

# Distribution of macrophages in the human fallopian tubes: an immunohistochemical and electron microscopic study

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*The fallopian tubes are essential for the normal transport of gametes, fertilisation and early embryonic development and transport. Their locomotive force is mainly due to the contractility of the smooth muscle cells, as well as to the ciliary activity of the tubal epithelium. Steroid hormones such as oestradiol and progesterone mediate changes in tubal morphology, in particular the tubal epithelium. It is well known that macrophages participate in the immune system, but recent studies have shown that they also play other roles under physiological conditions. They are known to be a source of prostaglandins of the E series, which influence the contractility of the uterine tube. Lymphocytes in the tubal mucosa can be involved in the process of immune tolerance, which could enable sperms and blastocysts to be transported through the oviduct under normal conditions without the activation of local immune mechanisms. Most of the evidence for mucosal immune responses in the female reproductive tract is related to the vagina, with less information available for the uterus. The less known segment in this regard is the oviduct, which prompted us to review and summarise the current state of knowledge of the immune system at the level of the human oviduct. The present study was therefore undertaken to examine the distribution and morphological properties of macrophages in the endosalpingeal stroma and smooth muscle layer of the human fallopian tubes. Thirty fresh fallopian tubes were examined, taken at the proliferative (7 cases) and secretory (12 cases) phases of the menstrual cycle, and during the postmenopausal period (11 cases). Sections were stained by immunocytochemistry with a primary antibody (CD 68) and were used for counting the macrophages. Ultrathin sections were stained with lead citrate and uranyl acetate and studied by means of electron microscopy to assess the ultrastructure of the macrophages. A significant difference was observed between reproductive and postmenopausal women in the number of macrophages ( $p < 0.05$ ). This study may help to clarify the possible role of macrophages of the uterine tubes in some cases of infertility in females. (Folia Morphol 2008; 67: 43–52)*

**Key words:** human, fallopian tube, macrophages

## INTRODUCTION

The human fallopian tube is a dynamic muscular tube that undergoes cyclic variation in its functional epithelium. It is the site of gamete transport, maturation, and fertilisation as well as early embryonic development and transport. Far from functioning as a passive conduit to gametes and embryos, the uterine tube actively secretes multiple substances. It has an active ciliated epithelium and undergoes muscular contractions [6]. The oviductal cells maintain the fertilising capacity of spermatozoa, whereas the human follicular fluid facilitates the fertilisation process of oviductal spermatozoa. Recently, an additional role for the entire female reproductive tract has emerged. It has a major role in mounting local immune responses against microbial pathogens [33].

The entire length of the human tubal epithelium undergoes cyclic variations across the menstrual cycle [3, 10, 17, 22]. Cell height and cell populations as well as mitotic activity vary significantly across the cycle. Circulating ovarian sex steroids may regulate these cyclic changes; however, the mechanism of action of these steroids (direct or indirect via growth factors and cytokines) remains largely unknown. In animals, Abe et al. [1] and Steinhauer et al. [28] showed that the goat and canine oviducts also revealed visible changes in the morphology of their epithelium during the oestrous cycle.

Tissue macrophages derive from monocytes. The functions of macrophages include phagocytosis, antigen presentation, cell and tissue regulation and remodelling in growth and repair and anti-tumour activity [20]. Recent studies have shown that macrophages also play other roles under physiological conditions. Macrophages are known to be a source of prostaglandins of the E series and of cytokines [9]. Prostaglandins can influence the smooth muscles of the uterine tubes [11]. It can be suggested that the lymphocytes in the tubal mucosa may be involved in the process of immune tolerance, which could ensure the transport of sperms and blastocysts through the oviduct under normal conditions without activation of local immune mechanisms. Thus the lymphoid tissue of the oviduct is a specialised form of mucosal-associated lymphoid tissue (MALT) [8, 14, 34]. Morris et al. [23], by using immunocytological labelling techniques and electron microscopy, have explored the possibility that MALT exists in the normal uterine tube and that epithelial cells form an integral part of this system.

Macrophages express Class II MHC molecules on their surfaces; they are also positive for the charac-

teristic (though not specific) molecular surface marker CD 14 and the specific cytoplasmic granules marker CD 68. Macrophages in certain regions express additional markers, for example CD 33 in myeloid tissue and CD 16 in pulmonary alveoli. The macrophages in the human uterine tubes were identified via immunocytochemistry staining with CD 68 that recognises human monocytes/macrophages [26].

The cytological properties of leukocytes in the female reproductive organs have been investigated by many researchers [2, 18, 27, 31]. More specifically, the presence and distribution of macrophages have been studied in relation to the different stages of the oestrous cycle in the ovary, endometrium, cervix, and vagina [19, 30, 32].

However, to the best of our knowledge, there have been few reports concerning the distribution of macrophages in the human tubal tissue [26], whereas other leukocyte populations, such as T lymphocytes, were identified in normal human uterine tubes by Otsuki et al. [25], Boehme and Donat [4], Hunt and Lynn [16]. These studies were based on immunolabelling and electron microscopy.

Most of the evidence for mucosal immune responses in the female reproductive tract is related to the vagina, with less information available for the uterus. The less known segment in this regard is the oviduct, which prompted us to review and summarise the current state of knowledge of the immune system at the level of the human oviduct.

Thus the present study was carried out to examine, by immunocytochemical staining, the distribution of macrophages in the endosalpingeal stoma and smooth muscle layer in different segments of the human fallopian tubes during various stages of the reproductive and postmenopausal periods. The ultrastructural morphological properties of macrophages were also investigated during the same periods.

## MATERIAL AND METHODS

### Fallopian tube specimen collections

A total of 30 fallopian tubes were obtained from women undergoing gynaecological operations such as total abdominal hysterectomy or salpingo-oophorectomy for benign conditions (excluding tubal pathology) at Almadinah Maternity and Child Hospital (MMCH). Approval for the study and informed consent was obtained from the patients according to the guidelines of the Ethics Standards Protocol. Of the 30 patients, 19 had had a regular menstrual cycle (25–35 days). Histological dating of the

endometrium was performed as described by Palter et al. [26]. The specimens were taken at the proliferative (7 cases) and secretory (12 cases) phases of the menstrual cycle. The remaining 11 patients were postmenopausal. Their ages ranged from 30 to 50 years in the proliferative cases, 20 to 47 years in the secretory cases and 50 to 65 years in the postmenopausal women.

Immediately after salpingectomy, oviductal samples were collected from three different parts of the uterine tubes (the isthmus, ampulla and infundibulum). Isthmic tubal segments were obtained 2 cm from the cornua, ampullary segments 2 cm from the fimbriated segment, and fimbriated segments were obtained from the distal 1 cm of the tube. Tubes with gross pathology were discarded from this study.

### Immunocytochemical staining

Immunocytochemistry was carried out using a labelled streptavidin-biotin immunoenzymatic antigen detection system (Lab Vision Corporation, USA) on formalin-fixed paraffin embedded tissue. One section from each block was stained with haematoxylin and eosin and the absence of histopathological lesions were confirmed by light microscopy. Tissue blocks were cut into 6  $\mu\text{m}$  thick sections, deparaffinised and rehydrated. Epitope retrieval was performed using the Tris-EDTA buffer epitope retrieval method. To reduce non-specific background staining due to endogenous peroxidase, the slides were incubated in hydrogen peroxide block for 10–15 minutes. They were then washed twice in a buffer. The primary antibody (CD 68), mouse monoclonal antibody (Lab Vision Corporation, USA), was applied and incubated overnight in a humidity chamber. The streptavidin peroxidase was applied and incubated for 10 minutes at room temperature. The biotinylated goat anti-polyvalent was then applied and incubated for 10 minutes at room temperature. After each step the sections were washed four times in phosphate-buffered saline. One or two drops of DAB Chromagen were added to 1 ml of DAB Substrate, mixed by swirling and applied to the tissues, which were then incubated for 5–15 minutes, depending on the desired stain intensity. Lastly, they were counterstained with Mayer's haematoxylin and cover-slipped using a permanent mounting media [21]. One of the positively stained fallopian tubes was used as a positive control. The numbers of macrophages were counted by light microscopy per ten high power fields ( $\times 400$ ) in the endosalpingeal stroma and in the

smooth muscle layer of each specimen by means of a micrometer. The counting of macrophages was performed by two observers blind to the sample's menstrual phase. The macrophages were expressed per area of the view field, which was randomly selected.

### Electron microscopy

Small pieces of each block of tissue were also fixed in a chilled 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer (PH 7.4) for 2 hours and then post-fixed for 1–2 hours in osmium tetroxide dissolved in the same buffer. Subsequently, they were dehydrated by passage through a graded ethanol series and in propylene oxide, and then embedded in epoxy resin. The embedded blocks were cut into ultrathin sections with an ultramicrotome, stained with lead citrate and uranyl acetate [5], and studied by means of an electron microscope (12-A or H-300: Hitachi. Tokyo. Japan).

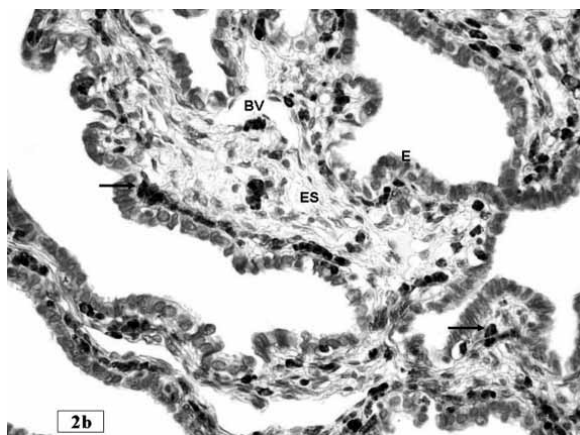
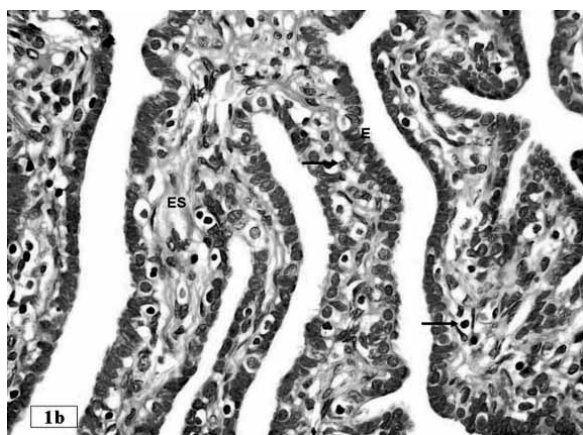
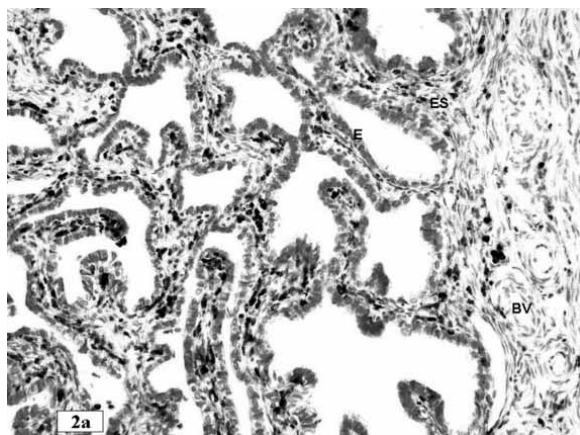
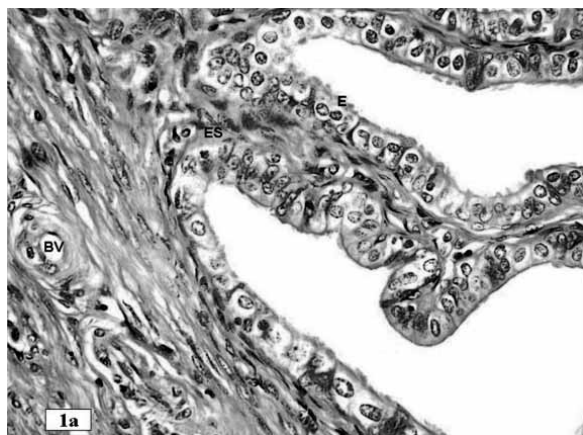
### Statistical analysis

Values are presented as means  $\pm$  standard error of mean (SEM). Means of the number of macrophages in the endosalpingeal stroma and smooth muscle layer in the different segments of the human fallopian tubes during the different phases of the menstrual cycle and postmenopausal period were analysed statistically by one way analysis of variance (ANOVA) followed by Tukey's HSD (honestly significant difference) pairwise comparisons using a current SPSS statistical package. Graph pad Prism 4 (Graphpad Software, Inc., San Diego, CA, USA, 2003) was used. The level of significance was set at  $p < 0.05$  throughout the study.

## RESULTS

### Immunocytochemical localisation of macrophages in the human fallopian tubes

The normal structure of the human fallopian tube during the proliferative and secretory phases of the menstrual cycle are shown in Figures 1A and 1B. Sections stained immunocytochemically with the use of CD 68 monoclonal antibody showed macrophages in the endosalpingeal stroma and in the smooth muscle layer of the uterine tubes during the secretory and proliferative phases of the reproductive period (Fig. 2, 3). In the postmenopausal period few or no CD 68 positive cells were found in the two layers of the uterine tube. Positive cells were also found in the vascular lumen of the endosalpingeal stroma and smooth muscle layer in the reproductive (Fig. 2, 3) and postmenopausal periods (Fig. 4).

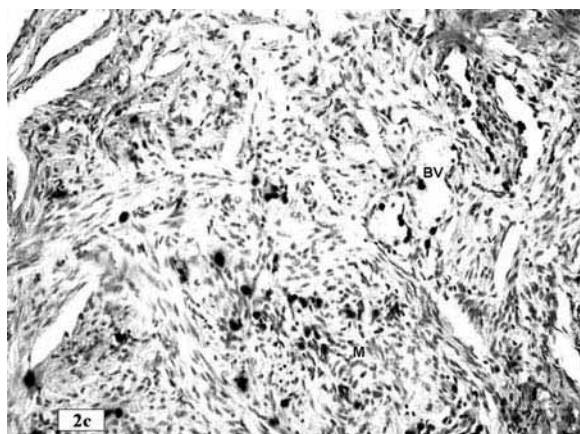


**Figure 1.** Normal human uterine tube during the proliferative (A) and secretory (B) phases of the menstrual cycle stained with haematoxylin and eosin stain. Mononuclear inflammatory cells were seen (→); E — epithelium, ES — endosalpingeal stroma, BV — blood vessel; ×400.

### Quantitative immunocytochemical analysis of macrophages in the human fallopian tubes

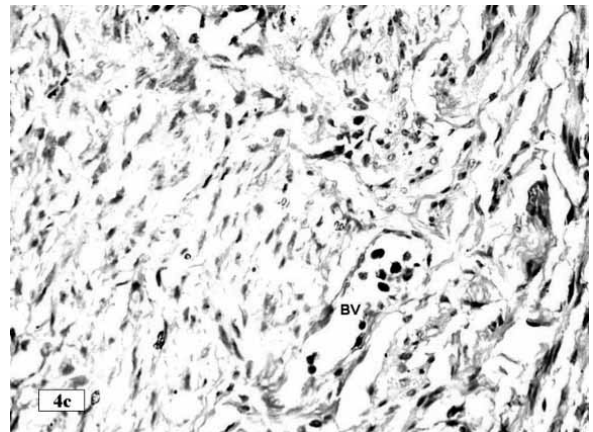
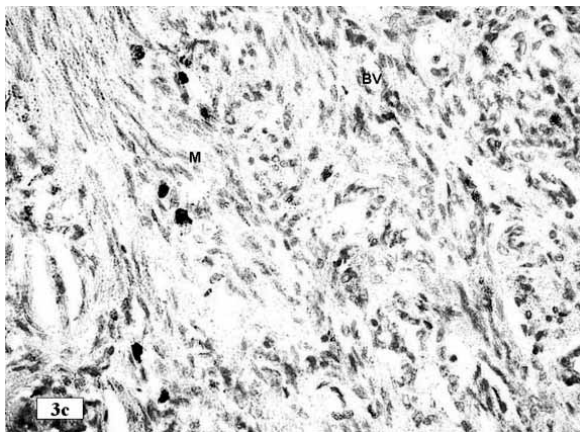
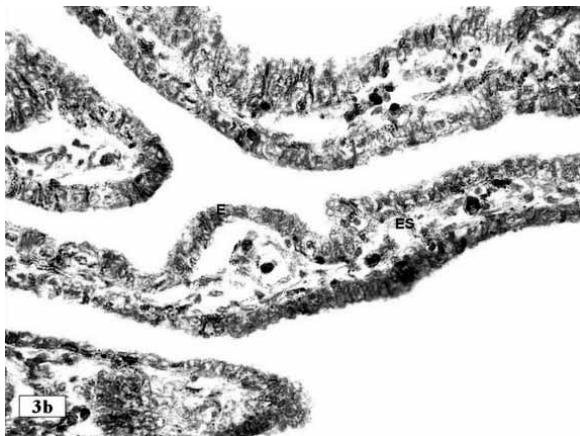
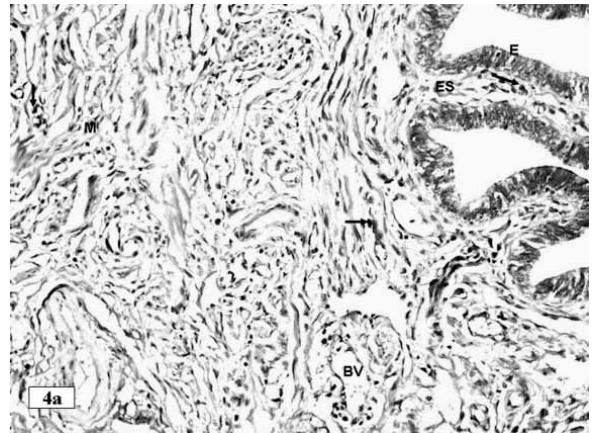
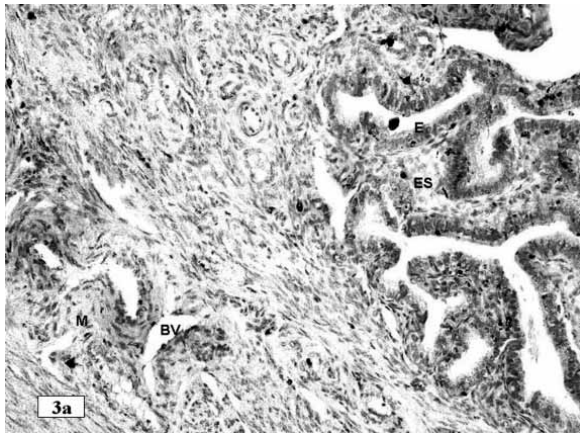
From the qualitative observations it was evident that the number of immunocytochemically stained macrophages during the secretory phase of the menstrual cycle was high in the endosalpingeal stroma (Fig. 2A, B) as well as in the smooth muscle layer (Fig. 2C) compared with that during the proliferative phase (Fig. 3A–C) of the menstrual cycle. However, in the postmenopausal period macrophages were few in number or absent (Fig. 4A–C). Most of the CD 68-positive cells in the postmenopausal specimens were found in the vascular lumen of the endosalpingeal stroma and smooth muscle layer (Fig. 4A, C). The quantitative analyses confirmed these observations.

The average number of immunocytochemically positive macrophage cells in the isthmus segment of the fallopian tubes during the postmenopausal period was significantly lower compared



**Figure 2.** During the secretory phase, CD 68-positive macrophages (→) were observed in the endosalpingeal stroma (ES) (A, B) as well as in the smooth muscle layer (M) and within the vascular lumen (BV) (C); E — epithelium; A: ×200; B, C: ×400.

with that during the proliferative and secretory phases of the menstrual cycle (Table 1, Fig. 5). In the secretory phase of the menstrual cycle a larger number of immunocytochemically CD 68-positive macrophages was found in the endosalpingeal stroma than in the smooth muscle layer of the fallopian



**Figure 3.** During the proliferative phase a smaller number of CD 68-positive macrophages was present in the endosalpingeal stroma (ES) (A, B) and in the smooth muscle layer (M) (C); E — epithelium, BV — blood vessel; A:  $\times 200$ ; B, C:  $\times 400$ .

**Figure 4.** During the postmenopausal period, CD 68-positive macrophages (arrows) were few or absent in the endosalpingeal stroma (ES) (A, B) and in the smooth muscle layer (M). Most of the macrophages were seen within the vascular lumen (BV) (C); E — epithelium; A:  $\times 200$ ; B, C:  $\times 400$ .

tube. There was no significant difference in their numbers in the endosalpingeal stroma and smooth muscle layer in the postmenopausal period or in the proliferative phase of the menstrual cycle (Fig. 5). With regard to the different segments of the fallopian tubes, there were significant differences in the

mean number of macrophages during the different phases of the menstrual cycle and postmenopausal period in the endosalpingeal stroma (Table 2) and the smooth muscle layer (Table 3). However, there was no significant difference in the mean number of macrophages present in the different

**Table 1.** Mean number of macrophages, at different phases of the menstrual cycle and postmenopausal period, of the endosalpingeal and smooth muscle layers in the isthmus segment of the fallopian tubes (n = 30); values are presented as means  $\pm$  SEM

	Secretory phase	Proliferative phase	Postmenopausal period	Significance
Endosalpingeal stroma	54.483 $\pm$ 4.901	6.143 $\pm$ 0.439	0.927 $\pm$ 0.109	< 0.000
Smooth muscle layer	17.900 $\pm$ 1.643	6.914 $\pm$ 0.483	1.055 $\pm$ 0.112	< 0.000

**Table 2.** Mean number of macrophages during different phases of the menstrual cycle and postmenopausal period in different segments of the fallopian tube in the endosalpingeal stroma (n = 30); values are presented as means  $\pm$  SEM

	Secretory	Proliferative	Postmenopausal	Significance
Isthmus	54.483 $\pm$ 4.901	6.143 $\pm$ 0.439	0.927 $\pm$ 0.109	< 0.000
Ampulla	54.667 $\pm$ 4.846	6.214 $\pm$ 0.475	0.936 $\pm$ 0.099	< 0.000
Fimbria	54.608 $\pm$ 4.874	6.200 $\pm$ 0.443	0.918 $\pm$ 0.112	< 0.000

**Table 3.** Mean number of macrophages during different phases of the menstrual cycle and postmenopausal period in different segments of the fallopian tube in the smooth muscle layer (n = 30); values are presented as means  $\pm$  SEM

	Secretory	Proliferative	Postmenopausal	Significance
Isthmus	17.900 $\pm$ 1.643	6.914 $\pm$ 0.483	1.055 $\pm$ 0.112	< 0.000
Ampulla	17.858 $\pm$ 1.645	6.929 $\pm$ 0.474	1.036 $\pm$ 0.102	< 0.000
Fimbria	17.875 $\pm$ 1.634	6.971 $\pm$ 0.483	1.046 $\pm$ 0.119	< 0.000

**Table 4.** Mean number of macrophages in different segments of the fallopian tube at different phases of the menstrual cycle and postmenopausal period in the endosalpingeal stroma (n = 30); values are presented as means  $\pm$  SEM

	Isthmus	Ampulla	Fimbria	Significance
Secretory	54.483 $\pm$ 4.901	54.667 $\pm$ 4.846	54.608 $\pm$ 4.874	< 1.000
Proliferative	6.143 $\pm$ 0.439	6.214 $\pm$ 0.475	6.200 $\pm$ 0.443	< 0.993
Postmenopausal	0.927 $\pm$ 0.109	0.936 $\pm$ 0.099	0.918 $\pm$ 0.112	< 0.993

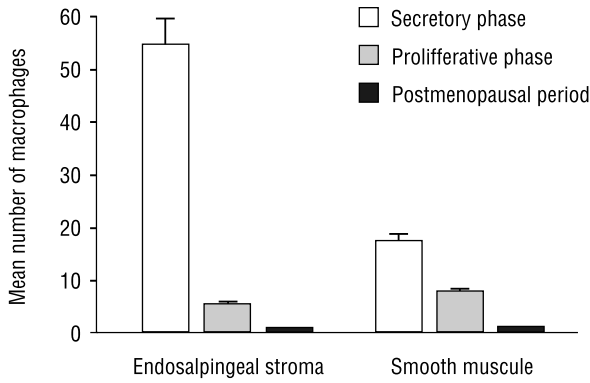
**Table 5.** Mean number of macrophages in different segments of the fallopian tube at different phases of the menstrual cycle and postmenopausal period in the smooth muscle layer (n = 30); values are presented as means  $\pm$  SEM

	Isthmus	Ampulla	Fimbria	Significance
Secretory	17.900 $\pm$ 1.643	17.858 $\pm$ 1.645	17.875 $\pm$ 1.634	< 1.000
Proliferative	6.914 $\pm$ 0.483	6.929 $\pm$ 0.474	6.971 $\pm$ 0.483	< 0.996
Postmenopausal	1.055 $\pm$ 0.112	1.036 $\pm$ 0.102	1.046 $\pm$ 0.119	< 0.993

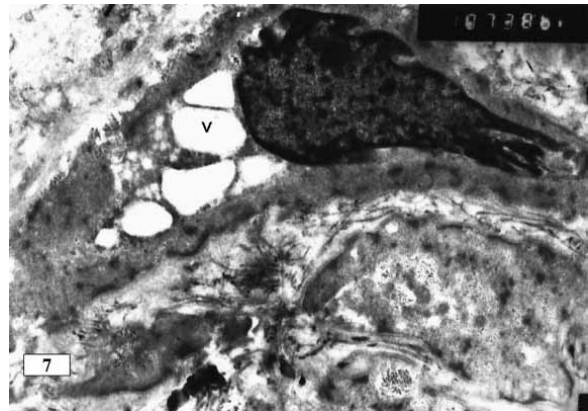
portions of the fallopian tubes during the reproductive and postmenopausal periods in the endosalpingeal stroma (Table 4) or in the smooth muscle layer (Table 5).

#### Electron microscopic examination in the reproductive and postmenopausal periods

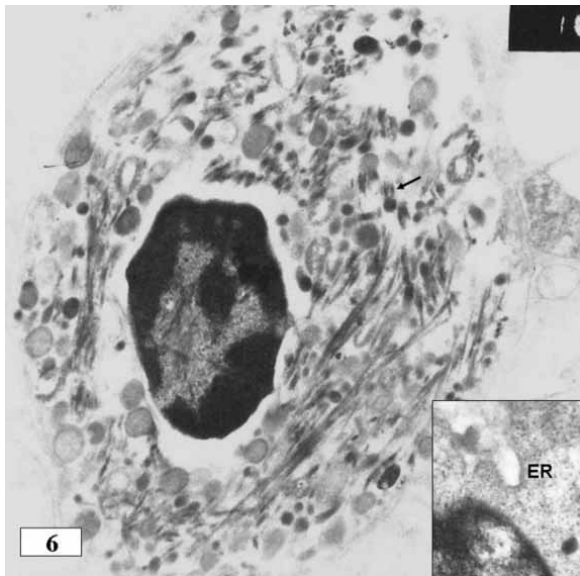
The macrophages within the fallopian tube in both the reproductive (Fig. 6, 7) and postmenopausal



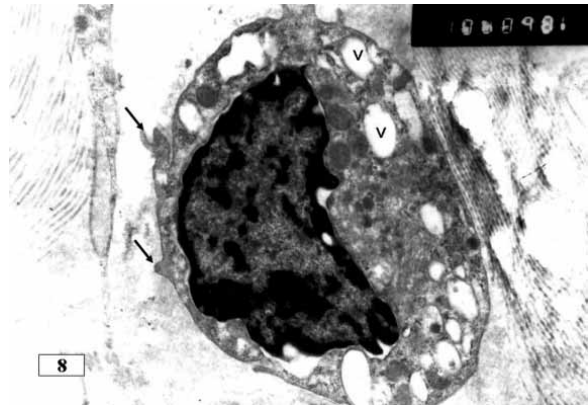
**Figure 5.** CD 68-positive macrophages were significantly increased in number during the secretory phase in the endosalpingeal stroma and smooth muscle layer as compared to the proliferative phase of the menstrual cycle and postmenopausal period.



**Figure 7.** A macrophage at the proliferative phase showing the indented nucleus, well developed organelles, and cytoplasmic vacuoles (V) of various sizes and configurations. Lead citrate and uranyl acetate;  $\times 10,000$ .

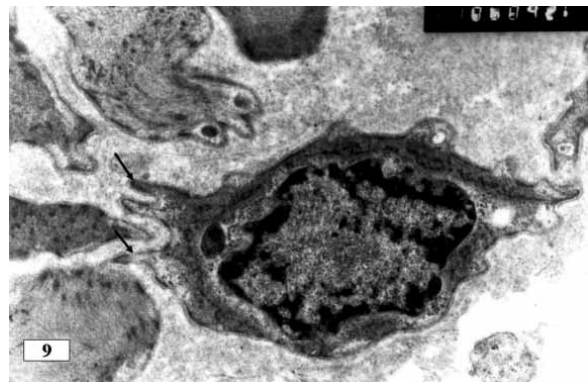


**Figure 6.** A macrophage at the secretory phase with an oval nucleus and well developed organelles ( $\times 10,000$ ). Many cisternae of rough endoplasmic reticulum stuffed with ribosomes were seen ( $\rightarrow$ ). An inset ( $\times 13,000$ ) showing extensive rough endoplasmic reticulum (ER). Lead citrate and uranyl acetate.



**Figure 8.** A macrophage at the postmenopausal period showing the indented nucleus, less well developed organelles, cytoplasmic vacuoles (V), and short cytoplasmic processes (arrows). Lead citrate and uranyl acetate;  $\times 10,000$ .

periods (Fig. 8) were round to oval and varied in dimensions from  $7.6 \mu\text{m}$  to  $9.5 \mu\text{m}$  transversely and from  $6.7 \mu\text{m}$  to  $10.9 \mu\text{m}$  longitudinally. They had oval or indented nuclei and well developed organelles with primary and secondary lysosomes, mitochondria, rough endoplasmic reticulum, centriole, Golgi apparatus and cytoplasmic vacuoles of various sizes and configurations, which were presumably lysosomal in nature. Cell processes were noted in many cells. However, the macrophages of the secretory phase showed more rough endoplas-



**Figure 9.** A macrophage at the secretory phase showing the cytoplasmic extension resembling pseudopods (arrows). Lead citrate and uranyl acetate;  $\times 10,000$ .

mic reticulum (Fig. 6) and extensions of the cytoplasm resembling pseudopods (Fig. 9).

## DISCUSSION

The oviduct is a key component of the reproductive system, where essential states such as spermatozoa capacitation, fertilisation and early embryonic development take place. The oocyte is picked up at the fimbriated portion and *in vivo* fertilisation occurs in the ampullary portion of the oviduct in humans. The functional state of the uterine tubes, therefore, has an intimate role in fertility and reproduction, and histopathological cytology may provide new information in this field. Recently, macrophages have been observed to play a role in the pathogenesis of infertility. Honda et al. [15] reported the enhancement *in vitro* of mouse embryonic development by culture with peritoneal macrophages. The macrophages might control fertilisation by secretion of several cytokines that are known to affect cell function [31].

There have been few reports concerning the distribution of macrophages in the human fallopian tubes. In this study the macrophages were found in the different parts of the fallopian tubes. There were no significant differences in the numbers of macrophages in the different tubal segments. Macrophages were found in the tubal stroma, smooth muscle, and arterial endothelial and smooth muscle cells. This is in agreement with Zhao and Chegini [34], Suenaga et al. [29] and Palter et al. [26]. In this work a significant difference in the number of macrophages was shown between the proliferative and secretory phases of the menstrual cycle as well as in the postmenopausal period. In the secretory phase the number of macrophages was significantly higher. This is in agreement with the quantitative studies of Suenaga et al. [29] and Zhao and Chegini [34]. These results have suggested that the macrophages are potentially regulated by ovarian hormones and imply their importance in a variety of tubal functions and possibly in early embryonic development. Givan et al. [13] have also observed that the uterine endometrium from post-menopausal women has fewer leukocytes than the uterine endometrium from pre-menopausal women. In contrast, the qualitative studies of Palter et al. [26] mentioned that there were no significant differences in the number of macrophages in the uterine tubes from the different phases of the menstrual cycle. With regard to the ovary the quantitative studies of Takaya et al. [30] demonstrated that the number of macrophages increased in the mid and late corpora lutea, and reached a maximum in the early degenerating corpus luteum, indicating their role as scavengers. In addition, Kaushic et al. [19] found that macrophages were present in large numbers in the

stroma of the endometrium and around the glandular epithelium in the uterus at oestrus, the stage of the reproductive cycle when oestradiol levels are known to be high, relative to those seen at diestrus, when oestrogen levels are low and progesterone is the predominant hormone. The differences between the number of macrophages in the uterine tube, the endometrium and the ovary at different stages of the oestrous cycle may be attributed to the differences in their role within the female reproductive system. In this work the macrophages were observed in the secretory phase to be in a higher number in the endosalpingeal stroma than in the smooth muscle layer. This observation was also reported by Suenaga et al. [29].

In the present study the macrophages in the secretory phase showed more numerous well-developed intracellular organelles, including rough endoplasmic reticulum and Golgi apparatus and cytoplasmic cell processes, than in the proliferative and postmenopausal phases. These ultrastructural findings were thought to be an indicator of activated macrophages [12]. Our findings are in agreement with those of Suenaga et al. [29], who mentioned that during this period the uterine tubes move by contraction of the smooth muscle. Prostaglandins might be produced by macrophages in the tubes and influence the smooth muscle.

Calogero et al. [7] have shown that several macrophage-derived cytokines are present in the follicular fluids of infertile women. Patients with infertility due to immunological causes had higher levels of interleukin-6 and lower concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF) compared to patients with a tubal factor of infertility. In addition, Mulayim et al. [24] found that the interleukin-8 expression of tubal epithelial and stromal cells in response to inflammatory cytokines such as interleukin-1 $\alpha$  and tumour necrosis factor  $\alpha$  were increased. This may be an important factor in the pathogenesis of salpingitis, which may result in infertility and ectopic pregnancy. Palter et al. [26] have mentioned that macrophages in the uterine tube produce interleukin-8, which may have an autocrine growth-regulating and/or chemokine activity that may participate in this condition.

The present work clarifies the detailed distribution of macrophages in normal fallopian tubes in order to build a base line for further studies of macrophages in pathological conditions of the uterine tubes. The findings of the present study also clarify the possible role of macrophages in human fallopian



tubes without histopathological changes. Macrophages are introduced via the bloodstream during the secretory phase, and the quantitative study in the present investigation shows that they are important components of the normal function of the uterine tube, including the fertilisation process.

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