

Ki-67, oestrogen receptor, and progesterone receptor proteins in the human *rete ovarii* and in endometriosis

M S Khan, A R Dodson, M K Heatley

Abstract

Aim—To examine proliferative activity using the Ki-67 protein, oestrogen receptor protein, and progesterone receptor protein expression in the *rete ovarii*, and to make comparisons with their expression in endometriosis.

Methods—Immunohistochemistry was used to study the *rete ovarii* in 24 cases and endometriosis in seven cases, using antibodies to Ki-67 protein (growth fraction (GF) quantified using a point score method) and oestrogen receptor and progesterone receptor (quantified using the H score method).

Results—There was no evidence of a significant difference in the Ki-67 protein, oestrogen receptor, and progesterone receptor in the *rete ovarii* in different phases of the menstrual cycle (proliferative phase: GF = 1.052, oestrogen receptor H score = 13.4, progesterone receptor H score = 15.32; secretory phase: GF = 0.736, oestrogen receptor H score = 7.5, progesterone receptor H score = 1.84). The expression of all three proteins was greater in the foci of endometriosis (GF = 6.99, oestrogen receptor H score = 152.02, progesterone receptor H score = 127.36) than in the *rete ovarii* ($p < 0.0005-0.0008$, Mann-Whitney U test).

Conclusions—There is a low rate of cellular proliferation in the *rete ovarii* and this structure shows less responsiveness to hormone stimulation than foci of endometriosis. These differences may provide a useful tool to distinguish the *rete ovarii* from endometriosis in cases of diagnostic difficulty.

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Keywords: *rete ovarii*; Ki-67; oestrogen receptor protein; progesterone receptor protein

The *rete ovarii* is a group of epithelial tubules located at the hilum of the ovary. It is

Table 1 Antisera and antibodies used to detect the proliferation marker Ki-67 protein, oestrogen receptor protein, progesterone receptor protein, and cytokeratin in five Fallopian tubes

Clone	Reagent type	Antigen detected	Pretreatment	Dilution	Supplier
NCL-Ki67p	Polyclonal	Ki-67 protein	Microwave	1:500	Novocastra, Newcastle upon Tyne, UK
IA6	Monoclonal	Progesterone receptor protein	Microwave	1:30	Novocastra, Newcastle upon Tyne, UK
ID5	Monoclonal	Oestrogen receptor protein	Microwave	1:30	Dakopatts, Glostrup, Denmark
MNF116	Monoclonal	Cytokeratin	Trypsin	1:50	Dakopatts, Glostrup, Denmark

commonly identified as an incidental finding in histological sections of the ovary obtained from patients with a range of gynaecological conditions. Rarely it gives rise to cysts and to benign and malignant tumours.¹⁻³ Its major importance in human diagnostic practice is said to be in distinguishing it from endometriosis.⁴ While there are histological criteria for distinguishing endometriosis from other conditions, the distinction may at times be difficult.⁵ The immunohistochemical profile of the *rete ovarii* has been studied in humans and animals and it has been shown to express cytokeratin and vimentin intermediate filament proteins,⁶⁻⁸ desmoplakin, and laminin. Although characterised in endometriosis,^{9, 10} the proliferative activity and expression of oestrogen receptor and progesterone receptor proteins have not been described in the *rete*. The capacity of different tissues to respond to steroid hormones has been evaluated mainly according to the concentration of oestrogen receptor and progesterone receptor. Assays of these receptors have been used in determining the best treatment of various diseases, in particular carcinoma of the breast.⁹

Our aim in this study was to examine proliferative activity of the cells composing the *rete ovarii* using the Ki-67 protein (which is expressed in all stages of the cell cycle except G0 and early G1, reaching a peak at G2 and M phases¹¹) together with oestrogen receptor and progesterone receptor proteins, and to establish their value in distinguishing the *rete ovarii* from foci of endometriosis.

Methods

Thirty one patients who had had total abdominal hysterectomies with bilateral salpingo-oophorectomies were selected for the study. Patients with malignant conditions were excluded. The specimens were dissected and blocks selected according to the departmental dissection protocol. Blocks were taken sagittally across the ovary and fallopian tube to include the mesovarium and mesosalpinx, and the *rete*

Department of Pathology, Royal Liverpool Hospital, Fifth Floor, Duncan Building, Prescot Street, Liverpool L7 8XP, UK
M S Khan
A R Dodson
M K Heatley

Correspondence to: Dr Heatley.

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ovarum was sought microscopically. In 24 cases in which there was no evidence of endometriosis or neoplasia in the specimen, the *rete ovarum*

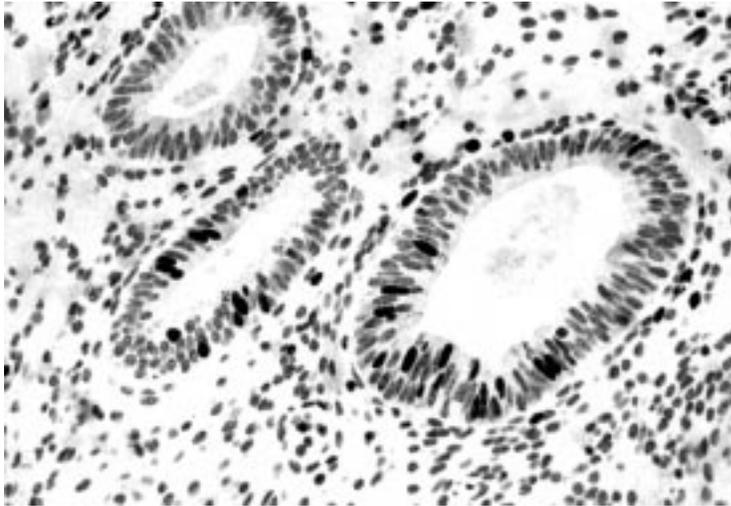


Figure 1 The antibody to Ki-67 has reacted with nuclei in the epithelium lining glands and the stroma in foci of endometriosis.

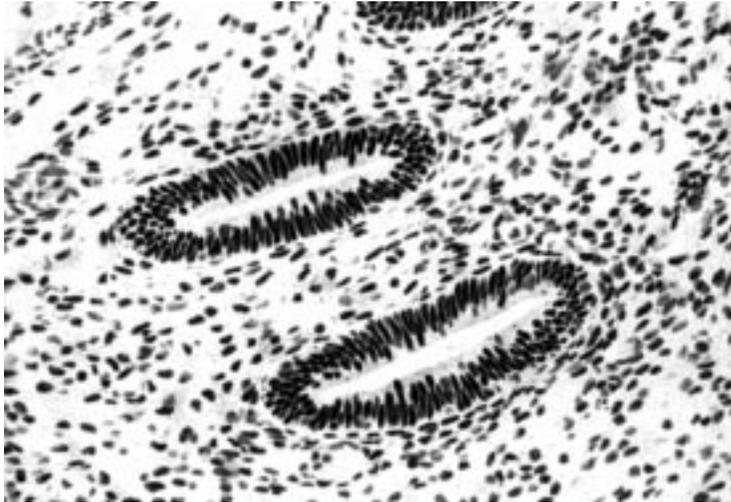


Figure 2 Extensive and intense reaction with the antibody to oestrogen receptor protein in endometriosis.

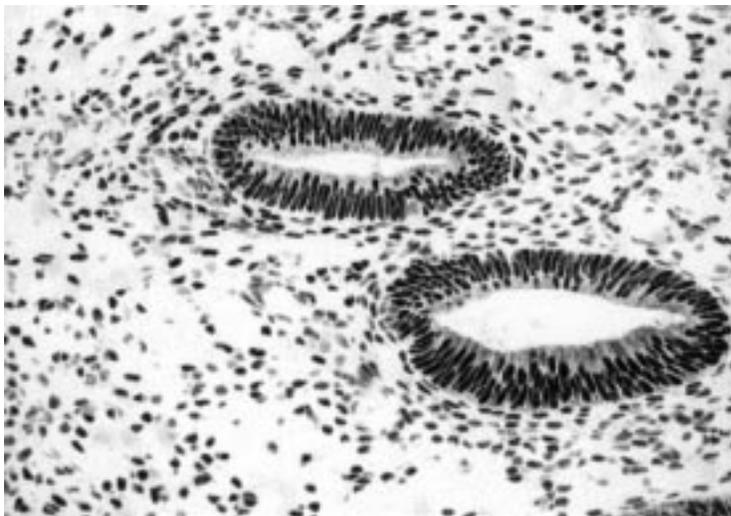


Figure 3 Extensive and intense staining with the antibody to progesterone receptor protein in endometriosis.

was identified for further study. In five patients the fallopian tube was extensively sampled to permit comparison between Ki-67, oestrogen receptor, and progesterone receptor expression in this structure, acting as a control, with that in the *rete ovarum*. Seven patients with endometriosis related to the ovary or fallopian tube were also studied.

Following routine processing, 5 µm sections were mounted on 3'3'-aminopropyl-triethoxysaline (APES) coated slides. The sections were incubated in primary antisera/antibodies at the stated dilutions (table 1) following antigen retrieval by either microwaving the sections in 10 mM ethylenediamine-tetra-acetic acid (EDTA) for 25 minutes at high power (600 W microwave) or trypsin pretreatment for 20 minutes at 37°C. The reaction was detected using the Dako Envision system (Dakoplats) and diaminobenzidine (DAB) with H₂O₂. Slides were counterstained with haematoxylin. A positive reaction was detected with each antibody when there was distinct brown staining in the nuclei (Ki-67, oestrogen receptor, progesterone receptor) or cytoplasm (MNF 116) of the epithelial cells, faint staining being regarded as negative.

The presence of a reaction was quantified in two ways, depending upon the antibody being examined. The Ki-67 growth fraction was quantified using a simple point scoring system, with the number of positive cells expressed as a percentage of the total counted.¹² The second method employed the H score technique. This method seeks to quantify the actual concentration of protein present in the tissue, because in addition to calculating the proportion of positive cells, each cell is assigned a score from 0 to 3 depending on the intensity with which it has reacted to the antibody, as indicated by its staining with DAB. The score (0–3) is multiplied by the percentage of cells which stain with each level of intensity, and the sum of these mathematical products is expressed as a score of 0–300. A close association between the results of biochemical methods of quantifying the concentration of hormone receptor proteins and the H score has been demonstrated,^{13 14} and has been used previously in the study of endometriosis.¹⁰ The combination of immunohistochemistry and the H score method permits the reaction of the antibody in epithelial cells to be distinguished from that in the stroma, as the distribution of hormone receptors may differ in epithelium and stroma.⁹ The calculation of a running mean determined the optimum number of cells to be counted.¹⁵ The results were analysed using the Kruskal–Wallis test and the Mann–Whitney U test on a Statview 4.0 program (Abacus Concepts).

In addition to the usual positive and negative controls, a section from each block was stained using the anticytokeratin antibody (MNF116). This was to ensure that the immunogenicity of the sections was preserved after processing.

Results

The intensity of the reaction in foci of endometriosis (figs 1–3) was greater than in the

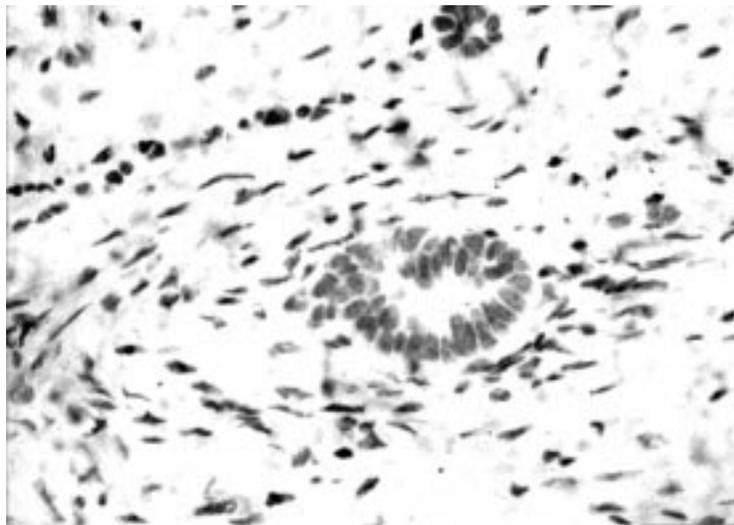


Figure 4 Very faint reaction of the antioestrogen receptor antibody to epithelial nuclei in a tubule from the rete ovarii.

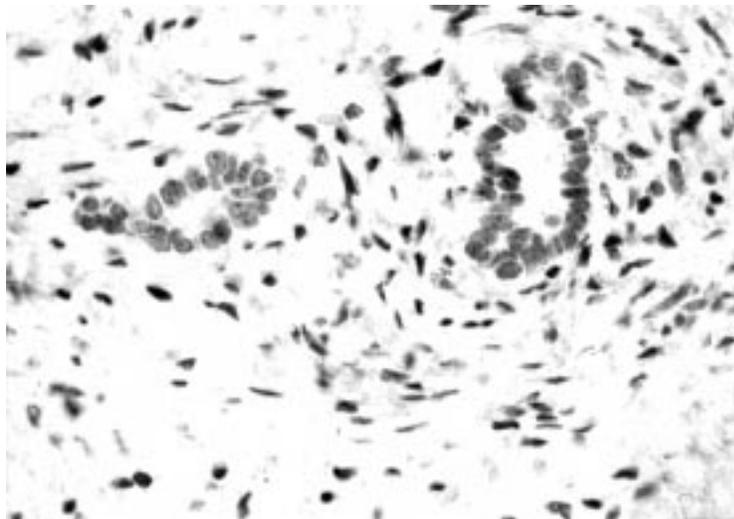


Figure 5 Only occasional epithelial cells in these tubules forming the rete ovarii have reacted with the antibody to progesterone receptors. The intensity of staining is less intense than in the foci of endometriosis.

rete ovarii (figs 4 and 5). The reactivity of the antibodies to Ki-67 protein, oestrogen receptor, and progesterone receptor is presented in table 2. The expression of these proteins in different phases of the menstrual cycle showed no evidence of a statistically significant difference. There was a greater expression of all three proteins in foci of endometriosis and in the fallopian tube, which acted as a control tissue, than in the rete ovarii. These results were statistically significant at the 5% level.

Of the seven patients with endometriosis, five were in the proliferative phase of the cycle and two were in the secretory phase. Two patients had been treated with intramuscular injections of leuporelin acetate up to three months before their operation. None was receiving hormonal therapy or treatment with hormone antagonists at the time of their operations. There was no evidence of a statistically significant difference in Ki-67, oestrogen receptor, or progesterone receptor reactivity in the endometriotic foci in the two phases of the cycle, or in patients who had or had not been treated.

Expression of cytokeratin intermediate filament proteins, as detected using the MNF116 antibody, was confirmed in the three tissue types in all the sections examined.

Discussion

The low rate of proliferation in the rete ovarii as detected using this antibody to Ki-67 suggests a lower growth rate in this tissue than in other tissues in the female genital tract, including the fallopian tube epithelium and more particularly in ectopic endometrium in foci of endometriosis. These findings may help to explain the comparative rarity with which benign and malignant tumours arise in this structure compared with other sites in the female genital tract.¹⁻³ The lower levels of oestrogen receptor and progesterone receptor in the rete also suggest a lesser degree of responsiveness to hormone stimulation in this

Table 2 Quantitation of the reaction by antibodies to Ki-67 protein, oestrogen receptor protein, and progesterone receptor protein in the rete ovarii from patients in different phases of the menstrual cycle, compared with the expression of these proteins in foci of endometriosis and the Fallopian tube

Structure	No of cases	Ki-67 point score	Antigen H score	
			Oestrogen receptor	Progesterone receptor
<i>Rete ovarii</i> (by phase of menstrual cycle)				
Proliferative	14	1.052	13.40	15.32
Secretory	8	0.736	7.50	1.84
Postmenopausal	2	0.135	2.79	1.08
All phases	24	0.870	10.84	9.87
Endometriosis	7	6.99	152.0	127.4
Fallopian tube	5	1.22	144.4	109.5
<i>Results of statistical analyses (p values)</i>				
<i>Rete ovarii</i> , in all phases of the cycle (Kruskal-Wallis test)		0.93	0.45	0.12
<i>Rete ovarii</i> , proliferative v secretory phases (Mann-Whitney U test)		0.78	0.74	0.067
<i>Rete ovarii</i> (all phases of cycle) v endometriosis (Mann-Whitney U test)		0.0005	0.0008	0.0007
<i>Rete ovarii</i> (all phases of cycle) v fallopian tube (Mann-Whitney U test)		0.043	0.0006	0.0006
Fallopian tube v endometriosis (Mann-Whitney U test)		0.062	0.94	0.81

The Ki-67 protein growth fraction was expressed as the proportion of positive cells as a percentage of the total. Oestrogen receptor and progesterone receptor expression was quantified using the H score method.

structure, which may explain the low proliferation rate.

There have been previous studies of proliferative activity using Ki-67 and oestrogen and progesterone receptor expression in eutopic endometrium and endometriosis.^{9 10 16} We are unaware of any study describing the proliferation index in the *rete ovarii* using Ki-67, or of oestrogen receptor or progesterone receptor expression in this structure. Substantial differences in Ki-67, oestrogen receptor, and progesterone receptor expression in the *rete ovarii* and in the apparently more active endometriotic foci may provide a useful tool for distinguishing these tissues in cases of diagnostic difficulty.

- 1 Scully RE, Mark EJ, McNeely BU. Case records of the Massachusetts General Hospital—case 23-1986. *N Engl J Med* 1986;**314**:1564–72.
- 2 Nogales FF, Carvia RE, Donne C, *et al.* Adenomas of the rete ovarii. *Hum Pathol* 1997;**28**:1428–33.
- 3 Rutgers JL, Scully RE. Cysts (cystadenomas) and tumors of the rete ovarii [abstract]. *Lab Invest* 1988;**58**:A79.
- 4 Russell P, Farnsworth A. *Surgical pathology of the ovaries*. Edinburgh: Churchill Livingstone, 1997:222–3.
- 5 Clement CP. Pathology of peritoneum including endometriosis and related lesions. In: Scully RE, Young RH, Crum CP, eds. *Gynecologic and obstetric pathology with clinical correlation*. Cambridge, MA: Harvard Medical School, 1995: 1–61.
- 6 Czernobilsky B, Moll R, Levy R, *et al.* Co-expression of cytokeratin and vimentin filaments in mesothelial, granulosa and rete ovarii cells of the human ovary. *Eur J Cell Biol* 1985;**37**:175–90.
- 7 Park SH, Kim I. Histogenetic consideration of ovarian sex cord–stromal tumors analyzed by expression pattern of cytokeratins, vimentin and laminin—correlation studies with human gonads. *Pathol Res Pract* 1994;**190**:449–56.
- 8 Van Den Hurk R, Dijkstra G, Van Mil FN, *et al.* Distribution of the intermediate filament proteins vimentin, keratin and desmin in the bovine ovary. *Mol Reprod Dev* 1995;**41**:459–67.
- 9 Bergqvist A, Ferno M, Skoog L. Quantitative enzyme immunoassay and semiquantitative immunohistochemistry of oestrogen and progesterone receptors in endometriotic tissue and endometrium. *J Clin Pathol* 1997;**50**:496–500.
- 10 Nisolle M, Casanas-Roux F, Donnez J. Immunohistochemical analysis of proliferative activity and steroid receptor expression in peritoneal and ovarian endometriosis. *Fertil Steril* 1997;**68**:912–19.
- 11 Gerdes J, Lemke H, Baisch H, *et al.* Cell-cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* 1984;**133**: 1710–15.
- 12 Heatley MK, Maxwell P, Whiteside C, *et al.* The diagnostic and prognostic value of the Ki67 antibody in breast disease. *Mod Pathol* 1994;**8**:889–90.
- 13 McCarty KSJ, Miller LS, Cox EB, *et al.* Estrogen receptor analyses—correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med* 1985;**109**:716–21.
- 14 Zidan A, Christie-Brown JS, Preston D, *et al.* Oestrogen and progesterone receptor assessment in core biopsy specimens of breast carcinoma. *J Clin Pathol* 1997;**50**:27–9.
- 15 Hall PA, Coates PJ. Assessment of cell proliferation in pathology—what next? *Histopathology* 1995;**26**:105–12.
- 16 Amso NN, Crow J, Shaw RW. Comparative immunohistochemical study of oestrogen and progesterone receptors in the Fallopian tube and uterus at different stages of the menstrual cycle and the menopause. *Hum Reprod* 1994;**9**: 1027–37.