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uterus in three different anatomical planes and thus has a high sensitivity and specificity in the diagnosis of congenital uterine anomalies (CUA) [3,4]. The relevance of detecting CUA lies on the fact that certain types seem to be associated with a decrease in pregnancy achievement and increase in miscarriage and obstetric complications rate [5-7]. Moreover, 3D-US is also useful in the measurement of uterine size, either endometrial volume through Virtual Organ Computer-aided AnaLysis (VOCAL, GE Medical Systems, Austria) system, or manual measurements in the coronal plane such us interostial distance, with high inter-observer agreement [8]. The impact of the uterine size and endometrial thickness and volume on the chances of pregnancy is still under research [9]. It has been shown that the highest live birth rate after IVF cycles is observed in patients with uterine length between 7 and 9 cm measured with a soft catheter [10, 11] and that implantation and ongoing pregnancies are significantly higher in patients with an endometrial thickness >7-8 mm measured by 2D-US [12]. Although there is high correlation between endometrial thickness measured on 2D-US and endometrial volume [13], the relationship between endometrial volume and pregnancy rates after ART is not so clear yet [14]. Some contradictory results could be explained because measurements have been taken at different moments of the menstrual cycle [15], and it is well known that both endometrium thickness and volume increase during the menstrual cycle and are greater in the mid-luteal phase [16], during the window of implantation. Those studies analyzing endometrial volume on the day of ET suggest a critical threshold below which pregnancy rates decrease [17-20], with no pregnancies described when endometrial volume is 1 ml or lower [21]. Given this background, we aimed to examine if uterine cavity measurements and endometrial volume obtained with 3D-US on the day of ET can predict chances in live birth in oocyte donation treatments.

Materials and Methods

Design and Setting

This is a post hoc analysis of a prospective cohort study conducted at IVI-RMA, Valencia, Spain.

This study was approved by the Institutional Review Board of IVI-RMA and registered on ClinicalTrials.gov (NCT02696694).

We aimed to investigate if serum progesterone levels and/or endometrial volume, both measured on the day of embryo transfer in oocyte recipients, had an impact on pregnancy rates. Regarding the first variable, our results showed that low serum P levels on the day of embryo transfer impaired significantly ongoing pregnancy and live birth rates [22]. In the present manuscript, we report the detailed results regarding the relationship between endometrial volume and other uterine cavity measurements on the day of embryo transfer and live birth.

Patient Population

This study included 244 infertile patients undergoing their first/second oocyte donation cycle. All the patients underwent a blastocyst transfer after an artificial endometrial preparation cycle. Inclusion criteria were age <50 years, BMI <30 kg/m^2, normal uterus according to 2D-US, no systemic diseases, a triple layer endometrium >6.5 mm after HRT, and good quality blastocysts transferred on day 5 of embryo development according to the Spanish ASEBIR classification [23]. Exclusion criteria were recurrent miscarriages, implantation failure, severe male factor, uterine diseases (e.g., fibroids, polyps, severe adenomyosis, previously diagnosed congenital uterine anomalies) or presence of hydrosalpinx.

Endpoints

We analysed the relationship between different uterine cavity measurements on the coronal plane of the 3D-US (interostial distance (IOD) and transverse diameter (TD) at 15 mm from the internal fundus) and endometrial volume (ml, measured by VOCAL) on the day of ET with live birth rate in artificial endometrial preparation cycles. We also analysed implantation rate; clinical pregnancy and miscarriage rate (any clinical pregnancy loss before pregnancy week 12).

Study Protocol

Endometrial Preparation

All the patients received HRT for endometrial preparation, as described elsewhere [22]. After 10-14 days on estrogens, endometrial thickness and pattern were assessed. Patients were considered ready for ET when endometrial thickness was greater than 6.5 mm, ultrasound detected quiescent ovaries, serum E2 was >100 pg/ml and the serum P level was <1 ng/ml. The luteal phase was supplemented with a vaginal administration of natural micronized P administered from 5 days before ET (400 mg/12h of Utrogestan®, SEID, Barcelona, Spain) until pregnancy week 12.

Selecting patients

Eligible patients were informed about the nature of the study and decided if they wanted to participate. After signing the informed consent (IC) form, a blood test and a 3D-US of the uterus were performed one or two hours before ET.

Three-Dimension Ultrasound

The 3D datasets were acquired using an ultrasound system (Voluson® S6 device, General Electric Healthcare Ultrasound, Milwaukee, WI, USA) by an experienced examiner (E.L) in the luteal phase (day of embryo transfer). Patients were scanned with an empty bladder in the dorsal lithotomy position. Women were asked to hold their breath and refrain from moving during 3D volume acquisition. A maximum sweep angle of 120° after obtaining a sagittal view of the uterus was adopted and the approximate angle between the ultrasound beam and the uterine axis was 90°. The Z

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technique was systematically used to obtain a good quality mid-coronal uterus plane display [24]. A single 3D volume of the uterus of each patient was obtained, anonymized, numbered, and sent to prepare the images of the coronal view. Endometrial thickness, interostial distance and transverse diameter were measured and description of the uterus was performed according to the ESHRE-ESGE classification [4]. According to this classification, arcuate uterus was considered when the interostial line was curved but with an internal indentation at the fundal midline not exceeding 50% of the uterine wall thickness, thus considered as Class U0 or normal uterus. When the internal indentation depth was ≥ 10 mm with an indentation angle < 140°, the uterus was considered as septate, following CUME (Congenital uterine malformation by experts) criteria [25]. Interostial distance, measured as the distance between the two ostia, was at the transition point between the endometrial cavity and the isthmic part of the Fallopian tubes [15]. The transverse diameter was the transversal distance in the coronal view at 15 mm from the internal fundus of the uterine cavity (see Figure 1A). We choose the 15-mm point to confer homogeneity to all types of uterus, to be able to make comparisons among them, and because it is the area where the embryo usually implants in the uterus [26, 27]. The stored uterine volumes were examined, and the 3D automated virtual organ computer-aided analysis (VOCAL) imaging program took the endometrial volume (ml) measurements with 15º degrees of rotation in the manual measurement mode.

Statistical Analysis

All the categorical variables were compared with a Chi-square test among groups. The student's *t*-test was used to compare continuous variables. To analyze the impact of endometrial volume the day of ET on cycle outcome, a multinomial logistic regression analysis including all potential confounding variables was performed, e.g., age, BMI, endometrial thickness, endometrial volume, serum P the day of ET and number of transferred embryos. To define the predictive capability of the different uterine measurements

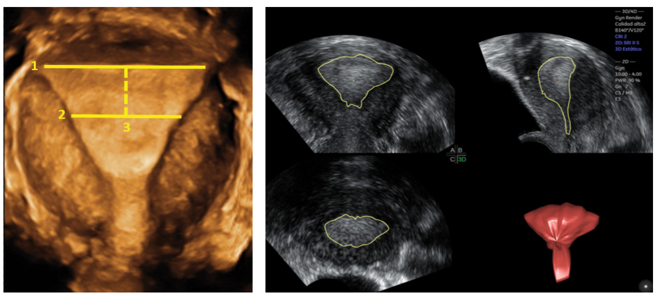

Figure 1a: 3D-US Measurements. A) Measurements taken in the coronal plane of the uterus (1: Interostial distance; 2: Transverse diameter is the parallel line to the interostial distance at 15 mm from the internal fundus or indentation´s edge at the cavity; 3: Distance that determines where to measure transversal diameter).

B) Endometrial volume (ml) measured with VOCAL

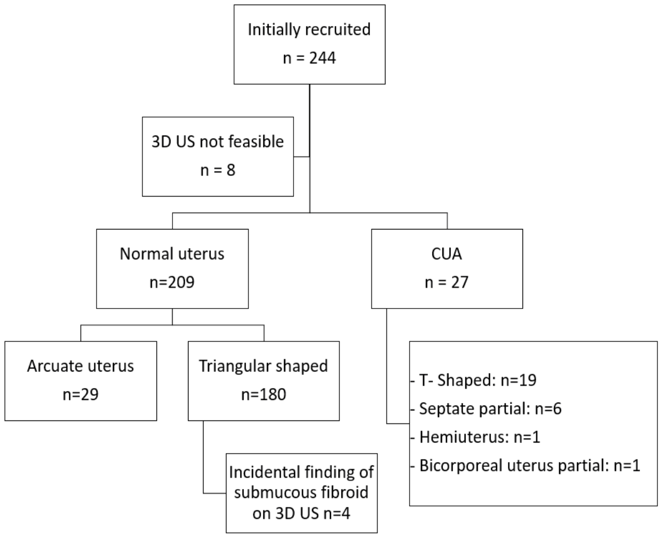

Figure 1B: Distribution of the patient population. 3D US could be performed in 236 women, with an incidental finding in 16.5% of the cases (CUA or submucous fibroid distorting the internal cavity).

on the OPR, the receiving operating characteristic (ROC) curve was defined and the area under the curve (AUC) was calculated.

Results

A total of 244 women were initially recruited. Patient´s mean age was 41.3±4.4 years, with a BMI of 22.3±2.6 kg/m^2 and an endometrial thickness of 8.9±1.7 mm before starting P.

Serum P and E2 on the day of ET were 12.7±5.4 ng/ml and 245±183 pg/ml, respectively.

General findings of 3D Ultrasound the day of embryo transfer

In 8 patients, 3D-US could not be performed due to bad image quality. Findings of 3D-US of the uterus were: triangular shaped (n=173, 73.3%), arcuate (n=29, 12.2%), infantilis (n=7, 2.9%), T-Shaped (n=19, 8.1%), Septate partial (n=6, 2.5%), hemi uterus without rudimentary cavity (n=1, 0.4%) and bicorporeal uterus partial (n=1, 0.4%).

Finally, 5 cases out of the 173 normal uteri had a submucous fibroid that slightly distorted the cavity (not seen in 2D but in 3D-US). Figures 1A and 1B show the images obtained and the distribution of the findings after 3D-US.

Overall, there were 16.5% of the cases (39/236) in which a uterine anomaly, either congenital or acquired, was seen by the 3D-US but not previously diagnosed by 2D-US.

Thirty-one patients had had a previous live birth, all of them showed a normal uterus (3 arcuate uteri included).

Features of uterine size and volume in normal uterus and with CUA

Endometrial volume was significantly higher in normal

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uterus than in CUA (3.5±1.9 ml *vs*. 2.5±1.7 ml, p=0.007), as well as endometrial thickness the day of embryo transfer (8.9±2.2 cm *vs*. 7.9±1.5 cm, p=0.015).

Regarding the measurements taken in the coronal plane of the uterus, transverse diameter was significantly higher in normal uterus than in CUA (1.5±0.5 cm *vs*. 1.0±0.4 cm, p=0.000), whereas interostial distance was similar between both groups (2.9±0.6 cm vs. 2.8±0.8 cm, p=0.6) (see Figure 2A). Table 1 shows the comparison of different uterine measurements between different types of CUA and normal uterus. Infantilis uterus had a significantly lower endometrial volume, interostial distance, transverse diameter and endometrial thickness, when compared with normal uterus. T shaped uterus had a significantly lower endometrial volume, transverse diameter and endometrial thickness, when compared with normal uterus, whereas interostial distance was similar. Septate partial uterus had a non-significantly higher endometrial volume and a significantly higher interostial distance, whereas transverse diameter and endometrial thickness, were similar to normal uterus. Normal uteri showed a triangular shape, with a ratio between interostial distance and transverse diameter of 2:1 whereas in CUA it was 3:1 (1.95±0.5 vs. 3.0±1.0, p=0.000). This finding was even more remarkable in T-Shaped uteri in which the ratio was higher due to a decrease in transversal diameter (1.95±0.5 vs. 3.5±0.9, p=0.000) (see figure 2B). Pearson´s correlation showed that all the parameters related with uterine measurements (endometrial volume and thickness, IOD and TD) related positively one to each other (p<0.001). Patient´s weight was positively related with endometrial volume (r=0.33, p=0.000) and thickness (r=0.22, p=0.001) whereas height was positively correlated with endometrial thickness (r=0.16, p=0.016). Neither the weight nor the height was related with the uterine measurements on the coronal plane (IOD, TD). Finally, patients with a previous live birth, showed a significantly higher endometrial volume (4.3±2.7 vs. 3.2±1.8, p=0.02).

Pregnancy outcome in normal uterus and with CUA

Positive pregnancy test was 73.3% (148/202) in normal uterus *vs*. 64.7% (22/34) in CUA (p=0.35).

Live birth rate (LBR) was 49.5% (100/202) in normal uterus whereas it was 35.3% (12/34) in CUA (p=0.14), with a miscarriage rate of 18.2% (27/148) and 31.8% (7/22), respectively (p=0.12). Detailed results according to the pregnancy outcome are shown in Table 2.

Patients with normal uteri and submucous fibroids seen on 3D US (n=5), had 2 live births, 1 biochemical pregnancy, 1 clinical miscarriage and 1 negative. LBR was significantly lower in infantilis uterus (14.3% vs. 49.5%, p=0.049), and clinically lower in T shaped uterus albeit not statistically significant (36.8% vs. 49.5%, p=0.3) when compared with normal uterus. On the contrary, live birth rate was comparable in septate partial uterus when compared with normal uterus (50% vs. 49.5%, p=0.98). In normal uterus, no significant association was observed between endometrial volume and clinical outcome. Nevertheless, the women with a very low endometrial volume (<p5=1.43 ml) (n=10) were associated with a clinically lower LBR (20.0% vs. 48.2%, p=0.06) and higher pregnancy loss rate -including biochemical and clinical miscarriages- (66.6% vs. 32.7%, p=0.08), albeit not significant.

Predictive value of uterine features on 3D-US on OPR

The ROC curve showed no predictive value of endometrial volume on the day of ET for LBR, with an AUC (95%CI) =0.51 (0.43-0.59); (p=0.78). Same analysis could not find a predictive value of interostial distance (AUC (95%CI) =0.48 (0.38-0.58); (p=0.72)), transverse diameter (AUC (95%CI) =0.54 (0.47-0.62); (p=0.26)) or endometrial thickness (AUC (95%CI) =0.52 (0.39-0.64); (p=0.80).

The multinomial logistic regression analysis after adjusting for age, BMI, endometrial thickness, serum P4 and E2, and number of embryos transferred, showed a significant relationship between the likelihood of live birth and endometrial volume on the day of ET in patients with CUA (OR:2.76: 95% CI: 1.14-6.69), p=0.025. On the contrary, the same analysis didn´t show a significant relationship between the likelihood of live birth and endometrial volume on the day of ET in normal uterus (OR:1.05: 95% CI: 0.85-1.29), p=0.65.

Table 1: Comparisons of the different uterine measurements according to the diagnosis of the uterus.

	Normal (n=202)	Infantilis (n=7)	T Shaped (n=19)	Septate partial (n=6)
Endometrial volume (ml)	3.5±1.9 ml	1.5±0.3 ml [a]	2.2±0.9[b]	4.9±2.9
Interostial distance (cm)	2.9±0.6	1.7±0.2[a]	2.9±0.2	3.4±1.0[c]
Transverse diameter (cm)	1.5±0.5	0.8±0.1[a]	0.9±0.2 [b]	1.5±0.7
Endometrial thickness (mm)	8.9±2.2	7.6±1.2[a]	7.5±1.2 [b]	9.9±1.3

a p<0.05 for comparison between uterus infantilis vs. normal and arcuate
b p<0.05 for comparison between T shaped uterus vs. normal and arcuate
c p<0.05 for comparison between septate partial uterus vs. normal and arcuate

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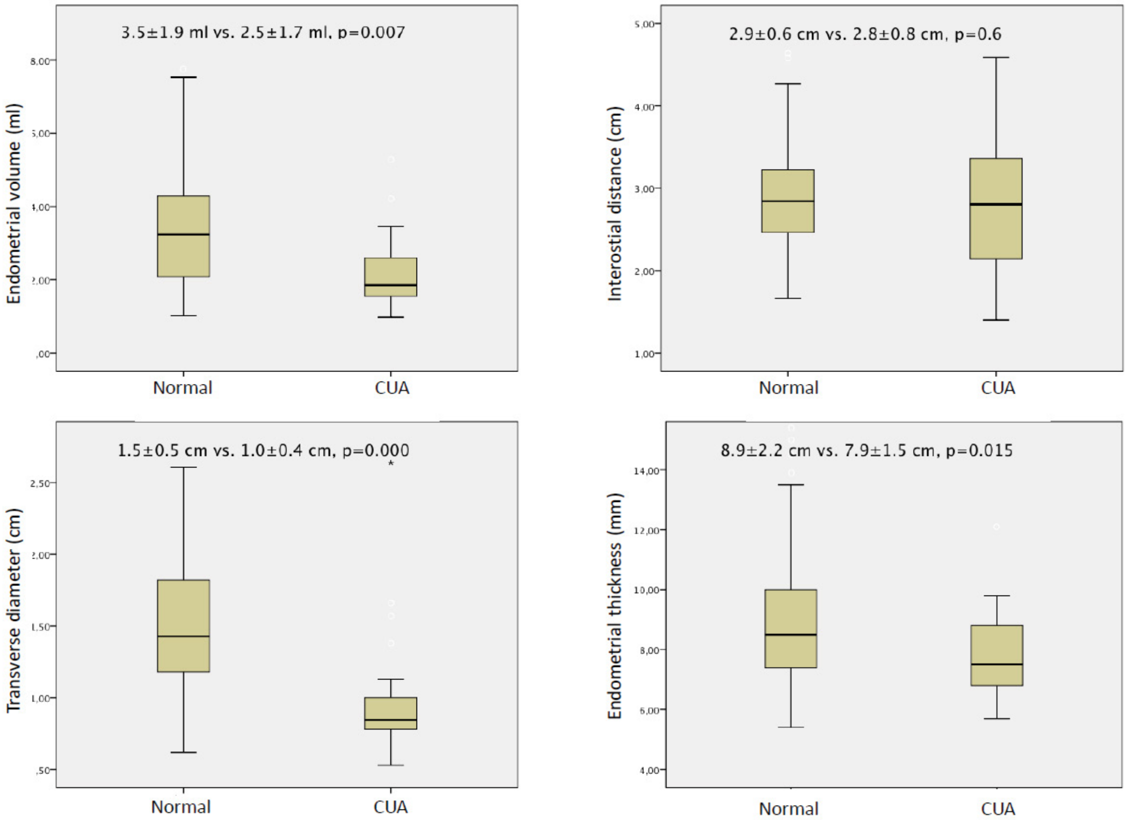

Figure 2: Box and whisker plot of the uterine measurements in normal and CUA. Upper text: Mean levels (± standard deviation) of each measurement in both groups, and p value of the comparison.

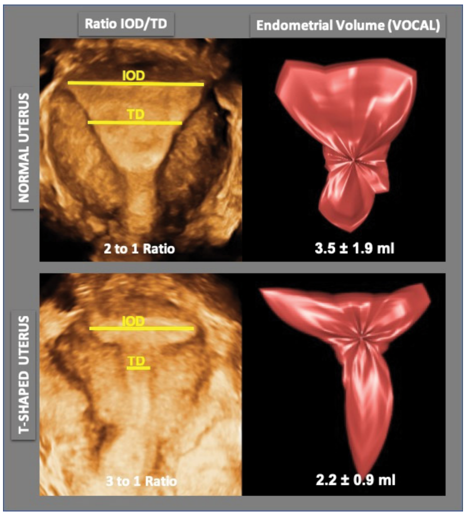

Figure 3: Main differences between a normal and a T-shaped uterus. Normal uteri showed a triangular shape, with a ratio between interostial distance and transverse diameter of 2:1 whereas in T-shaped uteri it was 3:1 due to a decrease in transversal diameter.

Discussion

Uterine evaluation by 3D-US has shown some interesting findings in this study.

First, we would like to point out the high utility of the 3D-US, as it detects acquired or CUA not previously shown in the routine 2D-US examination. In fact, an unsuspected finding was present in 16.5% of the 3D-US performed the day of ET. This is of relevant interest as it is well known that ongoing pregnancy rates seem to be lower when these anomalies are present [6].

Secondly, patients with CUA showed a decreased, albeit not significant, probability of live birth and an increase in the miscarriage rate, but results must be interpreted with caution due to the small number of patients with uterine abnormalities in this sample.

We also found that some measurements differ among different type of uterus. Interestingly, endometrial volume, endometrial thickness and transversal diameter in the coronal plane were significantly lower in patients with CUA in comparison with those with normal uterus. In any case, the distribution of these measurements was not similar among different CUA as their shapes are completely different. While infantilis uteri show an harmonic decrease in all measurements, T-Shaped uteri have all measurements reduced, except the interostial distance, which confers it the T shape. On the contrary, septate partial uteri do not show a decrease in uterine measurements.

Our results show that, in patients with normal uteri in

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the 3D ultrasound, no association for endometrial volume with the LBR was present; but we found a threshold of endometrial volume below which the LBR were clinically and relevantly lower. In any case, there were very few patients with a very low volume as we included only those patients with a minimum endometrial thickness of 6.5 mm. Hence no conclusions were drawn from this finding but highlights the fact that once endometrial thickness reaches satisfactory levels, the measurement of endometrial volume by VOCAL is not relevant. Some studies have correlated endometrial volume measured by 3D-US with pregnancy outcomes after ART, but with conflicting results [28] probably because the volume was taken in different cycle times across distinct studies. In some of those studies, a correlation with pregnancy rates was found when measuring the endometrial volume on the day of ET. Most importantly, it has been shown that a minimum volume is required to achieve pregnancy [20, 21]. This agrees with our findings because those women with endometrial volume <1.43 ml had lower LBR. But is not only endometrial volume but also the shape of endometrial cavity. In fact, mean endometrial volume was 2.2 ml in T Shaped uteri (vs. 3.5ml in normal uteri), which showed a high miscarriage rate (41.7%). In fact, some prospective studies have shown that the increase of endometrial volume (measured by 3D-US) through metroplasty in T Shaped uteri improves pregnancy outcome in patients with background of recurrent miscarriage, implantation failure or unexplained infertility [29, 30].

One of the strengths of this study is that all the uterine measurements were taken on the same cycle day, which is the best time for this; e.g., during the window of implantation [15], as it is well-known that measurements across the menstrual cycle can vary [13]. It has been demonstrated that the estimation of both endometrial volume (through VOCAL) and interostial distance [8, 31] has satisfactory high levels of intra- and inter-observer reproducibility [32]. The measurements taken in the coronal view allowed us to know about the normal size and shape of the endometrial cavity. We found a mean of interostial distance of 2.8 cm, which agrees with the results previously published by Benacerraf who found that the width of the normal uterus in nulliparous women was 2.7cm [33]. We have introduced a new measurement in the coronal view of the uterus, that we have called it the "transversal distance", at ≈15 mm from the internal fundus of the uterine cavity and parallel to the interostial distance. This was done like this for two reasons. First, is that the 15mm reference point avoid any subjective evaluation as the decision making on when to draw the line depends on the volume of the uterus so we wanted to be homogeneous in all determinations. Second, because this is the area where the embryo usually implants [34, 35]. We observed that, in normal uterus, the ratio between interostial and transversal distance is usually 2 to 1, as the uterus is triangular shaped; whereas in other anomalies, e.g., in the T-Shaped uterus, the ratio increases to 3 to 1, as the interostial distance is higher, whereas the transversal distance is narrower. According to these observations, we suggest these two determinations as additional tools to better categorize the different CUA once the reference measurements have been established. The impact of endometrial thickness and endometrial volume continues to be a black box. Although a positive relationship was suggested between endometrial thickness and outcome after FET cycles [36], a recent retrospective analysis on 959 single euploid frozen embryo transfers couldn´t identify a threshold of the ET that either precluded live birth or under which the LBR decreases perceivably [9]. Moreover, given the fact that no clear correlation has been found between endometrial volume and pregnancy rates, and that there is high correlation between endometrial thickness (on 2D-US) and endometrial volume (R^2= 0.767, p<0.001) [13] it seems that 3D-US assessment of endometrial volume is not recommended in the routine practice. Furthermore, the results can be heterogeneous between different groups as there is not an established technique, although it has been published that the inter-observer agreement seems to be high [37].

The limitations of our study are that only the women with a minimum endometrial thickness of 6.5mm were included. Thus, extrapolation of the effect of endometrial volume to patients with a thinner endometrium needs to be validated. To conclude, we found a significant relationship between the likelihood of live birth and endometrial volume on the day of ET in patients with CUA, but not in normal uteri. Patients with CUA showed a decreased, albeit not significant, probability of live birth and an increase in the miscarriage rate. This study demonstrates that 3D-US is a very useful tool to diagnose in advance any congenital or acquired pathology that can be misdiagnosed by doing 2D-US alone. Likewise, we propose a double measurement of the endometrial cavity in the coronal view of the uterus (interostial and transversal distance), to better define the shape and size of the uterus. This standardization would allow to stablish relationships between 3D-US findings and pregnancy outcome, analyze the impact of interventions such us surgery to reshape the uterine cavity, inform and advice the patients about its prognosis, mainly in the scenario of infertility in which a single embryo transfer is strongly recommended in cases of CUA due to the risk of premature labor.

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Authors´ Roles

E.L., G.M. and P.C. contributed equally to recruit patients and capture endometrial volumes. E.L. performed the VOCAL analysis and took uterine measurements. E.L. and E.B. were involved in the study design, undertook the

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literature review. E.B. performed the analysis and interpreted the data. E.L. drafted the manuscript, reviewed by E.B. All the authors approved the final manuscript for submission.

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