Continuous Endometrial Volumetric Analysis for Endometrial Receptivity Assessment on Assisted Reproductive Technology Cycles

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Research

Keywords: Endometrial receptivity, Assisted reproductive technology, Endometrial volume, Adjusted endometrial volume, Embryo implantation

Posted Date: February 10th, 2020

DOI: https://doi.org/10.21203/rs.2.22964/v1

Version of Record: A version of this preprint was published at BMC Pregnancy and Childbirth on November 3rd, 2020. See the published version at https://doi.org/10.1186/s12884-020-03372-2.

Abstract

Background: Human implantation is a complex process requiring synchrony between a healthy embryo and a functionally competent or receptive endometrium. In order to assess endometrial receptivity in Assisted Reproductive Technology (ART) cycles continuous evaluation of endometrial biophysical markers may have a predictive value on a positive outcome.

Design: Serial 3D transvaginal ultrasound performed in women on ART cycle to evaluate a pattern that better predicts implantation rates. 169 subjects on a prospective case control study were assessed. Endometrial pattern, endometrial thickness, endometrial volume and adjusted endometrial volume (ratio between endometrial volume and uterine volume) was performed to all subjects on a continuous process from baseline (prior to ovarian controlled stimulation), day 6, 8 and 10 of controlled ovarian stimulation, trigger day with human chorionic gonadotropin hormone (hCG) and at embryo transfer day.

Results: No statistical difference was noted between the two groups in terms of demographics and ART procedures and scores. Endometrium morphology also didn't show any difference between the two groups. Endometrial volume and adjusted endometrial volume was significantly higher in the positive group as soon as day 6 of ovarian controlled stimulation.

Conclusions: Continuous serial 3D endometrial volume and adjusted endometrial volumes may be a useful tool for clinicians in predicting endometrial receptivity enhancing elective embryo transfers in fresh ART cycle, and thus providing a non-invasive continuous technique for endometrial receptivity assessment that reflects endometrial changes during ART procedures.

Background

Successful assisted reproductive technology cycles outcome depends on the intricate interplay between embryo quality and endometrial receptivity. Endometrium is a dynamic tissue that grows, differentiates and suffers regression throughout the menstrual cycle in response to hormonal regulation to prepare the uterus for embryo implantation (1). Therefore endometrium is a highly dynamic tissue undergoing physiological changes in response to ovarian steroid hormones. It has been proven that the supraphysiological hormonal levels has a harmful effect on endometrial receptivity. Endometrial characteristics compatible with a successful pregnancy have proven to be difficult to be properly assessed. To prepare for pregnancy, the endometrial lining in the uterus thickens and becomes receptive to implantation of a fertilized egg. Adequate endometrial development seems to be important for implantation given that previous studies have shown an association between abnormal glandular or vascular development and defective placentation disorders. The window of implantation (WOI) is defined as a short period of time while the endometrium is receptive to the embryo (2).

Diagnosis of endometrial receptivity (ER) has posed a challenge and so far, most available tests have been subjective and lack accuracy and a predictive value. (3,4)

The use of transcriptomic signature of the WOI by microarray technology is possible however it demands an endometrial biopsy. (5) This requires an invasive procedure and it has an associated cost. In women with irregular cycles it may not prove to be cost-efficient. (6)

Ultrasound is a non-invasive technique that can assess changes in the endometrium during stimulated cycles. It also has minimal inter-observer and intra-observer variability. Monitoring of both the endometrial and ovarian response to ovarian stimulation on ART cycles with transvaginal ultrasound has become an important predictor of the success of ART. Many published studies have conflicting results on this subject but the common feature in all, is the lack of continuity on endometrial assessment. (7) The use of high-resolution transvaginal probes makes it possible to follow endometrium changes throughout the cycle. (8) From a clinical point of view some objective parameters must be obtained in order to ascertain the likelihood of an ongoing pregnancy in ART cycles, preferably in a non-invasive and cost-efficient way. (9) Some published work has recently proven a pattern of hemodynamic changes in utero-ovarian arteries during ART cycles with predictive value on endometrial receptivity. (10) Hou et al. have also confirmed the possibility of non-invasive prediction of success in ART cycles, with serial assessments of the echogenicity pattern transformation, after human recombinant gonadrotropin hormone. (11) The aim of this prospective study is to further evaluate the capability of serial and continuous evaluation of biophysical markers as a non-invasive procedure to determine endometrial receptivity. (12–13)

Material And Methods

Prospective case control study of 169 women in ART cycles. Infertile couples undergoing ART treatment at our institution were included. Canceled treatments prior to oocyte pickup; cycles with donated gametes; cryopreserved oocyte treatments; cycles for genetic disease screening and embryo selection; cycles with missing or erroneous data; and cycles with elective single embryo transfer were excluded.

The primary data source for this study was the local databases routinely used in the participating centre in ongoing treatments. The data output was anonymized in the extraction for statistical treatment purposes. All data collected and written informed consent was obtained according to the Ethics Committee of our Institution.

Only subjects with viable good grade embryos for transfer (double embryo transfer on day 3 of embryo development) were selected. All subjects have been in a short protocol regimen with antagonist for ovarian controlled stimulation using gonadotropins. All used recombinant human chorionic gonadotropin hormone (rhCG) for induction of ovulation 36 hours prior to oocyte pick up.

Demographics data was collected for all patients and serial ultrasound analysis (endometrial morphology, endometrial thickness, endometrial volume and uterine volume) was performed using the same protocol for all participating subjects.

During ovarian controlled stimulation serial ultrasound exams were performed and serum oestradiol levels obtained for all participants.

Biophysical markers were obtained in all evaluations (Basal moment – day 2 or 3 of women menstrual cycle and prior to begin of ovarian controlled stimulation; at day 6, day 8 and day 10 after initiating ovarian controlled stimulation; at Trigger day with recombinant human gonadochorionic hormone; and at embryo transfer day).

Endometrial morphology was based on the two grade system by Sher et al. (14): non-multilayered homogeneous hyperechogenic or iso-echogenic endometrium compared with the myometrium and multilayered triple-line pattern, 'halo pattern' with an outer peripheral layer of denser echogenicity and a central sonolucent area.

Endometrial thinckness was obtained in milimeters (mm) on the long axis or sagittal plane, with the entirely of the endometrial lining through and endocervical canal in view. The measurement was taken of the thickest echogenic area from one basal endometrial interface across the endometrial canal to the other basal surface.

Endometrial volume calculation by 3D ultrasound presented as voxels and geometric information of surfaces in a 3D dataset. The results obtained are then converted to mililitres. Adjusted Endometrial volume was also obtained as a ratio between endometrial volume calculated on 3D analysis and uterine volume based on 3D volumetric acquisitions which then generated an estimated uterine volume (also in mililiters). Adjusted endometrial volume deflects the potential difference in uterine volume from each single individual.

At day 12 after successful embryo transfer, human gonadochorionic sub-unit B serum levels were obtained, and groups were set: positive results (for values over 5 International Units - IU) and negative results (for values under 5 IU).

All data collected was analysed between these two set groups and compared.

Data was analysed in Excel 2019 (Microsoft Corp, Redmond, WA) and IBM SPSS statistics v25 (IBM Corp. Armonk, NY). Continuous variables were analysed with Levene's test (equality of variances) and visual assessment of the histogram (normality).

For analysis of parametric continuous variables, a t-student test for independent samples was used. Chi-square and Fisher's exact tests were used to analyse associations between categorical variables. Endometrial thickness, endometrial volume and adjusted endometrial volume were analysed using analysis of variance for repeated measurement data.

Value of $p < .05$ was considered statistically significant.

The authors do not report any conflict of interest.

The study protocol has been approved by the Ethics Committee of our Institution (CHCB 22/2017), in accordance with the relevant guidelines and regulations. This study has been conducted in accordance with legal and regulatory requirements, as well as follow generally accepted research practices described in International Conference Harmonisation (ICH) guidelines, Good Clinical Practices (GCP) and the Declaration of Helsinki.

Results

Clear morphology and volume of the endometrium was obtained in all 169 cycles using 3D transvaginal ultrasound in continuous observations. Demographics characteristics and ART parameters are shown in Table 1.

Women were divided into two groups depending on the value of hCG at Day 12 after embryo transfer: 123 on the negative group (72.8%) and 46 on the positive group (27.2%).

There were no statistical difference between the two set groups in terms of demographics and ART parameters: mean age of female partner or mean age of male partner, duration and type of infertility, total drug dose used for ovarian stimulation, overall median number of harvested oocytes per cycle defined as the total number of oocytes harvested during oocyte pick up procedure, rate of collected metaphase II (MII) oocytes. Also the mean number of cleaved embryos at day 3 of embryo development, and mean number of blastocysts for cryopreservation showed no significant statistical difference between the two set groups.

Endometrial morphology showed no statistical significant difference between the two set groups. Endometrial thickness on a single 2D sagittal profile showed no statistical difference at baseline and at Day 6 after ovarian controlled stimulation. Statistical significant difference was only met at a later phase of ovarian controlled stimulation – at day 8 and on following evaluations. Uterine Volume was comparable between the two Groups with no statistical difference between the two. (Table 2)

Endometrial Volume and Adjusted Endometrial Volume showed statistical difference from Day 6 after Ovarian controlled stimulation (Table 3 and Graph 1 and 2). Consistently higher values were seen for both of these biophysical markers on the positive group. In terms of endometrial volume the positive group versus the negative group has statistically significant higher values in all observations except the basal moment prior to ovarian controlled stimulation (2.77±0.63 vs 2.52±0.71 for p value of 0.54 at basal observation, 3.33±0.57 vs 3.08±0.66 for p value 0.024 at day 6 after ovarian controlled stimulation, 4.40±0.71 vs 3.90±0.94 for p value of 0.002 at day 8 after ovarian controlled stimulation, 4.91±0.82 vs 4.12±1.01 for p value of 0.001 at day 10 after ovarian controlled stimulation, 5.33±0.76 vs 4.52±1.00 for p value of 0.001 at trigger day with recombinant human chorionic gonadotropin, and 5.59±0.77 vs 4.84±1.01 for p value of 0.001 at embryo transfer day; respectively). Similar findings were noted on adjusted endometrial volume (Table 3).

By comparing the variations between two consecutive measurements in terms of endometrial and adjusted endometrial volumes, and the overall variation between the final value and the initial basal measurement we were able to note with statistical difference that values were higher on the positive group on initial phases of endometrial development (variation 2 for endometrial volume and variation 2 and 3 for adjusted endometrial volumes) (Table 4). Also overall variation was statistical higher on the positive group when compared with the negative one. (Graph 4 and 5)

In this study the intra-observer reliability was 0.96. In addition, because all measurements were performed by the same operator in this study there was no inter-observer variability.

Discussion

Although several parameters have been used to assess the pregnancy rate in ART cycles, there is still some controversy about its efficacy, and underlying mechanisms in endometrial receptivity (15-18). Vaginal 3D ultrasound is a non-invasive and an inexpensive tool at clinicians disposal. (19) The process of endometrial transformation from proliferative phase to secretory phase under the steroids hormonal influence, called endometrial decidualization is a set goal for optimal implantation. The cyclic changes of endometrium are regulated by ovarian hormones and its receptors, and endometrial luteal phase development may alter in ART cycles due to supraphysiological hormone levels.

Single analysis of endometrial pattern at trigger day has been the most used, with contradictory findings. Recent studies (*Silva Martins, R. et al.*) have proven that perhaps serial evaluations provide better understanding rather than a single scoop at a pre-determined phase of the process. It has been proven that in terms of angiogenesis that there is a certain pattern of evolution that one should expect from a transforming living tissue and its natural adaptations in need to further assist on the complex binding process of implantation. The main purpose of this study was to further evaluate other potential biophysical markers that might be evaluated in the continuous changes that endometrium has to go through during an ART cycle. With a possible non-invasive tool to further acknowledge those changes in better predicting a receptive endometrium may be developed, providing clinicians with a significant and powerful tool to decide either to proceed to transfer a healthy embryo on that given cycle or not.

In this study we aimed to assess endometrial evolution in order to ascertain a plausible predictive non-invasive diagnostic tool for clinicians to better understand endometrium changes.

Endometrial morphology proven not to be useful and no significant difference was found between the two groups. Also endometrial thickness showed no difference at early stages of ovarian controlled stimulation but significant difference could be seen after day 8 of stimulation. These findings are compatible to provided literature as they are by definition also subjective tools and as such, conflicting results were not able to provide an accurate diagnostic tool for endometrial receptivity assessment.

Endometrial volume and adjusted endometrial volume proven to be more effective with differences shown since the beginning of ovarian controlled stimulation. Both groups were similar at baseline but as

soon as controlled ovarian stimulation started, the differences between those with a positive outcome and those without were clearly met. We have also been able to show differences between the two groups in terms of endometrial and adjusted endometrial volume in early stages of endometrial development under the influence of controlled ovarian stimulation. Higher volumes were seen in the positive controls, but the changes were more evident in early stages (between day 6 and day 8 of ovarian controlled stimulation for endometrial volume, and between day 8 and day 6 and also between day 10 and day 8 of ovarian controlled stimulation in adjusted endometrial volumes). All of these findings may prove to be a useful management tool for clinicians in order to establish yet another diagnostic tool for better decision making in selective embryo transfer. The possibility of real time non-invasive continuous assessment of the endometrium further induces clinicians to better decision making choices.

Nevertheless, one must always be cautious that artefacts during 3D analysis may occur due to 2D imaging process, patient motion during rendering of images and artefacts due to operator choice in the selection of which part of the volume to display. (20)

We could not refrain to uphold expectation of these results as they show a serial of values, demonstrating a certain pattern of evolution on a transforming living tissue and its natural adaptations to a complex and yet unknown process.

Conclusions

Endometrial receptivity plays an important role in the successful outcome in ART cycles. Many have been gained in the area of embryo transfer, and embryo cultures. Yet the underlying mechanism that results in failure of implantation of a good quality embryo on a supposed receptive endometrium is still unclear.

Many techniques have been developed but results are still controversial, or in some cases proven to be too invasive and lacking reliability especially in women with irregular menstrual cycles.

The continuous evolution of endometrium and its adaptive changes makes it difficult to establish a pattern that might be useful in identifying a receptive endometrium.

Ultrasound developments have been able to clarify and make aware more information about the morphokynetics of this tissue and its changes throughout the cycle. Better understanding of the role that makes an endometrium receptive may be the key in solving these issues, providing a diagnostic tool that will enhance ART cycles and elective embryo transfers more effective in producing better outcomes. Also the possibility to determine in real time endometrial receptivity will shorten the time to birth lapse, thus improving quality of life for infertile couples.

This study showed that endometrial 3D volume analysis as well as adjusted 3D endometrium volume may identify a receptive endometrium as soon as day 6 of ovarian controlled stimulation. In this way clinicians may be made aware of this possibility and further enhance its procedures with better knowledge weather or not to perform embryo transfer on that given cycle.

Abbreviations

ART	Assisted Reproductive Technology
CHCB	Centro Hospitalar Cova da Beira
ER	Endometrial Receptivity
ERA	Endometrial Receptivity Array
GCP	Good Clinical Practices
hCG	human chorionic gonadotropin
ICH	International Conference Harmonisation
IU	International Units
ML	Multi-layered
NM	Non Multi-layered
SD	Standard Deviation
WOI	Window of Implantation
2D	Two Dimensions
3D	Three Dimensions

Declarations

Ethics approval and consent to participate.

The study has been approved by the Ethics Committee of our Institution (CHCB 22/2017). Oral and written consent was obtained for all willing participants prior to registering for this study. Patient Informed consent to participate in this study CHCB 22/2017.

The authors have consented for publication.

Availability of Data and Materials

Encrypted non-disclosure data available at Open Science Framework database for peer review purpose only. Project name Physical Biomarkers in Endometrial Receptivity with access link: https://osf.io/hr25m/?view_only=8d5f6dcb8b25420bbd9188382163e7d7

Competing interests

The authors do not report any conflict of interest.

Funding

No Grant support on this study.

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Author's Contribution

RSM, AHO and JMO are responsible for the study design. RSM has been the principal investigator and the principal collector of data. RSM has been responsible for data analysis. DVO, AHO and JMO have been responsible for reviewing the article for publication.

Acknowledgements

We would like to appraisal M.S. for the help with the statistical analysis of data.

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Tables

Table 1. Demographics and ART parameters between two Groups. (Positive Group, N = 46 and Negative Group, N = 123). Descriptive statistics between two Groups. Mean values with standard deviation (SD).

	Negative Group N= 123 (72.8%)	Positive Group N= 46 (27.2%)	t-Test p value
Female Age (in years)	34.94±4.03 (19-39)	34.28±3.35 (25-39)	0.290
Male Age (in years)	36.14±4.76 (22-46)	37.19±5.91 (29-62)	0.832
Time of Infertility (in months)	54.46±33.82 (12-204)	60.22±38.49 (14-192)	0.375
Type of Infertility: • Primary • Secondary	95/123 (77.2%) 28/123 (22.8%)	38/46 (82.6%) 8/46 (17.4%)	0.297
Antimullerian hormone (pg/mL)	2.45±2.45 (0.09-16.65)	2.62±2.46 (0.04-13.56)	0.679
Antral follicle count	8.43±5.07 (2-40)	8.63±3.74 (2-20)	0.801
Total dose of gonadotropins (in International Units)	2500.81±812.19 (300-4500)	2508.15±757.91 (450-4500)	0.956
Progesterone levels at Trigger day (ng/mL)	0.88±0.44 (0.01-2.20)	0.78±0.47 (0.01-2.10)	0.188
Number of collected Oocytes	8.25±5.14 (2-22)	10.50±5.20 (2-23)	0.140
Metaphase II Oocytes	6.57±4.22 (2-17)	7.06±4.77 (2-21)	0.150
Number of day 3 embryos	3.18±2.40 (2-12)	3.84±2.65 (2-12)	0.120
Number of blastocyst for vitrification	0.65±1.51 (0-6)	0.86±1.71 (0-9)	0.200

Table 2. Ultrasound parameters between two Groups - Endometrial morphology and Endometrial thickness at baseline, at day 6, 8 and 10 after controlled ovarian stimulation, at trigger day and at embryo transfer day. Ratios in percentages (%) and mean values with standard deviation (SD). NM – Non multi-layered endometrium; ML – Multi-layered endometrium; rhCG – recombinant human chorionic gonadotropin; NS – No statistical analysis performed.

		Negative Group N=123 (72.8%)	Positive Group N= 46 (27.2%)	p Value
Basal	Endometrial Morphology (ML/NM)	0% / 100%	0% / 100%	NS
	Endometrial Thickness (in mm)	4.32±0,72	4.22±0,51	0.387
Day 6 after Controlled Ovarian Stimulation	Endometrial Morphology (ML/NM)	78.9% / 21.1%	93.5% / 6.5%	0.15
	Endometrial Thickness (in mm)	6.32±0.96	6.28±0.75	0.827
Day 8 after Controlled Ovarian Stimulation	Endometrial Morphology (ML/NM)	100% / 0%	100% / 0 %	NS
	Endometrial Thickness (in mm)	7.47±0.80	7.96±0.79	**0.01**
Day 10 after Controlled Ovarian Stimulation	Endometrial Morphology (ML/NM)	100% / 0%	100% / 0 %	NS
	Endometrial Thickness (in mm)	8.01±1.04	8.61±0.98	**0.01**
Trigger Day with rhCG	Endometrial Morphology (ML/NM)	100% / 0%	100% / 0 %	NS
	Endometrial Thickness (in mm)	8.53±1.32	9.59±1.44	**0.001**
Embryo Transfer Day	Endometrial Morphology (ML/NM)	4.1% / 95.9%	4.3% / 95.7%	0.613
	Endometrial Thickness (in mm)	9.06±1.30	10.15±1.35	**0.001**

Table 3. Ultrasound parameters between two groups - Endometrial volume and adjusted endometrial volume at baseline, at day 6, 8 and 10 after controlled ovarian stimulation, at trigger day and at embryo transfer day. Ratios in percentages (%) and mean values with standard deviation (SD). rhCG – recombinant human chorionic gonadotropin.

		Negative Group N=123 (72.8%)	Positive Group N= 46 (27.2%)	t-Test p Value
Basal	Endometrial Volume (in mm3)	2.52±0.71	2.77±0.63	0.54
	Adjusted Endometrial Volume	4.60±1.42	5.51±1.28	0.21
Day 6 after Controlled Ovarian Stimulation	Endometrial Volume (in mm3)	3.08±0.66	3.33±0.57	**0.024**
	Adjusted Endometrial Volume	5.63±1.50	6.67±1.38	**0.001**
Day 8 after Controlled Ovarian Stimulation	Endometrial Volume (in mm3)	3.90±0.94	4.40±0.71	**0.002**
	Adjusted Endometrial Volume	7.28±2.67	8.98±2.47	**0.001**
Day 10 after Controlled Ovarian Stimulation	Endometrial Volume (in mm3)	4.12±1.01	4.91±0.82	**0.001**
	Adjusted Endometrial Volume	7.60±2.54	9.99±2.61	**0.001**
Trigger Day with rhCG	Endometrial Volume (in mm3)	4.52±1.00	5.33±0.76	**0.001**
	Adjusted Endometrial Volume	8.30±2.52	10.76±2.62	**0.001**
Embryo Transfer Day	Endometrial Volume (in mm3)	4.84±1.01	5.59±0.77	**0.001**
	Adjusted Endometrial Volume	8.32±2.58	10.83±2.73	**0.001**

Table 4. Endometrial and Adjusted Endometrial volume variance between two consecutive continuous evaluations, and Overall variation set as the difference between final and first evaluation.

		Negative Group N=123 (72.8%)	Positive Group N= 46 (27.2%)	t-Test p Value
Variation 1 Day 6 after Controlled Ovarian stimulation versus Basal	Endometrial Volume (in mm3)	0.5533	0.5632	1.34
	Adjusted Endometrial Volume	0.0102	0.0115	1.43
Variation 2 Day 8 versus Day 6 after Controlled Ovarian Stimulation	Endometrial Volume (in mm3)	0.8275	1.0657	**0.01**
	Adjusted Endometrial Volume	0.165	0.0231	**0.01**
Variation 3 Day 10 versus Day 8 after Controlled Ovarian Stimulation	Endometrial Volume (in mm3)	0.4357	0.5686	0.47
	Adjusted Endometrial Volume	0.0077	0.0112	**0.01**
Variation 4 Trigger Day with rhCG versus Day 10 after Controlled Ovarian Stimulation	Endometrial Volume (in mm3)	0.3626	0.4108	0.987
	Adjusted Endometrial Volume	0.0063	0.0083	0.873
Variation 5 Embryo Transfer Day versus Trigger Day with rhCG	Endometrial Volume (in mm3)	0.2861	0.3260	0.567
	Adjusted Endometrial Volume	0.005	0.0067	0.678
Overall Variation Basal versus Embryo Transfer Day	Endometrial Volume (in mm3)	2.4654	2.9367	**0.01**
	Adjusted Endometrial Volume	0.0459	0.061	**0.01**

Figures

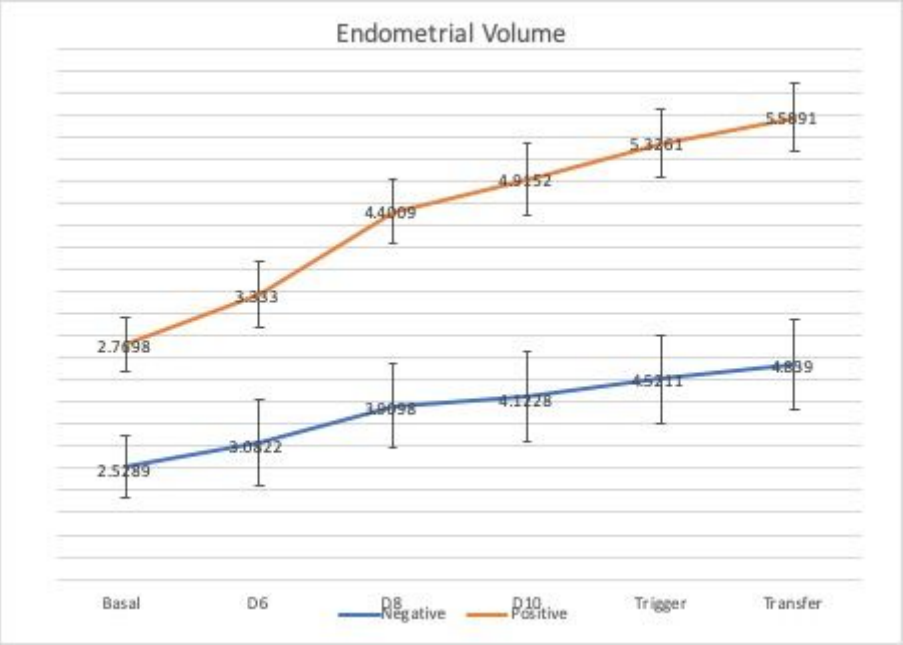

Figure 1

Graph 1. Continuous endometrial volume analysis (Mean values with Standard Deviation)

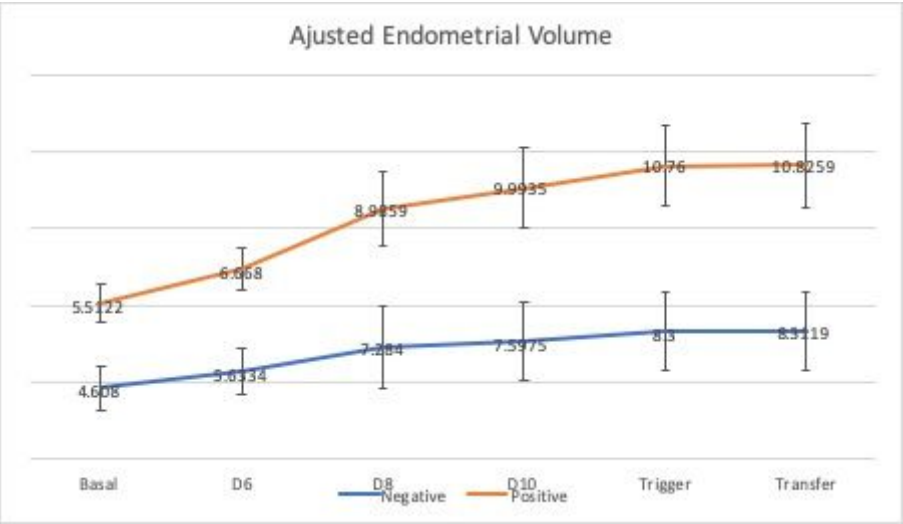

Figure 3

Graph 2. Continuous adjusted endometrial volume analysis (Mean values with Standard Deviation)

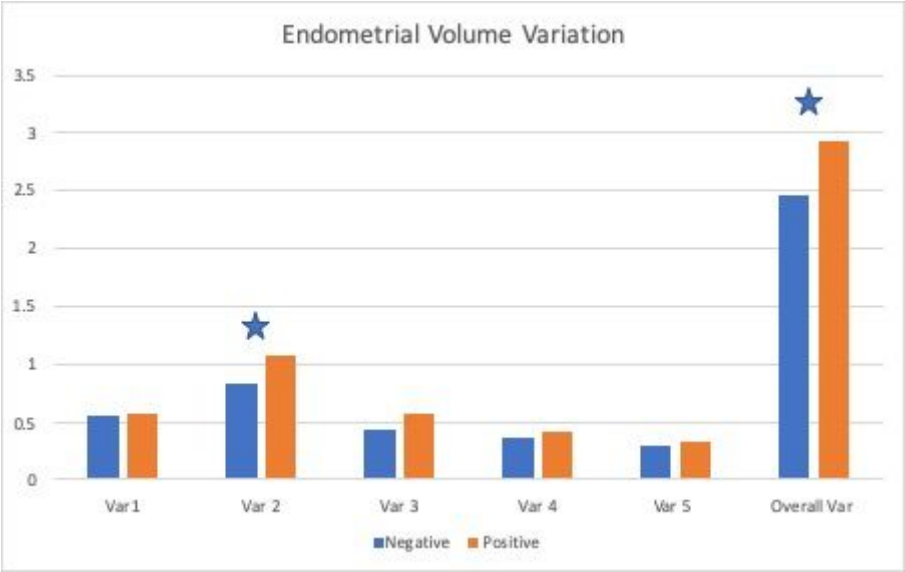

Figure 5

Graph 3 – Endometrial Volume Variation (difference between two consecutive measurements and Overall variation as a difference between last and first evaluation) (signalled with a star the moments were statistical difference between the two set groups was met $p<0.05$)

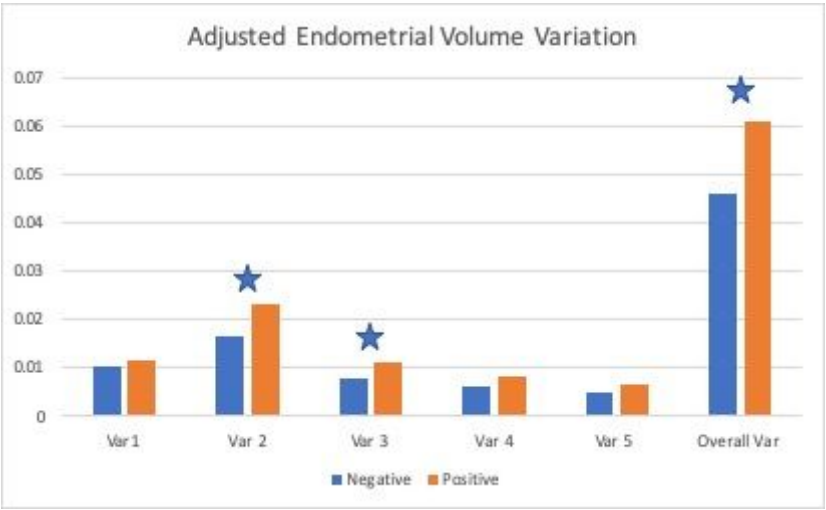

Figure 7

Graph 4 – Adjusted Endometrial Volume Variation (difference between two consecutive measurements and Overall variation as a difference between last and first evaluation) (signalled with a star the moments were statistical difference between the two set groups was met $p<0.05$)