 6.3±0.2
Growth phase (days)	*3.6±0.5* 3.1±0.4	*2.5±0.4* 3.2±0.4	*4.2±0.5* 3.0±0.3	- 3.6±0.1
Static phase (days)	*3.0±0.6*^{a} 2.7±0.7^{a}	*1.8±0.6*^{b} 1.7±0.3^{b}	*1.7±0.2*^{b} 1.4±0.3^{b}	- 1.5±0.2^{b}
Regressing phase (days)	*3.7±0.4* 4.5±0.4^{a}	*3.0±0.6* 4.2±0.4^{a}	- 2.7±0.3^{b}	- -
Growth rate (mm/days)	*1.1±0.1* 1.2±0.1	*1.2±0.2* 1.1±0.1	*0.8±0.1* 1.1±0.1	- 1.0±0.1
Inter-wave interval (days)	*6.2±0.3*^{A} 4.5±0.2^{B}	*4.2±0.4*^{A} 3.1±0.3^{B}	- 3.7±0.3	- -

*Day 0 = ovulation; regular font: 4 waves; italics: 3 waves; AB = differences (p<0.05) between groups (3 vs. 4 waves); ab = differences (p<0.05) within groups; transrectal ovarian ultrasonography was performed daily over the entire the estrous cycle in the mid-breeding season (November-December).

DISCUSSION

Our present analyses revealed that although progesterone (P_4) concentrations did not differ significantly on any specific day of the estrous cycle, the mean daily concentration of P_4 were significantly higher in ewes with 4 waves as compared to ewes with 3 follicular waves per estrous cycle. Hence, even with a slight increase in P_4 concentration, the inter-peak intervals for serum FSH concentrations were shorter in ewes with 4 waves resulting in an earlier emergence of antral follicles in Waves 2 and 3. This is in agreement with our previous findings in cyclic ewes treated with exogenous P_4 between days 0 and 4 of the interovulatory interval; the creation of mid-luteal phase levels of P_4 early in metestrus advanced the first post-ovulatory FSH peak and follicle wave emergence [20]. However, this finding lacks support from a previous study in which there was no difference in P_4 concentrations between cycling WWF ewes with 3 or 4 antral follicular waves [19]. This discrepancy in results may be due to differences in the sample size; Seekallu *et al*. [19] had a smaller sample size (n=19 in total; n=9 with 3 waves and n=10 with 4 waves) as compared to our retrospective analysis (n=29 in total; n=10 with 3 waves and n=19 with 4 waves). In addition, Seekallu *et al*. did not state at which stage(s) of the breeding season the study had been conducted; serum P_4 concentrations are higher during the mid-breeding season than during the transition to and from the seasonal anestrus [4, 5]. However, the most crucial difference between the two studies was in the classification of the 3- and 4-wave cycles. In the study by Seekallu *et al*. [19], the authors classified the 3- or 4-wave cycles based solely upon ultrasonographically observed numbers of follicle cohorts reaching ≥5 mm in diameter. As some follicular waves consist of only one or two follicles attaining this size range, with ultrasonography performed on a daily basis it is feasible to "miss" the antral follicles that remain at this ostensibly ovulatory size for a relatively short duration. Hence, in our retrospective study, serum FSH concentrations were also used to ensure each antral follicular wave was preceded by an FSH peak and, in case of any discrepancy between the numbers of FSH peaks and emerging follicular waves, the ewes were withdrawn from analyses.

As expected, the post-ovulatory FSH peaks occurred approximately 2 days earlier in WWF ewes with the 4-wave cycles as compared to those with the 3-wave estrous cycles. Interestingly, sheep with 4 follicular waves had lower concentration of FSH as compared to ewes with 3 waves; a similar difference was observed in the study by Seekallu *et al*. [19]. Mean peak concentrations and basal FSH levels (i.e. nadir FSH concentrations) were also consistently greater during the 3-wave cycles. These differences in mean daily FSH concentrations and FSH peak characteristics may be attributed to an increased negative feedback on the hypothalamus and/or anterior pituitary from elevated P_4 and E_2 concentrations in ewes exhibiting the 4-wave pattern or differences in the synthesis of follicular inhibitors of FSH release (e.g., inhibin; [22]).

The E_2 peaks in ewes with 4 waves occurred earlier than in ewes with 3 follicular waves per cycle. In addition, the ewes with 4 follicular waves had higher E_2 concentrations than ewes with 3 waves for the majority of the estrous cycle; the difference was significant on days – 1, 2 and 10. Mean and basal E_2 secretion was greater from early to mid-luteal phase, whereas the duration of E_2 fluctuations was significantly shorter during diestrus in ewes with 4 waves per cycle. In contrast, the study recently performed by Seekallu *et al.* [19] did not report any significant differences in mean daily E_2 concentrations between cyclic WWF ewes with 3 or 4 follicular waves. These differences in serum E_2 concentrations are difficult to explain. It is possible that higher numbers of small (2–3-mm) antral follicles and/or differences in follicular estrogenicity (since there were no significant differences in populations of medium-sized and large antral follicles) contributed to the differences in serum E_2 concentrations between the 3- and 4-wave groups.

Follicle wave characteristics determined in the present study are generally in agreement with previously reported observations on antral follicular turnover in cyclic ewes [3, 9]. Ovulation rates did not differ between ewes with 3 or 4 waves per cycle, which is similar to the findings of a previous study [19]. In the present study, no difference was observed in the maximum follicle diameter between the 3- and 4-wave cycles, including the comparisons within respective waves. In earlier studies in goats [12], cattle [11] and sheep [3, 19], the same trend in maximum follicle diameter was seen throughout the estrous cycle. The inter-wave interval between Waves 1 and 2, in both the 3- and 4-wave cycles, were longer as compared to subsequent waves, which is also in agreement with an earlier study [3]. When antral follicles in different size categories were analyzed, the most apparent difference between ewes with the 3- or 4-wave cycles was noted for 3-mm follicles; animals with 4 waves of follicular growth had more 3-mm follicles as compared to ewes with 3 follicular waves. Differences in numbers of 3-mm follicles could be due to a direct effect of progesterone [8]. A larger number of small antral follicles have been found to grow on the CL-bearing ovary during the ovine luteal phase/early pregnancy, suggesting a local influence of the CL on follicular development [7, 18]. However, in a previous ultrasonographic study, an increase in the number of small antral follicles was observed mainly at the beginning and end of the ovine estrous cycle when serum P_4 concentrations are low [9]. The situation in cattle also offers no obvious insight. Cattle can be classified as having either high or low number of small follicles [21]. It was recently found that high number of small follicles in cows did not have an effect on FSH concentrations produced in the pituitary gland [17]. It is evident that more studies are needed to clarify the relationships between P_4 and FSH concentrations, and the initial growth of ovarian antral follicles in ewes. This may be of interest since animals that have a high number of small, gonadotropin-responsive follicles tend to respond better to superovulation treatments than those with low numbers of such follicles [14, 16].

In conclusion, the evidence from the retrospective data analyses provides strong support for implicating P_4 as the key regulator of circulating FSH concentrations in ewes and in determining the number of antral follicular waves per estrous cycle in ewes [20]. This mechanism might also be in effect in other species that exhibit a wave-like pattern of antral follicular development. The specific mechanism whereby P_4 regulates periodic increases in serum FSH concentrations remains to be elucidated.

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