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Case Report

Fertile window and biophysical biomarkers of cervical secretion in subfertile cycles: a look at biotechnology applied to NaProTechnology

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Abstract

Background: The principal objective of this study was to correlate biophysical properties of vaginal discharge present in the cervical mucus with the timing of the fertile window. In particular, we produce measures of the viscoelasticity of the cervical secretion using two methods. The first uses only the elasticity extracted from the Creighton Model Fertility Care System (CrMs) scale, calculated P-6 ovulation estimated day (OED) with respect to the peak day of the CrMs. The second uses a numerical method that takes into account the changes in viscoelasticity, but without reference to the peak day calculated using the CrMs model. Using both methods, twelve records were obtained from a single female subject. **Methods**: The methodology used to evaluate the viscoelasticity factor was by measuring the approximate length in centimeters (cm) of the vaginal discharge of cervical discharge. For this, the scale of the stretching graph established by observing the stretching of CrMS was used, taking into account the previous 6 days at peak day P-6. The first method, which we termed CFW (Clinical Fertile Window), uses a measure based on the approximate length (cm) of the maximal stretchiness of the vaginal discharge. The second method we termed SFW (Software-CrMS/strectching) (Software-based Fertile Window). **Results**: The fertile window was detected correctly in 100% of the cases using either method, and a correlation value of 0.71 was observed between the two methods. **Conclusions**: We conclude that the assessment of viscoelasticity using SFW algorithm allowed in this pilot study to detect the fertile window and to describe the evolution pattern of cervical discharge throughout the fertile window. Our study provides support for the use of computational methods in detecting the fertile window, taking only into account the time evolution of the cervical discharge throughout the menstrual cycle.

Keywords: Fertile window; NaProTechnology; Cervical secretion; Ovulation; Fertility awareness; Subfertility; Biophysical biomarkers; Biotechnology

1. Introduction

Determination of the fertile window through the observation of the length of the menstrual cycle is an already established procedure. Each month, the ovary has a cohort of antral follicles sensitive to a certain concentration of follicle-stimulating hormone (FSH) [1]. At the follicular stage, the antral follicles need to be sensitive to the action of FSH, in order to be rescued from the follicular pool, and have receptors to FSH for adequate aromatization to occur in the antral follicle [2]. These physiological changes are decisive for an increase in the production of estrogens. Thus, around 5 to 12 days of the menstrual cycle, the preantral follicle presents a significant increase in the number of stromal cells [3–5]. This event is essential for endocervical cells to become stimulated to produce type-L and type-S secretions. As a result, a significant increase in the biophysical properties of cervical secretion occurs around the estimated day of ovulation (OED), changes which correlate with the progressive elevation of steroid derivatives in blood and urine, in synchrony with follicular development [6–9].

Herein we review the main concepts relevant to understanding the changes observed in the cervical secretion, focusing on the production of estrogen by endocervical cells. In particular, we review the basic physiology of ovulation concerning the evolution of the biophysical characteristics of the cervical secretion with the fertile window, and its impact for the processes of follicular growth, selection, recruitment and maturation.

The purpose of this work is to review the role of the viscoelasticity and transparency of cervical mucus can play as signals and biophysical markers of changes in cervical secretion properties in order to recognize the occurrence of the fertile window. Viscoelasticity often refers to many terms that describe the rheological properties of cervical mucus, including viscosity, elasticity, plasticity, shrinkage, adherence, spinbarkheit and stretchiness, among others. The ability to lengthen or stretch the cervical discharge has been one of the classical parameters used to assess the progression of cervical discharge throughout the menstrual cycle. That the elasticity is greater on the days preceding the peak day, and during the peak day, has been reported in previous studies [10–17]. Accordingly, assessing the biophysical properties through the CrMs method [10] is well supported by the literature. Thus, it has been possible to recognize the viscoelasticity of the cervical discharge as a clinical sign to detect the fertile window [17–19].

This allows us to systematically track the progressive changes related to the fertile window, from the left kurtosis phase to the postovulatory phase, after the peak day, in both normal and subfertile cycles [10]. Using this scale as a normalizing artifact, we are able to gauge the effectiveness of our detection methods as applied to ovulatory cycles in subfertile populations.

Therefore, it is possible to apply concepts derived from ovulatory cycles in subfertile patients. It is estimated that around 12% to 15% of couples may experience infertility. The fertile window in subfertile patients has proven efficacy in spontaneous pregnancies [14]. The changes observed in the biophysical parameters of the cervical secretion: as well as the volume, the viscoelasticity or spinnbarkheit, the transparency, and the crystallization of the cervical secretion, are likely to be used to identify the fertile window in subfertile patients [20,21].

2. Materials and methods

2.1 Menstrual cycles

We analyzed biophysical parameters obtained from observations of a total of 12 menstrual cycles that occurred in a 29-year old female patient between 2017 and 2018. The subject gave their informed consent for inclusion before they participated in the study, which was conducted in strict accordance with the Declaration of Helsinki, and whose protocol was approved by the Ethics Committee of CEImLAR, Center for Biomedical Research of La Rioja (CIBIR) (approval number P.I.339).

The shortest cycle was 29 days, and the longest was 39 days. The first day of the cycle was defined as the first day of menstruation, and the last day of the cycle, the day before the start of the next menstruation. Twelve records of the typical evolution of cervical mucus were documented by recording the charts of the observation of vaginal discharge from cervical secretion with the Creighton Model Fertility Care System (CrMS).

A single individual carried out extraction and analysis of data obtained from the survey records. This was done to reduce inter-observer variability because the clinical evidence taken into account in the measurements carried out by the patient were then extracted and corroborate by the same single individual who performed the clinical study.

The patient received a clinical diagnosis of infertility after more than one year of regular sexual intercourse without contraception. During initial diagnosis, we ruled out various gynecological pathologies that could affect follicular development, such as hipertiroidism, hipotiroidism, hiperprolactinemia, hirsutism, ehiperandroegenemia, and ovarian polycystic syndrome, as well as other functional causes as anorexia and obesity.

A basic infertility study was performed, in which no detectable cause of female sterility was documented, including the exploration of the genital tract, transvaginal echography, Pap smear, colposcopy, histerosalpinografy and analytical functional test in which no detectable causes of female sterility were detected. The presence of male factor of treated oligoastenozoospermia was confirmed without significant improvement despite an effective sperm count (ESC) of 627.000 sperm.

The medical examination also included a follicular follow-up study, which was carried out at the beginning of the clinical evaluation.

Signs compatible with ultrasound ovulation were documented by observing the sign of the double peri-follicular contour, follicular rupture and emptying registered by the presence of irregular walls and mixed echo images inside the follicle, and postovulatory mixed echo image compatible with corpus luteum was verified.

2.2 Criteria for establishing clinical fertile window and software-based fertile window

The objective of the study was to model the evolution and progression of the viscoelasticity of the cervical secretion in the fertile window of ovulatory menstrual cycles using a computational method. We validate the method by means of the elasticity of the cervical mucus, as measured in cm according to the CrMs scale, taking into account the data calculated from day P-6 as estimated day of ovulation (OED) retrospectively. The computational method accounts for changes in the measurement of viscoelasticity of the vaginal discharge of cervical secretion throughout the menstrual cycle. The method also uses the CrMs scale, but does not take into account the peak day calculated from the CrMS model. The Spinbarkheit assessment of cervical discharge was coded using the following guidelines: A: Sticky: 0.5 cm (Less than 0.65 cm, equivalent to ¼ inch). B: Tacky: 1 cm, equivalent to the lower value of the CrMS classification interval between (1–2 cm); (1.27–1.905 cm), which corresponds to (½–¾ inch for the CrMS system). C: Stretchy: 2.5 cm was recorded when the yarn was equal to or greater than 1 inch. The days on which

Table 1. Spinbarkheit and cervical secretion scale extracted from creighton model fertility care system (CrMS)* for CFW: (CrMS/stretching).

A: Sticky: 0.5 cm CrMS: (Less than 0.65 cm / ¼ inch)
B: Tacky: 1 cm CrMS: (1.27–1.905 cm / ½–¾ inch)
C: Stretchy: 2.5 cm CrMS (2.5 or greater / 1 inch or greater)

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there were dryness and no secretion were tabulated as 0. Table 1 summarizes the scale used to evaluate the parameter of viscoelasticity. Fig. 1 describes the distribution of the mean viscoelasticity values in cm throughout the menstrual cycle. Within the methodology used by the algorithm, the aforementioned scale was chosen, because this scale according to CrMS describes better than other scales, the variation that occurs from the follicular phase of cervical secretion. In this way, it was possible to record the progressive evolutionary change in a systematic way from the left kurtosis of the fertile window to the postovulatory phase after the peak day as seen in Graph 1. The clinical window in this article was established as CFW: (CrMS/stretching) by means of the retrospective assessment of the mentioned scale taking into account the 6 days prior to the peak day: P-6. The methodology used consisted in comparing the two fertile windows with ODE. To check the evolution in the characterization of the viscoelasticity parameter of the cervical secretion, the last day of maximum fertility was established, with the letter "P" for the peak day, according to the recognition rules for the Peak day of CrMS. The correlation test was performed to calculate the percentage of coincidence of the intervals, and the points of change (POC) between both windows. The interval between both windows was evaluated CFW: (CrMS/stretching) and SFW: (Soft-CrMS/stretching) by means of a graph of middles points, and correlation test of overlap of intervals. The correlation test was performed to calculate the percentage of coincidence of the intervals, and the points of change (POC) between both windows.

The maximum transparency was registered with the letter "k" extracted from the CrMS registry, which was not taken into account to define, nor was the clinical fertile window CFW: (CrMS/strectching), nor ODE nor was it considered in counts in the SFW calculation: (Soft-CrMS/stretching). The k "clear" is the maximum transparency standard on the CrMs scale. To assess the contribution of the variants of the peak day, Last Max Spinbarkheit (LMS) was considered, for which the estimation of the variations of Peak Day was taken into account in a descriptive way through the concept of: LMS & Transparency by "K" from CrMS. Different from the Peak Day concept, which is defined as any type of discharge that is clear, stretches or has isolated lubricative properties, or in combination with each other, according to CrMS.

2.3 Software implementation phase and statistical study

The software to calculate SFW was made up of two paired applications following an object-orientation design, and programmed in C# and Java. Unit tests were done using JUnit and NUnit. The first application, running under Windows 10, was used to collect the measurements and store them in a database. The second application followed a three-tier design. The first tier supported data access and included mechanisms for data query, modification and insertion given the database constructed by the first application.

The second tier implements all necessary functions to manipulate the logical entities abstracted from the data model. The third layer implemented the presentation logic, which allows the end user to interface with the system. Finally, an exhaustive study was carried out to find out the necessary algorithms, also carrying out a formal verification. A normality test was carried out to describe the study sample with the Shapiro-Wilk test. The correlation study was performed with Excel in conjunction with "R"; environment and programming language focused on statistical analysis of the correlation test. An intuitive graphical interface was developed.

3. Results

The fertile window using CFW: (CrMS/stretching) it was possible to detect them in 100% of the cycles. Using the present series, it was possible to evaluate the elasticity of the vaginal discharge by estimating the length in cm. The CFW: (CrMS/stretching); P-6 interval was distributed between days 10 and 25 of the menstrual cycle as shown in Fig. 1. In Fig. 2, the records that make up each cycle with the two fertile windows are observed. The days in blue are those referring to the stretching described in the clinical scale of Table 1. This figure describes the days before the beginning of the fertile window, the fertile window and the stretching the days after the end of the fertile window of CFW (CrMS/stretching). The clinical window of the SFW software (Soft-CrMS/stretching) presented a normal distribution with a mean of 5.08 days (SD $\pm$ 2.87 days). The days detected by the algorithm are graphed in yellow as SFW: (Soft-CrMS/stretching). It was possible to identify the days of the fertile window in yellow, both at the beginning and at the end of it, identified as points of change (POC). A midpoint concordance is displayed between each cycle of the two windows equivalent to the correlation coefficient of 0.71, as observed in Fig. 3. The concordance between the two fertile windows coincides in a percentage greater than 50% in 75% of the cycles, as shown in Table 2. In Fig. 4, a graph of the middle points of each cycle of average stretching is made for both clinical fertile window, as for the fertile window of the algorithm.

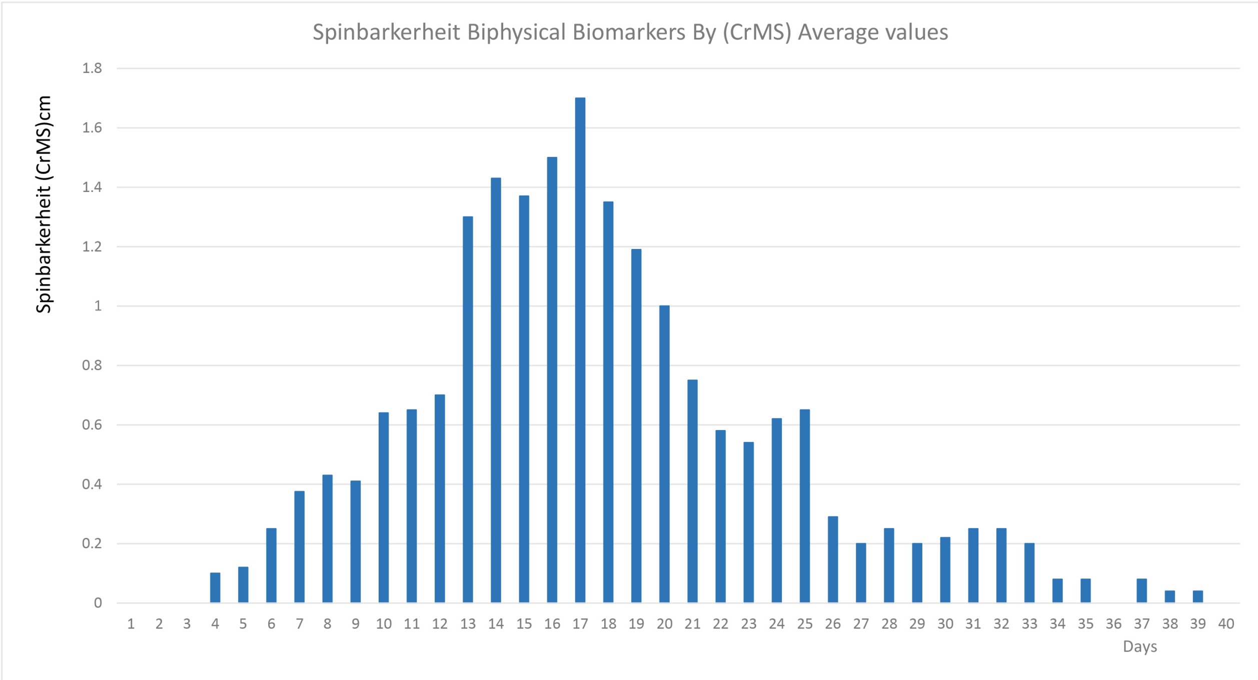

Fig. 1. The mean values of the stretching data of each day of the menstrual cycle were represented. The evolution of the stretching according to A: Sticky: 0.5 cm is recorded. Tacky: 1 cm. Strechy 2.5 cm: The typical course of cervical mucus is documented by observing the vaginal discharge of cervical discharge (VDRS) based on the classification system of the Creighton Model Fertility Care System (CrMS).

Last Max Spinbarkheit (LMS) is related to the maximum progression of, stretching and transparency by "K" according to CrMS. A systematic concordance between the K and the relationship with P was found. Apparently, the characteristics of the stretching and lubrication capacity are possible thanks to the physical properties of the endocervical crypts type L and S. In this series, all clinical windows registered this gradual rise in relation to LMS. LMS and K was observed in 41.6%, and LMS + (K $\leq$1 Day) was found in 4 cycles (33.3 %), and Spinbarkheit $\leq$ LMS + (K $\leq$1 Day) = 3 cycles (25%). In less percentage, in three cases did not coincide with the peak day, or with the day of greatest transparency or greatest elasticity, but was very near (-1 day OED).

4. Comments

The fertile window was detected in 100% of the cases with both methods. A correlation coefficient of 0.71 was obtained, between the clinical fertile window detected by means of the parameter of viscoelasticity of the cervical secretion, and the prediction of viscoelasticity by means of a software computer tool.

In this context, it is evident that as soon as the cervical mucus stretches more, and the elasticity increases, both the days before the peak day and the days after the viscoelasticity parameter. It was possible to record it. Within another of the biophysical parameters, the characteristics of the last days of maximum fertility were taken into account through the concept of LMS & Transparency by "K" of CrMS. Twelve records of the typical evolution of cervical mucus were documented by recording the observation graphs of the vaginal discharge of cervical discharge, with CrMS. In this way, it was possible to record the progressive evolutionary change in a systematic way from the left kurtosis of the fertile window to the postovulatory phase after the peak day as seen in this series. The purpose of the present work consisted in comparing the biophysical variable of the viscoelasticity of the cervical secretion, by comparing the clinical window of the CrMS model that uses the peak day according to the CrMs model. Thus, the main of outcome is chosen as the peak day of the CrMs scale. And there are two ways of measuring, two, valuations; one uses only the elasticity extracted from the CrMs scale, as described in detail in the manuscript, which refers to the peak day of the CrMS, and the other is the computer tool. A computer tool is used that determines the progression of viscoelasticity without any reference point, by means of a Neperian algorithm that takes into account the means of the previous values, it evaluates each new value that is presented, until a significant lag is detected, with a change between 25–50% in the measurements between the previous values, and the consecutive ones, in this way it detects the POC at the beginning and at the end to indicate the fertile window called by the software. The scale of the CrMs model was cho-

Table 2. Fertile windows in 12 cycles according to SFW and CFW.

Cycle	Inicial Day SFW	End Day SFW	Inicial Day CFW	End Day CFW	Precision Test	Overlap %
1	16	20	14	20	0.67	66.67
2	13	17	10	16	0.50	50.00
3	15	21	14	20	0.83	83.33
4	14	18	16	22	0.33	33.33
5	17	20	17	23	0.50	50.00
6	14	18	13	19	0.67	66.67
7	12	26	19	25	1	100
8	13	19	12	18	0.83	83.33
9	15	18	11	17	0.33	33.33
10	16	21	15	21	0.83	83.33
11	8	12	10	16	0.33	33.33
12	14	17	14	20	0.50	50.00

Days	Filance (cm)
1	0
2	0
3	0
4	0
5	0
6	0.5
7	1
8	0.5
9	0.5
10	1
11	1
12	0.5
13	2.5
14	2.5
15	2.5
16	2.5
17	1
18	1
19	0.5
20	0.5
21	0.5
22	0
23	0.5
24	0.5
25	0.5
26	0
27	0.5
28	0.5

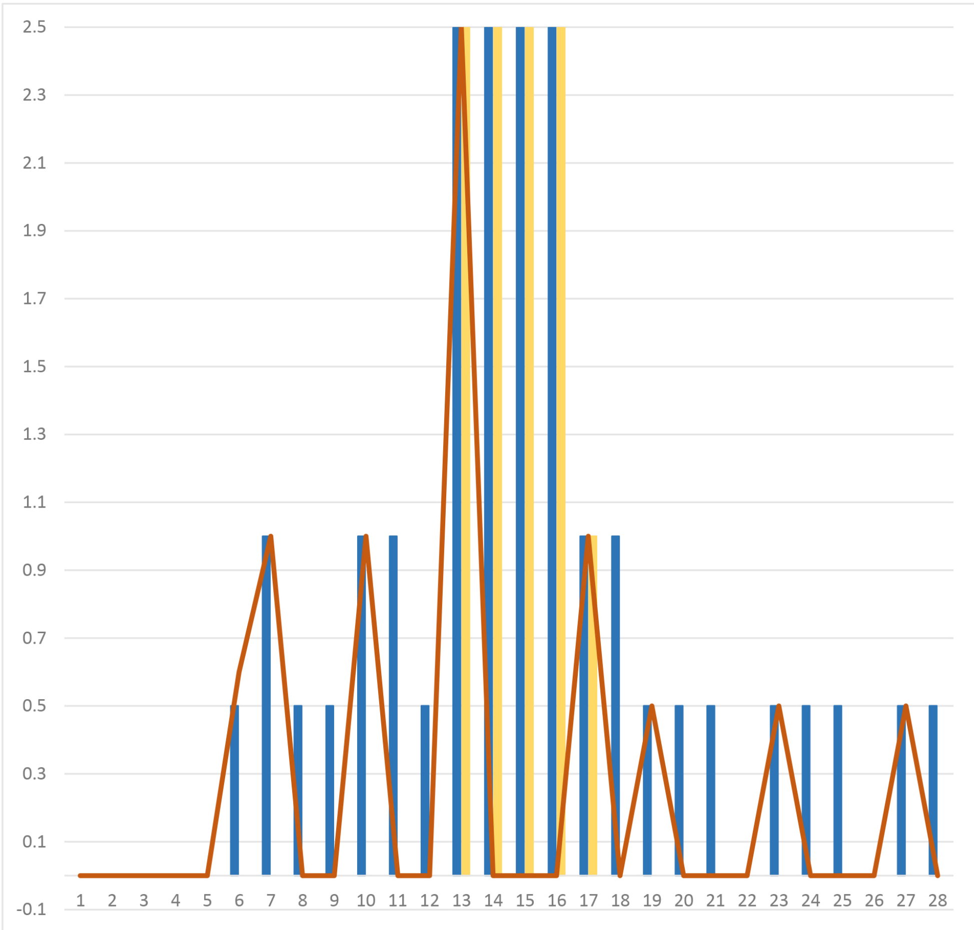

Fig. 2. Example of one record. Stretching in centimeters (cm) is made for both fertile windows; for clinical fertile windows CFW: (CrMS/stretching) as for the fertile window of the algorithm SFW: (Soft-CrMS/stretching). Mean value of SFW: (Soft-CrMS/stretching) was: 5.08 days ($\pm$ 2.87 SD). The days in blue are those referring to the stretching described in the clinical scale of Table 1, it describes the days before the beginning of the clinical fertile window, the fertile window and the stretching the days after the end of the clinical fertile window of CFW (CrMS/stretching). The days detected by the algorithm are graphed in yellow as SFW (Soft-CrMS/stretching). It was possible to identify the days of the fertile window in both at the beginning and at the end of it, identified as points of change (POC).

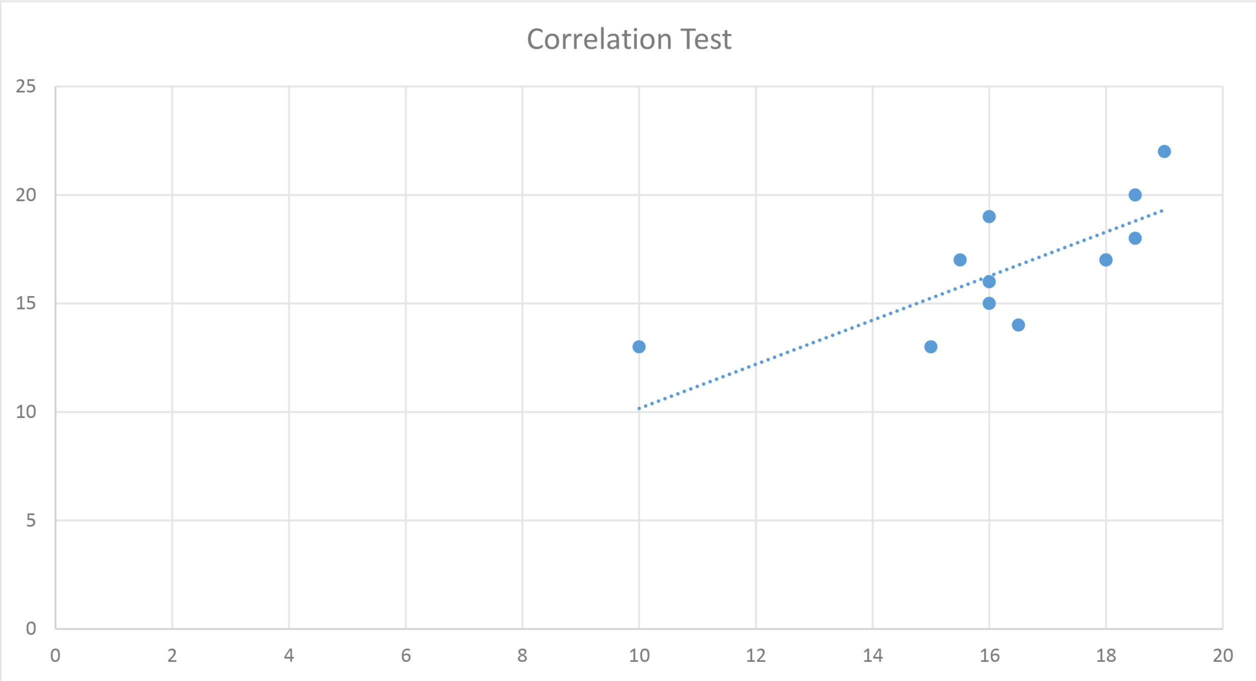

Fig. 3. Graph plotted showing the correlation coefficient between SFW and CFW (r = 0.71).

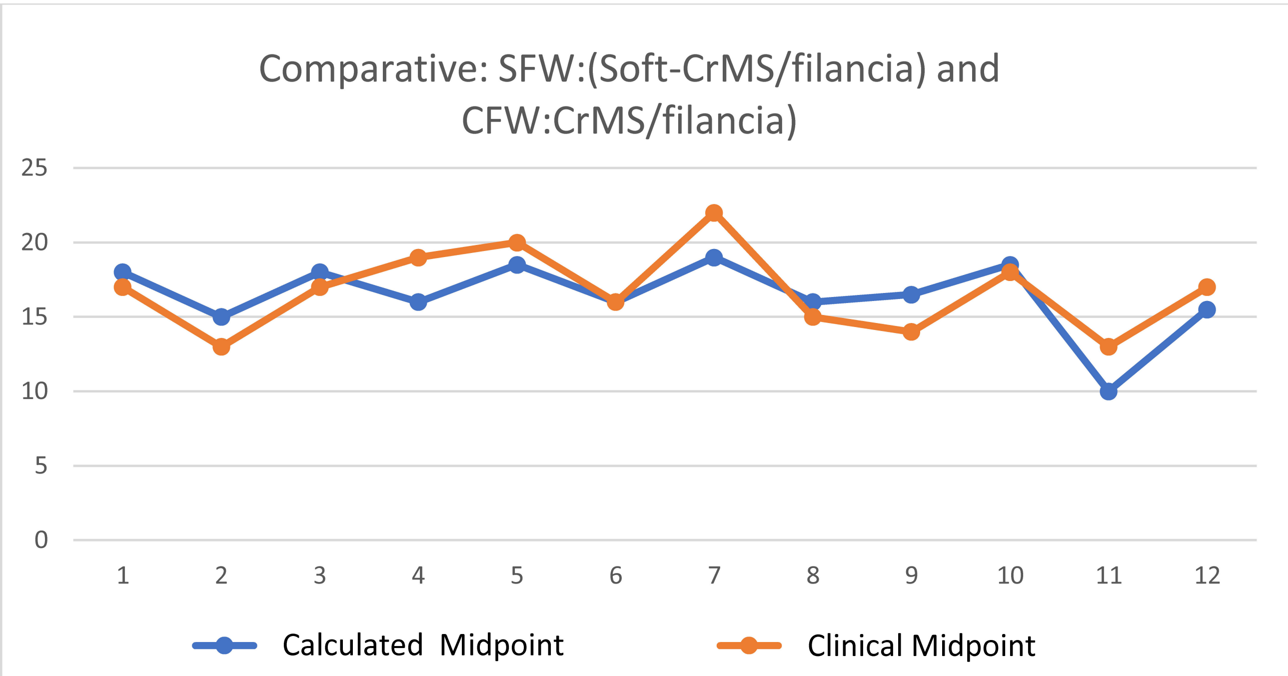

Fig. 4. A graph of the middle points of each cycle of average stretching is made for both clinical fertile windows CFW: (CrMS/stretching), as for the fertile window of the algorithm SFW: (Soft-CrMS/stretching).

sen for several reasons, because it is reproducible, because in patients trained in the CrMS method, and effectively allows the reproducibility of clinical assessments, and is recognized worldwide.

The fertile window recorded by the length of the menstrual cycle is a procedure already studied by Wilcox [22]. In Wilcox's study [22], he identifies in his series of regular cycles the possibility of finding the self-reported fertile window between days 7 and 21 of the menstrual cycle [22]. The possibility of being in a fertile window on the 4th day of the cycle was 2%, by interpreting the duration of the menstrual cycle, 17% on the 7th day of the cycle, and 54% between the 12th and 13th of the cycle [22]. However, in his study, the self-reported possibility of the menstrual cycle to be in a fertile window has a correlation index (CI) of 0.55, which makes it possible for the fertile window to be between days 7 and 21 of the cycle in 50 % of cases in the fertile window. The CrMs scale allows recording the progression and variability of the elasticity and transparency of the cervical secretion [10,12–14].

Within the clinical classifications, Scarpa [23] describes the symptom of vaginal discharge mucus in four ranges. He manages to identify a mucus of greater fertile characteristics (a mucus 4) within the fertile window range between days 10 and 17 days. He finds in 50% of cases the presence of this type of mucus around the 13th day of the cycle, and outside this interval less than 20%. Likewise, he associates a 30% probability of pregnancy, when he is within the range of greater probability of finding type 4 mucus. Fehring manages to frame by assessing the cervical mucus gradient (MMG) the detection of the peak of cervical mucus in 97% between the days (–4 and +4) around ovulation [24]. These results are similar to those described by Hilgers [25] in 1978 where he shows evidences a peak day between (–3 and +3) in relation to the peak day of ovulation. Bigelow describes the characteristics of cervical discharge on a fourth scales [26]. He found a relationship between the quality of cervical mucus and the probability of pregnancy, being the day –3 OED higher for a type 4 mucus, which decreased as he moved away from OED. Although the highest amount of type 4 mucus was evident in –2 OED. These findings have also been corroborated with the Creighton Model Fertility Care System. In such a way it has been possible through cervical secretion to identify and describe cervical secretion from the early follicular phase to the peak day of cervical mucus [27].

The restorative and restorative methodology of the menstrual cycle makes it possible to recover the natural biological approach related to the pathophysiological process of ovulation, in the field of fertility recognition [14]. The estimated day of ovulation (OED) has been recognized as one of the fundamental strategies to determine the signs that define or frame the fertile period. And the peak day of cervical secretion has been considered a point of reference that allows locating the evolutionary clinical changes of the fertile window [28].

It was observed that the peak day in some cycles did not coincide with the day of maximum elasticity, which was taken in counts according to the standards of the CsMS model. These mentioned changes are well correlated with the dynamics of follicular growth, and the elevation of the determinations in urine of estrone-3-glucuronide (E3G). The difference between the number of estrogens produced at the beginning of the cycle and at the ovulatory level is significant, which makes up the aforementioned physiological findings [4,5].

The E3G determination increases from 20–30 ng/mL from day -6 relative to the estimated day of ovulation (OED), until the day of ovulation, at which point the LH surge is normally detected greater than 30 IU/L [6–10]. This peak generally occurs 24 to 48 hours before ovulation but is never after it. The basal body temperature (BBT) curve was not taken into account in this serie, but a high correlation is known to detect the fertile window [28].

One outcome of the validation of a computational method for measuring the biophysical properties of cervical secretion is its possible inclusion as an essential component in a clinical apparatus, as part of a more comprehensive mechanism that also takes into account sperm swimming properties [29,30].

Currently several biomarkers are known which assist in the determination of the fertile window, which open the door for the development of future biotechnological applications based on Brown's theory [1,21]. Aldecrewtz reports a difference in the production of estrogen that exists between the initial follicular phase and the middle of the cycle, and describes a POC which indicates the start of the fertile window [6]. Similarly, the peak of LH [7,8,10] has been used to determine the end of the fertile window, the LH peak Is found between 24 and 48 hours before ovulation, but never before, and therefore can be used as a predictor. Those two well-established points in time bound the interval -6 of the fertile window with respect to the Expected Day of Ovulation (OED). Ecochard [17] relates detection of the fertile window to basal temperature changes by echography [17] and report a detection accuracy of the fertile window on the order of 98 to 99%. Currently there exist several scales [10,15,23,24] which provide a clinical assessment of the changes in the properties of the cervical secretion all throughout the menstrual cycle, which were initially described by Billings in his 1972 publication in The Lancet [12].

The mucin filaments tend to align longitudinally within the cervical canal, creating aqueous channels between the filaments. It seems that the effect of the parallel fibers of the glycoprotein bands can exert a driving effect on the gametes themselves. Natural swimming channels are formed from the interaction between the non-ciliated cylindrical epithelial cells which secrete mucin granules, and the hair cells that drive cervical mucus from the crypt of origin to the external cervical orifice. This process is considered to be dependent on the rheological forces associated with mucus flow from the cervical crypts, which tend to align the mucin filaments longitudinally within the cervical canal.

The integration of sperm swimming in the fertile window approach allows the development of new technologies that integrate the joint assessment of cervical factor and sperm swimming. It is necessary to consider more advances in biotechnological devices that can contribute to giving information to the study on the total interaction of the transport factor and the capacity for sperm rise.

Further studies are needed to further evaluate the efficacy of the method, by introducing not only more data points, but by also incorporating other biophysical characteristics for the cervical secretion, such as measures of fluidity or elasticity, as well as other factors, such as sperm rise [29,30].

The k "clear" is the maximum pattern of clarity, on the CrMs scale, its evidence is constant and may improves the result of the algorithm, which has not been calculated in this study. The use of K rating in the Creighton scale may also improve the algorithm, and we plan to conduct such studies in the future. The incorporation of transparency as part of the biophysical assessment, would also be beneficial [28–30].

Finally, given the increasing use of Machine Learning techniques in the sciences, all across the board, it would be useful to determine whether one could extend the computational method used herein, to include other observed quantities, in order to produce biophysical feature vectors that could be used to construct training data. Such data could be used to train a neural network that could be used to predict the fertile window.

5. Conclusions

The rating scale used in the CrMs model allowed us to use the elasticity and transparency parameters to help identify and characterize the peak day. We found a strong correlation between the fertile windows obtained through clinical measures compared to those obtained from the computational method. Therefore, since our method is solely based on changes observed at the vulvar level in the elasticity of the cervical secretion, we expect it could be used to the beginning of the fertility window and the end of the fertile interval in both fertile and sub-fertile patients.

The assessment of the midpoint of the clinical window in the series allowed to extract the elasticity and transparency parameters, to identify and characterize POCs, at the beginning, at the midpoint, and at the end of the fertility window in conjunction with a computer tool.

The evaluation of the term "last value of maximum elasticity" (LMS), was a parameter that allowed to characterize the progression of viscoelasticity in conjunction with the "K" of transparency of cervical secretion of CrMs, in relation to the peak day of the fertile window.

Author contributions

JMML developed study concept, data acquisition, study design, methodology, project administration, performed data acquisition and data interpretation, and wrote manuscript and draft. OMM, MMC, AFA all contributed to software development, draft elaboration, stadistic validataion, and writing original draft. JEM Preparation including pre-publication stages. DS and JS preparation, creation and presentation of the published work, commentary and revision-including pre-publication stages. JLA reviewed the manuscript and final topics to editing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The subject gave their informed consent for inclusion before they participated in the study, which was conducted in strict accordance with the Declaration of Helsinki, and whose protocol was approved by the Ethics Committee of CEImLAR, Center for Biomedical Research of La Rioja (CIBIR) (approval number P.I.339).

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Conflict of interest

The authors declare no conflict of interest.

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