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ARTICLE

Uterine contractile activity in healthy women throughout the menstrual cycle measured using a novel quantitative two-dimensional transvaginal ultrasound speckle tracking method

BIOGRAPHY

Connie Rees received her degree in Medicine from Utrecht University. Subsequently, she started her PhD on adenomyosis and uterine contractility at Catharina Hospital Eindhoven, the Netherlands. The research is led by Professor Dick Schoot, Professor Massimo Mischi and Professor Huib van Vliet from Eindhoven University of Technology and Ghent University.

Connie Odette Rees[1,2,3,*], Anna de Boer[1], Yizhou Huang[2], Blijke Wessels[2], Celine Blank[2,3], Nienke Kuijsters[2], Aleida Huppelschoten[1], Brunella Zizolfi[4], Virginia Foreste[4], Attilio Di Spiezio Sardo[4], Nikos Christoforidis[5], Hubertus van Vliet[1,3], Massimo Mischi[2], Benedictus Christiaan Schoot[1,2,3]

KEY MESSAGE

Uterine contractility measured by objective transvaginal ultrasound speckle tracking included the novel features coordination, direction and velocity. Menstrual cycle uterine contractility was highest in the periovulatory phase, and lowest in the late luteal phase. Future studies could investigate uterine contractility by this method in women with infertility or abnormal uteri.

ABSTRACT

Research question: To explore normal uterine contractile function across the menstrual cycle using a novel quantitative ultrasound method.

Design: This multicentre prospective observational study took place in three European centres from 2014 to 2022. Uterine contraction frequency (contractions/minute), amplitude, direction (cervix-to-fundus, C2F; fundus-to-cervix; F2C), velocity and coordination were investigated. Features were extracted from transvaginal ultrasound recordings (TVUS) using speckle tracking. Premenopausal women ≥18 years of age, with normal, natural menstrual cycles were included. A normal cycle was defined as: regular (duration 28 ± 2 days), no dysmenorrhoea, no menometrorrhagia. Four-minute TVUS were performed during the menstrual phase, mid-follicular, late follicular phase, early luteal phase and/or late luteal phase. Of the 96 recordings available from 64 women, 70 were suitable for inclusion in the analysis.

Results: Contraction frequency (for the posterior wall) and velocity (for the anterior uterine wall in the F2C direction) were highest in the late follicular phase and lowest in the menstrual and late luteal phases (1.61 versus 1.31 and 1.35 contractions/min, $P < 0.001$ and 0.81 versus 0.67 and 0.62 mm/s, $P < 0.001$, respectively). No significant difference was found for contraction amplitude. Contraction coordination (simultaneous contraction of the anterior and posterior walls in the same direction) was least coordinated in the mid-follicular phase ($P = 0.002$).

Conclusions: This is the first study to objectively measure uterine contraction features in healthy women during the natural menstrual cycle on TVUS. Likewise, it introduces contraction coordination as a specific feature of uterine peristalsis. Differences in uterine contractility across the menstrual cycle are confirmed, with highest activity seen in the late follicular phase, and lowest in the late luteal phase.

[1] Department of Obstetrics and Gynaecology, Catharina Hospital, Eindhoven, the Netherlands
[2] Laboratory of Biomedical Diagnostics, Department of Electrical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands
[3] Department of Reproductive Medicine, Ghent University Hospital, Ghent, Belgium
[4] Department of Public Health – School of Medicine, University Federico II, Naples, Italy
[5] Embryolab Fertility Center Thessaloniki, Greece

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*Corresponding author. E-mail address: connie.rees@catharinaziekenhuis.nl (C. O. Rees). https://doi.org/10.1016/j.rbmo.2022.08.104 1472-6483/© 2022 Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.
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KEYWORDS
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Transvaginal ultrasound
TVUS
Uterine contractile function
Uterine peristalsis

INTRODUCTION

In a healthy uterus, rhythmic contractions change in rhythm and intensity during the menstrual cycle to support sperm propagation and embryo implantation (*Bulletti and De Ziegler, 2006*; *Fanchin and Ayoubi, 2009*; *Kuijsters et al., 2017*; *van Gestel et al., 2003*). However, no study thus far has been able to comprehensively characterize all aspects of uterine contractions during the menstrual cycle; therefore, these characteristics remain largely speculative, based on heterogenous studies. Furthermore, research into their characteristics have been hampered by the subjectivity of the available measurement tools (*Kuijsters et al., 2017*).

It has been suggested that aberrant uterine peristalsis or 'dysperistalsis' is associated with reduced fertility and/or symptoms such as dysmenorrhoea (*Kissler et al., 2007*). Up to now, there exists no quantifiable marker for dysperistalsis, which is variously defined by previous investigators.

Multiple methods have been used to visualize and assess uterine contractions and their different characteristics, one of which is transvaginal ultrasound (TVUS). A recent study, however, showed that medical professionals shared only mild agreement on the direction and timing of uterine peristalsis by subjective visual inspection of TVUS recordings (*Kuijsters et al., 2020*). Although visual inspection of TVUS can provide a number of peristalsis parameters (frequency and direction), it is generally qualitative and unsuitable to quantify contraction amplitude or velocity. Furthermore, contraction coordination – the synchronized movement of the anterior and posterior walls of the uterus – has never been investigated. There is thus a need for an objective, quantifiable method of uterine contraction assessment, preferably in a non-invasive operator- and patient-friendly way.

Recently published data by the current author group presented a novel method for assessing uterine contractility, using two-dimensional (2D) TVUS and speckle tracking techniques (*Huang et al., 2018b*; *Sammali et al., 2018a*, *2018b*, *2019*). This has been tested and (externally) validated in IVF patients prior to embryo transfer (*Blank et al., 2020*; *Sammali et al., 2021*). This method is able to quantitatively assess features such as contraction frequency and amplitude, in addition to a novel set of features: contraction coordination, direction and velocity. Coordination is defined as the synchronized simultaneous movement of the anterior and posterior uterine walls, where the value reflects the degree of synchronicity of coordination. This aspect of uterine movement is potentially of clinical relevance for the assessment of (normal) uterine function.

Differences in uterine contractility have been shown to have a strong association with ongoing pregnancy in an IVF population (*Blank et al., 2020*). This study explores the characteristics of normal uterine contractile function across the menstrual cycle in healthy, nulliparous women using this quantitative method, with a focus on the novel feature of coordination as a possible measure of dysperistalsis.

MATERIALS AND METHODS

Study objectives
To evaluate uterine contraction features (frequency, amplitude, velocity, direction and coordination) using a dedicated speckle tracking algorithm by 2D TVUS measurement in healthy women throughout the menstrual cycle.

Study design and setting
Multicentre observational prospective cohort study carried out in the outpatient gynaecology departments of the Catharina Hospital in Eindhoven, the Netherlands; the University of Naples, Federico II Naples, Italy; and the Embryolab Fertility Centre in Thessaloniki, Greece.

Participants
Between September 2014 and January 2022, 74 healthy women were included from the gynaecological outpatient departments of the participating centres. Women were included if they were ≥18 years of age, premenopausal and had a normal, natural menstrual cycle. A normal cycle was defined as: regular (duration 28 ± 2 days), no dysmenorrhoea and no menometrorrhagia. Exclusion criteria were: (i) pregnancy, (ii) diagnosed with a mental disorder, (iii) significant language barrier, (iv) use of oral hormonal contraceptives or intrauterine device, (v) use of other (hormonal) medication affecting the uterus, or (vi) uterine pathology (congenital or otherwise, e.g. leiomyomas, adenomyosis), based on morphological uterine sonographic assessment (MUSA) (*Van den Bosch et al., 2019*) criteria. Ultrasound scans of the included women were also assessed retrospectively by experts to confirm the absence of uterine abnormalities.

Seventy-four women were enrolled in the study, of which 64 ultimately underwent TVUS recording at different phases of the menstrual cycle. This resulted in a total of 96 completed TVUS recordings across cycle phases. Eighteen recordings were subsequently excluded due to insufficient ultrasound quality for the analysis. Reasons for exclusion due to recording quality included: shadow across the endometrial lining, out-of-plane motion or insufficient resolution of the images. Additionally, eight recordings were excluded due to suspected uterine abnormalities or use of hormonal contraceptive methods. FIGURE 1 presents a flow diagram of patient inclusion. Overall, 70 out of 96 conducted recordings from 64 women were included in the analysis.

Data sources and measurements

TVUS measurement
TVUS was performed during several phases of the menstrual cycle: the menstrual phase (cycle days 1–5), mid-follicular phase (cycle days 6–10), late follicular phase (cycle days 11–14), early luteal phase (cycle days 15–20) and late luteal phase (cycle days 21–28). During each session, 4-min video recordings of the uterus in the mid-sagittal section were made. The ultrasound machines used were an Accuvix WS80A with Elite for Women's Health (Samsung Medison, Seoul, Korea) equipped with a V5-9 transvaginal probe (bandwidth 5–9 MHz) or a Voluson S10™ Expert (GE Healthcare, Zipf, Austria) equipped with a RIC5-9W-RS probe (bandwidth 3.8–9.3 MHz).

Feature extraction
Various uterine contractility features were extracted from the gathered ultrasound recordings using a quantitative dedicated speckle tracking algorithm previously developed and implemented in Matlab software (Mathworks, Natick, USA). The full details of the methodology of feature extraction have been described in detail in previously published works

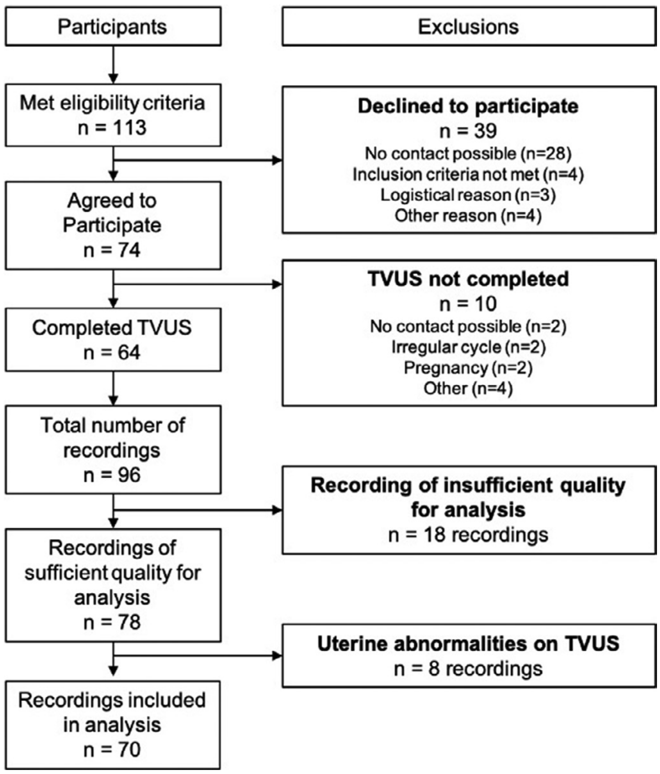

FIGURE 1 Flow chart of patient recruitment and inclusion. TVUS = transvaginal ultrasound.

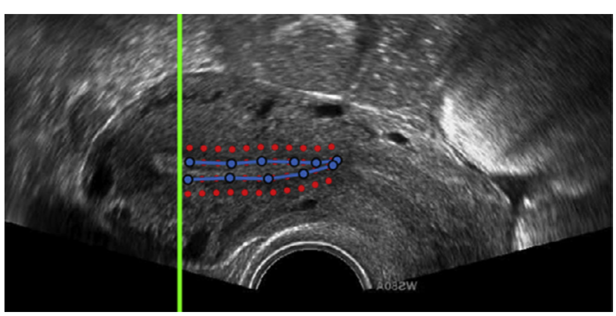

FIGURE 2 Ultrasound image of the uterus in the mid-sagittal section. Placement of the speckle tracking grid (red dots) along the endometrial border (blue line) at 5 mm (green line) from the apex of the fundus.

(*Blank et al., 2020*; *Huang et al., 2018a*, *2022*; *Sammali et al., 2018a*, *2019a*, *2019*b). Simply put, speckle tracking measures the displacement of image 'speckles' (such as those seen in various shades of grey on ultrasound images) over time. Speckle movement reflects movement of the imaged tissue, which in this case is movement of the uterine myometrium.

For each ultrasound recording, a grid of tracking markers was manually positioned over the uterine junctional zone along the endometrium, known to be the most contractile part of the uterus (see FIGURE 2 for an illustrative example). Grid markers were placed 5 mm from the fundus along the endometrial border. The grid markers were coupled in pairs, and distance and strain signals were derived between each pair in both the longitudinal and radial directions (FIGURE 3). Several contraction features were extracted from the measured strain signals as described below. Previous analyses of inter- and intra-observer variability in the placement of the grid markers showed a high level of correlation, making the method both reproducible and reliable (*Huang et al., 2022*) (see Appendix A).

Euclidean distance was used to derive the distance between each pair, resulting in absolute motion estimates. The strain, $\in$, was defined as the relative variation of the distance, d, between the tracked blocks as: $\in = d(j)-d(j-1)/d(j-1)$, where $d(j)$ and $d(j-1)$ indicate the distance between the tracked blocks at the current frame (j) and previous frame ($j-1$), respectively.

Contraction frequency

Frequency features were analysed separately for the anterior and posterior

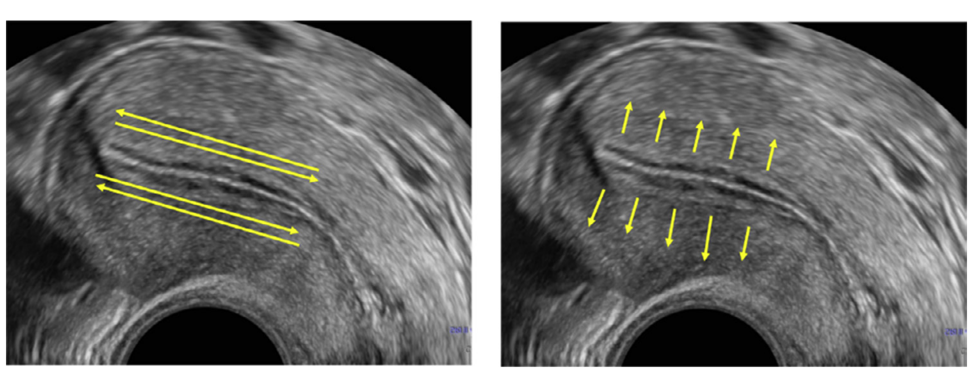

FIGURE 3 Ultrasound images of the uterus in the mid-sagittal section with depiction of contractions in the longitudinal (left) and radial (right) direction.

walls of the uterus, in the longitudinal and radial directions. Only the longitudinal direction of contractions is presented here. Frequency-related features are reported as contractions per minute (*Sammali et al., 2019b*). Further technical details about feature extraction, as well as pre-processing analysis, can be found in *Sammali et al. (2018a)*.

Contraction amplitude

Contraction amplitude features reflect the relative strength of uterine contraction. Amplitude of contraction was assessed by calculating the SD of the strain signal in the longitudinal and radial directions from its frequency spectrum (Parseval's theorem) (*Hu and Fan, 2010*). A higher value reflected stronger uterine contractions. Results are reported for contractions in the longitudinal direction, separately for the anterior and posterior uterine walls.

Contraction direction

Uterine peristalsis is thought to propagate mainly in one of two directions: either fundus-to-cervix (F2C) or cervix-to-fundus (C2F). The contraction direction was estimated by analysis of the radial strain signal representation in the spatiotemporal frequency domain, where the spatial domain is intended along the longitudinal direction of the uterus (*Huang et al., 2022*). The ratio between the strain signal energy in the quadrants corresponding to the two propagation directions (C2F and F2C) provided a global measure of the dominant propagation direction in each wall (posterior and anterior) separately (*Huang et al., 2022*). Basically, a more positive value represented movement predominantly in the F2C direction, whereas a more negative value represented movement predominantly in the C2F direction. A value around zero represented movement that did not show a predominant direction, being either a circular movement, or standing or opposing contractions.

Contraction velocity

Velocity, the propagation speed of the peristaltic waves in a certain direction (C2F or F2C, in mm/s), was calculated for movement in the anterior and posterior walls. This was again done by analysing the radial strain signal representation in the spatiotemporal frequency domain, where the spatial domain is intended along the longitudinal direction of the uterus (*Sammali et al., 2018a*). The analysis was performed over a window of 20 s sliding over the full recording time. Subsequently, the median velocities in the C2F and F2C directions were calculated by averaging velocities over time in the corresponding directions; a high value reflected increased velocity in the reported direction. Results were reported separately for the anterior and posterior uterine walls.

Contraction coordination

In addition to the features described above, the aim was to also assess the coordination of uterine contraction. This is the first attempt at a quantifiable measurement of coordination of uterine movement to date. In order to quantify this, an assessment was done of whether the anterior and posterior walls of the uterus were moving synchronously or asynchronously. This was accomplished by estimating the time evolution of the estimated propagation direction over the anterior and posterior walls using a running window of 20 s. The two resulting evolutions were then compared using a similarity measure. This resulted in a feature defining the uterine contraction coordination depending on the adopted similarity measure: the mean square error (MSE). Two additional coordination features (Hausdorff distance metric and cross-correlation) were also investigated, and are shown in Appendix A. Again, full details of the technical background of these units have been published elsewhere (*Huang et al., 2022*). A higher value reflected decreased contraction coordination.

Study outcomes

The primary outcomes investigated were the following uterine contraction features, compared between the four menstrual phases: (i) frequency, in contractions/minute; (ii) amplitude (unitless); (iii) direction (unitless, whereby >0.0 globally represents F2C movement, and <0.0 represents C2F movement); (iv) median velocity (mm/s); (v) coordination, in MSE.

Statistical methods

Statistical analysis was performed using SPSS Statistics for Windows, Version 27 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was first employed to test the normality of the distributions. Comparison of the outcome measures (frequency, amplitude, direction, coordination and velocity) between the various phases was done using the Kruskal–Wallis test if abnormally distributed, and a one-way analysis of variance (ANOVA) if normally distributed (with Bonferroni correction). Statistical significance was defined as a P-value <0.05. This study is reported according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (*Von Elm et al., 2007*).

Ethical approval

This study received ethical approval from the local and regional ethical committees of participating centres, with study number NL52466.100.15 on 15 July 2020 (the Netherlands), 12 May 2021 (Greece) and September 9th, 2021 (Italy). All participants gave informed consent prior to study participation.

RESULTS

Patient characteristics and recruitment

Ultimately (see FIGURE 1), 70 recordings from 64 women were available for analysis. TABLE 1 presents an overview of the characteristics of these women.

Uterine contraction features

TABLES 2–6 present an overview of the values found per contraction feature across the menstrual cycle phases, for the features of frequency, direction, velocity, amplitude and coordination.

Contraction frequency, velocity and coordination differed significantly between menstrual phases. No significant differences were found between cycle phases for amplitude or direction.

Contraction frequency

The overall values of contraction frequency per menstrual phase can be seen in TABLE 2. The highest mean contraction frequency (1.61, SD 0.17, $P < 0.001$) was found in the late follicular phase in the posterior wall. The phase with the lowest mean contraction frequency was the late luteal phase (1.28, SD 0.13) in the anterior wall, $P = 0.003$). The early luteal and menstrual phases had comparable contraction frequencies ($P > 0.05$).

Contraction amplitude

The overall values of contraction amplitude per cycle phase can be seen in TABLE 3. No significant differences were found between cycle phases. The mean contraction amplitude was 0.044–0.062

TABLE 1 PATIENT CHARACTERISTICS FOR ANALYSED PATIENTS (*N* = 64)

Characteristic	
Patients per participating centre	
Netherlands	33 (51.6)
Italy	24 (37.5)
Greece	7 (10.9)
Age (years)	34.1 (6.3)
BMI (median, IQR)	23.0 (3.75)
Parity	
Nulliparous	34 (53.1)
Multiparous	9 (14.1)
Missing	11 (17.2)
Cycle duration (days)	28.1 (1.7)
Cycle day menses measurement	2.42 (1.13)
Cycle day mid-follicular measurement	8.33 (0.94)
Cycle day late follicular measurement	12.31 (0.85)
Cycle day early luteal measurement	16.33 (3.95)
Cycle day late luteal measurement	27.50 (1.00)
Uterine measurements	
Uterine length (mm)	71.44 (10.77)
Uterine height (mm)	35.96 (6.18)
Uterine width (mm)	63.10 (1.06)
Endometrial thickness (mm) (median, IQR) per cycle phase	Menses: 2.00 (0)
	Mid-follicular: 5.34 (1.52)
	Late follicular: 7.46 (2.77)
	Early luteal: 10.02 (3.92)
	Late luteal: 6.60 (2.44)

Data are presented as *n* (%) or mean (SD) unless otherwise stated.

BMI = body mass index; IQR = interquartile range.

(SD 0.011–0.016) in the late follicular phase and 0.036–0.062 (SD 0.013–0.024) in the late luteal phase ($P > 0.05$).

Contraction direction and velocity

Contraction direction did not seem to differ significantly between menstrual phases (TABLE 4, $P > 0.05$). During the menstrual phase, direction of contraction showed a trend towards F2C contractions. In other phases mainly C2F contractions were seen. Contraction velocity overall differed significantly across cycle phases. The velocity of contractions was significantly higher in the late follicular phase in all directions (see TABLE 5, $P < 0.001$, $P = 0.021$, 0.004 and 0.026, respectively), and lowest in the late luteal phase.

Contraction coordination

The contraction coordination values are shown for all cycle phases in TABLE 6. MSE showed a significant difference across the cycle phases, with a significantly ($P = 0.011$) reduced coordination of contractions during the late follicular phase compared with the menstrual and late luteal phases. Further coordination parameters did not differ significantly between cycle phases (see Appendix B).

DISCUSSION

The results of this exploratory study suggest a preliminary range of normal reference values in a healthy population of women without hormonal contraception

TABLE 2 CONTRACTION FREQUENCY ACCORDING TO MENSTRUAL CYCLE PHASE

	Menstrual (n = 4)	Mid-follicular (n = 11)	Late follicular (n = 26)	Early luteal (n = 14)	Late luteal (n = 15)	P-value (one-way ANOVA)
Contraction frequency, longitudinal, anterior wall (contractions/min)	1.31 (0.08)	1.46 (0.12)[a]	1.46 (0.14)[a]	1.40 (0.14)	1.28 (0.13)	0.003
Contraction frequency, longitudinal, posterior wall (contractions/min)	1.31 (0.13)	1.54 (0.14)[a,b]	1.61 (0.17)[a,b]	1.45 (0.17)	1.35 (0.19)	<0.001

Data are presented as mean (SD).

[a] Significant difference versus late luteal phase.

[b] Significant difference versus menstrual phase.

TABLE 3 CONTRACTION AMPLITUDE ACCORDING TO MENSTRUAL CYCLE PHASE

	Menstrual (n = 4)	Mid-follicular (n = 11)	Late follicular (n = 26)	Early luteal (n = 14)	Late luteal (n = 15)	P-value (one-way ANOVA)
SD in strain longitudinal direction anterior	0.050 (0.015)	0.049 (0.010)	0.062 (0.016)	0.056 (0.013)	0.062 (0.024)	0.141
SD in strain longitudinal direction posterior	0.042 (0.006)	0.036 (0.010)	0.043 (0.012)	0.041 (0.014)	0.040 (0.014)	0.240
SD in strain radial direction anterior	0.041 (0.014)	0.038 (0.010)	0.045 (0.012)	0.047 (0.021)	0.038 (0.013)	0.266
SD in strain radial direction posterior	0.041 (0.011)	0.037 (0.010)	0.044 (0.011)	0.044 (0.023)	0.036 (0.013)	0.218

Data are presented as mean (SD).

TABLE 4 CONTRACTION DIRECTION ACCORDING TO MENSTRUAL CYCLE PHASE

	Menstrual (n = 4)	Mid-follicular (n = 11)	Late follicular (n = 26)	Early luteal (n = 14)	Late luteal (n = 15)	P-value (one-way ANOVA)
Direction anterior wall[a]	0.085 (0.288)	–0.100 (0.379)	–0.032 (0.396)	–0.054 (0.297)	0.084 (0.153)	0.669
Direction posterior wall[a]	0.013 (0.279)	–0.270 (0.252)	–0.207 (0.180)	–0.206 (0.179)	–0.061 (0.242)	0.300
Predominant direction	F2C	C2F	C2F	C2F	None	n/a

Data are presented as mean (SD).

[a] A value under 0.0 reflects movements predominantly in the cervix-to-fundus (C2F) direction, whereas a value higher than 0.0 reflects movement predominantly in the fundus-to-cervix (F2C) direction. Values between –0.1 and 0.1 reflect no predominant direction, or standing/opposing contractions.

TABLE 5 CONTRACTION VELOCITY ACCORDING TO MENSTRUAL CYCLE PHASE

	Menstrual (n = 4)	Mid-follicular (n = 11)	Late follicular (n = 26)	Early luteal (n = 14)	Late luteal (n = 15)	P-value (Kruskal–Wallis)
Fundus-to-cervix propagation anterior (mm/s)	0.67 (0.10)	0.77 (0.26)	0.81 (0.31)[a]	0.71 (0.17)	0.62 (0.08)	<0.001
Fundus-to-cervix propagation posterior (mm/s)	0.69 (0.09)	0.73 (0.23)	0.85 (0.21)	0.73 (0.12)	0.67 (0.16)	0.021
Cervix-to-fundus propagation anterior (mm/s)	0.68 (0.06)	0.80 (0.30)	0.82 (0.29)[a]	0.71 (0.14)	0.65 (0.13)	0.004
Cervix-to-fundus propagation posterior (mm/s)	0.65 (0.11)	0.78 (0.31)	0.86 (0.33)[a]	0.74 (0.11)	0.66 (0.15)	0.026

Data are presented as median (IQR).

[a] Significant difference versus late luteal phase.

TABLE 6 CONTRACTION COORDINATION ACCORDING TO MENSTRUAL CYCLE PHASE

	Menstrual (n = 4)	Mid-follicular (n = 11)	Late follicular (n = 26)	Early luteal (n = 14)	Late luteal (n = 15)	P-value (one-way ANOVA)
Mean square error	0.15 (0.04)[b]	0.28 (0.75)[c]	0.24 (0.12)[a,c]	0.20 (0.08)	0.18 (0.07)	0.011

Data are presented as mean (SD).

[a] Significant difference versus late luteal phase.

[b] Significant difference versus late follicular phase.

[c] Significant difference versus menstrual phase.

and normal uteri. A novel, reproducible and objective method based on ultrasound speckle tracking is presented. It is possible to characterize uterine contraction amplitude and frequency, as well as coordination, direction and velocity. Coordination, direction and velocity of uterine contractions are features that have never before been quantified in this context. The current results show that contraction frequency and velocity are highest in the late follicular phase and lowest in the menstrual and late luteal phases. Coordination seems to be negatively affected by contractions with higher frequency and velocity in the late follicular phase compared with other phases. Amplitude and contraction direction in this population do not show significant variations across the menstrual cycle.

The findings are generally in accordance with the existing literature concerning uterine contractile activity in the healthy uterus. Previously described methods to assess uterine contractility have assessed some subsets of the features presented here (Blank et al., 2020); however, this is the first study where all the presented features are quantified and evaluated (Kuijsters et al., 2017). The novel features for the characterization of different uterine activity and associated patterns – coordination, direction and velocity – could form a new avenue for research and knowledge into uterine function. The TVUS method presented for the quantitative analysis of uterine contractions is also easily reproducible (Huang et al., 2022): quick, objective and patient-friendly. It is potentially possible to integrate into routine gynaecological practice (after sufficient training), and does not require extensive skill or expertise.

The main limitation of the results presented here is the small sample size of the study population. However, it is believed that the results presented are valid due to their general accordance with the currently accepted patterns of uterine peristalsis throughout the menstrual cycle. In addition, most patients received an ultrasound in only one phase of the menstrual cycle for a 4-minute time frame, and therefore it was not possible to conduct a within-subjects comparison. It could be debated how far this relatively short recording is representative of the behaviour of the uterus during this phase in general, however a subanalysis with repeated recordings within subjects was conducted in previous work (Huang et al., 2022), with comparable results. Additionally, the majority of ultrasounds were conducted in the late follicular phase, which may affect the significance of results. Furthermore, due to the novel and still experimental nature of the quantitative analysis employed in this study, its clinical application in routine practice is not yet possible. It was also necessary to exclude a significant number of recordings from analysis (n = 18) due to insufficient quality of the ultrasounds, which indicates that there is a learning curve which could (initially) affect clinical useability. In some cases this was avoidable (e.g. insufficient resolution or out-of-plane motion), but incidentally it is not possible to analyse contractions despite good ultrasound technique (for instance due to the orientation of the uterus, or shadows caused by intestinal contents, for example). It is also not yet feasible to gain contraction feature results in real time while performing the ultrasound scan, as the implemented analysis still relies on offline, post-ultrasound data processing. In the future, steps need to be taken to make this TVUS speckle tracking method for quantitative analysis of uterine contractions usable in daily clinical practice.

The results presented here provide further insight into uterine behaviour at different phases of the menstrual cycle, whereby each cycle phase shows an individual contraction pattern. The results clearly show that the late follicular phase is the most active, with the highest contraction frequency and velocity. One could surmise that these features are thus important for the sperm transport and ovulation that occurs in this period of the menstrual cycle. Furthermore, the relatively reduced activity in the late luteal to menstrual phases suggests a relevance of these characteristics with regard to facilitation of embryo implantation and/or menstruation symptoms. The coordination feature has not been investigated before; however, these initial results show that increased contraction frequency and velocity seem to be accompanied by reduced coordination of contractions. The clinical importance of simultaneous (coordinated) anterior and posterior contractions and how this could relate to fertility outcomes or clinical symptoms, for example, merits further investigation. Potentially, this coordination feature could be seen as a measure of uterine dysperistalsis, which has been previously described in patients with infertility and endometriosis (*Kissler et al., 2006*, *2007*; *Leyendecker et al., 1996*) with significant clinical consequences, especially with regards to fertility.

Now that it is possible to suggest preliminary reference values for uterine peristalsis in a normal menstrual cycle, it is possible to better assess how and if uterine contractile activity is abnormal in different populations. Previous work by this research group has assessed uterine peristalsis in IVF patients, which showed promise with regards to prediction of IVF treatment success (*Blank et al., 2020*). Future works will be able to compare how uterine contractions differ between fertile and infertile populations, also relative to the preliminary reference values in a normal menstrual cycle presented here, potentially identifying treatment target points, and perhaps uncovering a new facet of infertility aetiological mechanisms.

Although this study focused on healthy women with normal uteri, uterine peristalsis assessment and characterization can also represent a valuable diagnostic tool in the context of common pathological conditions of the uterus, such as adenomyosis, endometriosis or uterine fibroids. The effect of these conditions on uterine function (and disease symptoms such as dysmenorrhoea and infertility) may in fact be reflected in altered uterine peristalsis patterns, such as coordination. Dedicated clinical trials can be designed to investigate the potential of the proposed features for the diagnosis of uterine diseases and dysfunctions. Differences in uterine contractions could be an explanatory factor for the symptomatology in certain uterine disorders, and thereby become a target for patient-tailored treatment.

In summary, preliminary reference values of uterine contraction features in healthy women during the natural menstrual cycle are suggested. The current study serves as a standard to which uterine peristalsis in infertile women or women with abnormal uteri can be compared, potentially identifying treatment targets and aetiological mechanisms yet unexplored. Furthermore, the study presents novel uterine contraction features that can be used to assess the presence (or absence) of normal uterine contractility, namely coordination, direction and velocity.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2022.08.104.

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