

*Original Research*

# Reduced Numbers of Nerve Fibers in the Oviduct Ampulla of Women with Tubal Ectopic Pregnancy

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## Abstract

**Background:** The oviduct plays a major role in fertility by facilitating the movement of gametes to promote fertilization and passage of the embryo for implantation. In the present study, we compared the distribution of nerve fibers in the ampulla of the oviduct tube in patients who presented with and without ectopic pregnancy (EP). Our aim was to acquire a better understanding of the pathologies underlying EP. **Methods:** We recruited 25 patients with EP (representing group A) and 25 patients who underwent hysterectomy for benign gynecological diseases (representing group B). None of the recruited women had a previous history of induced abortion. We acquired the oviduct ampulla from each patient in the two groups and used immunohistochemistry to detect S100 and protein gene product 9.5 (PGP9.5) and reveal abnormal distributions of nerve fibers within the fallopian tubes. **Results:** There was no statistical difference between the two groups in terms of the proportion of S100- and PGP9.5-immunoreactive nerve fibers in the mucosal, muscular and serosal layers of the oviduct ampulla. However, the proportions of S100- and PGP9.5-immunoreactive nerve fibers were reduced in the mucosal, muscular and serosal layers of the oviduct ampulla in women with EP when compared to women without EP. **Conclusions:** The reduced density of nerve fibers in the endometrial and muscular layer of the ampulla of the fallopian tube, as detected by PGP9.5 and S100, may play a significant role in the pathologies underlying ectopic pregnancy.

**Keywords:** ectopic pregnancy; fallopian tube; PGP9.5; S100

## 1. Introduction

Ectopic pregnancy (EP) is a form of pregnancy in which the zygote is implanted outside of the normal uterine cavity and accounts for approximately 1.5 to 2% of all pregnancies; over 95% of EPs are located in the fallopian tubes (FTs) [1]. The maternal morbidity and mortality arising from cases of EP has created a significant health and economic burden worldwide. Our knowledge of the underlying causes of this condition has increased over recent years and the most accepted hypothesis is retention of the fertilized egg within the FTs due to alterations in the tubal condition and impaired embryo-tubal transport, thus resulting in early embryo implantation. Over recent years, the rates of medical abortion, artificial abortion and previous chronic pelvic inflammatory disease (PID) as increased; this is associated with a mild increase in the incidence of EP [2]. The exact mechanism underlying EP induced by artificial abortion remains unclear. If the cervix is pulled excessively during artificial abortion, it can easily damage the uterosacral ligament and may damage nerves on the ligament surface. Excessive suction or curettage can also readily cause damage to the nerves between the endometrium and the myometrium. In a previous study, Neamtu *et al.* [3] suggested that the motility of the FTs was influenced by both nerves and hormones. Several neuropeptides, such as neuropeptide Y-immunoreactivity (NPY), have been de-

tected in nerve fibers in the FTs [4]. For example, Jankovic *et al.* [5] detected some nerves fibers and neuropeptides in the FTs which were considered to regulate the surface epithelium, vasculature and smooth muscle, thus controlling the beat activity of cilia and the contractility of the smooth muscle in the FTs [6]. Moreover, the abnormal distribution of nerve fibers in the FTs may result in dysfunctional motion in the tube; this condition is closely related to EP.

Based on studies published by the pioneers mentioned above, we hypothesized that EP was also associated with nerve fiber damage. In our study, we detected damaged nerve fibers in the FTs of patients by immunohistochemical staining methods using highly specific biomarkers of nerve fibers, a polyclonal rabbit protein gene product 9.5 (PGP9.5) antibody and an S100 antibody.

The purpose of our study was to compare the distribution and density of nerve fibers in FTs between patients with and without EP. Our aim was to acquire a better knowledge of tubal pathologies such that we can improve the reproductive and sexual health of women.

## 2. Material and Methods

### 2.1 Patient Selection

This was a retrospective immunohistochemical study carried out in a single center. Oviductal ampulla tissues were obtained from 50 patients undergoing laparoscopic



**Table 1. Patient's demographics, and PGP9.5 and S100-immunoreactive nerve fibers in the oviduct ampulla.**

Characteristics	Group A (n = 25)	Group B (n = 25)	p value
Age (years)	26.68 ± 3.30	28.84 ± 4.47	0.058
BMI (kg/m <sup>2</sup> )	20.68 ± 1.49	21.17 ± 1.81	0.307
Abortion	1.5 (0–5)	1 (0–4)	0.628
S100 percentage			
Mucosal layer	80% (20/25)	100% (25/25)	0.059
Muscular layer	88% (22/25)	100% (25/25)	0.234
Serosal layer	92% (23/25)	100% (25/25)	0.470
PGP9.5 percentage			
Mucosal layer	84% (21/25)	100% (25/25)	0.118
Muscular layer	88% (22/25)	100% (25/25)	0.234
Serosal layer	84% (21/25)	100% (25/25)	0.118
S100 nerve fiber density (/mm <sup>2</sup> )			
Mucosal layer	6.51 ± 3.52	11.84 ± 3.28	0.007
Muscular layer	11.29 ± 4.18	19.89 ± 4.12	0.001
Serosal layer	7.86 ± 3.12	14.48 ± 4.02	<0.001
PGP9.5 nerve fiber density (/mm <sup>2</sup> )			
Mucosal layer	5.98 ± 2.32	10.21 ± 3.02	0.008
Muscular layer	10.58 ± 3.19	18.49 ± 3.82	0.002
Serosal layer	6.22 ± 2.95	11.42 ± 3.24	0.006

PGP9.5, protein gene product 9.5; BMI, body mass index.

salpingectomy for EP (group A; n = 25) and due to other benign gynecological diseases (group B; n = 25), who were treated at the Jinhua Municipal Central Hospital between January 2022 and December 2022. Prior to recruitment, all women provided written and informed consent, and our study was approved by the Ethics Committee of Jinhua Municipal Central Hospital (ethical approval number: 2022-16). The inclusion criteria were as follows: (1) patients aged between 20 and 45 years; (2) patients with no previous history of induced abortion; (3) patients with benign gynecological diseases, including severe squamous intraepithelial neoplasia, cervical carcinoma *in situ*, adenomyosis and myoma, and patients in the luteal phase; (4) patients in which the appearance of the FTs was normal, and post-operative pathology confirmed that the FTs were normal. Patients with a previous history of FT disease or surgery, pelvic inflammation or chronic pelvic pain were excluded.

## 2.2 Histology and Immunohistochemistry

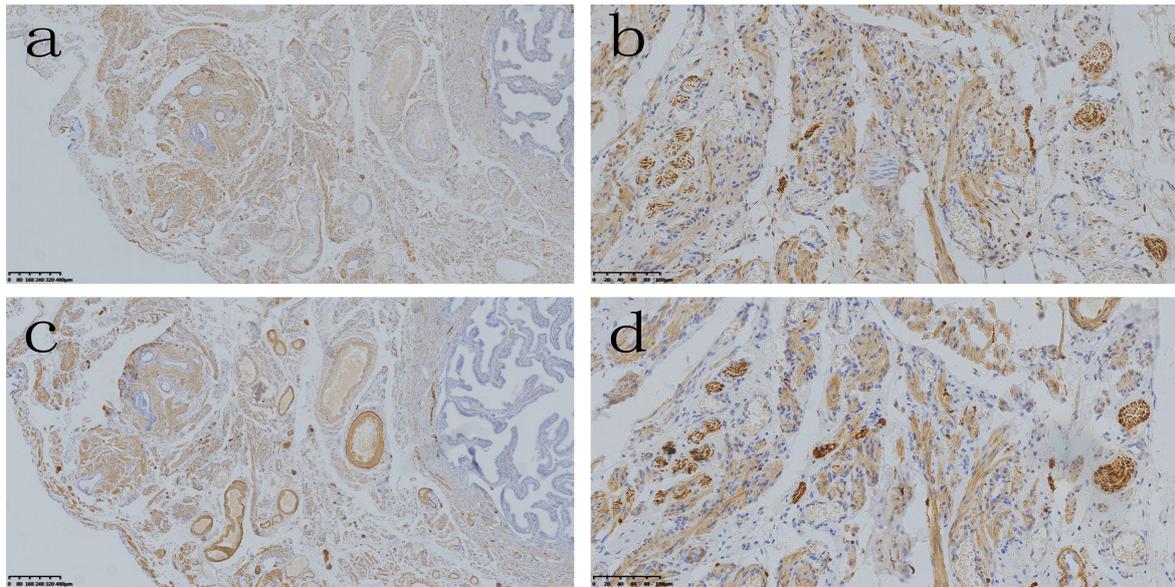
After surgery, all oviduct ampulla tissues were fixed in formalin for 18–24 h and then processed with paraffin wax. Then, we cut five tissue sections (6 µm thick) from each tissue which were then stained with hematoxylin and eosin and stained by immunohistochemistry. Specific markers (S100 and PGP9.5) were used in the immunohistochemistry experiments to determine the presence of nerve fibers in the ampulla.

Immunohistochemistry was performed as described previously by Kelm *et al.* [7]. The tissue sections were immunostained with a polyclonal rabbit PGP9.5 antibody (1:200 dilution; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) and a monoclonal mouse anti-

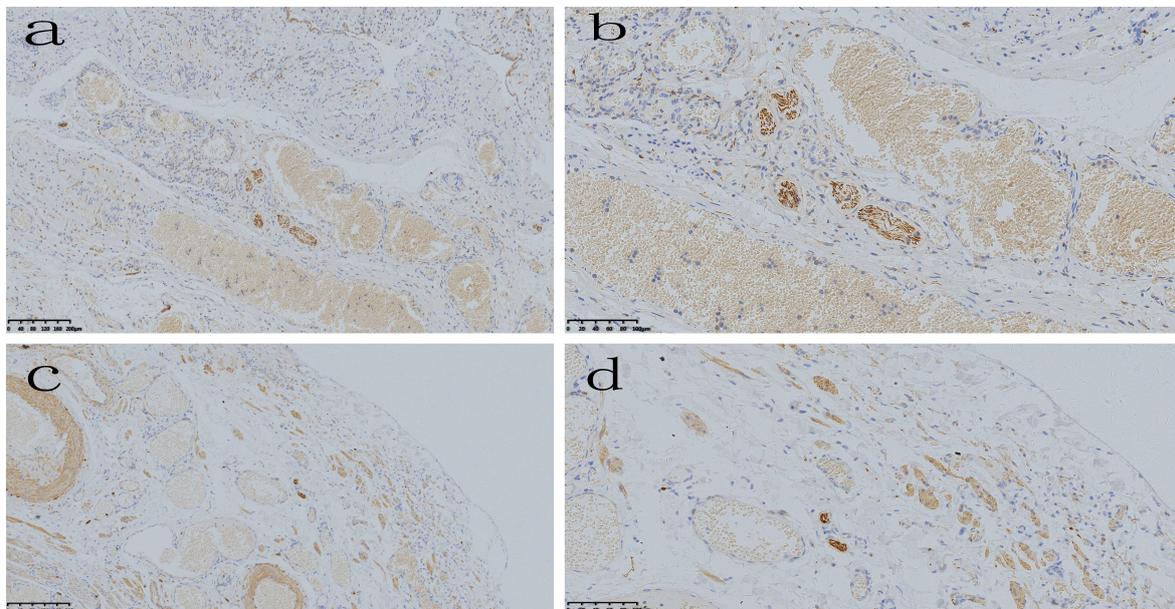
human S100 antibody (1:200 dilution; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) at room temperature for 60 min. Then, the tissue sections were washed in phosphate-buffered saline (PBS) and incubated with Pv-6000 goat anti rabbit/D and mouse IgG/HRP polymer (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) for 60 min. The antigen-antibody reaction was detected by diaminobenzidine (DAB) as a chromogen (GK346810; Novocastra, Beijing, China). Following washing, the sections were counterstained with hematoxylin, dehydrated, and mounted with medium. If no immunoreactive nerve fibers were detected, then the case was designated as a negative case; otherwise, cases were designated as being positive; in such cases, we quantified the proportion of immunoreactive nerve fibers as a proportion of the total number of nerve fibers.

## 2.3 Comparison of Nerve Fiber Density

First, we counted the total number of positive nerve fibers identified by S100 and PGP9.5 staining in ampulla [8–10]. After staining, we scanned the tissue sections at low magnification (40× or 100×) (Leica Microsystems, Wetzlar, Germany) to confirm “hot spots”, in other words, the areas with most significant nerve innervation. Within this defined area, the selected nerve fibers were counted at high power (200×) to yield an accurate nerve count. The total number of fibers was then divided by the total number of hot spots in each area, thus yielding an average number of nerve fibers per hot spot. The final results were given as the mean number of nerve fibers in each section from all tissues. Hot spots were detected in the mucosal, muscular and serosal layers of the FTs.



**Fig. 1.** PGP9.5 (a 40 $\times$ , b 200 $\times$ ) and S100 (c 40 $\times$ , d 200 $\times$ ) nerve fibers in the oviduct ampulla of women without tubal ectopic.



**Fig. 2.** PGP9.5 (a 100 $\times$ , b 200 $\times$ ) and S100 (c 100 $\times$ , d 200 $\times$ ) nerve fibers in the oviduct ampulla of women with tubal ectopic.

#### 2.4 Statistical Analysis

SPSS version 20.0 (SPSS, Inc., IBM, Chicago, IL, USA) was used to perform all statistical analyses. Data are described as the means  $\pm$  standard deviations (SDs), medians (with range), or absolute proportions (%). Differences in the means between the two groups were tested by analysis of variance (ANOVA). All  $p$  values were two-tailed, and  $p < 0.05$  was considered as statistically significant.

### 3. Results

No statistical differences were detected between the two groups in terms of body mass index (BMI), age, or the number of previous abortions ( $p > 0.05$ , Table 1). PGP9.5-

immunoreactive nerve fibers were detected in all 25 patients of group B in the mucosal, muscular and serosal layers of the oviduct ampulla (100%, 25/25). PGP9.5-immunoreactive nerve fibers were also detected in women with EP, accounting for 84%, 88% and 84% in the mucosal, muscular and serosal layers of the ampulla tissue, respectively (Figs. 1,2). However, we found no statistical difference between the two groups in terms of the proportion of PGP9.5-immunoreactive nerve fibers in the mucosal, muscular and serosal layers of the oviduct ampulla ( $p > 0.05$ , Table 1). There were significant differences in the density of PGP9.5-immunoreactive nerve fibers in women with EP when compared to women without FP in the serosal ( $6.22 \pm$

2.95/mm<sup>2</sup> versus 11.42 ± 3.24/mm<sup>2</sup>,  $p = 0.006$ ; Table 1), muscular (10.58 ± 3.19/mm<sup>2</sup> versus 18.49 ± 3.82/mm<sup>2</sup>,  $p = 0.008$ ; Table 1), and mucosal layer (5.98 ± 2.32/mm<sup>2</sup> versus 10.21 ± 3.02/mm<sup>2</sup>,  $p = 0.002$ ; Table 1).

Similar to PGP9.5, there were no significant differences between the two groups in the serosal, muscular and mucosal layers in the density of S100-immunoreactive fibers in the oviduct ampulla ( $p > 0.05$ ; Figs. 1,2). However, there was a significant reduction in the density of S100-immunoreactive nerve fibers in the mucosal, muscular and serosal layers of the ampulla in group B (the EP group;  $p < 0.05$ , Table 1).

#### 4. Discussion

It is reported that over 98% of EPs occur in the FTs and that a proportion of these patients are difficult to diagnose until symptoms occur, mostly because of intra-abdominal bleeding [6]. The FTs is the most common site for EP, although other reported sites include the cornua uteri, cervix, ovary, broad ligament, and the interstitial portion of the uterus [1]. The current therapies for EP are drug therapy (mifepristone or methotrexate) and surgical interventions; however, acquiring a more enhanced knowledge of the pathogenesis of EP could avoid these situations by providing better prediction and prevention for at-risk patients [11]. In a previous study, Shao *et al.* [12] performed animal experiments related to FT biology and provided an enhanced knowledge of the mechanisms underlying EP; however, such studies cannot be performed in humans. Thus, the most significant area of confusion relates to why some women develop EP while others do not. Understanding the molecular and cellular mechanisms underlying the implantation of embryos in the FTs will lead to the development of better diagnostics and therapies for EPs [13].

The uterine nerves pass through the pelvic cavity and then through the uterosacral-cardinal ligament complex and uterine vessel before entering the uterus. The myometrium receives sparse innervation from endometrial-myometrial and sub-serosal nerve fibers, which also supply plexus to the muscle layers of the FTs. Furthermore, the distal FTs receive additional innervation from the ovarian nerves through the mesosalpinx. Therefore, injuries to the endometrial-myometrial nerves would have significant impact on the nervous supply to the FTs [14,15]. A reduction of nerve fibers in the FTs may affect the secretory function of epithelial cells and the activity of ciliary cells; these events may play a significant role in the pathogenesis of EP [6].

S100 is an immunohistochemical marker of Schwann cells and represents a reliable and sensitive marker of nerve damage [16]. In a previous study, Bacchi and Fleury [17] visualized peripheral nerves in tuberculoid granulomas by staining for S100. In another study, Zhang *et al.* [10] reported that PGP9.5 was a highly specific pan-neuronal marker that stained both sensory nerve fibers and autonomic

nerve fibers. S100 and PGP9.5 may, therefore, be considered as reliable diagnostic markers for nerve damage in the FTs. However, only a few of the existing have attempted to determine the role of S100 in the diagnosis of nerve damage in EP. Our present study showed that nerve fibers that were positive for S100 and PGP9.5 staining were present in the serosal, muscular and mucosal layers of the oviduct ampulla. Furthermore, when compared with women without EP, S100- and PGP9.5-immunoreactivity were both significantly reduced in each layer of the ampulla tissue in cases with EP. Moreover, Zhu *et al.* [18] reported the presence of PGP9.5-positive nerve fibers in smooth muscle strands, beneath the surface epithelium of the ampulla tissue, and in the vascular smooth muscle, thus suggesting that these may supply the surface epithelium, the smooth muscle, and the vasculature of the FTs. Abnormalities of the nerve plexus may be involved in the mechanisms that facilitate movement of the gametes; this could result in the pathogenesis of EP. On the other hand, the reduction of PGP9.5 and S100 in the mucosal and muscular layers of the FTs suggests that injury to the nerve fibers in the ampulla tissue may negatively impact on the release of transmitters including acetylcholine (ACh), adrenaline, noradrenaline, and the modulation of the nerve reflex. These events may reduce oviduct motility by influencing the vascular muscle contractility, the tubal smooth muscle, ciliary beat activity and the secretory processes of the mucosal epithelial cells [3], thus resulting in the pathogenesis of FP.

However, our study was retrospective, thus creating a notable limitation. Furthermore, most tissues were fixed and stored for approximately 1–2 years prior to analysis. This may be the reason why some tissues were negative for PGP9.5 and S100 staining. Further prospective studies need to be performed to overcome these limitations.

#### 5. Conclusions

Our analysis showed that the density of nerve fibers stained with S100 and PGP9.5 in the oviduct ampulla tissue were significantly reduced in patients with EP. Our results suggested that nerve injury in the walls of the FTs may represent one of the causes of EP although further prospective studies are needed to confirm these findings.

#### Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available, because we still have some research on ectopic pregnancy in the future, but are available from the corresponding author on reasonable request.

#### Author Contributions

FT: substantial contributions to the conception; FT, LJi: design of the work; LJin, MH: the acquisition, analysis, and interpretation of data for the work; LJi, LJin: drafting

the manuscript; FT, MH: revising it critically for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

### Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Affiliated Jinhua Hospital, Zhejiang University School of Medicine (ethics approval number: 2022-16). The Statements of Informed Consent was provided and written by the patients before surgery.

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### Conflict of Interest

The authors declare no conflict of interest.

### References

- [1] Vang R, Wheeler JE. Diseases of the Fallopian Tube and Paratubal Region. In Kurman RJ, Ellenson LH, Ronnett BM (eds.) *Blaustein'S Pathology of the Female Genital Tract* (pp. 529–578). Springer: Boston, MA. 2011.
- [2] Walker JJ. Ectopic pregnancy. *Clinical Obstetrics and Gynecology*. 2007; 50: 89–99.
- [3] Neamțu MC, Neamțu RL, Avramescu ET, Vrabete M., Călina LM, Mindrilă I. Contributions to myometrium study in uterine-tubal junction. *Romanian Journal of Morphology and Embryology*. 2009; 50: 675–681.
- [4] Heinrich D, Reinecke M, Gauwerky JFH, Forssmann WG. Immunohistochemical and biological evidence for a neuromodulator function of neuropeptide Y in the human oviduct. *Archives of Gynecology and Obstetrics*. 1987; 241: 127–132.
- [5] Jankovic SM, Protic BA, Jankovic SV. Contractile effect of acetylcholine on isolated isthmic segment of fallopian tubes. *Methods and Findings in Experimental and Clinical Pharmacology*. 2004; 26: 87–91.
- [6] Shaw JLV, Dey SK, Critchley HOD, Horne AW. Current knowledge of the aetiology of human tubal ectopic pregnancy. *Human Reproduction Update*. 2010; 16: 432–444.
- [7] Kelm AR, Lancellotti CLP, Donadio N, Auge APF, Lima SMRR, Aoki T, *et al.* Nerve fibers in uterosacral ligaments of women with deep infiltrating endometriosis. *Journal of Reproductive Immunology*. 2008; 79: 93–99.
- [8] Weidner N. Current pathologic methods for measuring intratumoral microvessel density within breast carcinoma and other solid tumors. *Breast Cancer Research and Treatment*. 1995; 36: 169–180.
- [9] Zhang X, Yao H, Huang X, Lu B, Xu H, Zhou C. Nerve fibres in ovarian endometriotic lesions in women with ovarian endometriosis. *Human Reproduction*. 2010; 25: 392–397.
- [10] Zhang X, Lu B, Huang X, Xu H, Zhou C, Lin J. Innervation of endometrium and myometrium in women with painful adenomyosis and uterine fibroids. *Fertility and Sterility*. 2010; 94: 730–737.
- [11] Shao R, Feng Y, Zou S, Weijdegård B, Wu G, Brännström M, *et al.* The role of estrogen in the pathophysiology of tubal ectopic pregnancy. *American Journal of Translational Research*. 2012; 4: 269–278.
- [12] Shao R. Understanding the mechanisms of human tubal ectopic pregnancies: new evidence from knockout mouse models. *Human Reproduction*. 2010; 25: 584–587.
- [13] Shao R, Zou S, Wang X, Feng Y, Brännström M, Stener-Victorin E, *et al.* Revealing the hidden mechanisms of smoke-induced fallopian tubal implantation. *Biology of Reproduction*. 2012; 86: 131.
- [14] Zhang X, Xu H, Huang X, Quinn M. Altered innervation of the fallopian tube in adenomyosis. *Journal of Obstetrics and Gynecology*. 2015; 35: 319–320.
- [15] Atwal G, du Plessis D, Armstrong G, Slade R, Quinn M. Uterine innervation after hysterectomy for chronic pelvic pain with, and without, endometriosis. *American Journal of Obstetrics and Gynecology*. 2005; 193: 1650–1655.
- [16] Miko TL, Gschmeissner SE. *Histological Methods for Assessing Myelin Sheaths and Axons in Human Nerve Trunks*. Biotechnic and Histochemistry. 1994; 69: 68–77.
- [17] Fleury RN, Bacchi CE. S-100 protein and immunoperoxidase technique as an aid in the histopathologic diagnosis of leprosy. *International Journal of Leprosy*. 1987; 55: 338–344.
- [18] Zhu L, Huang Q, Huang X, Zhang J, Xu H, Zhang X. Decreased nerve fibers in the oviduct isthmus of women with endometriosis. *Acta Histochemica*. 2014; 116: 871–877.