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Assessment of Fallopian Tube Cytology for the Diagnosis of Endometriosis and Hydrosalpinx

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Abstract

Fallopian tube cytology has been used as a useful tool in the diagnosis of infertility.

In this study, we developed an intra-fallopian tube cell collection method with the simultaneous use of a laparoscope and hysteroscope, and examined its safety and applicability for the diagnoses of endometriosis and hydrosalpinx. Fallopian tube cells were collected at laparoscopy and hysteroscopy from 20 volunteer patients who visited the infertility clinic. There were 10 patients with normal fallopian tubes (Group 1), 7 with pelvic endometriosis (Group 2), and 3 with hydrosalpinx (Group 3). The collected cells were fixed onto glass slides using an auto-smear method, stained by the Papanicolaou method and subjected to detailed cytomorphological examinations. In each case, an adequate number of cells with well-preserved morphology for a reliable evaluation was obtained. Cells from normal fallopian tubes were mainly fallopian tube epithelial cells including ciliated columnar cells and secretory cells. The number of inflammatory cells was quite low. A characteristic feature in cases with pelvic endometriosis was the presence of a large number of macrophages, some of which showed hemosiderin phagocytosis. In contrast, cases with hydrosalpinx showed an extremely low cellular component. No complications were found in any of the patients. Our study indicates that the present intra-fallopian tube cell collection method using a laparoscope and hysteroscope is a reliable and safe method that can be applied to the diagnosis of endometriosis, hydrosalpinx, as well as pelvic infertility. (J Nippon Med Sch 2002; 69: 445–450)

Key words: fallopian tube, cytology, endometriosis, hydrosalpinx, diagnosis

Introduction

Hysterosalpingography (HSG), hydrotubation and pneumotubation have been used in the diagnosis of infertility resulting from fallopian tube disorders. Recently, clinical studies on the fallopian tube using a tuboscope[1], 3D-HSG[2] or ultrasound with contrast medium[3] have been reported. However, few clinical hystocytologic studies on fallopian tubes[4–6] have been performed. Although morphological studies on fallopian tube epithelial cells and methods of fallopian tissue collection have been reported previously, there have been no reports on how to collect live fallopian tube cells without intra-fallopian tube instrumentation. Recently, we established and reported a method for fallopian tube cell collection without intra-fallopian tube instrumentation under laparoscopic and hysteroscopic control[7]. The present study is the first report describing a detailed cytomorphological examination of fallopian tube cells obtained by our method from normal oviduct, pelvic

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Table 1 Characteristics of group 1 patients (normal fallopian tubes)

Case	Age	Preoperative diagnosis	Laparoscopic diagnosis
1.	29	unexplained infertility	normal pelvic
2.	33	unexplained infertility	normal pelvic
3.	29	unexplained infertility	normal pelvic
4.	37	unexplained infertility	normal pelvic
5.	28	unexplained infertility	normal pelvic
6.	32	unexplained infertility	normal pelvic
7.	39	benign ovarian cyst	Dermoid cyst
8.	28	benign ovarian cyst	Dermoid cyst
9.	36	benign ovarian cyst	Dermoid cyst
10.	37	benign ovarian cyst	simple serous cyst

Table 2 Characteristics of group 2 (endometriosis) and 3 (hydrosalpinx)

	Case	Age	Preoperative diagnosis	Laparoscopy diagnosis	Tube patency	Re-AFS stage
Group 2	11.	36	unexplained infertility	endometriosis	Bilateral	Ⅰ
	12.	30	unexplained infertility	endometriosis	Unilateral	Ⅱ
	13.	32	benign ovarian cyst	endometriosis with chocolate cyst	Bilateral	Ⅱ
	14.	34	benign ovarian cyst	endometriosis with chocolate cyst	Bilateral	Ⅱ
	15.	33	benign ovarian cyst	endometriosis with chocolate cyst	Bilateral	Ⅲ
	16.	29	benign ovarian cyst	endometriosis with chocolate cyst	Bilateral	Ⅲ
	17.	23	benign ovarian cyst	endometriosis with chocolate cyst	Bilateral	Ⅲ
Group 3	18.	34	unexplained infertility	hydrosalpinx	Unilateral	
	19.	29	unexplained infertility	hydrosalpinx	Bilateral	
	20.	20	unexplained infertility	hydrosalpinx	Unilateral	

Re-AFS: Revised American Fertility Scietry

endometriosis and hydrosalpinx. The objective was to assess the clinical applicability of fallopian tube cytology for the detection of endometriosis and hydrosalpinx.

Materials and Methods

Fallopian tube cells were collected at laparoscopic and hysteroscopic surgery from 20 volunteer patients visiting the infertility clinic at Nippon Medical School 2 nd Hospital between February 2000 and February 2001. Informed consent was obtained from all patients before inclusion in the study and the Declaration of Helsinki (Revised 1975) was strictly observed. The mean age of the patients was 32.2 years old (range: 23 to 39 years old). All the laparoscopic and hysteroscopic surgeries were performed under general anesthesia in the operating room.

Preoperative diagnoses in these cases were unexplained infertility in 11 cases and benign ovarian cyst in 9 cases. Laparoscopic findings were normal pelvic in 6 cases, pelvic endometriosis without ovarian chocolate cyst in 2 cases, ovarian chocolate cyst with pelvic endometriosis in 5 cases, simple serous ovarian cyst in 1 case, dermoid cyst in 3 cases and hydrosalpinx in 3 cases. Of the 40 fallopian tubes, 37 were patent and 3 were obstructed. The degree of pelvic endometriosis was assessed according to the American Fertility Society staging classification[8]; 1 case was classified as revised-American Fertility Society (r-AFS) I and 3 cases each as r-AFS II and r-AFS III. Overall, 10 patients had normal fallopian tubes (Group 1), 7 suffered from pelvic endometriosis (Group 2) and 3 from hydrosalpinx (Group 3) (**Tables 1, 2**).

The procedure for fallopian tube cell collection with simultaneous use of a laparoscope and hysteroscope has been reported previously[7]. The schematic representation is shown in **Fig 1**. Briefly,

10 ml of physiological saline containing 5% (v/v) of indigo carmine was injected into the fallopian tube from the uterotubal ostium using a teflon catheter passed through the hysteroscope. The saline solution containing released fallopian tube cells was retrieved from fallopian tube fimbria using a pouch. This step was monitored with a laparoscope. The pouch was pulled out from pelvic cavity. The solution in the pouch was aspirated into a 10 ml syringe and taken to the laboratory as soon as possible for cytological processing. The cells were fixed onto glass slides using an auto-smear method [9]. Each preparation was stained by the Papanicolaou method and subjected to a thorough cytomorphological examination.

In this study, a Frespout hysterofiberscope catheter (Kitazato Sapply Co. Ltd, Shizuoka, Japan) was used as the teflon catheter for saline injection. An HYF X-P hysteroscope (Olympus Co. Ltd, Tokyo, Japan) was used for hysteroscopy. An Endopouch (Johnson & Johnson Co. Ltd, OH, USA) or Slim bag (Hakko Medical Co. Ltd, Nagano, Japan) was used as the pouch for fallopian tube cell retrieval. A Stryker laparoscopic system (Stryker, MI, USA) was used as the laparoscope for monitoring the cell collection in the pelvic cavity.

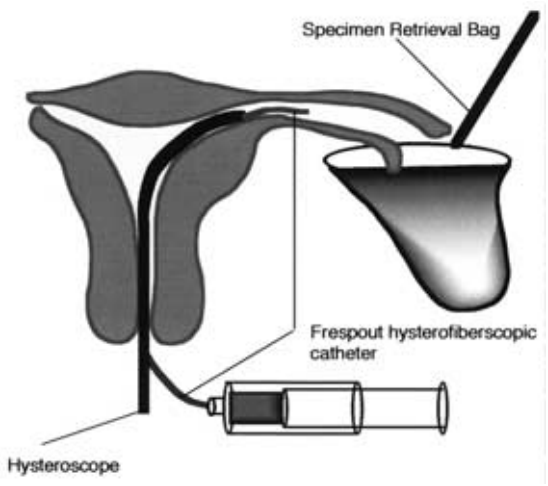

Fig. 1 The schematic representation of fallopian tube cells collected with laparoscope and hysteroscope. Physiological saline (10 m*l*) containing 5% (v/v) of indigo carmine is injected into fallopian tube from uterotubal ostium using a Teflon catheter through hysteroscope. Fallopian tube cells released in the saline are retrieved from fallopian tube fimbria with a pouch under laparoscopic monitoring.

Results

All the hysteroscopic and laparoscopic procedures were performed safely without any complications. The total time for fallopian tube cell collection from the hysteroscopic procedure to the extraction of the pouch containing the cell solution from the pelvic cavity was within 10 min. The macroscopic findings of the retrieval solution from fimbria including saline, indigo carmine and fallopian tube components were normal without bloody discharge. The mean volume of the retrieval solution was 3.5 m*l*.

In order to analyze the cytomorphological results of the fallopian tube cells, we classified the patients into 3 groups. Group 1 consisted of patients with normal fallopian tubes; Group 2 constituted patients with pelvic endometriosis; and Group 3 was composed of patients with hydrosalpinx. In all cases, an adequate number of cells with well-preserved morphology allowing a reliable evaluation was obtained (**Table 3**). In Group 1 patients (**Fig. 2**), the retrieved cells were mainly epithelial type by morphology and included ciliated columnar cells and secretory cells. In addition, the number of inflammatory cells including neutrophils was quite small and red blood cells were seldom observed. In Group 2 patients (**Fig. 3a, b, c**), numerous macrophages and normal numbers of epithelial cells

Table 3 Cytological findings in the 3 groups

Group	Total cell counts	Tubal epithelial cells	Macrophages	Other inflammatory cells
1	++	++	+	+
2	+++	++	+++	+++
3	+	+	−	+

−: Scarce +: Mild ++: Moderate +++: Numerous

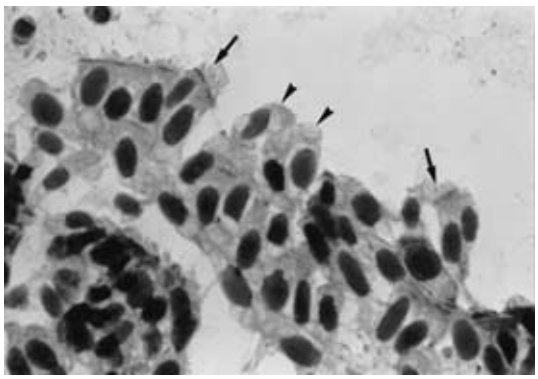

Fig. 2 Cytological findings in normal fallopian tubes (Group 1) showing mainly fallopian epithelial cells including ciliated columnar cells (arrows) and secretory cells (arrow heads). Inflammatory cells including neutrophils are very few. (Pap. stain × 40)

were recognized in the preparations from all 7 patients. The macrophages appeared at various morphological stages including foamy cells and some with hemosiderin phagocytosis. Red blood cells were scanty. In Group 3 patients (**Fig. 4**), very small numbers of cells were obtained. However, almost all were inflammatory cells including neutrophils, monocytes and lymphocytes. Few normal fallopian tube cells or red blood cells were found.

Discussion

The oocyte is picked up at the fimbria portion and in vivo fertilization occurs in the ampulla portion of the oviduct in humans. Therefore, the functional state of the fallopian tubes has an intimate role in fertility and reproductive medicine. However, very few studies have addressed this issue. In general, not only conventional HSG but also the latest methods of ultrasound with contrast medium may confirm the patency of the fallopian tubes. Yet, these methods do not offer detailed information on fallopian tube function as related to fertility. In order to assess the fallopian tube function for oocyte retrieval, we developed a pattern analysis of intra-oviduct injection pressure[10]. However, the system did not provide information on the status of the inside of the fallopian tube. The Falloposcope, a recently developed instrument, enables us to observe the internal condition of the oviduct, but it

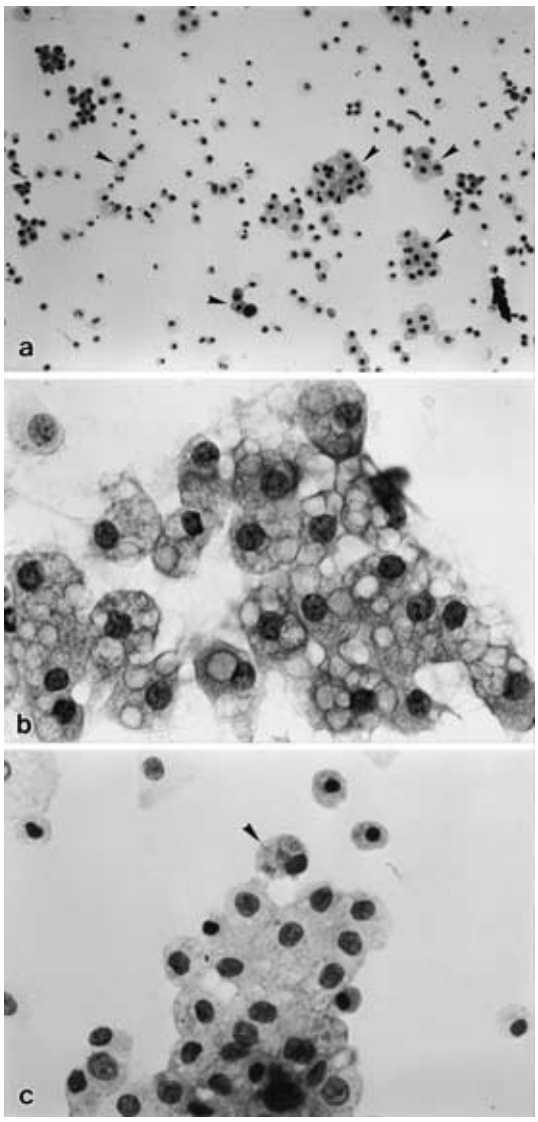

Fig. 3 Cytological findings in pelvic endometriosis (Group 2).
a: Numerous macrophages (arrow heads) are recognized. (Pap. stain × 10)
b: A proportion of macrophages appeared as foamy cells. Pap. stain × 40)
c: A macrophage showing hemosiderin phagocytosis (arrow head). (Pap. stain × 40)

requires intra-fallopian tube instrumentation and carries the risk of tubal perforation.

In the present study, we developed a method for intra-fallopian cell collection using a laparoscope and hysteroscope simultaneously. The histopathological cytology of the fallopian tube may provide new information in the field of reproductive medicine. Especially the mechanisms of and relationship between infertility and endometriosis are important issues. In this respect, peritoneal inflammatory

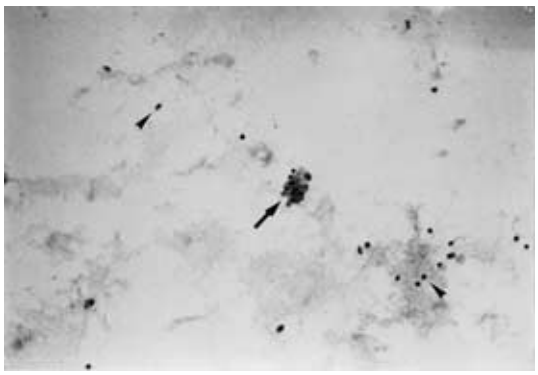

Fig. 4 Cytological findings in hydrosalpinx (Group 3). Very small number of collected cells including both epithelial cells (arrow) and inflammatory cells (arrow heads) are seen. (Pap. stain × 10)

mediators[11-14], cellular immune mechanisms[15] and natural killer cell activity[16] have often been reported on. Supplementary to these data, we found a large number of macrophages in collected fallopian tube cells from endometriosis-associated infertile women. Our results suggested that fallopian tube macrophages may play an intimate and causative role in endometriosis-associated infertility. Indeed peritoneal macrophages in endometriosis-associated infertility have recently been shown to express a high level of inducible nitric oxide synthase which adversely affects sperm, embryos, implantation, and oviduct function[17] Further characterization of the fallopian tube macrophages by an immunocytological method is in process.

It has been reported using salpingoscopy[1] that fallopian tube mucosal folds are pressed and flattened against the tubal wall in hydrosalpinx. Our study showed that the cellular component including fallopian tube epithelial cells and inflammatory cells are insignificant in hydrosalpinx. These results may reflect thin fallopian mucosa, reduced mucosal folds and mild inflammation in hydrosalpinx. In addition, two of the 6 fallopian tubes in 3 cases with hydrosalpinx were completely obstructed. It was impossible to retrieve saline with fallopian tube cells from these obstructed tubes. Therefore, no information on the internal condition of these fallopian tubes could be obtained by our method. However, after the fallopian tube patency is restored by laparoscopic surgery, our method may well provide such information.

Our method is considered non-invasive to the internal condition of the oviduct, because no intra-fallopian tube instrumentation is administered and the retrieval solution contains no bloody discharge; in addition, cytological preparations showed no red blood cells. Furthermore, the same type of catheter as in our study has been used for hysteroscopic GIFT procedure in infertility clinics and successful pregnancies have already been reported[18]. However, in our method, the laparoscopic and hysteroscopic procedures for collecting fallopian tube cells had to be performed in the operating room. We intend to further develop a simple fallopian tube cell collection method using only a hysteroscope and teflon catheter.

In conclusion, our study indicates that the present intra-fallopian tube cell collection method using a laparoscope and hysteroscope is a reliable and safe method that can be applied to the diagnosis of endometriosis, hydrosalpinx, as well as pelvic infertility.

Acknowledgements

We thank Dr. Matsumoto, Assist. Prof. Surgical Pathology Section, for his helpful discussions and Mr. Saito and Ms. Nagasawa (cytotechnologists) for their helpful collaboration in presenting this study. We also thank Dr. Mohammad Ghazizadeh, Assoc. Prof. Department of Molecular Pathology, Institute of Gerontology, for reviewing and revising the manuscript.

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(Received, April 25, 2002)
(Accepted, May 23, 2002)