Breast volume fluctuations are associated with oestradiol and progesterone changes across the menstrual cycle

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Abstract

Background:Breast volume changes across the menstrual cycle, but the relationship of this to oestradiol, progesterone and awareness of this change has yet to be characterised. Across the menstrual cycle, this study investigated relationships between breast volume, oestradiol, progesterone, and perceptions of volume change, with associated behaviour change.

Methods:Fifteen women undertook saliva hormone testing bidaily throughout one menstrual cycle. Women attended six laboratory appointments across their cycle (day 2, ovulation-2, ovulation, ovulation+7, menses-3, day 2 (month two)) for 3D surface scanning (breast volume), perception of volume and behaviour change was recorded.

Results:Breast volume changed by +7.3%, but up to -41.7% in one woman. Volume asymmetry increased around ovulation (5.1%). Breast volume change strongly correlated with oestradiol and progesterone, but was delayed by three appointments (left r=0.85; right r=0.95) and one appointment (left r=0.84; right r=0.84), respectively. For women whose volume decreased in follicular and increased in luteal phases (typical pattern; n=11) oestradiol decreased by 13.3%, compared to those who did not (n=4) (14.2%). Breast volume was not associated with perceived breast volume change.

Conclusion:

Average breast volume increases of ~one-third bra cup might not be meaningful; however, inter-participant variability was large, with up to ~1 bra cup change. As women did not accurately perceive their volume changes, measuring individuals' cyclical breast volume would be useful for bra fitting. Volume peaked ~13 days after oestradiol and ~four days after progesterone peak. However, large variability makes it difficult to predict this; studies should take multiple samples across the cycle.

Introduction

It is reported that breast volume changes across the menstrual cycle (1–5), yet the extent of change remains inconclusive. Early studies suggested breast volume change could be as much as 44% when measured using plaster cast breast models (4). However, more recent technology of Magnetic Resonance Imaging (MRI) and three-dimensional (3D) surface scanning have reported less change in breast volume decreasing from onset of menses to ovulation (follicular phase) by 4% (6) to 9.8% (2); and increasing from ovulation to menses (luteal phase) by 3% (2) to 21% (6). Additionally, it is not clear whether left and right breast volumes change equally across the menstrual cycle (1, 7).

The literature exploring the mechanisms behind breast volume changes across the menstrual cycle is reasonably limited. Little is reported on the relationship between menstrual cycle hormones and changes in breast volume, which could be mechanistic. Earlier research indicates that as breast volume increases in the luteal phase, so does breast water volume (6, 8), suggesting that most of the cyclical breast volume

change may be due to water fluctuations in breast tissue. Although there is some glandular volume change, this is thought to be relatively small (6, 8).

Oestradiol and progesterone are thought to impact the hormonal and neural systems which control water and sodium balance (9). It is thought that the oestradiol peak before ovulation (late follicular phase), and the increase in oestradiol in the mid-luteal phase, may lower the osmotic threshold for the release of arginine vasopressin, promoting water retention (9, 10). It is also thought that the peak of progesterone in the mid-luteal phase may influence adrenal aldosterone production, also promoting water retention (9, 11). However, Stachenfeld and Taylor (12) reported that clinically water retention did not increase when oestradiol peaked in the follicular phase, but rather when oestradiol and progesterone peaked in the mid-luteal phase. They also reported a delayed response between the peak of oestradiol and progesterone in the mid-luteal phase resulting in water retention in the late luteal phase (12).

Oestradiol and progesterone are also thought to impact glandular breast tissue morphology which may have an effect on breast volume (13). When oestradiol peaks before ovulation, it triggers glandular epithelial cell growth in the luteal phase of the menstrual cycle (13, 14), which early researchers termed the 'proliferation phase' (15, 16). In the mid-luteal phase of the menstrual cycle, progesterone peaks, which regulates the proliferation of breast epithelia in the subsequent follicular phase of the menstrual cycle (13, 17), which early researchers termed the 'regression phase' (15). It is suggested that breast morphological changes may take time to develop within each menstrual cycle following the peak of oestradiol and progesterone (13), but this has yet to be investigated empirically. Previous literature has reported substantial inter-participant variability in breast volume changes across the menstrual cycle (1, 5). Equally, previous literature has reported substantial inter-participant variability in oestradiol and progesterone changes across the menstrual cycle (18).

Any changes in breast volume across the menstrual cycle are likely to be associated with changes in bra size and fit (19). It is not known whether women perceive changes in breast volume across their menstrual cycle and whether they make any associated behavioural changes (bra size). Consequently, women could be wearing an appropriate bra size at certain time points in their menstrual cycle, but not at other time points.

The aim of this study was to investigate the menstrual cycle hormones of oestradiol and progesterone, and breast volume across the menstrual cycle. It is hypothesised that;

1. Breast volume will change significantly across the menstrual cycle, decreasing between menses and ovulation, and increasing between ovulation and the end of the cycle.

2. There is a paucity of research investigating whether all women conform to this typical pattern of breast volume change across the menstrual cycle, therefore, it is hypothesised that there will be a difference in oestradiol and progesterone between the participants who demonstrate this typical pattern of breast volume change and those who do not.

3. There will be a strong relationship between breast volume, and oestradiol and progesterone across the menstrual cycle, but the relationship will be delayed.

4. Participants who perceived a change in breast volume will show an actual change in measured breast volume, compared to those who do not.

Methods

Recruitment

The study received institutional ethical favourable opinion (SHFEC 2022-050). Participants were recruited via advertising posted on University staff and student portals. Female participants, aged between 18 to 39 years of age, were recruited to join the study. Participants were required to have a regular menstrual cycle (similar number of days per menstrual cycle) for the previous six months, and not be on hormonal contraception within the previous six months. Additionally, to standardise the group, participants were sought who were a bra size of 32D, 34C and 36B (UK bra sizing). Women suffering from vaginal infections and pelvic pathology were unable to participate in the trial due to the effect this may have on the menstrual hormones (20). Participants who were pregnant or breast feeding, had breast surgery or who were currently on medication which may affect the menstrual cycle hormones (antidepressants, antibiotics) were also excluded. Participants with photosensitive epilepsy or migraines were excluded due to possible side effects from the trial equipment used. Signed informed consent was sought before participants began the trial and participants were compensated for their time.

Fifteen women were recruited onto the study, with a mean age of 28 (SD 6) years, height of 1.64 (SD 0.08) meters; and mass of 65.8 (SD 9.2) kilograms. Ten women were fitted to a UK bra size of 34C, and five women were fitted to a 36B. An experienced bra fitter carried out a professional bra fit, using the criteria outlined by McGhee and Steele (21).

Protocol

Laboratory appointments were personalised for each participant according to their self-reported number of menstrual cycle days and ovulation. The laboratory appointment schedule coincided with the peaks and troughs of oestradiol and progesterone across one menstrual cycle (Fig. 1).

Figure 1: Trial protocol; research activity and how it maps to the peaks and troughs of oestradiol and progesterone across one hypothetical 28-day menstrual cycle. The percentage of participants who attended each research visit is represented as adherence.

Hormone Saliva Testing

Saliva sampling is a popular method of determining menstrual cycle hormone profiles as it has sampling advantages of being convenient for the participant and non-invasive (22–24). More recent studies have reported saliva sampling to have a very strong correlation to serum progesterone (24), and a strong

correlation to serum oestradiol (23). Participants were provided with a saliva hormone testing kit (Hormonix pack from Mint Diagnostics, UK). The participants carried out their own saliva sample collection at home. This involved downloading the Hormonix App onto a mobile phone or computer to set up a sample collection schedule. Participants selected their own saliva collection schedule which consisted of four saliva collections per seven days, for the duration of their menstrual cycle, with an additional sample on day two of the next menstrual cycle. Participants provided a 1 mL passive drool saliva sample in a small plastic vial on the morning of their selected day, before eating, drinking or brushing their teeth. Each vial was stamped with a QR code for ease of tracking. Saliva samples were registered on the Hormonix App by scanning the vial QR code, this allowed each vial to be time and date stamped on the App. Saliva samples were frozen in the participants' home freezer until completion of the trial, whereby they were collected by courier for processing by Mint Diagnostics. Upon receipt of the saliva samples, Mint Diagnostics either analysed the samples immediately or stored the acellular samples in accordance with the Human Tissue Act 2004 Code of Practice and Standards (2017). Analysis of saliva samples measured hormone concentrations of 17beta-estradiol and progesterone with CE-marked enzyme linked immunosorbent assay (ELISA) test kits. All assays were conducted using commercially available immunoassay kits without modifications to the manufacturer's recommended protocol (IBL International GMBH, Tecan Trading AG, Switzerland). The test kits were validated using mass spectrometry and a quality control procedure was performed for every test run in accordance with the manufacturer's instructions for use.

Ovulation Test

Participants began ovulation testing using Clearblue Digital Ovulation Test Sticks (Swiss Precision Diagnostics, Switzerland) by urinating on the test stick, starting from the day of their menstrual cycle indicated by the manufacturer's instructions for the length of their cycle. Clearblue ovulation tests are easy to carry out and the results are easy to interpret for the participants (25), they have also been reported to be 99% accurate when compared to the reference method of urine ovulation testing (26). Participants recorded the day of the menstrual cycle when a 'flashing smiley face' was first seen (Fig. 1). The Ovulation Tests show a constant 'smiley face' when ovulation occurs, participants recorded the day of the menstrual cycle when this occurred (Fig. 1). One participant did not observe a constant 'smiley face'. This participants ovulation lab appointment was carried out on the first day the ovulation test sticks went from a 'flashing smiley face' to low ovulation, symbolised by a circle on the test stick. In this case, a luteal phase rise in progesterone (a peak of 295.6 pg/mL) suggested ovulation had occurred and the data set was included in the analyses.

Laboratory Appointment

The breast boundary was identified using the folding lines method (27) and marked with Henna (or surgical marker if the participant reported a skin reaction to Henna). To ensure the breast boundary remained constant throughout the menstrual cycle, the Henna was reapplied at each laboratory appointment on top of the fading marks when required.

Breast volume was obtained by 3D scanning both breasts (Artec Eva, Artec 3D, Luxembourg) while the participant lay prone, with the breasts hanging between two physiotherapy tables. The body below the breasts was stabilised with one physiotherapy table; while the upper shoulders and head were stabilised on the second physiotherapy table, with hands next to the participants' head (28).

At each laboratory appointment participants completed a questionnaire on Google Forms (Google, USA) which assessed whether the participants perceived a change in their breast volume since their last laboratory appointment. Participants also reported behavioural changes associated with their perceived breast volume change (bra style, bra size, activity level and other (free text box)).

Data Analysis

Artec Studio 17 Professional (Artec3D, Luxembourg) was used to process the prone breast scans in high-definition with a 3D resolution of 0.5 mm. Using the Henna marked breast boundary each breast was removed from the thorax. The posterior of the breast was filled using the 'fix holes' function (flat filling) and volume was obtained using the 'measurements' function in Artec Professional 17 (29). Volume of each breast at each laboratory appointment was recorded, as well as percentage volume change between laboratory appointments.

Left and right breast volume were normally distributed determined by Kolmogorov-Smirnov, Shapiro-Wilk, P-P plot which demonstrated data did not deviate significantly from normal (30). To compare breast volume change across the menstrual cycle, left breast volume and right breast volume were analysed separately (hypothesis one). A one-way analysis of variance (ANOVA), followed by pairwise t-tests, which calculated multiple t-tests between all possible combinations of laboratory appointments, was carried out. A Bonferroni correction ($p < 0.01$) was applied to adjust probability values.

Unpaired t-tests were carried out to analyse differences between left and right breast volume at each laboratory appointment. A paired t-test was used to analyse percentage difference between left and right breast volume between lab appointments.

To test hypothesis two, participants were divided into two groups, those who followed the typical reported pattern of breast volume change where breast volume decrease in the follicular phase and increase in the luteal phase (1, 2)) (Group A), and those who did not demonstrate this pattern (Group B). Percentage changes between consecutive laboratory appointments were calculated for left and right breast volume, oestradiol and progesterone and compared across the groups using an unpaired t-test (data were normally distributed; Kolmogorov-Smirnov, Shapiro-Wilk, P-P plot).

Oestradiol and progesterone were normally distributed (Kolmogorov-Smirnov, Shapiro-Wilk, P-P plot). The relationship between breast volume, and oestradiol and progesterone across the menstrual cycle (hypothesis three), was assessed using Pearson correlation coefficients. As a delayed response between the menstrual cycle hormones and breast volume had been reported in the literature (9, 13), cross-

correlations by appointment were carried out using unbiased normalisation in MATLAB (MathWorks Inc., UK). Group mean oestradiol and progesterone (separately) from each laboratory appointment were cross-correlated with means of left and right breast volume (separately). Pearson correlation coefficients were used to determine the best fit appointment lag, with zero lag being the original data. A relationship of 0 to 0.3 was considered poor; a relationship of 0.3 to 0.5 was considered fair; a relationship of 0.6 to 0.79 was considered moderately strong; 0.8 and above was considered very strong (31).

As volume and hormone data were highly variable between participants, individual's data were also cross-correlated by appointment in MATLAB to determine best fit lag between oestradiol and breast volume (left and right), and progesterone and breast volume (left and right).

To test hypothesis four, percentage differences between laboratory appointments were calculated for left and right breast volume, oestradiol and progesterone for the follicular phase and the luteal phase and divided into two groups; participants who perceived a decrease in breast volume in the follicular phase, and those who did not, and participants who perceived an increase in breast volume in the luteal phase, and those who did not. An unpaired t-test compared participants who perceived a change in their breast volume, and those who did not.

Results

Breast Volume

The mean (standard deviation) menstrual cycle length was 28 (4) days. Left breast volume changed significantly across the menstrual cycle ($F = 13.88$, $p = 0.016$) (Fig. 2a), but right breast volume did not ($F = 3.44$, $p = 0.633$) (Fig. 2b). Post-hoc analysis of left breast volume revealed that there were significant increases ($p = 0.003$) in breast volume between ovulation and predicted menses-3 days. The greatest percentage change in volume was in the luteal phase of the menstrual cycle, with a mean increase of 7.3% for the left breast, and 5.8% for the right breast (Fig. 2). There were large individual differences in breast volume change across the menstrual cycle, ranging from 0 mL to 108 mL (41.7%) in the left and 28 mL (1.1%) to 93 mL (43.2%) in the right breast. There also appeared to be a difference between cycle day 2 in month one, and cycle day 2 in month two, however this was not significant.

The difference between left and right breast volume at each laboratory appointment was not significant. However, there was an increasing percentage difference between left and right breast volume across the menstrual cycle (Fig. 3), this percentage difference was significant between ovulation-2 and ovulation ($t = 2.830$, $p = 0.005$).

Patterns of Change

Eleven participants (73%) (Group A) demonstrated the hypothesised pattern of breast volume change (decreasing from cycle day 2 to ovulation, and increasing from ovulation to menses-3 days (1, 2)). Four participants (27%) (Group B) did not demonstrate this pattern of change (Fig. 4).

The participants who demonstrated the hypothesised pattern of breast volume change (Group A) showed less of an oestradiol peak at ovulation-2 (5.1 pg/mL) and a greater peak of progesterone at ovulation + 7 (104.8 pg/mL) than group B. The participants who did not demonstrate the hypothesised pattern of breast volume change (Group B) showed a higher oestradiol peak at ovulation-2 (7.0 pg/mL) and a greater peak of progesterone at period-3 (113.0 pg/mL) than group A (Fig. 4).

When comparing percentage change in oestradiol and progesterone between consecutive laboratory appointments, overall Group A demonstrated a decrease in oestradiol between laboratory appointments (mean of the percentage change across all appointments = -13.3%, SD 6.0), whereas Group B demonstrated an increase (mean = 14.2%, SD 7.5), this difference was significant ($t = 2.738$, $p = 0.004$). Group A demonstrated no change in progesterone across the cycle (mean = -2.3%, SD 11.0), whereas visually Group B demonstrated a larger decrease (mean = -32.4%, SD 31.9), however, this was not significant.

Oestradiol, Progesterone and Breast Volume

There was inter-participant variability in oestradiol and progesterone across the menstrual cycle (Fig. 5). Mean oestradiol ranged from 2.1 pg/mL to 11.2 pg/mL across the menstrual cycle, peaking two days before ovulation (determined by ovulation testing), although there was large variability in the data (Fig. 5a). Mean progesterone ranged from 17.6 pg/mL to 350.2 pg/mL across the menstrual cycle, peaking seven days after ovulation, but the data demonstrated large variability (Fig. 5b). There were four participants who demonstrated progesterone profiles which were substantially greater than the mean. Three of these participants demonstrated the hypothesised typical pattern of breast volume change, and one did not. The volume change in the four participants were not greater than the mean.

Mean oestradiol measured on the days of the laboratory appointments ranged from 3.8 pg/mL to 5.5 pg/mL (Fig. 6a). With zero appointment lag, there was a moderately strong negative relationship between oestradiol and left breast volume ($r=-0.67$) and right breast volume ($r=-0.77$) (Fig. 6a). However, cross-correlation identified a very strong positive relationship between oestradiol and breast volume when a three-appointment lag was applied (left breast volume, $r = 0.85$ and right breast volume, $r = 0.95$) (Fig. 6b). When percentage change between appointments was utilised to determine the relationship between oestradiol and breast volume, the relationship was reduced (left breast $r=-0.69$; right breast $r=-0.58$) and cross-correlation did not improve outcomes.

Mean progesterone measured on the days of the laboratory appointments ranged from 42.7 pg/mL to 104.1 pg/mL (Fig. 7a). With zero appointment lag, there was a fair relationship between progesterone and left breast volume ($r = 0.40$), and a poor relationship between progesterone and right breast volume ($r = 0.12$) (Fig. 7a). However, cross-correlation identified a very strong relationship between progesterone and breast volume when a one-appointment lag was applied (left breast volume, $r = 0.84$ and right breast volume, $r = 0.84$) (Fig. 7b). When percentage change between appointments was utilised to determine a relationship between oestradiol and breast volume, the relationship was reduced (left breast $r = 0.45$; right breast $r = 0.25$) and cross-correlation did not improve outcomes.

Given the inter-participant range of breast volume and hormone data, individual participant data were explored to understand the variability in the relationship between breast volume and hormone changes across the menstrual cycle. Pearson correlation coefficients (with a zero-appointment lag) identified the relationships between volume and hormones in individual participants. Cross-correlations improved the outcomes in relationships; however, the number of appointment lag was variable between individuals (Table 1).

Table 1
Best fit appointment lag and range of Pearson Correlation Coefficient's between menstrual cycle hormones (oestradiol and progesterone) and breast volumes (left and right) for each participant. Participants who did not demonstrate the hypothesised pattern of breast volume change have been denoted by *.

Participant	Appointment lag (r-value)			
	Oestradiol and left breast volume	Oestradiol and right breast volume	Progesterone and left breast volume	Progesterone and right breast volume
1	2 (r = 0.57)	3 (r = 0.74)	0 (r = 0.58)	1 (r = 0.65)
2	3 (r = 0.73)	3 (r = 0.74)	1 (r = 0.59)	2 (r = 0.56)
3	1 (r = 0.67)	1 (r = 0.49)	0 (r = 0.72)	1 (r = 0.54)
4*	2 (r = 0.6)	2 (r = 0.53)	0 (r = 0.41)	4 (r = 0.44)
5*	4 (r = 0.39)	0 (r = 0.63)	3 (r = 0.6)	0 (r = 0.46)
6	5 (r = 0.56)	5 (r = 0.56)	2 (r = 0.59)	4 (r = 0.85)
7*	2 (r = 0.43)	0 (r = 0.44)	5 (r = 0.28)	0 (r = 0.54)
8	2 (r = 0.61)	3 (r = 0.63)	2 (r = 0.64)	2 (r = 0.79)
9	4 (r = 0.99)	4 (r = 0.99)	4 (r = 0.99)	0 (r = 0.98)
10	5 (r = 0.56)	5 (r = 0.56)	2 (r = 0.79)	2 (r = 0.7)
11*	2 (r = 0.33)	0 (r = 0.61)	1 (r = 0.48)	0 (r = 0.82)

Participant	Appointment lag (r-value)			
	Oestradiol and left breast volume	Oestradiol and right breast volume	Progesterone and left breast volume	Progesterone and right breast volume
12	0 (r = 0.82)	1 (r = 0.93)	2 (r = 0.84)	4 (r = 0.79)
13	1 (r = 0.95)	2 (r = 0.55)	5 (r = 0.68)	3 (r = 0.67)
14	4 (r = 0.8)	4 (r = 0.8)	1 (r = 0.74)	1 (r = 0.66)
15	3 (r = 0.46)	2 (r = 0.6)	2 (r = 0.8)	1 (r = 0.68)
Median	**2 (r = 0.78)**	**2 (r = 0.77)**	**2 (r = 0.73)**	**1 (r = 0.74)**
Range of Pearson Correlation Coefficients	**0 to 5**	**0 to 5**	**0 to 5**	**0 to 4**
Range of r-values	**0.33 to 0.99**	**0.44 to 0.99**	**0.28 to 0.99**	**0.44 to 0.98**

Perceived Changes in Breast Volume

In the follicular phase, four participants (27%) perceived a decrease in breast volume, and demonstrated a mean decrease in left and right breast volume of -4.0% (SD 17.0) and − 6.2% (SD 16.7), respectively. However, participants who did not perceive a change in breast volume across the follicular phase demonstrated a mean decrease in left and right breast volume of -11.2% (SD 6.9) and − 7.0% (SD 6.1), respectively. There were no significant differences in breast volume between participants who perceived a change in breast volume, and those who did not. There were no behavioural changes in response to a decrease in perceived breast volume.

In the luteal phase, seven participants (47%) perceived an increase in breast volume, and demonstrated a mean increase in left and right breast volume of 6.3% (SD 6.1) and 5.1% (SD 4.6), respectively. Participants who did not perceive a change demonstrated an increase in left and right breast volume of 6.2% (SD 6.3) and 2.3% (SD 4.4), respectively. These differences in breast volume between participants who perceived a volume change, and those who did not, were not significant. Of the participants who perceived an increase in breast volume, one participant reported altering bra size to a larger cup size

when an increase in breast volume was perceived, this participant also experienced an actual increase in breast volume of 16% in the left breast and 12% in the right breast.

Discussion

This study investigated the relationship between menstrual cycle hormones (oestradiol and progesterone) and breast volume across the menstrual cycle. The novel findings of oestradiol and progesterone being related to a delayed response in breast volume change has implications for the timing of data collection in future menstrual cycle research.

Breast Volume

It was hypothesised that breast volume would change significantly across the menstrual cycle, decreasing in the follicular phase and increasing in the luteal phase of the menstrual cycle, which is in keeping with previous literature (1, 2, 5, 6). In this study, on average the left breast was larger than the right and the right breast volume did not change significantly across the cycle, however left breast volume changed significantly, increasing from ovulation to menses (7.3%), equating to an average breast volume increase of a third of a cup size (37 ml) (32) or 35 g (33). These findings partially accept hypothesis one.

It has been reported that two mechanisms may result in breast volume changes across the menstrual cycle; water retention and glandular tissue morphological changes. Both water retention and glandular tissue changes have been related to oestradiol and progesterone changes (9, 13). Using MRI across the menstrual cycle, Fowler, Casey (6) and Graham, Stanchev (8) theorised that most of the cyclical breast volume changes were due to water fluctuations, and that volume changes in the glandular tissue were relatively small.

Hudson, Wilkinson (34) reported that in women with breast asymmetry, the larger breast contains more radiologically dense tissue. Whilst the change in glandular tissue volume across the menstrual cycle may not be significant, the amount of glandular tissue in the breast may affect the way it responds to fluctuations in menstrual cycle hormones. Oestradiol α and β receptors are found primarily in the mammary gland (35) along with the progesterone receptors (PR-a and PR-B) (36), which indicate that the more glandular tissue, the more responsive the breast may be to oestradiol and progesterone. This may explain why the results of this study show significant changes in breast volume of the larger breast (left side).

Literature suggests that changes in oestradiol and progesterone drive changes in breast tissue development (both glandular and adipose) (37, 38). Therefore, it is reasonable to assume that the relationship reported in this study is directional, with hormonal changes occurring first and driving the changes reported in breast volume. This directional relationship has implications in situations where these hormones are altered (either naturally or artificially), as this has the potential to cause changes in breast volume. Such situations may occur during puberty (38), pregnancy (39), menopause (40), and hormone medication (41). Whilst the current study reports a directional relationship between oestradiol,

progesterone and breast volume across the menstrual cycle, this should also be considered across a women's life cycle.

Patterns of Change

Hypothesis two stated that there would be differences in oestradiol and progesterone between participants whose breast volume decreased between menses and ovulation, and increased between ovulation and cycle end (hypothesised typical pattern of change; Group A) compared to those who did not (Group B). There were significant differences between the groups in oestradiol, but not progesterone, partially accepting hypothesis two. In the follicular phase, those who demonstrated breast volume decrease (Group A) had lower oestradiol levels. As such it is possible that participants who demonstrated no change or an increase in volume (Group B) were responding to their higher oestradiol peak and levels. It is thought that a peak in oestradiol in the follicular phase promotes glandular tissue proliferation and water retention, but that this response it delayed (12, 13).

In the luteal phase, participants whose breast volume increased (Group A) demonstrated smaller decreases in oestradiol and smaller increases in progesterone than women whose volume remained the same or decreased (Group B). Group A, did however, demonstrate a peak of oestradiol and progesterone at ovulation plus seven days, whereas Group B peaked approximately five days later at estimated menses minus three days. As high levels of oestradiol and progesterone in the mid-luteal phase are thought to promote water retention (12), it was thought that this would result in a greater increase in breast volume towards the end of the luteal phase, which was consistent with our findings. For Group B, as their peak in menstrual hormones occurred later, their volume change may be later still suggesting that future studies should track breast volume and menstrual cycle hormones for longer than one cycle.

Oestradiol, Progesterone and Breast Volume

Despite large individual variability in oestradiol, progesterone and breast volume changes across the menstrual cycle, there was a strong positive relationship between oestradiol and breast volume when a three-appointment lag was applied. The peak of oestradiol occurred two days before ovulation, however breast volume did not peak until three days before estimated next menses, which equated to a delay of thirteen days in an average 28-day cycle. Furthermore, there is a strong positive relationship between progesterone and breast volume when a one-appointment lag was applied. Progesterone peaked seven days after ovulation and breast volume peaked four days later in an average 28-day cycle. These findings accept hypothesis three.

Individual hormone and breast volume data were highly variable. Individual cross-correlation between the peak in oestradiol and left and right breast volume, reported a median of a two-appointment lag. Following individuals' progesterone peak, the peak in left and right breast volume lagged by a median of two-appointment and one-appointment respectively. However, there was a range in individuals' appointment lag from zero to five appointments. The current study is the first to present empirical data to quantify a delay in breast volume change associated with hormone change across the menstrual cycle.

Individual data were highly variable, including the magnitude of this delay. This outcome has important implications for the timing of data collection in future research projects, which given the variability, needs to be as regular as possible to ensure the capturing of these key events.

Perceived Changes in Breast Volume

It was hypothesised that participants who perceived a change in breast volume would also show actual changes in measured breast volume, however the data did not support this, rejecting hypothesis four. Cyclical water retention occurs throughout the body, and is not isolated to the breast tissue (9), thus water retention elsewhere may promote perceived breast volume change, even if actual breast volume change was little to none. Conversely, women who experienced an actual volume change may not perceive the change in their breasts if their whole body is changing. These results suggest that women are not good at detecting breast volume change across the menstrual cycle, which is worrying if, for some women their volume change is up to ~ 43%. In this study only one participant altered her bra size, due to her perceived changed in breast volume (which was an actual change). This suggests that while women may perceive a change in their breast volume, most do not alter their bra size as a result. Women would benefit from objective assessment of their typical magnitude of breast volume change across their cycle and if this is greater than one cup size (which was seen for two women in this study), they should be advised to make changes to their bra size.

Conclusion

In the luteal phase of the menstrual cycle, this study reports average breast volume increases of 7.3%, which equates to approximately one-third of a bra cup size. However, there was large inter-participant variability with one women's breast volume increasing by 43.2% or one cup size. The strong positive relationship of oestradiol and progesterone to breast volume has implications for other life stages where these hormones may change, leading to a change in breast volume. In this study, the women whose breast volume did not decrease in the follicular phase and increase in the luteal phase (which would be a typical response), also showed differences in their hormone profiles, which may be linked to a greater delay in volume change. On average, the peak in breast volume occurred 13 days after oestradiol peaked and four days after progesterone peaked. However, large inter-participant variability would make it difficult to predict this delay and therefore future research in this area should consider multiple sampling point across the cycle. Despite the breast volume changes being large for some women in this study, the cohort were not good at perceiving their volume changes, which is worrying considering the changes were at least one cup size in two women. This suggest women would benefit from breast volume monitoring across a menstrual cycle to help them determine if they need a bra size change.

Declarations

Ethics approval: All experimental protocols were approved by the institutional ethical committee (Faculty of Science and Health, University of Portsmouth, SHFEC 2022-050). Signed informed consent was sought

and obtained from all participants before they began the trial.

Consent for publication: Not Applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests: The authors declare that they have no competing interests

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Authors Contribution:

- JR, CM, ER, SA, AKML, JWS conceived and designed the research
- JR conducted the trial
- JR processed and analyzed the data
- JR, CM, JWS wrote the manuscript
- JR, CM, ER, SA, AKML, JWS read and approved the manuscript

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Figures

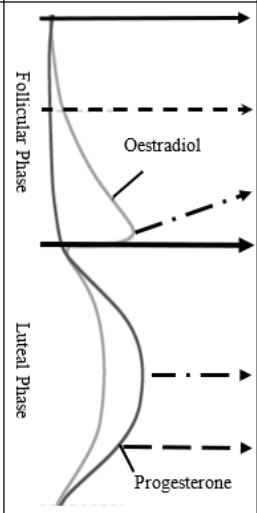
	Hormone profile	Cycle Day	Research Activity	Adherence
Pre-trial			Bra Fit (and consent)	100%
Month 1		**2**	Lab Appointment 1 (Cycle day 2); Saliva test	100%
		4	Saliva test	
		6	Saliva test	
		7	Start ovulation testing	
		8	Saliva test	
		10	Record 'flashy smiley face' (approx.)	
		12	Lab Appointment 2 (Ovulation-2 days); Saliva test	87%
		14	Record first constant 'smiley face'; Lab Appointment 3 (Ovulation); Saliva test	
		16	Saliva test	
		18	Saliva test	
		20	Saliva test	
		21	Lab Appointment 4 (Ovulation+7 days); Saliva test	100%
		22	Saliva test	
		24	Saliva test	
		25	Lab Appointment 5 (Menses-3); Saliva test	93%
		26	Saliva test	
		28	Saliva test	
Month 2		**2**	Lab Appointment 6 (Cycle day 2 (month 2)); Saliva test	93%
Mean adherence				96%

Figure 1

Trial protocol; research activity and how it maps to the peaks and troughs of oestradiol and progesterone across one hypothetical 28-day menstrual cycle. The percentage of participants who attended each research visit is represented as adherence.

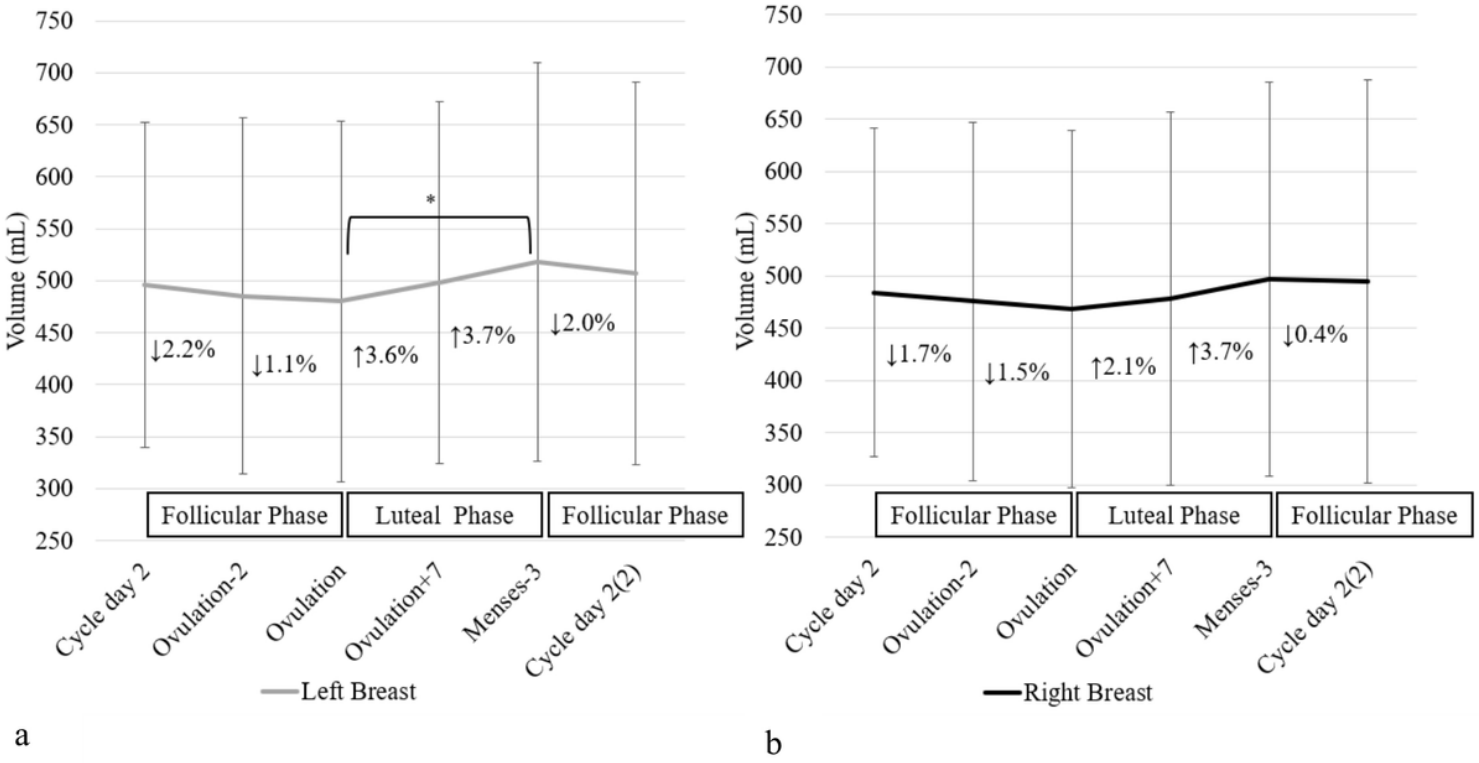


Figure 2

Left (a) and right (b) breast volume (mL) across the menstrual cycle (error bars show standard deviation). Significant changes ($p<0.01$ Bonferroni Correction) in breast volume are denoted by * (Paired t-test); percentage change is indicated by ↑ increase or ↓ decrease.

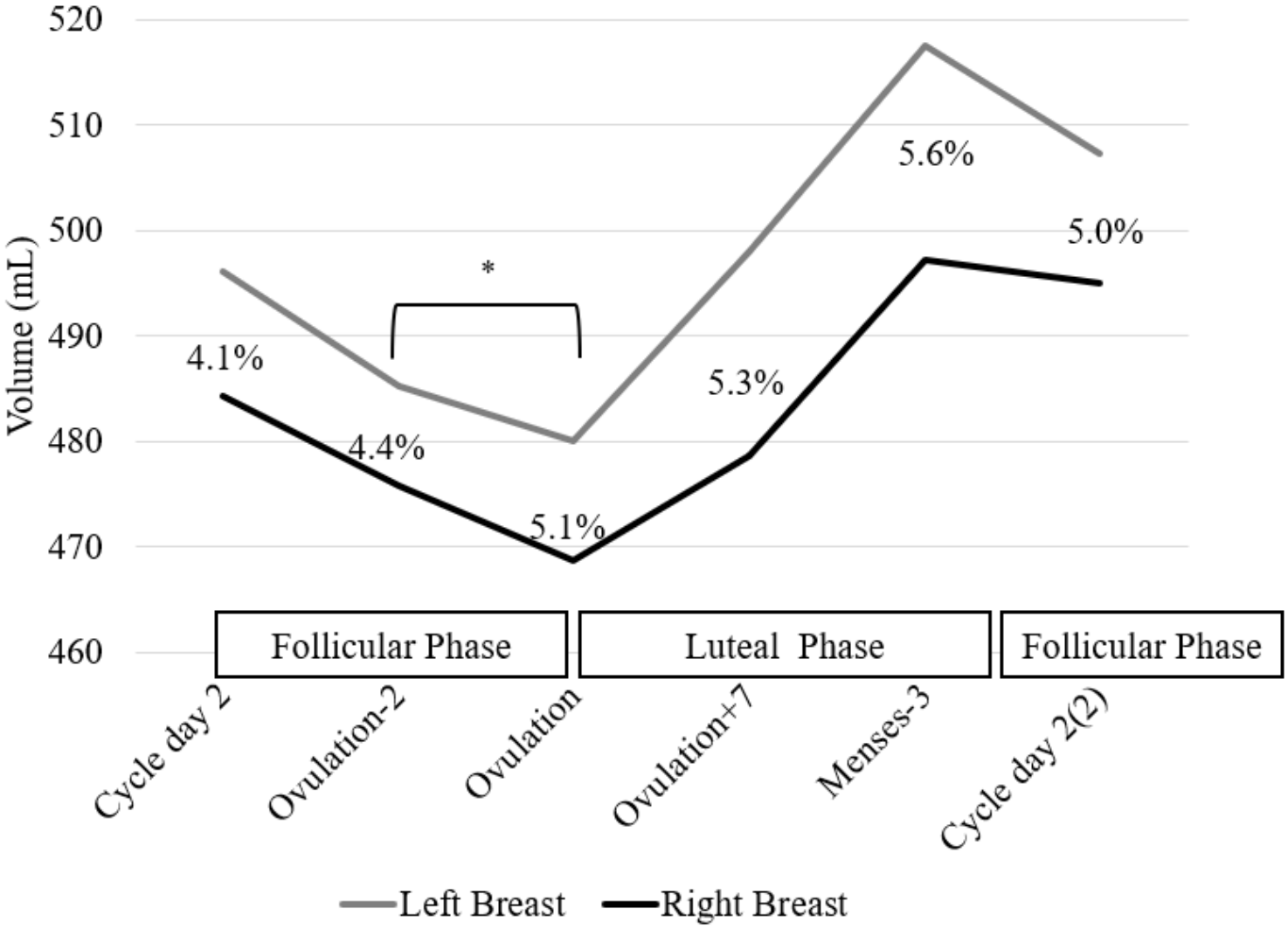


Figure 3

Percentage difference in breast symmetry throughout the menstrual cycle. Significant differences ($p<0.05$) in percentage asymmetry between research appointments is denoted by * (Paired t-test).

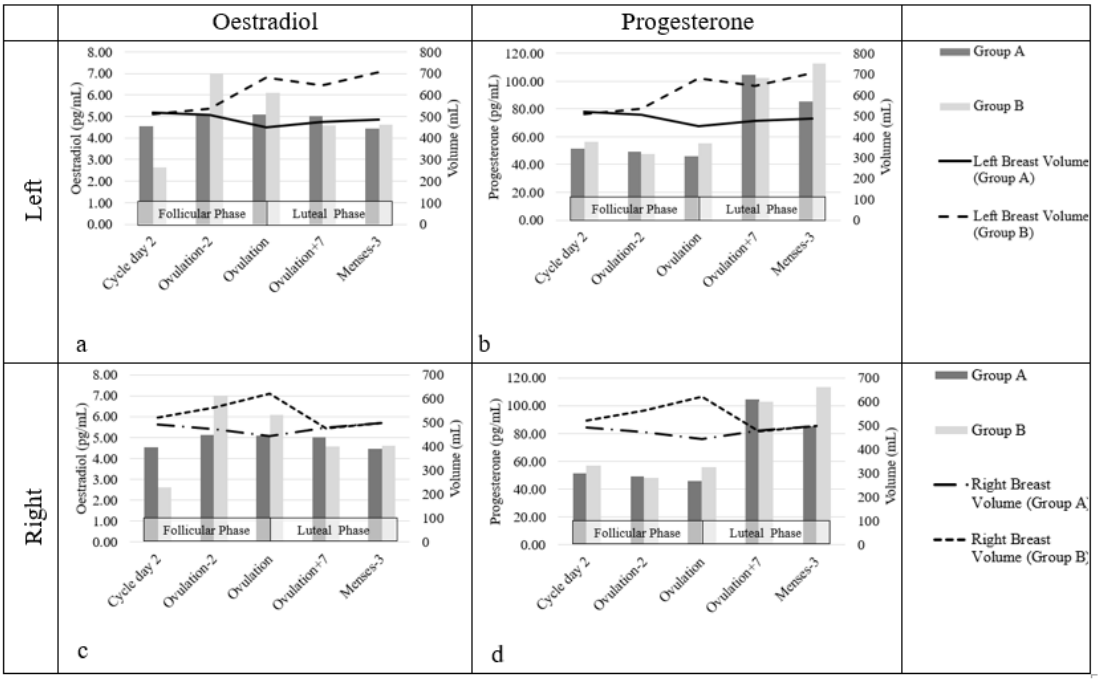


Figure 4

Oestradiol and progesterone levels for Group A and Group B across the menstrual cycle. (a) Oestradiol and left breast volume, (b) progesterone and left breast volume, (c) oestradiol and right breast volume, (d) progesterone and right breast volume.

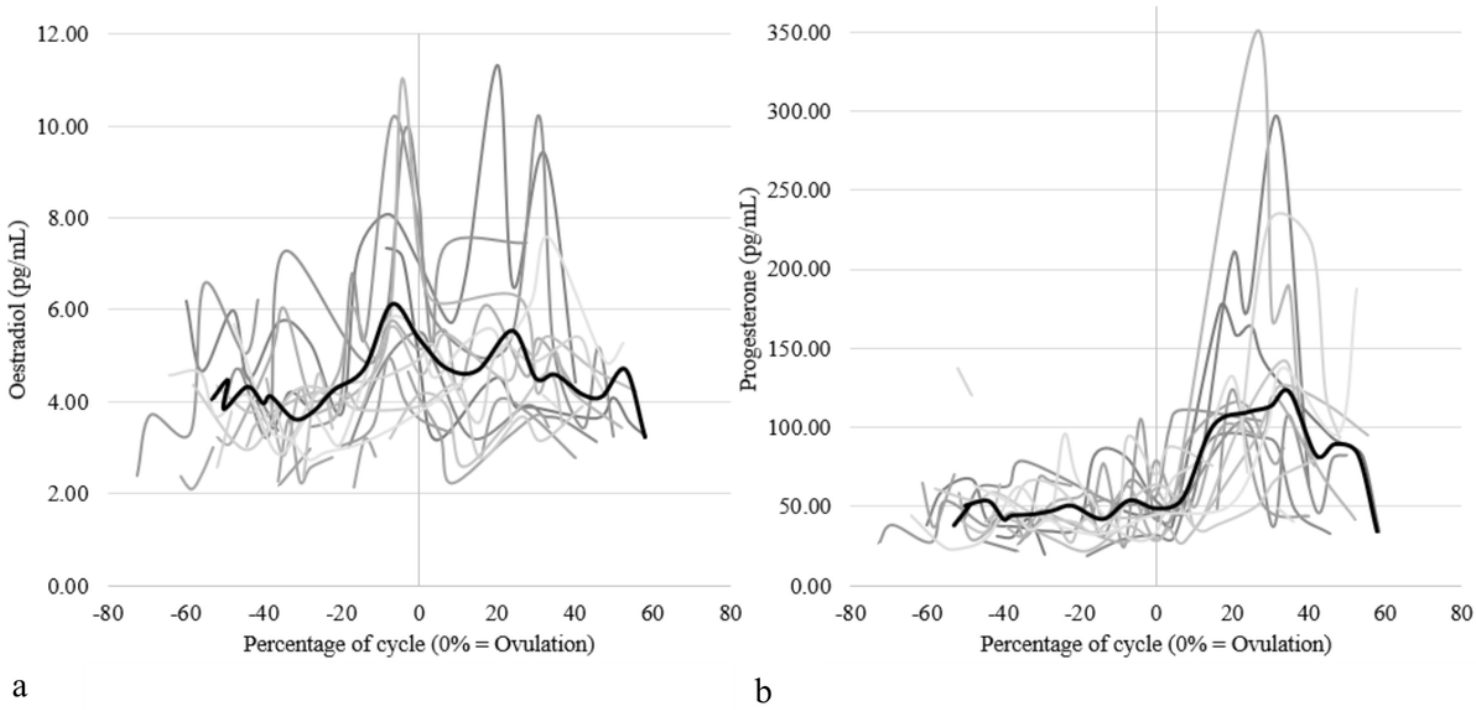


Figure 5

Inter-participant variability in oestradiol and progesterone across the menstrual cycle. Oestradiol and progesterone were normalised around ovulation; the grey lines indicate individual participants; the black line indicates the mean.

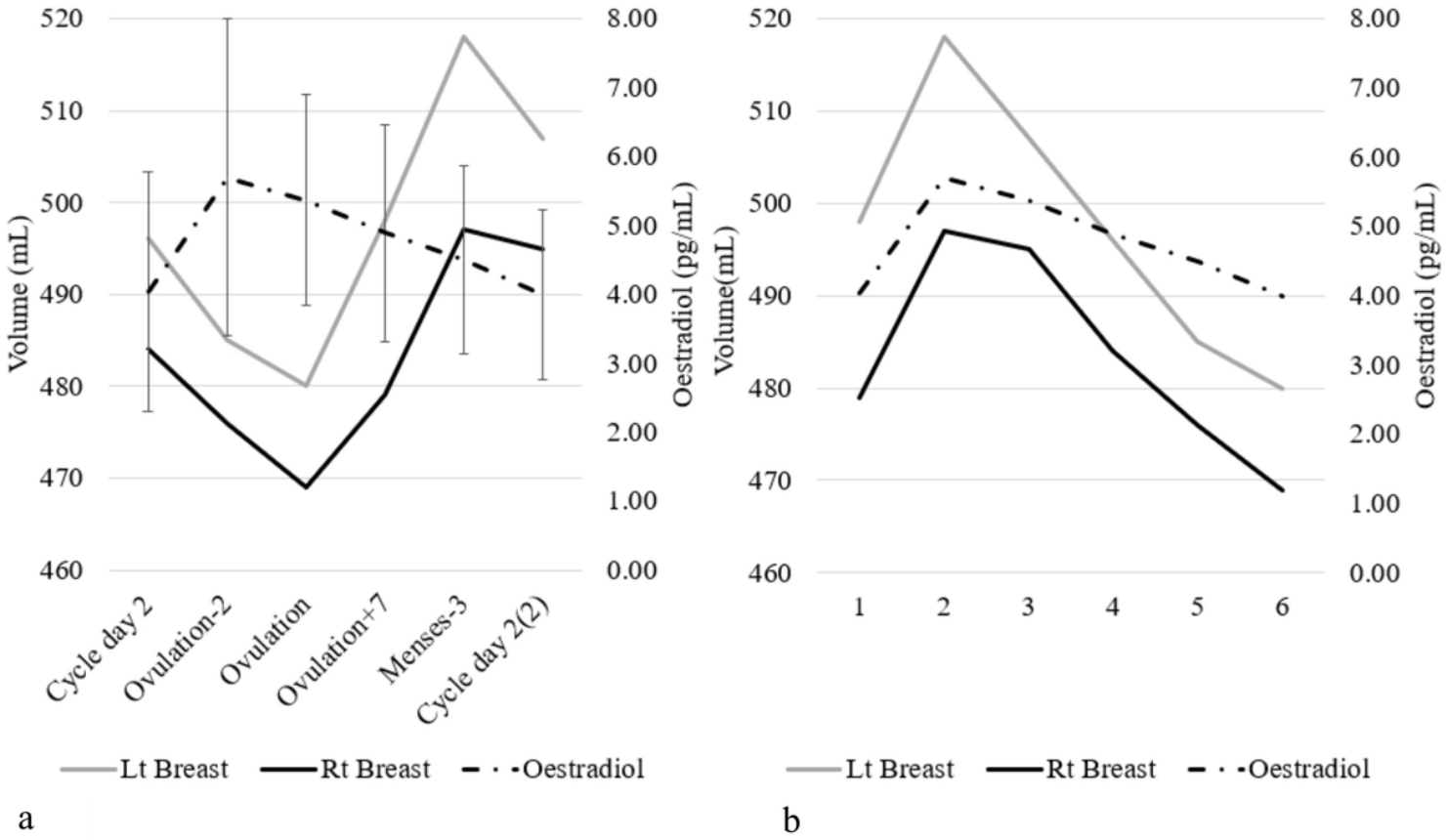


Figure 6

(a) Mean oestradiol and breast volume at zero lag (error bars represent standard deviation of oestradiol); (b) and mean oestradiol and breast volume when a 3-appointment lag was applied.

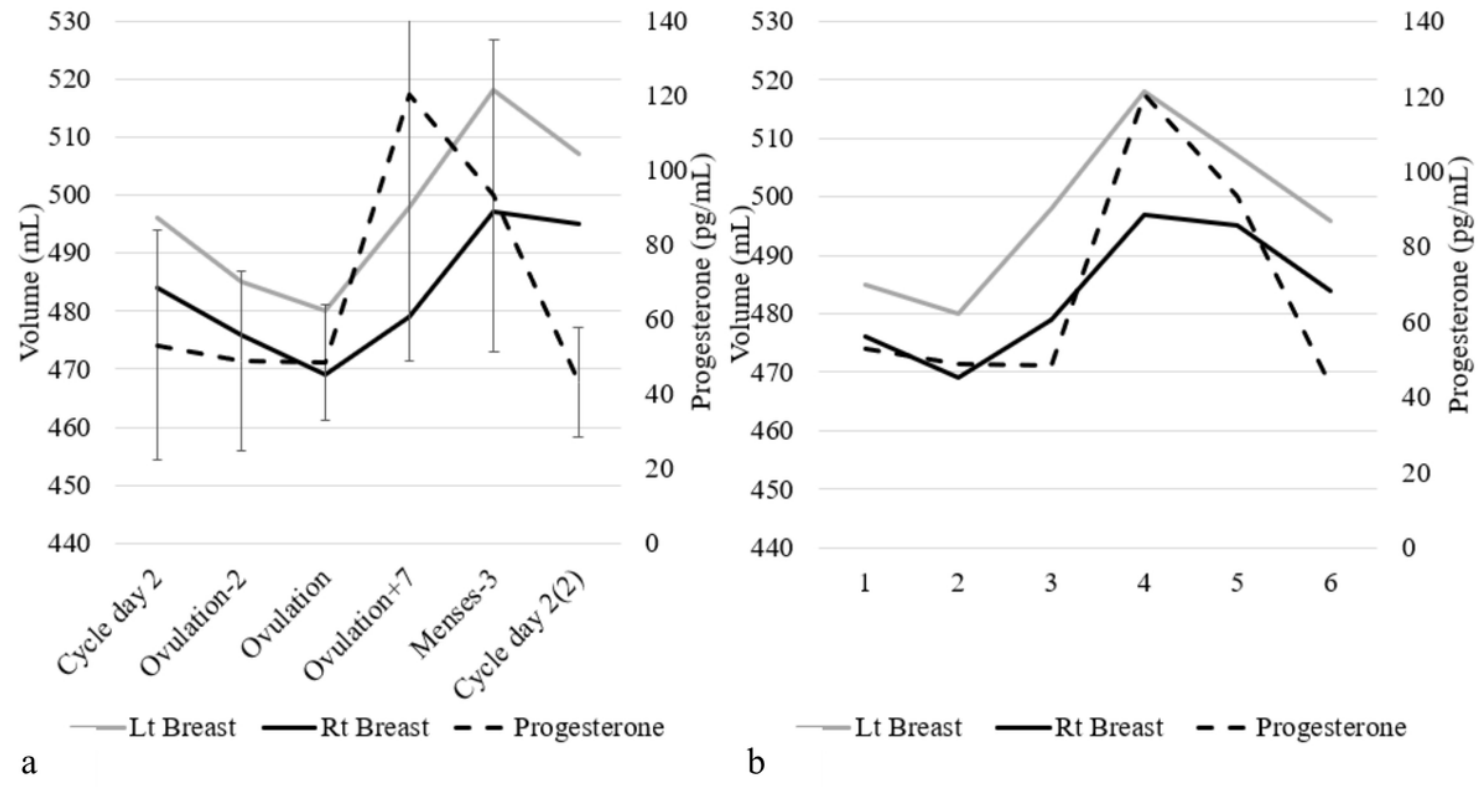


Figure 7

(a) Mean progesterone and breast volume at zero lag (error bars represent standard deviation of progesterone); (b) and mean progesterone and breast volume when a 1-appointment lag was applied.