

## Article

# Variability of endometrial glandular opening count in infertile patients prior to first IVF treatment



João Sabino Cunha-Filho obtained his MD (1993) and his specialism in Obstetrics and Gynecology (1997) from the Faculty of Medicine, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Brazil. His PhD degree was granted to him by the same University in 2000. He continued with post-doctoral studies at Clamart, France (2001). He is currently Professor in Obstetrics and Gynaecology at the Faculty of Medicine—Hospital de Clínicas de Porto Alegre in Brazil. His current research interests include assisted reproduction, endometriosis and ovarian stimulation.

Dr João Sabino Cunha-Filho

JS Cunha-Filho<sup>1,3</sup>, E Arbo<sup>1</sup>, V Rosa<sup>1</sup>, CR Sloczynski<sup>1</sup>, G Berton<sup>1</sup>, WP Gavião Neto<sup>2</sup>, VK Genro<sup>1</sup>

<sup>1</sup>Ob/Gyn Department, Faculdade de Medicina, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul; <sup>2</sup>Instituto de Informática, Universidade Federal do Rio Grande do Sul, Brazil

<sup>3</sup>Correspondence: e-mail: sabino@via-rs.net

## Abstract

The aim of the present study was to evaluate the number of endometrial glandular openings, using previously reported software that provides an objective count, and to assess the variability of this parameter during the luteal phase in a population of women who had no hormonal abnormalities presenting with tubal infertility or male factor infertility. A cross-sectional study was performed comprising 561 patients selected for a diagnostic hysteroscopy for the investigation of infertility. Hysteroscopy was performed during the mid-secretory phase prior to the first IVF treatment cycle. A total of 561 image frames from all patients were analysed. All images were automatically selected by the software, which also evaluated the number of endometrial glandular openings. The mean  $\pm$  SD glandular opening count was  $53.2 \pm 30$  (range 4–158). The analysis of variation showed a significant difference ( $P = 0.001$ ) among all video frames. In conclusion, endometrial glandular opening count, as measured by the method described, can be used in investigations during the luteal phase. Although a lack of pattern was observed in endometrial maturation, this feature should be explored further in this subgroup of patients.

**Keywords:** digitalization, endometrial maturation, glandular opening, hysteroscopy

## Introduction

Morphological, hormonal, biochemical and ultrastructural changes of the mid-secretory endometrium are essential for embryo implantation and the successful progress of pregnancy. During the luteal phase, several steroid hormones, peptides, growth factors and cytokines mediate endometrial maturation and development (Damario *et al.*, 2001; Lindhard *et al.*, 2002). Implantation is one of the most important steps in achieving pregnancy after an IVF attempt. However, even in the most successful assisted reproduction centres the implantation rate does not exceed 35% (after day2 or -3 fresh embryo transfers), even in the presence of a fertilization rate of 70% or more. The disparity between fertilization and implantation rates reveals our ignorance in understanding the mechanisms involved in the dialogue between the embryo and the endometrium during the luteal phase (Boomsma and Macklon, 2006; Edwards, 2006).

The study of an adequate endometrial environment ('in phase endometrium') was first proposed by Noyes in 1950 (Noyes *et al.*, 1950); since then investigators have tried to determine a useful and accurate method for the evaluation and diagnosis of endometrial changes during the luteal phase. In search of an appropriate method, some investigators have confirmed the results of Murray *et al.* (2004) that histological analysis of the endometrium is not related to fertility status (Coutifaris *et al.*, 2004; Murray *et al.*, 2004). In clinical practice, endometrial development is usually assessed by histology of biopsy samples or by ultrasound (Fanchin *et al.*, 2000). In addition, an adequate luteal phase can be indirectly studied by assay of serum progesterone levels (Soules *et al.*, 1989; Jordan *et al.*, 1994).

The development and adenogenesis of endometrial glands are

among the most important and evident physiological events that occur during this period (Gray *et al.*, 2001; Burton *et al.*, 2002; Demir *et al.*, 2002; Hempstock *et al.*, 2004). Glandular openings can be visualized during the entire menstrual cycle, but are more evident during the secretory phase. The changes in glandular opening (from dot and punctuate-type to ring-type) can be visualized during the cycle, and this is related to reproductive status and prognosis (Johannisson *et al.*, 1982; Sakumoto *et al.*, 1992). These structures play an important role in the establishment of initial pregnancy, acting as a source of nutrients during organogenesis, when metabolism is basically anaerobic (Lindhard *et al.*, 2002). Additionally, glandular density (number of glands per mm<sup>2</sup>) is strongly associated with endometrial status. The administration of clomiphene citrate or combined oral contraceptives (Ludicke *et al.*, 2000; Sereepapong *et al.*, 2000) reduces the glandular density by a direct anti-oestrogenic or antiprogesterational effect, demonstrating the importance of hormonal modulation in endometrial maturation.

Hysteroscopy has been utilized to evaluate functional modifications of the endometrium (Sakumoto *et al.*, 1992; Masamoto *et al.*, 2000). These authors also described some vascular and glandular characteristics, and showed a good correlation between the 'good' and 'poor' endometrium, based on these endoscopic aspects, with the respective reproductive outcomes. Nevertheless, this classification is entirely subjective and operator dependent.

A software program that analyses all relevant image frames during diagnostic hysteroscopy, and that also counts the glandular openings, has been reported previously (Cunha-Filho *et al.*, 2004). This software can be used easily and applied to evaluate the mid-secretory endometrium objectively in several situations.

Considering the low implantation rate after assisted reproduction treatment, even in infertile patients without any clinical or hormonal abnormality, it was decided to investigate this group of patients using a simple and accurate method to study the pattern of mid-secretory endometrium.

## Materials and methods

A cross-sectional study was performed on 561 infertile patients attending the Obstetrics and Gynaecology Service at Hospital de Clinicas de Porto Alegre, Brazil. Infertile patients included in the study demonstrated: normal serum FSH, thyroid-stimulating hormone and prolactin concentrations; body mass index less than 27 kg/m<sup>2</sup>; regular menstrual cycles; anatomical or male factor infertility; and they were seen before their first IVF or ICSI attempt. No patients were receiving hormonal treatment. All patients included in the study signed an informed consent form, and this research protocol was approved by the hospital ethical committee (IRB-equivalent, #02-333).

## Hysteroscopy

Diagnostic hysteroscopy was carried out in all patients during the mid-secretory phase, 7 days after ultrasonographic evidence of ovulation. The endoscopic procedure was performed on an outpatient basis and without anaesthesia. All examinations

were performed by the same investigator (JSCF), using a 4-mm Hamou II hysteroscope (Karl Storz, Tuttlingen, Germany), and carbon dioxide as the uterine distention medium. A total of 561 images from all patients were included; all images were selected by the software for retrospective analysis. The method (Cunha-Filho *et al.*, 2004) consists of two distinct modules: semi-automatic hysteroscopy video summarization; and key-frame processing for detection of glandular openings.

## Video digitalization

The proposed system was tested using a set of different hysteroscopy videos, acquired under different conditions. The purpose of video feature extraction was to obtain a compact representation for each frame of the video that was robust to changing conditions during video acquisition.

## Key-frame selection

When performing a hysteroscopy, little time is spent observing images that are not relevant, and the micro-camera is relocated seeking relevant images. However, when suitable images to support the diagnosis/prognosis are found, the micro-camera is focused on the centre of the region of interest, or the camera is moved slowly, producing redundant video segments (i.e. where video frames are similar). Thus, the 'important' segments present a significant redundancy (i.e. are virtually static); the main purpose of the video summarization procedure was to detect these redundant video segments.

In order to achieve this goal, the correlation co-efficient that would detect these video segments was computed. Key-frames were extracted from each video segment using a methodology based on the singular value decomposition (Gong and Liu, 2000). This technique is used to obtain a refined feature space, where visually similar feature vectors (i.e. frames) are more easily clustered and their relative positions indicate their degree of correlation and redundancy. In other words, by monitoring the distance that the frame feature vector is projected with respect to the origin in a refined feature space, the degree of redundancy of each frame can be estimated. Therefore, the selected key-frames are the most redundant frame feature vectors within their respective clusters. In order to evaluate the visual content of each cluster quantitatively, a content measure (*CON*) is computed, which is the mean of frame feature vector distance to the origin. Lower values mean that the respective cluster, and consequently its key-frame, is more redundant (more relevant).

The selected key-frames were assessed by a specialist, who then manually selected images for the next stage from the video segment represented by those key-frames. The key-frames to be evaluated were ordered according to the *CON* measure, i.e. key-frames associated with the more static clusters were evaluated first. Next, the image analysis of the frames selected by the specialist was detailed.

## Detection of glandular openings

Glandular openings appear as relatively brighter spots arising from the background of the image. These spots are not homogeneous in intensity, and it is assumed that there is at least

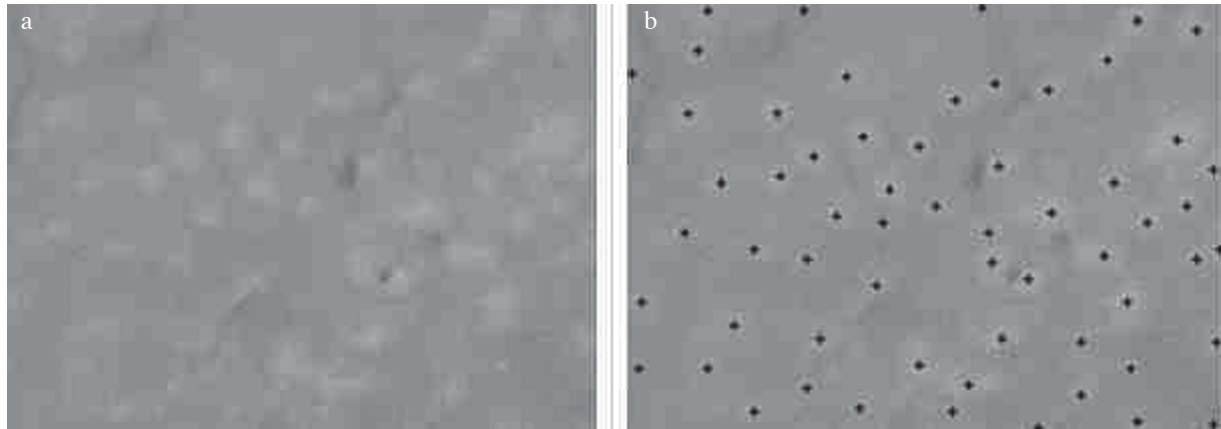
one intensity maximum inside each of them. The estimated number of glandular openings in each video frame was then evaluated automatically by the software.

## Statistics

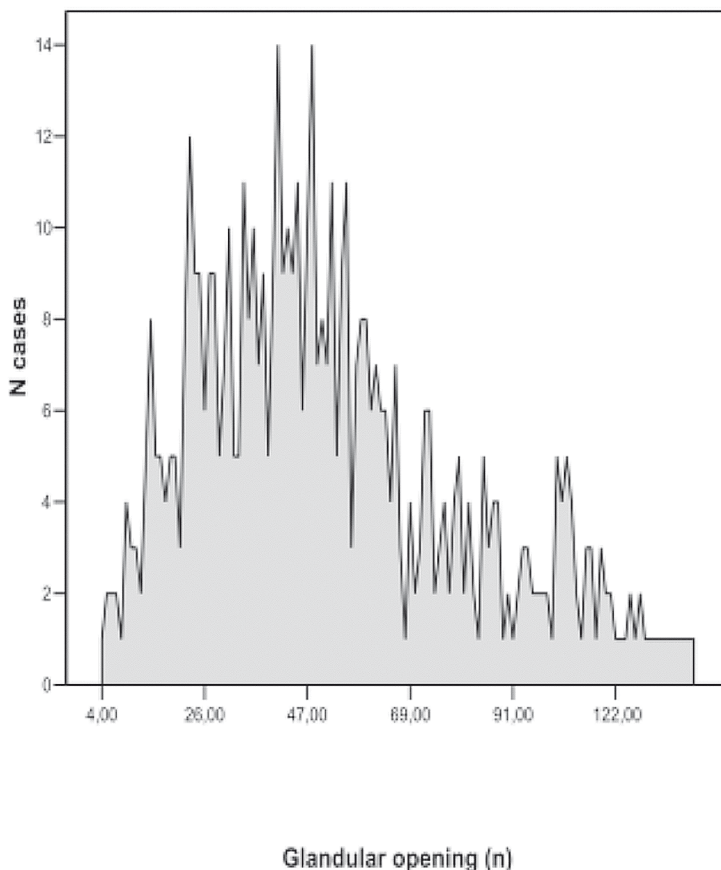
The endometrial glandular openings from all video segments were compared using the analysis of variance (ANOVA) test, with  $P < 0.05$  considered as statistically significant. SPSS statistical software was used for analysis.

## Results

The mean and SD for age (years) and body mass index (kg/m<sup>2</sup>) were  $33.7 \pm 3.3$  and  $22.35 \pm 0.95$ , respectively. **Figure 1** shows an example of the detection of glandular openings by the system. The mean  $\pm$  SD glandular opening count was  $53.2 \pm 30$  (range 4–158). The analysis of variation showed a significant difference ( $P = 0.001$ ) among all video frames (**Figure 2**). The median was 47 and the 95% confidence interval was 15–112 counts. The 25, 50 and 75 percentiles



**Figure 1.** (a) Image from a standard diagnostic hysteroscopy. (b) The same image showing glandular openings (crosses) detected by the method described.



**Figure 2.** Glandular opening detection among all cases. The median was 47 and the 95% confidence interval was 15–112 counts.  $P = 0.001$ , ANOVA test.

were, respectively, 31, 47 and 69 glandular openings. A total of 25 images (9%) presented the glandular openings as lower than the fifth percentile (15).

## Discussion

This study demonstrates for the first time that an objective method (digital hysteroscopy) for the analysis of endometrial glandular openings showed an important variability in terms of number of endometrial glandular openings in infertile patients with tubal aetiology or male factor infertility.

Currently, the glandular density in the endometrium can only be evaluated by using a histological sample to count the number of glandular openings during anatomopathological analysis. The method reported here is much easier, is simple to use, and yields more information regarding shape and other uterine abnormalities than does endometrial biopsy. However, the method evaluates only the density of endometrial glandular openings. The processes of embryo implantation and endometrial maturation are highly complex and require considerable further study.

Glandular openings were chosen for evaluation because they are associated with endometrial maturation. Although the appearance of the opening changes from a small circle to a more elongated orifice as the cycle advances (Masamoto *et al.*, 2000), the number of glandular openings was selected as the primary end-point because glandular density is associated with the reproductive status of the endometrium.

Morphologically, endometrial glands are most active and developed during early human pregnancy, and could provide an important source of growth factors, nutrients and cytokines (Hempstock *et al.*, 2004). In addition, over the first trimester of pregnancy they regress only after fetoplacental microvascularization has improved (Gray *et al.*, 2001).

The importance of endometrial glands has been confirmed by the demonstration of mucin MUC-1 and glycodeilin-A synthesis (Burton *et al.*, 2002). In addition, immunohistochemical and ultrastructural study of glandular epithelium shows the importance of these structures during early pregnancy (Demir *et al.*, 2002).

This study has, for the first time, demonstrated that there is a wide variability in the density of glandular openings among the subjects included (infertility patients prior to the first IVF treatment), which raises several questions about the real role of these structures in endometrial maturation and, consequently, implantation.

The mid-secretory period is important for the normal development of pregnancy; embryo implantation takes place approximately 7 days after the LH surge (Wilcox *et al.*, 1999). Morphological alterations during the mid-secretory endometrium are characterized by glandular and stromal modifications, which are expressed by several anatomical and molecular markers. In infertile patients receiving IVF treatment, implantation is still one of the most important factors that limit the pregnancy rates to below 30% in almost all assisted reproduction centres.

Several strategies have been used to increase the implantation rate: catheter selection, use of ultrasound, gentle transfer, progesterone/oestrogen supplementation, blastocyst transfer; however, even in apparently ideal conditions the implantation rates are low. The explanation for this low implantation rate could lie in the endometrium.

Others investigators, using an oocyte donor model, have studied the expression of glycodeilin and integrin ( $\alpha v \beta 3$ ) during the implantation window (Damario *et al.*, 2001; Edwards, 2006). These authors showed a strong correlation between those markers and glandular/stromal modification; however, the study design was not able to validate the association of those biomarkers with the dialogue between the embryo and the endometrium.

The utility of endometrial histology for the diagnosis of luteal phase defects was not strongly recommended in the reports of some well-designed studies (Coutifaris *et al.*, 2004; Murray *et al.*, 2004). The prevalence of out-of-phase endometrial biopsies was not different between fertile or infertile populations (Coutifaris *et al.*, 2004) and even the accuracy of histological endometrial dating for a proper luteal phase defect is questioned (Murray *et al.*, 2004). The present study concurs with those authors and the findings demonstrate an important variability in endometrial glandular opening count during the luteal phase, which confirms the flaw in assessing endometrial morphology on its maturation.

In addition to endometrial maturation, embryo quality and controlled ovarian stimulation protocols also have an important effect on implantation rates. These parameters are difficult to evaluate and compare, owing to the sample size and design needed to reach a reliable conclusion.

The present study opens a new perspective regarding endometrial assessment. This is a reproducible, cheap, easy and sensible method that could provide essential information for the correct diagnosis and prognosis for endometrial maturation. It has been demonstrated clearly that there is a wide variation in endometrial glandular opening count in this selected population (infertile patients with no hormonal dysfunction receiving their first IVF treatment).

The question needs to be raised whether the study of endometrial maturation before IVF transfer is currently well performed. The implantation rate could be improved if there was a better understanding of all mechanisms involved in endometrial preparation and, consequently, the embryo-uterine dialogue.

The scope of this research was to evaluate the endometrial glandular density as an important variable in endometrial maturation. However, several other variables are also related to endometrial maturation and, consequently, implantation rates.

In conclusion, a new and accurate method for the study of mid-secretory endometrium has been demonstrated. This method is based on the density of glandular openings as evaluated during hysteroscopy. In the future, this new method could aid better understanding of the dialogue between the embryo and the endometrium during the implantation window, an important factor in the success or failure of pregnancy.



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