associated migraine symptoms was reported by 7 (70.0%) subjects in group EM, 3 (25.0%) in group EH and none in group CH (p = 0.004).

Conclusions: Surgical removal of endometriosis and post-operative treatment with GnRH analogs may reduce migraine severity.

P-496 Real-time PCR analysis for estrogen receptor beta and progesterone receptor in menstrual blood samples – a new approach to a non-invasive diagnosis for endometriosis

S. Kissler1, M. Schmidt2, N. Keller1, I. Wiegratz1, T. Tonn3, W.K. Roth3, E. Seifried2, R. Baumann3, E. Siebzehnruebl1, G. Leyendecker4, M. Kaufmann1
1 University of Frankfurt, Department of Obstetrics/Gynecology, Frankfurt am Main, Germany; 2 University of Frankfurt, Institute of Transfusion Medicine and Immunohaematology, Frankfurt am Main, Germany; 3 Buergerhospital, Section Gynaecologic Endoscopy, Frankfurt am Main, Germany; 4 University of Frankfurt, Staedisches Klinikum Darmstadt, Frankfurt am Main, Germany

Introduction: Endometriotic and adenomyotic tissue mimic the cyclical changes of basal endometrium concerning hormone receptors. Since, there is a high prevalence of shed basal tissue in patients with endometriosis, the hypothesis was tested, whether the analysis of hormone receptors in menstrual blood by real-time NAT could serve as a new approach for the diagnosis of endometriosis.

Materials and methods: Twenty women with endometriosis and 22 control women were analysed for estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), cyclooxygenase-2 (COX-2), cytochrome P450 (CYP1A1) and progesterone receptor (PR) by real-time NAT. Samples were examined from menstrual blood as well as from peripheral blood. Endometriosis was verified performing in situ hybridization (ISH) with a VEGF-C specific probe. Immunohistochemical analysis demonstrated the presence of nume-

Results: Patients with endometriosis showed significant increased levels of estrogen receptor beta and progesterone receptor in menstrual blood samples whereas no differences were recognized between women with endometriosis and the control group in peripheral blood samples.

Conclusion: These data strongly indicate that endometriosis results from the dislocation of basal endometrium. The real-time PCR screening for estrogen receptor beta and progesterone receptor in patients with strong suspicion for endometriosis provides the opportunity to verify the diagnosis without invasive laparoscopic examinations. This study demonstrates the principle of proof, examinations in a larger population are eagerly awaited.

P-497 Endometrial uNK cells are associated with α1β1 integrin

C. Kalumbi1, R. Farquharson2, M. Anim-Somuah1, M. Bates3, G. Vince4, S. Quenby1
1 Liverpool Women’s Hospital, University of Liverpool, Liverpool, England, UK

Introduction: Uterine natural killer cells (uNK) have been associated with pregnancy implantation and Recurrent Miscarriage (RM). However, the exact role of uNK cells in implantation and pregnancy has not been elucidated. There are two competing hypotheses: either uNK cells are hostile to invading tropho-

Results: Staining of the endometrial stroma occurred in patches. Patches of endometrium with high numbers of NK cells also showed high intensity of integrin expression. When assessing all 51 samples αβ1 and α4β1 were not correlated with NK cell number (P = 0.17 and P = 0.08, respectively). However, α1β1 was strongly correlated with NK cell number (P < 0.0001). Furthermore, when NK cell number was decreased with prednisolone administration α1β1 integrin expression was also decreased (P = 0.011), with no significant differences in αβ3 and α4β1 (P = 0.59 and P = 0.20, respectively).

Conclusions: Endometrial stromal α1β1, but not αβ3 and α4β1 integrin expression, was strongly associated with uNK cell number. This could be explained either by the uNK cells adhering to the α1β1 integrin or by the uNK cells directly or indirectly influencing α1β1 expression. The fact that prednisolone administration aimed at decreasing uNK number, also decreased α1β1 suggested the latter. Further research is necessary to determine whether uNK cells exert their effect on early pregnancy by controlling α1β1 integrin expression.

P-498 VEGF-C/flt-4 mediates endometrial lymphangiogenesis

1 Radboud University Nijmegen Medical Centre, 415 Obstetrics and Gynaecology, Nijmegen, The Netherlands; 2 Radboud University Nijmegen Medical Centre, 437 Pathology, Nijmegen, The Netherlands

Introduction: After menstruation, the endometrial tissue of the uterus starts to regenerate. After reformation of the epithelial lining, endometrial glands and stroma regrow. The newly formed stroma consists of extracellular matrix, fibroblasts, blood vessels and lymphatic vessels. Angiogenesis, or the formation of new vessels (either blood vessels or lymphatic vessels), is regulated by members from the VEGF (Vascular Endothelial Growth Factor) family consisting of five related proteins, designated VEGF-A to -E. Whereas VEGF-A is the main angiogenic factor for blood vessels, VEGF-C and -D induce lymphangiogenesis, mainly via binding to VEGF-receptor 3 (VEGF-R3, also called flt-4).

To date it is unknown which growth factor(s) regulate the proliferation of lymphatic endothelial cells in human endometrium. We have now investigated the occurrence of lymphangiogenesis, and linked our findings to the expression of VEGF-C and its receptor flt-4 in different menstrual phases of the endometrium.

Materials and methods: Paraffin sections of human endometrium samples representing the proliferative (n = 7), secretory (n = 8) and menstrual (n = 4) phase of the menstrual cycle were stained immunohistochemically for proliferating lymphatic endothelium. Lymph vessels were detected with anti-bodies directed against prox-1 or podoplanine, whereas proliferating cells were stained with antibody Ki67. Proliferating lymph-endothelial cells were visual-

Results: Immunohistochemical analysis demonstrated the presence of numerous lymph vessels in the myometrium and in the basal (non-cyclic) layer of the endometrium, and, to a somewhat lesser extend, in the cyclic part of the endometrium.
endometrium. In proliferative endometrium, numerous Ki67-positive cells were demonstrated in the stroma, in glandular and surface epithelium, and in vessels. Proliferating lymph-endothelial cells were observed in lymph vessels located in the basal and cyclic part of endometrium in the proliferative phase. All lymphatic vessels expressed flt-4 (VEGF-C), irrespective of the menstrual phase of the endometrium. Remarkably, glandular epithelial cells in the cyclic part of the endometrium also stained strongly positive for flt-4. This staining was largely confined to endometrium in the proliferative phase.

Finally, large amounts of VEGF-C mRNA were demonstrated in the glandular epithelium, in the cyclic section of endometrium in the proliferative phase.

**Conclusions:** Our results have demonstrated extensive lymphangiogenesis in endometrium in the proliferative phase. The formation of new lymphatic vessels most likely originates from the pre-existing vessels in the basal layer of the endometrium. The combined expression of flt-4 and VEGF-C mRNA in endometrium in the proliferative phase suggests a role for the VEGF-C/flt-4 pathway in endometrial lymphangiogenesis.

Remarkably, the fact that both VEGF-C and its receptor flt-4 were abundantly expressed by glandular epithelial cells indicates that this growth factor/receptor may also be involved in the regeneration of glandular structures in endometrium in the proliferative phase, possibly via an auto- or paracrine mechanism. Our findings indicate that the glandular epithelium is a key player in the (re)construction of the endometrium after menstruation.

**P-499 Expression of the androgen receptor-related transcription factor RNF4 in human endometrium is not influenced by androgens**

B. Cloke1, R. Margara1, G. Trew1, S. Laverty1, J. Brosens1, S. Franks1, J. White2

1 Institute of Reproductive and Developmental Biology, Department of Obstetrics and Gynaecology, Hammersmith Hospital, Imperial College, London, UK; 2The Clinical School, Department of Obstetrics and Gynaecology, Swansea University, Swansea, Wales, UK

**Introduction:** Polycystic ovary syndrome (PCOS) is the commonest cause of anovulatory infertility but the prevalence of polycystic ovaries is also high (>50%) in women with regular cycles who have unexplained or tubal infertility. Hyperandrogenaemia is a feature of both ovulatory and anovulatory women with PCO. Androgens exert their effect on target cells predominantly through the activation of the androgen receptor (AR). Increased expression of AR in the endometrium has been reported in women with PCO, suggesting a direct adverse effect of androgens on endometrial function but the mechanism of action remains unknown. The transcriptional regulation of AR depend on the presence of transcription factors and co-factors. One of the most recently identified transcription factors implicated in expression of androgen expression is RNF4. RNF4 is involved in germ cell development. It has been shown to interact with AR, tethering it within the nucleus and acting as cofactor of AR-dependent gene expression. RNF4 has been shown to interact with other transcription factors that may be involved in AR-related gene regulation. The aim of this study, therefore, was to examine the expression of RNF4 in human endometrium and to assess the role that androgens might play in its regulation.

**Materials and methods:** All patients were recruited from Hammersmith, Queen Charlotte’s and Chelsea Hospital (with informed consent and approval of the local Ethics Committee). Timed endometrial biopsies were obtained for in vivo experiments. In vitro experiments were carried out in primary cultures of endometrial stromal cells (ESCs) processed from endometrial biopsies taken during the proliferative phase. Cultured ESCs can then be induced to decidualize in the presence of cyclic AMP and medroxyprogesterone acetate (MPA). ESCs were treated with dihydrotestosterone (DHT) as an in vitro model for PCO endometrium. Expression of RNF4 mRNA was assessed by RT–PCR and of RNF4 protein by immunohistochemistry (IHC) or western immunoblotting.

**Results:** AR transcript levels were significantly higher in normal proliferative than in secretory biopsies (9 versus 10 samples; p=0.016) confirming a previous report that AR is downregulated in the luteal phase. Treatment of ESCs with dihydrotestosterone in the presence of cAMP did not increase transcript levels, but did increase the nuclear translocation of AR.

IHC revealed that RNF4 is indeed expressed in endometrial stromal cells. This was confirmed by western and RT–PCR analysis. RNF4 protein levels did not fluctuate through the normal menstrual cycle, as revealed by IHC of timed biopsies. Furthermore, RT–PCR comparing proliferative to secretory biopsies revealed no significant differences (p>0.05). Lastly, RNF4 protein levels in decidualized ESCs were similar to those in untreated cells. However the transcript levels did show an early temporally-dependent increase upon decidualization treatment (p=0.015). IHC comparing endometrium from timed biopsies in fertile women with those in ovulatory PCO, revealed no differences in RNF4 expression. We were also unable to establish any pattern of RNF4 mRNA or protein expression upon treatment of ESC cultures with DHT.

**Conclusions:** We have shown, for the first time that RNF4 is transcribed and translated in human endometrium. However it does not appear to be regulated through the menstrual cycle, and remains unaffected by androgens or whether or not the patient had PCO. A functional role of RNF4 in human endometrium remains to be established.

**P-500 Thyroid hormone receptors in human endometrium**

L. Aghajanova1, A. Stavreus-Evers1, B. Landgren1, O. Hovatta1, L. Skjoldeland Sparre2

1 Karolinska University Hospital Huddinge, Department of Obstetrics & Gynecology, Stockholm, Sweden; 2 Danderyd Hospital, Department of Obstetrics & Gynecology, Stockholm, Sweden

**Introduction:** Thyroid disease is much more common in women than in men. It can cause disturbances in the menstruation and ovulation. We reported previously cell-type specific expression of thyroid stimulating hormone receptor (TSHR) in human endometrium from healthy fertile volunteers and infertile IVF patients and correlated it with endometrial ultrastructure (pinopode formation). Now we studied the expression of thyroid receptors (TR) α1, α2 and β1 in human endometrium from both fertile and infertile women in order to identify mechanisms behind the reproductive disturbances among women.

**Materials and methods:** Endometrial samples from 33 healthy fertile women were obtained throughout the menstrual cycle with days at luteal phase timed to the day after LH surge. The biopsies from 31 infertile IVF patients (10 with male factor of infertility, 8 with tubal factor and 13 with unexplained infertility) were obtained at the LH days 6 to 9 in the luteal phase. Cellular localization of thyroid hormone receptors proteins was studied using immunohistochemistry on paraffin-embedded sections. Reverse-transcriptase–polymerase chain reaction (RT–PCR) was performed to evaluate mRNAs levels. Scanning electron microscopy was used to observe the appearance of pinopodes on endometrial surface. TSH and T4 serum levels were measured by electrochemiluminescence immunoassay.

**Results:** We found TR α1 and TR β1 protein expression in all cell types of human endometrium. In healthy women there was a significant increase of TR α1 and β1 expression in the glandular and luminal epithelium on LH days 6–9, coinciding with the appearance of pinopodes and supposed period with uterine receptivity for blastocyst implantation, compared to early secretory phase (before pinopode formation). TR α1 and β1 immunostaining was weaker in late secretory phase and in proliferative phase. Stromal staining was faint throughout the menstrual cycle. Staining in luminal epithelium was stronger than in glandular epithelium for both antibodies. Endometrium from IVF patients showed lower luminal and glandular TR α1 and β1 expression compared to normal endometrium. Decrease in thyroid receptors expression in patients with tubal factor of infertility was significant compared to healthy patients, while in groups with unexplained and male factor of infertility such decline was nonsignificant, except for the TR α1 expression in luminal epithelium in women with male factor. Both TSH and T4 levels in serum were significantly higher in fertile women than in infertile, but still within reference values. No immunostaining was observed with TR α2 antibody. RT–PCR showed the presence of mRNA for all four receptors studied in human endometrium. TR α1 mRNA was most abundant while TR β1 was most scarce. The amount of TR α1 mRNA was significantly higher during the mid-luteal phase compared to other phases, while the opposite has been seen for TR α2. The highest TR β1 values were seen in the proliferative and early luteal phase. Diminished TR mRNA expression was observed in endometrium of infertile patients.

**Conclusions:** TR α1 and β1 are expressed in human endometrium. Coincidence with pinopode appearance in healthy women, low expression of TR β1 and α1 and higher TSH and T 4 serum levels in infertile patients suggest an importance of the endometrial thyroid receptors for human reproduction.