IODINE CONTRAST IN HUMAN GYNECOLOGICAL SPECIMENS ANALYZED BY MICROTOMOGRAPHY

P.T. Castro[1,2], O.L. Aranda[1,3], A.P. Matos[4,5], E. Marchiori[2], H. Alves[7], R.T. Lopes[8], A.L Machado[8], H. Werner[4,5], E. Araujo Junior[6]

[1]Radiology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.
[2]Alta Diagnóstico, Rio de Janeiro, Brazil.
[3]Obstetrics and Gynecology, Vassouras University, Vassouras, Rio de Janeiro, Brazil.
[4]Fetal Medicine, Alta Excelência Diagnóstica, Rio de Janeiro, Brazil.
[5]CDPI, Rio de Janeiro, Brazil.
[6]Obstetrics, UNIFESP, Escola Paulista de Medicina, São Paulo, Brazil.
[7]Applied Physics and Thermodynamics Department, State University of Rio de Janeiro, Rio de Janeiro, Brazil.
[8]Nuclear Engineering Program, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

RESUMO

A perspectiva tridimensional (3D) da anatomia humana é essencial para o conhecimento da fisiologia e mecanismo de doenças. A microscopia tradicional apresenta limitações que prejudicam a perspectiva espacial real da microanatomia. Para a formação tridimensional de imagens em espécimes humanos, é necessário que o método seja não destrutivo, a preparação da amostra não pode alterar as características químicas das células e não pode alterar a proporção entre os diferentes tecidos. Para superar esses obstáculos, buscamos métodos para o estudo de espécimes benignos ginecológicos, utilizando solução de Lugol e Micro-CT. O objetivo deste estudo é relatar nossa experiência com a solução de Lugol como coloração de espécimes ginecológicos analisados por Micro-CT.

ABSTRACT

The tridimensional (3D) perspective of the human anatomy is essential for the knowledge of physiology and mechanism of diseases. The traditional microscopy presents limitations that impair the real spatial perspective of the micro anatomy. For the tri-dimensional formation for images in human specimens, it is necessary for the method to be non-destructive, the specimen preparation cannot alter the chemical characteristics of the cells and cannot alter the proportion between the different tissues. To overcome these obstacles, we searched for methods for the study of gynecological benign specimens, using Lugol solution and Micro-CT. The objective of this study is to report our experience with the Lugol solution as staining of gynecological specimens analyzed by Micro-CT.

INTRODUCTION

The tridimensional (3D) perspective of the human anatomy is essential for the knowledge of physiology and mechanism of diseases. The technologies used in clinical setting provide good correlation between *in vivo* and imaging. The traditional microscopy provides images in high resolution and is used for centuries to analyze human specimen. However, the traditional microscopy presents limitations that impair the real spatial perspective of the micro anatomy. During the specimen preparation, the structures may suffer fractures, dislocations and compression due to traction and friction of the microtome. This is an obstacle for the real tri-dimensional perspective of the micro-anatomy.

For the tri-dimensional formation for images in human specimens, it is necessary for the method to be non-destructive, the specimen preparation cannot alter the chemical characteristics of the cells and cannot alter the proportion between the different tissues. These requirements are necessary for the posterior study of the specimen by traditional microscopy. Differently from animal studies, even normal human tissues must be analyzed by traditional microscopy, mainly to exclude diseases in the specimen donated. This factor turns limited the technologies available for the 3D microscopic representation of the human anatomy. Microtomography has been used

in human specimens since early 1980's, however its use was restricted to mineralized specimens, mostly human bone and teeth [1].

For the study of soft tissues, it was necessary to increase contrast between the different soft tissues in the specimen. The staining solutions like gold and Osmium solution have high cost, are toxic and difficult to manipulate [2]. With the introduction of the iodine solution as staining by Metscher [3], the technical impairments were diminished, increasing the possibility of the micro-CT in human soft tissue specimen. Studies using Lugol solution as staining for Micro- CT studies in human soft tissue has been published in the last years, from embryos to congenital malformations [4,5].

The 3D micro anatomy of human body actually described is based on reconstructions biased by the observer interpretation. These reconstructions are also constructed over the lack of knowledge of the technical limitations over his analysis. To overcome these obstacles, we searched for methods for the study of gynecological benign specimens, using Lugol solution and Micro-CT. The objective of this study is to report our experience with the Lugol solution as staining of gynecological specimens analyzed by Micro-CT.

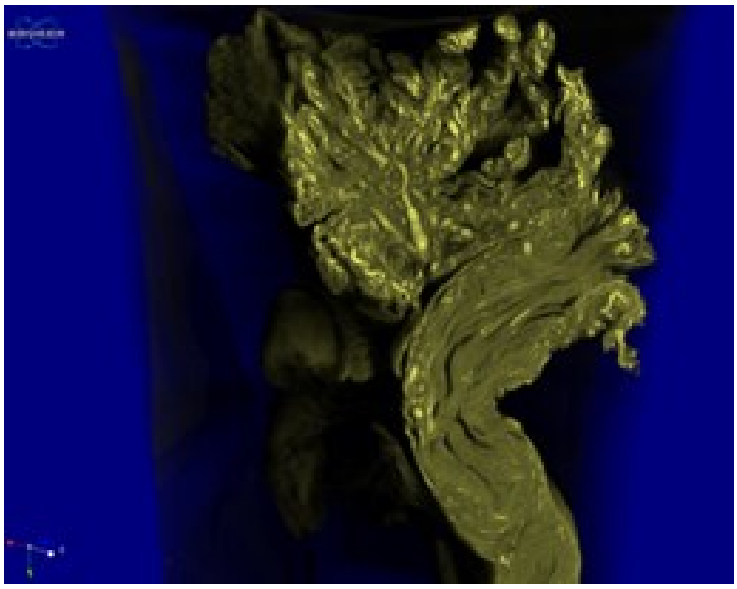

Figure 1: 3D reconstruction of the fallopian tube, demonstrating the abdominal ostium (arrow)

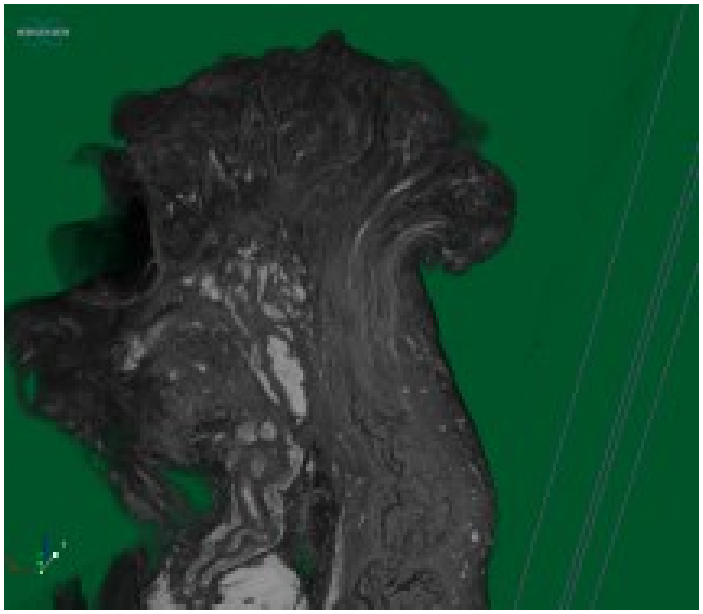

Figure 2: 3d reconstruction of the distal part of the fallopian tube. The fringes were well-defined, as the mucosal folds. Vessels presented more contrast.

METHODS

This study is part of a larger study involving the role of micro-CT in the study of human tissues, with special attention to gynecological conditions. The study was approved by the Ethics Committee of Vassouras University (#56031916.0.0000.5290). In a prospective selection of cases, patients scheduled to undergo surgical procedures for benign gynecological conditions (including adenomyosis, fibroids, and EP) in the Universitary Hospital of Vassouras (Vassouras, Rio de Janeiro, Brazil) agreed to participate in the study and provided written informed consent for the use of their tissues for research purposes. Patients with suspected malignant conditions were not included. The Ethical Committee of the University of Vassouras approved the study (#56031916.0.0000.5290).

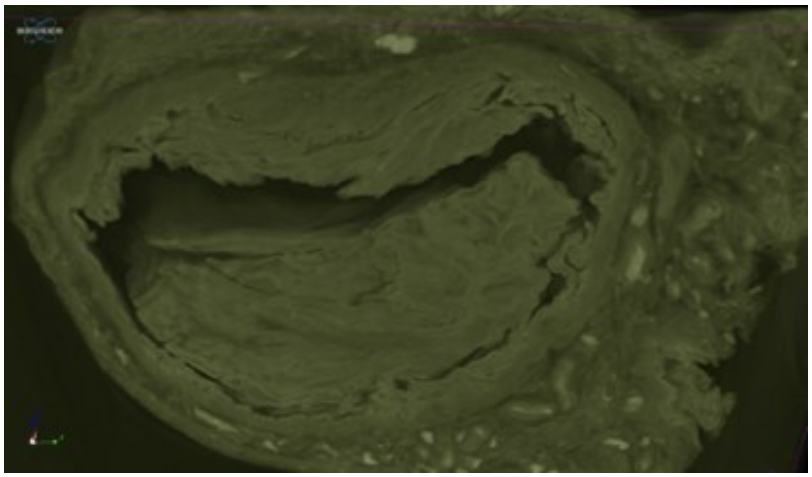

Figure 3: tridimensional image of the fallopian tube ampulla: the folds are complexes and unpredictable, the serosal part of the tube (arrow) has less foldings than the medular part.

SPECIMEN PREPARATI□ON

20 gynecological specimens, 14 fallopian tubes extracted for sterilization purposes, four uterine cervix and two uterus and cervix were removed for benign conditions. The specimens were fixed in formalin solution concentration for >24 h at room temperature for preservation of the tissue and cellular architecture [6]. The specimens were washed twice with distilled water.

In an adaptation of a previously described protocol, iodine staining was performed to enhance tissue contrast using different Lugol concentration diluted in physiological solution for different period of time. Each specimen was removed from the staining solution, rinsed with buffer to remove excess stain solution and prevent surface saturation, and dried with paper tissue.

The specimen was packed in vacuum sealed bags and fixed on Styrofoam to support the specimen, ensuring mechanical stability and avoiding movement artefacts during the X-ray examination. After the micro-CT study, to prevent specimen degradation and remove the stain of the iodine solution, the specimens were returned to 10% formalin solution for 7 days and followed for traditional histological analysis.

MICRO-CT AND IMAGE ANALYSIS

The SkyScan 1173 (version 1.6.9.4; Bruker micro-CT, Kontich, Belgium) system was used to acquire the FT images. X-ray energies, currents, and exposure times were adapted individually to optimize image acquisition for each specimen. Energy ranged from 40 to 130 kV, current from 61 to 200 μA, exposure time from 250 to 100 ms, scan duration between 13 and 88 min, and voxel size between 9.26 and 39.45 μm. Post- procession analysis and image reconstructions were performed using the Bruker micro- CT (version 1.16; Kontich, Belgium).

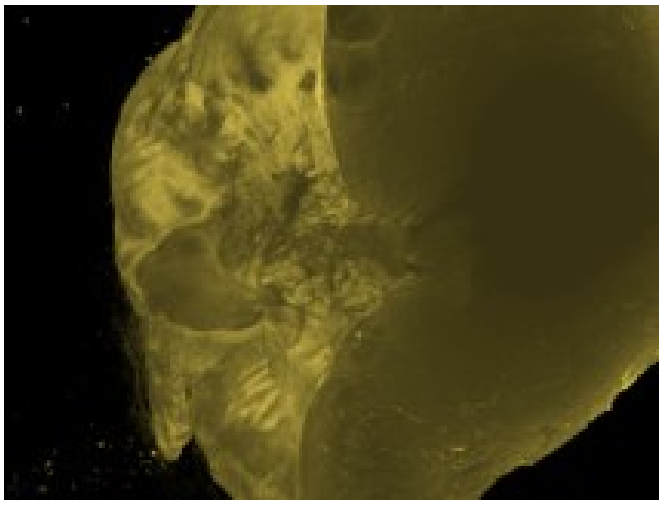

Figure 4: uterine cervix. the glandular external Os was clearly visible. The transition between cervical glands and cervical interstitial is not very clear.

RESULTS

All the specimens were successfully examined. The iodine solution contrast was noticed in all the specimen in different concentrations, according to the cellular characteristics of the tissue.

The fourteen fallopian tubes presented good contrast impregnation between the different tissues (Figure 1-3). The mucosa and the serosa were clearly seen, the muscular segments were visible, however not possible to reconstruct in three dimensions.

The microvascularization was perceived in many segments, despite the empty vessels, the endothelium was also defined by contrast impregnation. Cervix presented a visible and distinct mucosa, with the internal glands also visible. The cervical canal was clearly visible, with the glands well-defined.

The transition between cervical glands and endometrium was not clearly visible (Figure 4). The cervical fibrous tissue was not well-defined. The uterus presented good contrast impregnation in only few millimeters after the serosa. However, presented a very good contrast in the outer myometrium.

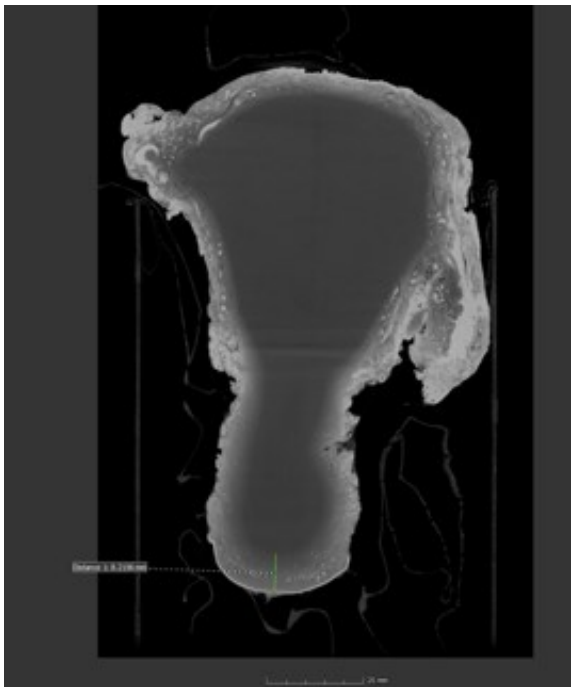

Figure 5: Human uterus. The outer myometrium presented good contrast. However, the amount of Iodine and time of contrast exposition was not sufficient for the entire impregnation of the organ

DISCUSSION

The Lugol solution presented good contrast in small specimens. The FT presented good contrast and tissue definition sufficient for 3D reconstruction in different segments. It is well described the relation between Lugol solution and specimen impregnation. Some authors described formulas for the standardization of the technique. However, in our specimen, the final histopathological result was indispensable, and caution was necessary during the experiments. There are articles using larger specimens, even entire fetuses using Lugol as staining. The time for iodine impregnation in larger specimens is wider than in small specimens, and time was against our study due the necessity of histopathological results for the patients who agreed to participate in the study.

In small specimens, the iodine in the Lugol's solution is absorbed until the equilibrium between solution and specimen. The capacity of penetration of the solution is more unknown in larger specimen and is recommended to develop an appropriate staining protocol for each experiment, and the interior staining was had limited passive diffusion of the uterus, also reported in other studies [7,8].

For larger specimen, the long time for staining is also necessary, and in our study, the requirement of the histological analyzes did not allow enough time for the staining. Also, the process of portion and absortion of iodine is related to the binding of the iodine to other molecules, mainly glycogen, lipids and other carbohydrates into the tissues [9].

In conclusion, we believe Lugol's solution is safe and can be used as staining in human specimen, and provide high quality images of gynecological organs and tissues. The contrast between tissues allow the reconstruction in 3D of many segments of gynecological microanatomy. However, despite the previously described in other studies, the myometrial and cervical tissues require more studies for better iodine impregnation, adding contrast between the tissues.

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