

Endometrial development during the transition to menopause: preliminary associations with follicular dynamics

A. Baerwald, H. Vanden Brink, C. Lee, C. Hunter, K. Turner & D. Chizen

To cite this article: A. Baerwald, H. Vanden Brink, C. Lee, C. Hunter, K. Turner & D. Chizen (2020): Endometrial development during the transition to menopause: preliminary associations with follicular dynamics, *Climacteric*, DOI: [10.1080/13697137.2020.1721455](https://doi.org/10.1080/13697137.2020.1721455)

To link to this article: <https://doi.org/10.1080/13697137.2020.1721455>



Published online: 20 Feb 2020.



Submit your article to this journal [↗](#)



Article views: 18



View related articles [↗](#)



View Crossmark data [↗](#)

Endometrial development during the transition to menopause: preliminary associations with follicular dynamics

A. Baerwald^a, H. Vanden Brink^b, C. Lee^c, C. Hunter^b, K. Turner^b and D. Chizen^b

^aDepartment of Academic Family Medicine, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada; ^bDepartment of Obstetrics, Gynecology and Reproductive Sciences, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada; ^cDepartment of Mathematics and Statistics, University of Calgary, Calgary, AB, Canada

ABSTRACT

Objective: This study aimed to test the hypothesis that the development of functional luteal phase dominant follicles (LPDFs) is associated with increased endometrial growth as women transition to menopause.

Methods: Endometrial thickness (ET), follicle development, and hormone production were characterized in ovulatory women of mid-reproductive age (MRA; 18–35 years, $n = 10$) and advanced reproductive age (ARA; 45–55 years, $n = 16$). Transvaginal ultrasonography was conducted every 1–3 days during one interovulatory interval to quantify ET and the diameters of follicles ≥ 2 mm. Blood was drawn at each visit to measure progesterone, estradiol, inhibin A, follicle stimulating hormone, and luteinizing hormone.

Results: In the MRA group, ET was lower (8.87 vs. 10.1 mm) in women with typical versus no LPDFs, in association with greater luteal phase estradiol (91.1 vs. 48.8 ng/l). In the ARA group, luteal phase endometrial growth was greater (12.0 vs. 10.4 mm) in women with typical versus no LPDFs, in association with lower progesterone (10.7 vs. 13.8 $\mu\text{g/l}$; LPDF effect $p < 0.1$) and inhibin A (35.6 vs. 51.17 ng/l; $p < 0.10$).

Conclusions: Preliminary findings suggest that ET may be increased in women who develop LPDFs, in association with reduced luteal phase progesterone and inhibin A, during the transition to menopause. Continued research is required to confirm these findings.

ARTICLE HISTORY

Received 26 August 2019
Revised 8 January 2020
Accepted 16 January 2020
Published online 20 February 2020

KEYWORDS

Age; endometrium; estradiol; follicle; inhibin A; menopause; progesterone; ovary

Introduction

The menopausal transition is a period of profound change in female reproductive physiology and menstrual cyclicity¹. Gradual depletion of the ovarian reserve with age corresponds to a reduction in the number of antral follicles emerging for cyclic growth during each menstrual cycle^{2–4}. A decrease in the number of preantral and antral follicles with age is thought to be related to a decrease in both anti-Müllerian hormone^{5–9} and inhibin B^{6,10,11}. The loss of inhibin B-mediated feedback on pituitary gonadotropin production results in a rise in systemic follicle stimulating hormone (FSH)^{10–12}. Luteinizing hormone (LH) concentrations rise gradually as women transition to menopause^{13,14}. Luteal phase progesterone and follicular phase estradiol gradually decline during the menopausal transition, with the fall in estradiol being one of the last changes to occur^{6,15–18}. Depletion of the ovarian reserve and resultant changes in hormone production may be accompanied by variability in menstrual cycle length^{19,20}.

In contrast to gradual changes in hormone production with age, acute and atypical elevations in luteal phase estradiol have been reported in approximately 30% of women of advanced reproductive age (ARA)²¹. These atypical elevations

in systemic estradiol have been termed luteal out-of-phase events, or ‘LOOP cycles’. The acute rise in estradiol during the luteal phase occurred simultaneously with a decline in progesterone in ARA women. The acute elevations in luteal phase estradiol have been associated with the development of anovulatory and ovulatory luteal phase dominant follicles (LPDFs)^{22,23}. In women of ARA versus mid-reproductive age (MRA), LPDFs grew atypically large and persisted for a long period of time. ARA women with LPDFs exhibited greater estradiol, lower progesterone, and lower inhibin A during the luteal phase compared to ARA women without LPDFs^{22,23}.

Ovarian hormone production regulates the growth dynamics of the endometrium throughout the menstrual cycle. The endometrium is generally thinnest at the end of menstruation, undergoes proliferation throughout the follicular phase under the influence of dominant follicle estradiol production, and reaches maximal thickness in the mid luteal phase due to progesterone-mediated increased glandular secretions^{24,25}.

Endometrial hyperplasia is a condition of endometrial overgrowth that becomes most prevalent in the early postmenopause²⁶. The diagnosis of endometrial hyperplasia is made clinically, following postmenopausal bleeding.

Transvaginal ultrasonography is the initial test for evaluating postmenopausal bleeding. Endometrial thickness (ET) >5 mm in women with postmenopausal bleeding^{27,28} and >11 mm in those without postmenopausal bleeding²⁹ has been correlated with an increased risk of endometrial cancer. Thus, measurements in these ranges warrant further investigation with endometrial biopsy. Endometrial hyperplasia is associated with obesity, and occurs in 6% of asymptomatic reproductive age women and 12% of asymptomatic postmenopausal women³⁰. Endometrial cancer has been associated with endometrial hyperplasia in 3–4% of asymptomatic postmenopausal women³¹ and in 15% of perimenopausal women with polymenorrhagia/menorrhagia³². A cumulative 23–28% risk of progression to carcinoma has been reported for women with atypical hyperplasia^{33,34}. The incidence of endometrial hyperplasia and carcinoma increases during the use of menopausal hormone therapy, more specifically with unopposed estrogen or sequential combined menopausal hormone therapy^{35,36}.

The underlying pathophysiology of the age-related increased incidence of endometrial hyperplasia is not well understood. In addition to exogenous hormones, insufficient endogenous progesterone and/or excess endogenous estrogen can result in endometrial hyperplasia or carcinoma in women of reproductive age³⁷. The recurrent development of anovulatory follicles and associated unopposed estradiol secretion in women with polycystic ovarian syndrome has been well documented to place women at increased risk of endometrial hyperplasia³⁸. Based on this knowledge, it is plausible that changes in ovarian follicular growth dynamics associated with atypical elevations in estradiol during the transition to menopause may result in increased endometrial development.

The objective of this study was to determine whether the development of LPDFs and associated hormone changes as women transition to menopause occur simultaneously with changes in endometrial growth. We tested the hypothesis that endometrial development would increase in the presence of an LPDF, in association with increased estradiol, inhibin A, FSH, and LH and reduced progesterone as women age.

Methods

A prospective, observational study was conducted from 2006 to 2011. The study protocol was approved by the Biomedical Research Ethics Board at the University of Saskatchewan and the Strategic Planning and Priorities Committee of the Saskatchewan Health Authority. Informed consent was obtained from each participant before study procedures were initiated. The study was conducted in accordance with the Tri-Council Policy Statement on the Ethical Conduct of Research Involving Humans.

Study sample

Thirty women were recruited in the following manner: MRA (18–35 years; $n = 10$) and ARA (45–55 years; $n = 20$). Inclusion

criteria included: a normal complete blood count; and normal serum thyroid-stimulating hormone, prolactin, and β -human chorionic gonadotropin. Women aged 18–35 years must have had a history of regular menstrual cycles in order to participate. Women aged 45–55 years must have experienced no more than 12 months of amenorrhea to be eligible. Exclusion criteria included: body mass index <18 or >35 , use of any hormone therapies within 2 months of enrollment, current smokers, women with documented ovarian failure and/or currently diagnosed infertility of unexplained or female origin, dysfunctional uterine bleeding, medical conditions or use of medications known or suspected to interfere with reproductive function, presence of only one ovary, inability to visualize the ovaries ultrasonographically, pregnancy, lactation within the last 12 months, surgery during the course of the study, and/or participation in an investigational drug trial within 30 days of study participation. Data from women with ovulatory cycles were included in our analyses ($n = 26$). Women were excluded from analyses due to anovulatory cycles ($n = 2$) and development of lag phases of follicle development ($n = 2$). A lag phase was defined as a period of no follicular development ≥ 6 mm for more than 20 days from menses or ovulation (i.e. based on 2 standard deviations above the mean follicular or luteal phase length).

Ultrasonography

The ovaries and uterus of each participant were evaluated every Monday, Wednesday, and Friday throughout one inter-ovulatory cycle (IOI) using two-dimensional transvaginal ultrasonography. An IOI was defined as the period from one ovulation to the subsequent ovulation; hence, an IOI was represented by the luteal phase followed by the follicular phase. When a follicle ≥ 14 mm was detected, ultrasonographic examinations were conducted daily until the follicle fate was determined (i.e. ovulation, regression). Video clips of both ovaries and the uterus were obtained at each visit. The numbers and diameters of all antral follicles >2 mm as well as the ET and pattern were measured retrospectively from ultrasonographic images. ET was measured as the distance from the anterior to posterior border of the stratum basalis layers in the mid-sagittal plane of the uterus, approximately 1 cm from the fundus (SanteSoft DICOM Editor 3.1.23)²⁴. Ultrasonographic examinations were performed by two observers (A.B., H.V.B.). Retrospective evaluations of ultrasonographic video clips were conducted by a single observer (K.T.) in order to ensure blinding.

Hormone analyses

Blood samples were collected every 1–3 days during the IOI, between the hours of 0800 and 1600. Serum was isolated and frozen at -80°C . After study completion, the samples were transported to the Prairie Diagnostics Services Laboratory at the University of Saskatchewan for quantification of FSH, LH, estradiol, progesterone, and inhibin A. The assays used were obtained as follows: FSH and LH (Immulite; Siemens Healthcare Diagnostics Inc.), 17β -estradiol and progesterone (Count-A-Coat; Siemens Healthcare Diagnostics

Inc.), and inhibin A (Gen II assay; Beckman Coulter Inc.). FSH and LH assays were performed in singlicate. The inhibin A, 17 β -estradiol, and progesterone assays were performed in duplicate. Intra-assay and inter-assay coefficients of variation were as follows: inhibin A, 5.5%, 7.8%; progesterone, 13.0%, 15.2%; estradiol, 9.9%, 12.9%; FSH, 6.7% (single assay); and LH, 3.4% (single assay). Sensitivity was detected as follows: inhibin A 5 ng/L; progesterone, 0.02 mcg/L; estradiol, 1.4 ng/L; FSH, 0.1 IU/L; and LH, 0.1 IU/L.

Statistics

The primary study endpoint was ET. Secondary endpoints included follicle diameter and serum concentrations of estradiol, progesterone, estradiol:progesterone ratio, FSH, LH, and inhibin A. Changes in ET and hormone concentrations over the IOI were tabulated and compared between MRA and ARA women, and within MRA and ARA women with and without LPDFs. An LPDF was defined as a follicle which grew to a diameter of ≥ 10 mm in the luteal phase and exceeded all other follicles in the wave by ≥ 2 mm, as previously reported²³. Typical LPDFs did not exceed the diameter of a normal preovulatory follicle. Atypical LPDFs were those that developed to a diameter exceeding that of a normal preovulatory follicle (≥ 26 mm). As previously described²³, MRA women developed either no or typical LPDFs; ARA women developed either no, typical, or atypical LPDFs.

The mean ET and hormone concentrations over the luteal and follicular phases were compared among groups using independent-sample t-tests and analysis of variance (ANOVA) (SAS version 9.4, 2013; SAS Institute, Cary, NC, USA).

Data across the IOI for each participant were aligned to the day of ovulation 1 to demarcate onset of the luteal phase, and aligned to the first day of menses to demarcate onset of the follicular phase. Growth curve modeling and repeated-measures ANOVA (PROC MIXED; SAS version 9.4) with mixed-effects models were conducted to determine the effects of age, day, LPDF, day \times age, and day \times LPDF interactions across the luteal and follicular phases. Mixed-effects models were fitted to determine whether changes in follicle and hormone endpoints occurred in a linear (x) or quadratic

(x^2) pattern over each phase. Significant main factors and interactions were determined and presented for each endpoint. In the model, 'age effect' refers to a change in outcome with age. A 'day effect' refers to a change in outcome over time. An 'LPDF effect' refers to a difference in mean outcome between women with no versus typical versus atypical LPDFs. A 'day by age interaction effect' indicates a difference in outcome patterns over time between the MRA versus ARA groups. A 'day by LPDF interaction effect' indicates differences in outcome patterns over time between women with no versus typical versus atypical LPDFs. All statistical analyses were conducted with a significance level of 0.10.

Results

Endometrial thickness and age

Changes in ET throughout the IOI in MRA versus ARA women are illustrated in Figure 1. Overall, the mean ET was greater during the luteal and follicular phases in ARA women versus MRA women, respectively (Figure 1, Table 1; $p < 0.1$). In both MRA and ARA women, the endometrium fluctuated during the luteal phase, reached a nadir during menses, and then increased throughout the follicular phase (Figure 1).

Endometrial thickness and antral follicular dynamics

In the MRA group, luteal phase endometrial growth was lower in women with typical versus no LPDFs (Figure 2(A), Table 2; $p < 0.1$), while the follicular phase ET did not differ between groups (Figure 2(A), Table 2; $p > 0.1$). In contrast, in the ARA group, women with typical LPDFs had greater ET than women with no or atypical LPDFs in both the luteal and the follicular phases (Figure 3(A), LPDF effect $p < 0.1$; Table 2, $p < 0.1$).

Endometrial thickness and estradiol

MRA women with typical LPDFs had greater luteal phase estradiol compared to women without LPDFs, in association

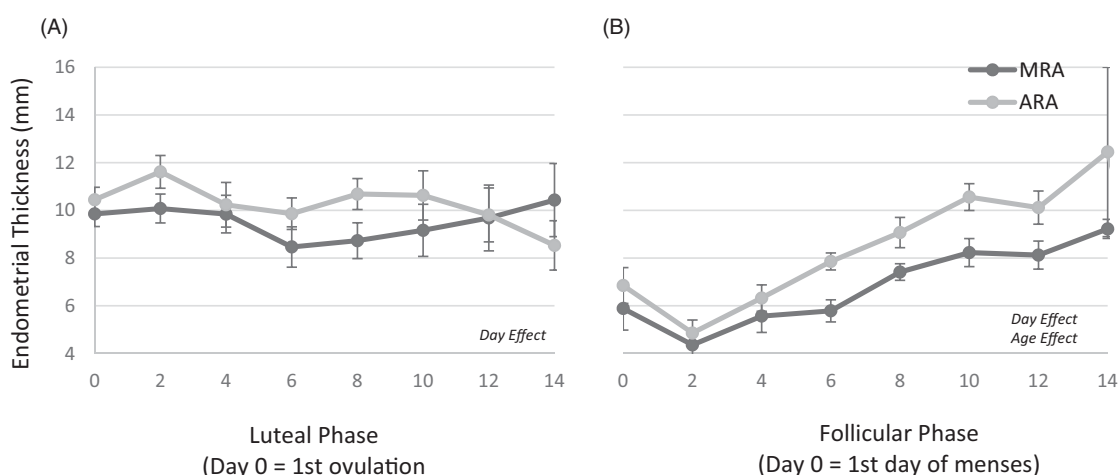


Figure 1. Endometrial thickness over the interovulatory interval in women of mid-reproductive age (MRA, dark line) and advanced reproductive age (ARA, light line) in the luteal phase (A) and follicular phase (B). Data are binned into 2-day intervals and centralized to the day of the luteal and follicular phases, respectively. Data are represented as mean \pm standard error. Significant outcome and interaction effects are indicated ($p < 0.10$).

Table 1. Mean endometrial thickness (ET) in mid-reproductive age (MRA) versus advanced reproductive age (ARA) women.

Mean ET (mm)	MRA women	ARA women	p-Value
Luteal phase	9.7 ± 0.32	11.3 ± 0.74	0.03
Follicular phase	6.96 ± 0.31	7.96 ± 0.43	0.03

Data are shown as mean ± standard error.

with lower ET (Table 2; Figure 2(B), LPDF effect $p < 0.1$). No differences in follicular phase estradiol or ET were detected in MRA women between LPDF groups (Table 2; Figure 2(B), LPDF effect $p < 0.1$).

ARA women with atypical LPDFs had greater luteal phase estradiol but lower ET compared to women with typical or no LPDFs (Figure 3(B), LPDF effect $p < 0.1$; Table 2).

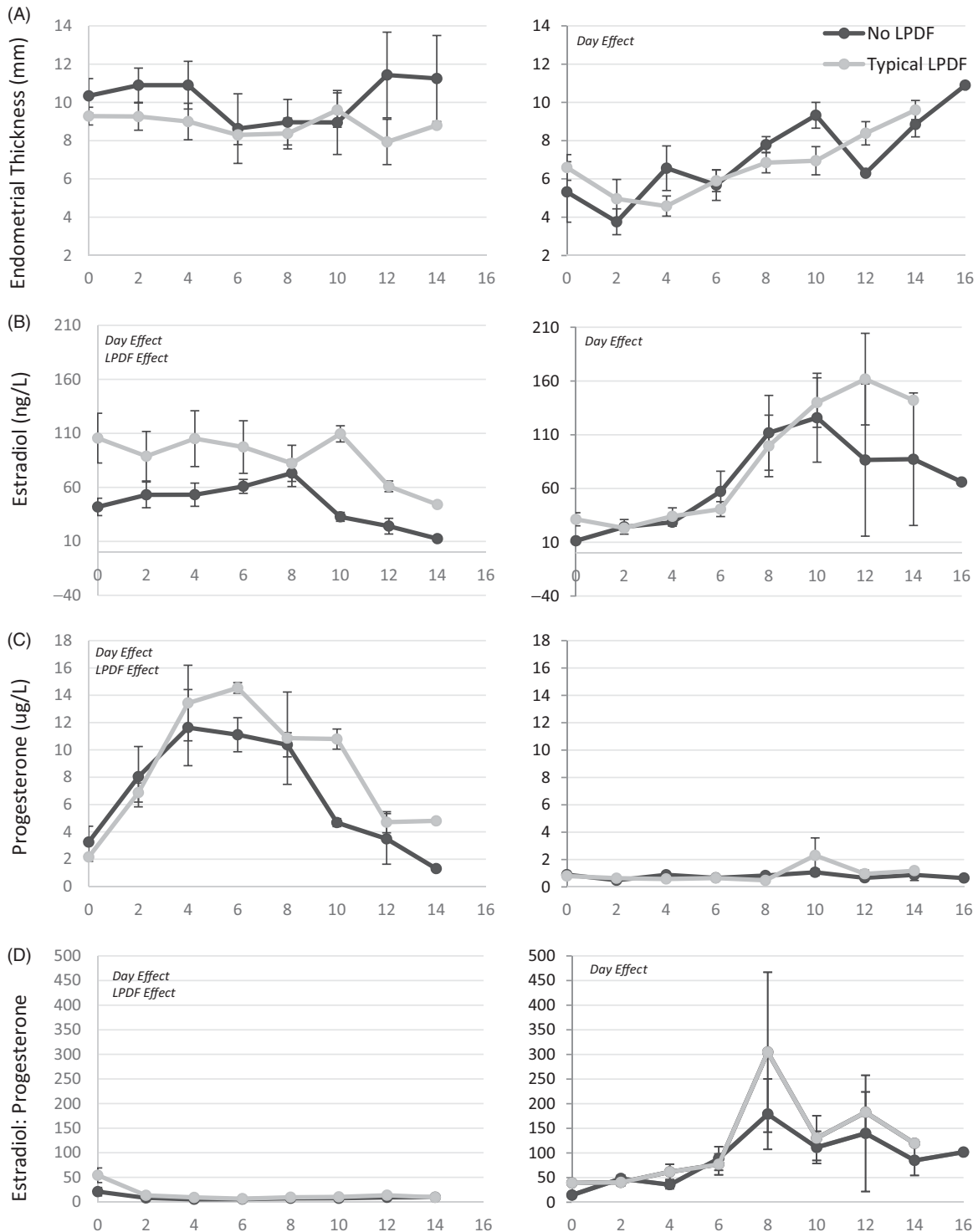


Figure 2. Endometrial thickness (A), estradiol (B), progesterone (C), estradiol:progesterone ratio (D), inhibin A (E), follicle stimulating hormone (FSH) (F), and luteinizing hormone (LH) (G) over the interovulatory interval among women of mid-reproductive age (MRA) with versus without luteal phase dominant follicles (LPDFs). Data are binned into 2-day intervals and centralized to the day of the first ovulation and day of menses, respectively. Data are represented as mean ± standard error. Differences between MRA women with no LPDFs (dark line) versus typical LPDFs (light line) are shown ($p < 0.10$).

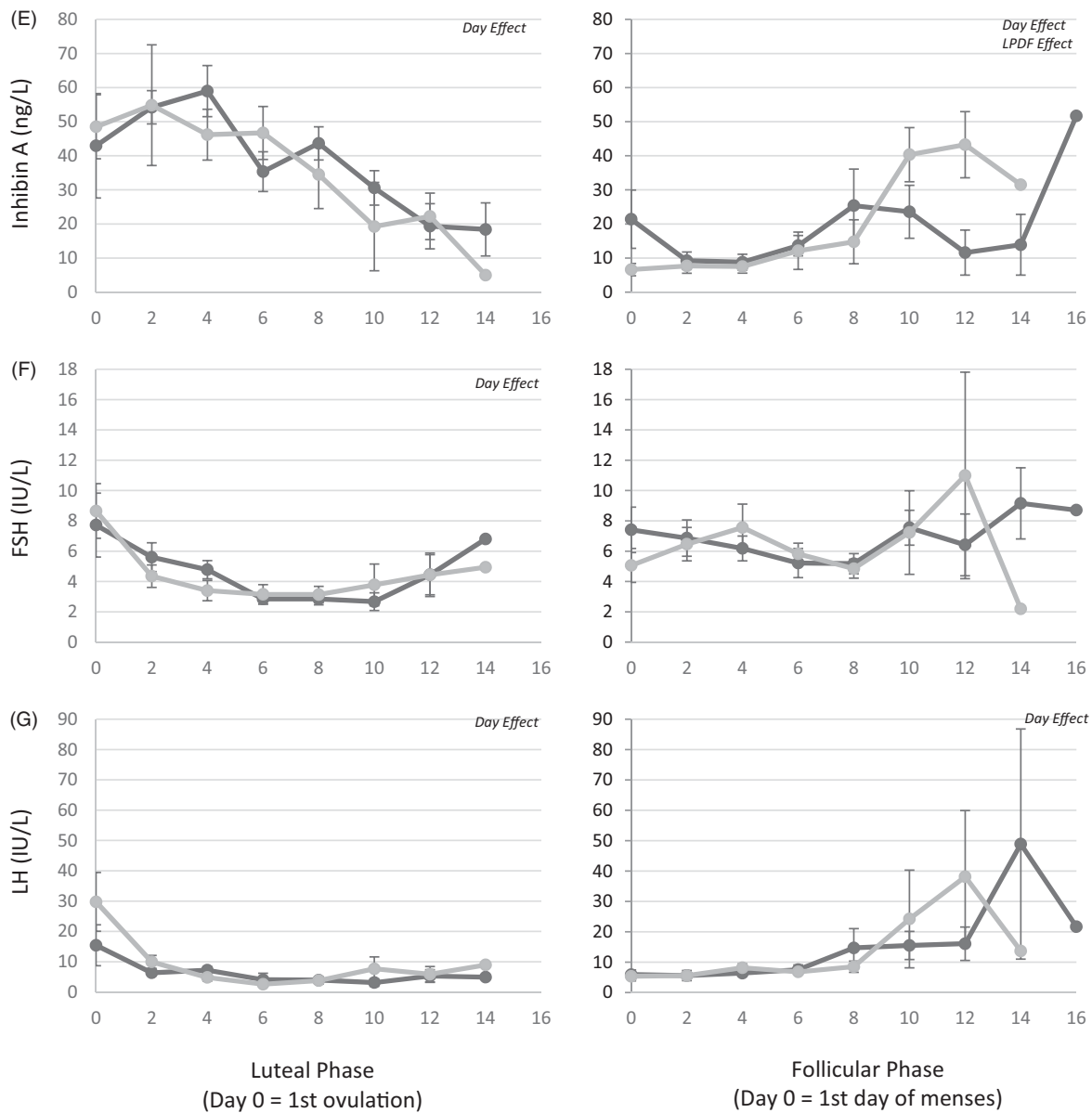


Figure 2. Continued.

Table 2. Endometrial and endocrine dynamics in women with and without a luteal phase dominant follicle (LPDF).

Age group	Cycle phase	LPDF	n	ET (mm)	Estradiol (ng/l)	Progesterone (μ g/l)	Estradiol:progesterone ratio	Inhibin A (ng/l)	LH (IU/l)	FSH (IU/l)
MRA	Luteal	None	5	10.08 \pm 0.46 ^a	48.75 \pm 4.79 ^a	7.32 \pm 0.83	9.17 \pm 1.44 ^a	40.76 \pm 3.4	6.32 \pm 1.1	4.56 \pm 0.45
		Typical	5	8.87 \pm 0.28 ^b	91.07 \pm 8.03 ^b	8.81 \pm 1.07	16.94 \pm 3.74 ^b	40.27 \pm 4.69	9.53 \pm 2.2	4.49 \pm 0.49
	Follicular	None	5	6.88 \pm 0.43	60.88 \pm 10.69	0.80 \pm 0.06	81.21 \pm 14.34 ^a	17.33 \pm 2.59	12.32 \pm 2.72	6.63 \pm 0.42
		Typical	5	6.59 \pm 0.33	72.58 \pm 11.17	0.89 \pm 0.17	111.51 \pm 24.18 ^b	18.02 \pm 3.06	12.94 \pm 3.5	6.64 \pm 0.92
ARA	Luteal	None	8	10.36 \pm 0.39 ^a	78.4 \pm 7.31 ^a	13.77 \pm 1.28 ^a	11.64 \pm 3.96 ^a	51.17 \pm 4.59 ^a	6.58 \pm 0.62	5.36 \pm 0.43
		Typical	4	12.02 \pm 0.51 ^b	68.97 \pm 8.97 ^a	10.66 \pm 1.08 ^a	10.24 \pm 3.05 ^a	35.55 \pm 4.62 ^b	6.48 \pm 1.02	8.22 \pm 1.2
		Atypical	4	9.17 \pm 0.49 ^a	198.57 \pm 31.72 ^b	7.38 \pm 1.05 ^b	41.27 \pm 10.94 ^b	36.28 \pm 4.51 ^b	9.07 \pm 1.53	5.99 \pm 0.73
	Follicular	None	8	7.48 \pm 0.35 ^a	95.41 \pm 14.23	0.73 \pm 0.07 ^a	391.23 \pm 230.55	22.38 \pm 2.71	11.87 \pm 1.72 ^a	10.53 \pm 0.88
		Typical	4	9.65 \pm 0.68 ^b	100.03 \pm 20.79	1.08 \pm 0.13	123.5 \pm 26.06	23.47 \pm 4.08	14.65 \pm 3.26 ^a	11.54 \pm 1.1
		Atypical	4	7.22 \pm 0.46 ^a	97.12 \pm 19.82	3.15 \pm 0.95 ^b	117.59 \pm 28.14	16.71 \pm 2.74	7.61 \pm 1.39 ^b	10.09 \pm 1.5

Data are shown as mean \pm standard error. Differences assessed by *t*-test and analysis of variance. ARA advanced reproductive age; ET, endometrial thickness; MRA, mid-reproductive age.

^a vs. ^b vs. ^c: within each age group and phase, *p* < 0.10.

Endometrial thickness and progesterone

In the MRA group, mid and late luteal phase progesterone were greater in women with versus without LPDFs

(Figure 2(C), LPDF effect $p < 0.1$); no differences in ET were observed in the LPDF groups.

In the ARA group, women with typical LPDFs had lower progesterone and greater ET compared to women with no

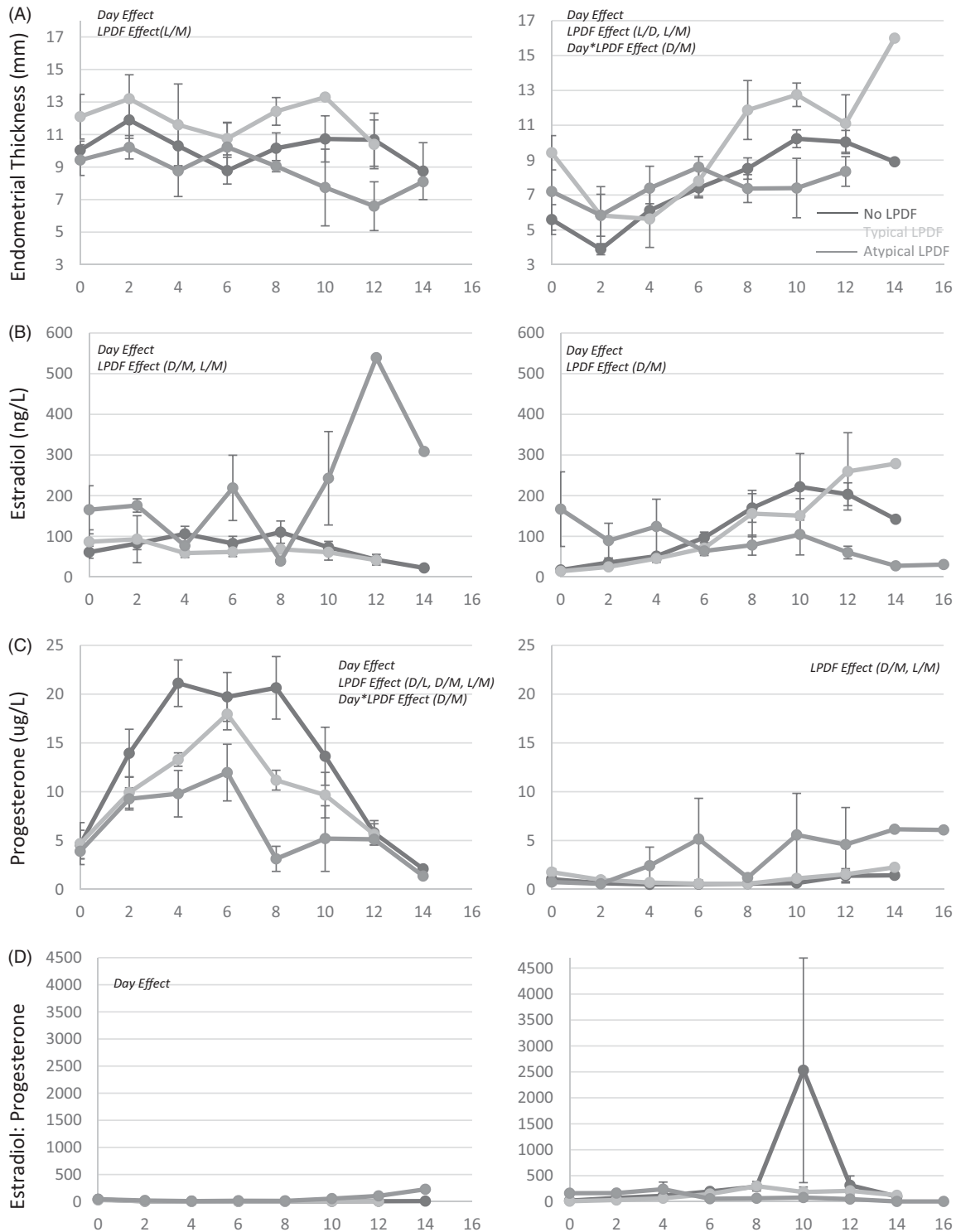


Figure 3. Endometrial thickness (A), estradiol (B), progesterone (C), estradiol:progesterone ratio (D), inhibin A (E), follicle stimulating hormone (FSH) (F), and luteinizing hormone (LH) (G) over the interovulatory interval among women of advanced reproductive age (ARA) with versus without luteal phase dominant follicles (LPDFs). Data are binned into 2-day intervals and centralized to the day of the first ovulation and day of menses, respectively. Data are represented as mean \pm standard error. ^{D/L}Differences between ARA women with no LPDFs (dark line, D) versus typical LPDFs (light line, L) are shown ($p < 0.10$). ^{D/M}Differences between ARA women with no LPDFs (dark line, D) versus atypical LPDFs (medium line, M) are shown ($p < 0.10$). ^{L/M}Differences between ARA women with typical LPDFs (light line, L) versus atypical LPDFs (medium line, M) are shown ($p < 0.10$).

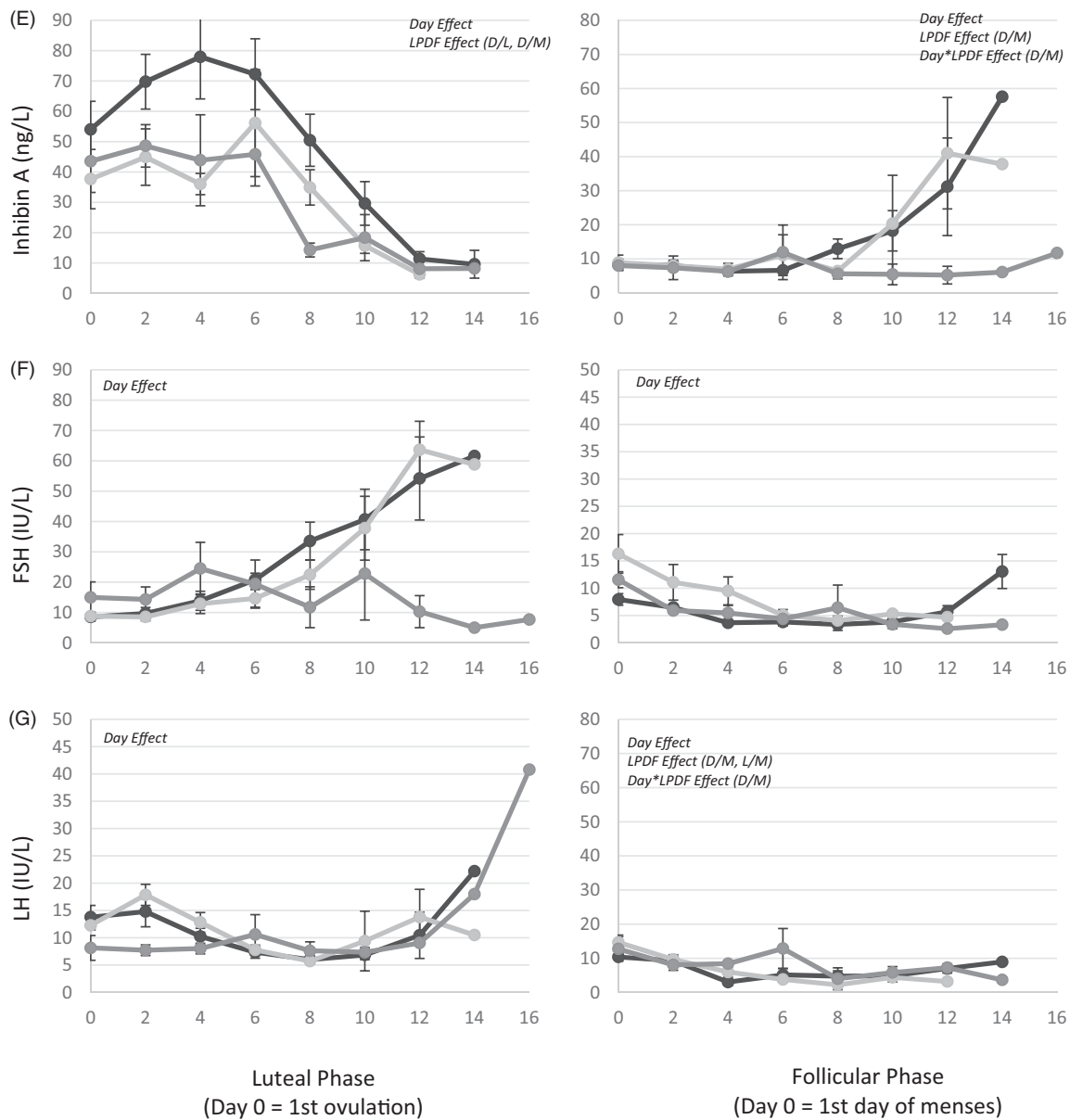


Figure 3. Continued.

LPDFs (Figure 3(C), LPDF effect $p < 0.1$). In contrast, ARA women with atypical LPDFs had lower luteal phase progesterone and lower ET compared to those women with typical LPDFs (Figure 3(C), LPDF effect $p < 0.1$; Table 2, $p < 0.1$).

Endometrial thickness and estradiol:progesterone ratio

In MRA women, the luteal phase estradiol:progesterone ratio was greater in women with versus without LPDFs, in association with lower ET ($p < 0.1$, Table 2, Figure 2(D)). The follicular phase estradiol:progesterone ratio was also greater in women with versus without LPDFs; however, no differences in ET were observed between groups.

In the ARA group, women with atypical LPDFs had a greater luteal phase estradiol:progesterone ratio but lower ET compared to women with typical LPDFs ($p < 0.1$, Table 2, Figure 3(D)).

Endometrial thickness and inhibin A

No differences in inhibin A were detected in MRA women with versus without LPDFs, in association with ET (Figure 2(E), Table 2; $p > 0.1$).

In the ARA group, women with typical LPDFs had greater luteal phase ET and lower inhibin A compared to those with no LPDFs (Figure 3(E), Table 2; LPDF effect $p < 0.1$).

Endometrial thickness and gonadotropins

In the ARA group, women with typical LPDFs had greater follicular phase ET and greater LH compared to women with atypical LPDFs (Figure 3(G), Table 2; $p > 0.1$). No differences in LH were observed between MRA women with versus without LPDFs (Figure 2(G), Table 2; $p > 0.1$). No differences in FSH were observed between MRA women (Figure 2(F) or ARA

women (Figure 3F) with versus without LPDFs (Table 2; $p > 0.1$).

Discussion

Acute and atypical elevations in luteal phase estradiol and decreases in progesterone and inhibin A have been reported to occur in association with increased variability in antral follicular dynamics as women age²³. In this study, we investigated potential associations between the development of LPDFs, reproductive hormone production, and endometrial growth in ovulatory women during the transition to menopause. We obtained preliminary data to suggest that the endometrium develops to a greater degree, in association with lower luteal phase progesterone and inhibin A in women of ARA who develop LPDFs to a typical preovulatory diameter. In contrast, the development of atypically large, persistent, and estrogenic LPDFs in women of ARA was not associated with increased endometrial growth. Thus, our hypotheses were partially supported.

When considering age alone, the endometrium developed to a greater degree throughout the IOI in older versus younger women. However, when antral follicular dynamics were considered within each age group, variable results were obtained. We expected that LPDF development in younger women would have led to increased estradiol, a greater estradiol:progesterone ratio, and greater inhibin A, thereby inducing endometrial growth. On the contrary, the endometrium developed to a lesser degree in the luteal phase in MRA women with LPDFs, in association with greater luteal phase estradiol, slightly greater progesterone, and a greater estradiol:progesterone ratio; no relationships with inhibin A, LH, or FSH were detected. Luteal phase estradiol may originate from either the corpus luteum or dominant follicles, while follicular phase estradiol originates from dominant follicles only^{39,40}. It is possible that the amplitude of increased estradiol in younger women with LPDFs was not sufficient enough or did not occur over a long enough period of time to influence the endometrium. Furthermore, it is likely that women with recurrent development of estrogenic LPDFs over multiple cycles would have a greater chance of endometrial overgrowth.

In contrast to younger women, the mean ET was greater in ARA women with typical LPDFs compared to those with atypical or no LPDFs in both the luteal and follicular phases of the IOI. Greater mean ET in this group occurred simultaneously with lower luteal phase inhibin A and progesterone, but not greater estradiol. The finding that the endometrium developed to a greater degree in ARA women with typical, but not atypically large, estrogenic dominant follicles in this study did not support our hypotheses. We attribute these findings to a small sample size. Luteal phase estradiol in ARA women with atypically large LPDFs was more than double and progesterone was less than half that of ARA women with typical or no LPDFs. However, endometrial development was not increased. The day of emergence and growth intervals of the atypically large LPDFs and the corresponding acute elevations in estradiol in ARA women varied across the

luteal phase, making it unlikely to detect changes in mean endometrial growth in this small group of women ($n = 4/20$).

Greater luteal phase endometrial development in ARA women with typical LPDFs, in association with lower inhibin A and progesterone production, is consistent with current notions of follicular versus luteal origins of luteal phase hormone production. Luteal phase inhibin A may originate from dominant follicles or the corpora lutea, while follicular phase inhibin A is produced solely by dominant follicles. Lower luteal phase inhibin A and progesterone but greater ET in ARA women with typical LPDFs suggests that LPDFs suppress luteal function but, at the same time, promote endometrial growth. Our data support the notion of decreased progesterone, rather than increased estradiol, as a contributing factor to endometrial growth as women age. Preliminary data from our laboratory have shown that variations in antral folliculogenesis contribute to luteal insufficiency as women age⁴¹. However, further research is required to elucidate the contributing factors of luteal insufficiency and declining progesterone on the increased risk of endometrial hyperplasia and malignancy during the transition to menopause.

The greatest strength of this study was the collection of follicular, endometrial, and hormone data every 1–3 days across an entire cycle. The biggest limitation of this study was the small sample size. The collection of serial ultrasonographic images and blood samples throughout an entire IOI, especially in women with longer cycles, is a very time consuming and expensive process. The collection of serial data is essential to observe patterns of change in outcomes across the IOI. A sample size of 30 subjects was a reasonable starting point to test our hypotheses. However, follicle dynamics among ovulatory women were more variable than anticipated, with increasing variability as women age. Due to the small number of ARA women with typical ($n = 4$) and atypical ($n = 4$) LPDFs, our findings were preliminary in nature. It is possible that nocturnal variations in hormone production (over 24 h) may have confounded the study findings; however, it is not practical to take blood samples more than once daily in research studies of this nature. We evaluated women with ovulatory cycles in the present study. It is likely that aberrancies in follicular and endocrine dynamics are more profound in women with anovulatory cycles during the menopausal transition. Continued research is required using a larger sample size of ovulatory and anovulatory women over multiple cycles to confirm how age-related aberrancies in antral follicle dynamics and hormone production influence the growth of the endometrium.

Another limitation of this study was the lack of statistical methodologies available to analyze variations in endpoints over time. Repeated-measures statistics require an assumption of variance structure. Due to variability in follicle dynamics and hormone production as women age, repeated-measures statistics may not adequately reflect clinically meaningful variability in outcomes. The development of additional methods for the statistical analyses of serial data with inherent variability are necessary to further research in this area.

Next steps should include histologic, ultrasonographic, and endocrinologic evaluation of endometrial dynamics as women approach menopause. The World Health Organization classification of endometrial hyperplasia lacks diagnostic reproducibility⁴². Therefore, future studies to evaluate endometrial hyperplasia should strive to maintain diagnostic agreement among pathologists. Knowledge in this area has implications for understanding the pathophysiology of endometrial hyperplasia, which may ultimately lead to strategies for preventing endometrial malignancy and improving the quality of life for women as they age.

Conclusion

Preliminary findings suggest that endometrial growth is increased in ovulatory women who develop LPDFs, in association with reduced luteal phase progesterone and inhibin A during the transition to menopause. The relative roles of luteal versus dominant follicle estradiol production on endometrial growth and associations with endometrial hyperplasia and malignancy as women age require further investigation.

Acknowledgements

The authors would like to thank the research volunteers, whose participation was invaluable for the conduct of this study. The authors would also like to thank Dr Roger Pierson at the University of Saskatchewan, Canada for assistance with facilitating data collection and Dr David Robertson at the Hudson Institute of Medical Research, Australia for assistance with data interpretation in this study.

Potential conflict of interest No potential conflict of interest was reported by the author(s).

Source of funding This work was supported by the National Health and Medical Research Council, Canadian Institutes of Health Research – Banting and Best Graduate Training Award, Canadian Foundation for Women's Health, Victorian Government's Operational Infrastructure Support Program and the University of Saskatchewan, Establishment Grant.

References

- Butler L, Santoro N. The reproductive endocrinology of the menopausal transition. *Steroids* 2011;76:627–35
- Block E. Quantitative morphological investigations of the follicular system in women: variations at different ages. *Acta Anat* 1952;14:108–23
- Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. *Biol Reprod* 1994;50:653–63
- Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. *Hum Reprod* 2008;23:699–708
- Dayal M, Sagar S, Chaurasia A, Singh U. Anti-mullerian hormone: a new marker of ovarian function. *J Obstet Gynecol India* 2014;64:130–3
- Robertson DM, Hale GE, Fraser IS, Hughes CL, Burger HG. A proposed classification system for menstrual cycles in the menopause transition based on changes in serum hormone profiles. *Menopause* 2008;15:1139–44
- Seifer DB, Maclaughlin DT. Mullerian inhibiting substance is an ovarian growth factor of emerging clinical significance. *Fertil Steril* 2007;88:539–46
- van Rooij IA, Broekmans FJ, Te Velde ER. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 2002;17:3065–71
- Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Mullerian hormone: a new marker for ovarian function. *Reproduction* 2006;131:1–9
- Burger HG, Dudley E, Mamers P, Groome N, Robertson DM. Early follicular phase serum FSH as a function of age: the roles of inhibin B, inhibin A and estradiol. *Climacteric* 2000;3:17–24
- Klein NA, Houmard BS, Hansen KR, et al. Age-related analysis of inhibin A, inhibin B, and activin a relative to the intercycle monotropic follicle-stimulating hormone rise in normal ovulatory women. *J Clin Endocrinol Metab* 2004;89:2977–81
- Robertson DM, Hale GE, Jolley D, Fraser IS, Hughes CL, Burger HG. Interrelationships between ovarian and pituitary hormones in ovulatory menstrual cycles across reproductive age. *J Clin Endocrinol Metab* 2009;94:138–44
- Ahmed Ebbiary NA, Lenton EA, Cooke ID. Hypothalamic-pituitary ageing: progressive increase in FSH and LH concentrations throughout the reproductive life in regularly menstruating women. *Clin Endocrinol* 1994;41:199–206
- Lee SJ, Lenton EA, Sexton L, Cooke ID. The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone in women with regular menstrual cycles. *Hum Reprod* 1988;3:851–5
- Miro F, Parker SW, Aspinall LJ, Coley J, Perry PW, Ellis JE. Sequential classification of endocrine stages during reproductive aging in women: the FREEDOM study. *Menopause* 2005;12:281–90
- Randolph JF Jr, Sowers M, Bondarenko I, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *J Clin Endocrinol Metab* 2005;90:6106–12.
- Santoro N, Brown JR, Adel T, Skurnick JH. Characterization of reproductive hormonal dynamics in the perimenopause. *J Clin Endocrinol Metab* 1996;81:1495–501
- Sherman BM, West JH, Korenman SG. The menopausal transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. *J Clin Endocrinol Metab* 1976;42:629–36
- Harlow SD, Gass M, Hall JE, et al. Executive summary of the stages of reproductive aging workshop +10: addressing the unfinished agenda of staging reproductive aging. *Climacteric* 2012;15:105–14
- Miro F, Parker SW, Aspinall LJ, Coley J, Perry PW, Ellis JE. Origins and consequences of the elongation of the human menstrual cycle during the menopausal transition: the FREEDOM Study. *J Clin Endocrinol Metab* 2004;89:4910–5
- Hale GE, Hughes CL, Burger HG, Robertson DM, Fraser IS. Atypical estradiol secretion and ovulation patterns caused by luteal out-of-phase (LOOP) events underlying irregular ovulatory menstrual cycles in the menopausal transition. *Menopause* 2009;16:50–9
- Vanden Brink H, Chizen D, Hale G, Baerwald A. Age-related changes in major ovarian follicular wave dynamics during the human menstrual cycle. *Menopause* 2013;20:1243–54
- VandenBrink H, Robertson DM, Lim H, et al. Associations between antral ovarian follicle dynamics and hormone production throughout the menstrual cycle as women age. *J Clin Endocrinol Metab* 2015;100:4553–62
- Baerwald AR, Pierson RA. Endometrial development in association with ovarian follicular waves during the menstrual cycle. *Ultrasound Obstet Gynecol* 2004;24:453–60
- Bree RL. Ultrasound of the endometrium: facts, controversies, and future trends. *Abdom Imaging* 1997;22:557–68
- Reed SD, Newton KM, Clinton WL, et al. Incidence of endometrial hyperplasia. *Am J Obstet Gynecol* 2009;200:678 e1–6

27. The role of transvaginal ultrasonography in evaluating the endometrium of women with postmenopausal bleeding. American College of Obstetricians and Gynecologists (ACOG) committee opinion. Number 734, May 2018. Available from: www.acog.com.
28. Goldstein RB, Bree RL, Benson CB, et al. Evaluation of the woman with postmenopausal bleeding: Society of Radiologists in Ultrasound-Sponsored Consensus Conference statement. *J Ultrasound Med* 2001;20:1025–36
29. Smith-Bindman R, Weiss E, Feldstein V. How thick is too thick? When endometrial thickness should prompt biopsy in postmenopausal women without vaginal bleeding. *Ultrasound Obstet Gynecol* 2004;24:558–65
30. Viola AS, Gouveia D, Andrade L, Aldrighi JM, Viola CF, Bahamondes L. Prevalence of endometrial cancer and hyperplasia in non-symptomatic overweight and obese women. *Aust NZ J Obstet Gynaecol* 2008;48:207–13
31. Novac L, Grigore T, Cernea N, Niculescu M, Cotarcea S. Incidence of endometrial carcinoma in patients with endometrial hyperplasia. *Eur J Gynaecol Oncol* 2005;26:561–3
32. Takreem A, Danish N, Razaq S. Incidence of endometrial hyperplasia in 100 cases presenting with polymenorrhagia/menorrhagia in perimenopausal women. *J Ayub Med Coll Abbottabad* 2009;21:60–3
33. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of “untreated” hyperplasia in 170 patients. *Cancer* 1985;56:403–12
34. Lacey JV, Jr., Sherman ME, Rush BB, et al. Absolute risk of endometrial carcinoma during 20-year follow-up among women with endometrial hyperplasia. *J Clin Oncol* 2010;28:788–92.
35. Lin MC, Gosink BB, Wolf SI, et al. Endometrial thickness after menopause: effect of hormone replacement. *Radiology* 1991;180:427–32
36. Roberts H, Hickey M, Lethaby A. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia: a Cochrane review summary. *Maturitas* 2014;77:4–6
37. Kim JJ, Chapman-Davis E. Role of progesterone in endometrial cancer. *Semin Reprod Med* 2010;28:081–90
38. Tokmak A, Kokanali MK, Guzel AI, Kara A, Topcu HO, Cavkaytar S. Polycystic ovary syndrome and risk of endometrial cancer: a mini-review. *Asian Pac J Cancer Prev* 2014;15:7011–4
39. Baerwald A, Adams G, Pierson R. Characteristics of ovarian follicular wave dynamics in women. *Biol Reprod* 2003;69:1023–31
40. Baerwald AR, Adams GP, Pierson RA. Form and function of the corpus luteum during the human menstrual cycle. *Ultrasound Obstet Gynecol* 2005;25:498–507
41. Baerwald A, Vanden Brink H, Hunter C, et al. Age-related changes in luteal dynamics: preliminary associations with antral follicular dynamics and hormone production during the human menstrual cycle. *Menopause* 2018;25:399–407
42. Allison K, Reed S, Voigt L, Jordan D, Newton K, Garcia R. Diagnosing endometrial hyperplasia: why is it so difficult to agree? *Am J Surg Pathol* 2008;32:691–8