

METHODOLOGY

Open Access

Changes in cell proliferation, but not in vascularisation are characteristic for human endometrium in different reproductive failures - a pilot study

Ariane Germeyer^{*1}, Michael von Wolff², Julia Jauckus¹, Thomas Strowitzki¹, Tanuj Sharma³ and Anna T Grazul-Bilska³

Abstract

Background: Reproductive failure, determined as recurrent spontaneous abortions (RSA) or recurrent implantation failure (RIF) in women is not well understood. Several factors, including embryo quality, and cellular and molecular changes in endometrium may contribute to the insufficient fetomaternal interaction resulting in reproductive failure. Prior clinical studies suggest an inadequate endometrial growth and development of the endometrium, leading to a lesser endometrial thickness.

Methods: We therefore aimed to determine the cellular proliferation using Ki67, and the expression of markers of vascularisation, such as factor VIII (a marker of endothelial cells) and smooth muscle cell actin (SMCA; a marker of pericytes and smooth muscle cells) in endometrium of healthy women and women with RSA or RIF. LH-dated mid-secretory endometrial biopsies of seven healthy women and twenty women with reproductive failure were examined via immunohistochemistry followed by image analysis.

Results: Cellular proliferation but not expression of factor VIII or SMCA was decreased ($P < 0.0004$) in endometrium of women with RSA and RIF compared to healthy controls. Conclusion: Our data indicate that reproductive failure is due to insufficient cell proliferation/tissue growth rather than inadequate vascularisation in the endometrium.

Background

Reproductive failure comprises idiopathic recurrent spontaneous abortions (RSA) as well as recurrent implantation failure (RIF) [1]. One of the current RIF definition is a failure to conceive after three transfers of one or more good quality embryos; however, this definition may vary depending on the center [2]. On the other hand, idiopathic RSA is defined as three or more consecutive pregnancy losses within the first 20 weeks of gestation after exclusion of known contributing factors including uterine malformations, bleeding abnormalities, hormone disequilibrium, parental chromosomal defects, infections and others [3].

Adequate implantation is a limiting factor in human reproduction. Despite major improvement in assisted

reproduction techniques (ART) the clinical pregnancy rate per embryo transfer in fresh ART cycles is 31%, and up to 41% in oocyte donation programs [4]. A number of etiologies, including decreased embryonic quality, endometrial receptivity, fetomaternal communication, endocrine, genetic and other factors have been suggested as causes for reproductive failure [5-7]. Even though intrauterine endometrium is not essential for embryo implantation, since ectopic pregnancies occur [8], several studies indicated an altered endometrial function in women with reproductive failure [9,10]. Changes at the cellular and molecular level in endometrium from women with reproductive failure have been reported [8,11,12]; suggesting an impact of specific genes on the success of implantation. However, these studies determine mostly gene expression profiles and only partially examine the transcribed products.

* Correspondence: cheesehopper@web.de

¹ Department of Gynecological Endocrinology and Reproductive Medicine, University Hospital Heidelberg, Heidelberg, Germany
Full list of author information is available at the end of the article



Although inadequate regulation of endometrial growth has been recognized as a possible factor in reproductive failures [11,13-15], limited data is available concerning endometrial cell proliferation and its regulation. Furthermore, inconsistent results were obtained from studies of the role of endometrial vascularisation, angiogenesis, blood flow and/or endometrial thickness in women with implantation failure; while some studies demonstrate differences between normal and reproductive failures subjects others do not [16-19].

We hypothesized that reproductive failures are due to altered uterine growth caused by changes in cell proliferation and vascularisation/angiogenesis in the endometrium. Therefore, the aim of this study was to examine cellular proliferation and expression of markers of vascularisation (factor VIII and smooth muscle cell actin [SMCA]) at the protein level in endometrial biopsies from the mid-secretory phase of healthy women and women with reproductive failure.

Methods

In this observational, non-therapeutic pilot study, endometrial biopsies were taken after informed consent according to the Ethical Committee of the University of Heidelberg, Germany. A total of 27 women, at age 25 to 42 years, who were not on any hormonal treatments were included in this study. All women exhibited regular 28 ± 1 day cycles. Pipelle biopsies were taken in the mid-secretory phase on day 8-9 after the LH surge (LH +8/+9) from 11 women with RIF, 9 women with RSA, and 7 healthy woman. RIF was defined by failure to detect a positive serum hCG after three transfers of two or three good quality embryos created through in vitro fertilization. RSA was defined as the appearance of three or more spontaneous abortions of unknown reasons during the first trimester. None of the women with RIF or RSA had ever had a successful term pregnancy. Control women delivered healthy babies at term, with one woman having had a preterm delivery due to premature rupture of membranes. None of the women included in the study had known contributing factors for pregnancy failure, including thrombophilia (Factor V Leiden mutation, prothrombin mutation, antithrombin III, as well as protein C or S deficiency), antiphospholipid syndrome (anticardiolipin antibodies, lupus anticoagulans, ANAs), uterine anomalies detected by ambulatory hysteroscopy, polycystic ovarian syndrome, hormone abnormalities (thyroid, prolactin etc.) and other endocrinopathies (e.g., diabetes). On the day of biopsy blood samples were collected for determination of estradiol-17 β (E2) and progesterone concentration in serum using a competitive immunoassay by the University of Heidelberg core facility. The LH surge was determined using a commercially available urine LH kit (Clear blue, WICK PHARMA/Procter &

Gamble GMBH, Schwalbach am Taunus, Germany). Furthermore, histology of the biopsies typical for the secretory phase was confirmed by two individual researchers according to the modified Noyes' criteria [20].

Tissue preparation

Immediately after the biopsies were performed, tissues were immersed in OCT, frozen in liquid nitrogen and stored at -80°C. Tissues were then sectioned at 8 μ m, mounted onto SuperFrost Plus slides (Menzel GmbH & Co, Braunschweig, Germany), fixed with 100% acetone at 4°C for 10 min, and stored at -70°C before immunohistological procedure.

Immunohistochemistry

Cell proliferation was determined based on immunolocalization of Ki67 protein (a marker of proliferating cells) and vascularisation was determined by immunolocalization of factor VIII (a marker of endothelial cells) and SMCA (a marker of pericytes and smooth muscle cells) as previously described [21,22]. Briefly, sections of cryopreserved endometrial tissues were rinsed several times in PBS containing Triton-X100 and were then treated for 20 min with blocking buffer [PBS containing normal goat serum (1-2%, v/v)] followed by treatment with primary antibody against Ki67 (1:100; mouse monoclonal; Vector Laboratories, Burlingame, CA, USA), factor VIII (1:100; rabbit polyclonal; Sigma, St. Louis, MO, USA) or SMCA (1:150; mouse monoclonal; Oncogene Research Products; San Diego, CA, USA) overnight at 4°C. Primary antibody was detected using a biotin-labeled secondary antibody (anti-mouse antibody for Ki67 and SMCA, and anti-rabbit antibody for factor VIII; Vector Laboratories) and the ABC kit (Vector Laboratories). For color development, Vector SG substrate kit (Vector Laboratories) was used. Sections stained for the presence of Ki67 were counterstained with fast red (Sigma) to visualize cell nuclei. Control sections were incubated with normal mouse IgG (4 μ g/mL) or rabbit IgG (diluted 1:100) in place of primary antibody.

Image analysis

Image analysis was performed as described in detail previously [22,23]. Endometrial images of randomly chosen areas (5-10 per uterine section/subject; 0.025 mm² per field) stained for Ki67, factor VIII or SMCA were taken at 400 \times magnification, using the Eclipse E600 Nikon microscope and digital camera. Labeling index (LI; proportion of proliferating Ki67-positive cells out of the total cells per area), vascularity based on relative expression of factor VIII (occupied by endothelial cells) and SMCA (located in smooth muscle cells and pericytes) were determined by using computerized image analysis (Image-Pro Plus, version 5.0; Media Cybernetics, Houston, TX, USA). For

factor VIII and SMCA, data are expressed as the total area that exhibited positive staining within tissue area.

Statistical Analysis

Data were analyzed using the general linear model (GLM) procedures of SAS (SAS Inst. Inc. Cary, NC). When the F-test was significant ($P < 0.05$), differences among means were evaluated by using the least square means procedure [24]. Data are expressed as mean \pm SEM.

Results

The mean age of the women in the control group (30.3 ± 1.9 years) was similar to the women with RSA (34.6 ± 1.2 years) and the women with RIF (34.3 ± 0.8 years). E2 and progesterone serum concentration were similar in control, RSA and RIF (E2: 146 ± 21 , 158 ± 25 and 128 ± 11 pg/ml; progesterone: 14.4 ± 4.3 , 11.0 ± 1.7 and 10.2 ± 0.8 ng/ml), respectively.

Ki67, factor VIII and SMCA were detected in uterine tissue sections in all groups (Fig. 1 and 2). Ki67 was detected in cells of uterine glands, stromal tissue and blood vessels (Fig. 1A-C), while factor VIII (Fig. 2A) and SMCA (Fig. 2B) were localized to blood vessels. Factor VIII was detected in small and larger blood vessels (Fig. 2A), and SMCA was detected mostly in the larger blood vessels, but also in some small blood vessels (Fig. 2B).

The labeling index was greater ($P < 0.0004$) in endometrium of the control group compared to the endometrium of women with RSA or RIF, which were both similar (Fig. 3). The cell proliferation index of the different groups was not significantly correlated to the estrogen serum concentration ($P = 0.369$). The expression of factor VIII or SMCA in endometrium was similar in all three groups (Fig. 3).

Discussion

The present data demonstrates that in two pathological groups, RSA and RIF cellular proliferation is less than in a control group. However, vascularisation of endometrium in these pathological groups was similar to the control group.

Although it is widely accepted that the quality of the embryo is the essential factor for successful pregnancy outcome, several studies have shown the importance of endometrial function in successful pregnancy outcome [5,14,25]. Numerous studies have focused on causes of implantation failures by examining embryo effects or endometrium at cellular and molecular levels [8,11,12,26,27]. The function of the endometrium is to allow implantation on one hand, but make sure that invasion of the uterine tissue is limited, as otherwise inadequate placentation occurs, including placenta accreta, increta, percreta [28]. Furthermore, endometrial cell proliferation and differentiation has to be tightly controlled

[14], as progression to endometrial cancer can be seen when uninhibited proliferation takes place [29]. In the present study, inadequate endometrial growth marked by reduced cell proliferation in endometrium could contribute to reproductive failure. Although very few studies investigated endometrial cell proliferation and its regulatory mechanisms, decreased proliferation is found in endometrium at the time of menopausal transition in women [30], while in endometriosis, cellular proliferation in the human endometrium was enhanced around the time of implantation and during late secretory phase [31]. Furthermore, Mertens et al have described ongoing tissue proliferation of endometrial stromal cells via Ki67 expression in the secretory phase despite rising progesterone levels [32]. In agreement with our results, Lee and colleagues [11] have indicated that defective cell growth, regulated by the differentially expressed genes controlling the cell cycle, is likely to contribute to implantation failure.

Numerous growth factors of uterine tissues including fibroblast growth factors, epidermal growth factors, vascular endothelial growth factor and others are involved in the control of endometrial growth and function [33,34]. In addition, estrogens and progesterone are also important regulators of endometrial function [15,35]. However, in our study as well as in other studies, concentration of E2 and progesterone were similar in normal and pathological conditions [36]. This suggests that inadequate expression of growth factor(s), rather than steroid hormones contributed to decrease the cell proliferation in reproductive failure cases. Nevertheless the effect of steroid hormones cannot be excluded, since a difference in distribution of nuclear and cytoplasmic progesterone receptors was found in women with early pregnancy loss compared to women with proven fertility [36]. In addition, the progesterone receptor is thought to play a role in the implantation defect of women with RIF [37]. Last but not least a reduction of estrogen receptor alpha at the time of implantation is described in women with RIF [10]. Therefore, further investigation is needed to evaluate the potential impact of the changes in hormone receptors on endometrial cell proliferation in women with reproductive failure.

Since cellular proliferation is reflected by tissue growth and development, endometrial thickness and/or volume is likely to depend on the rate of cell proliferation at specific reproductive stages. In fact, several studies have demonstrated a positive association between pregnancy rate and endometrial volume and/or thickness [38,39]. Thus, these observations are in agreement with our data showing reduced cell proliferation in reproductive failures. On the other hand, several studies did not observe any association between endometrial volume or endometrial thickness and pregnancy outcome [16,40]. These discrepancies are likely due to different timing and

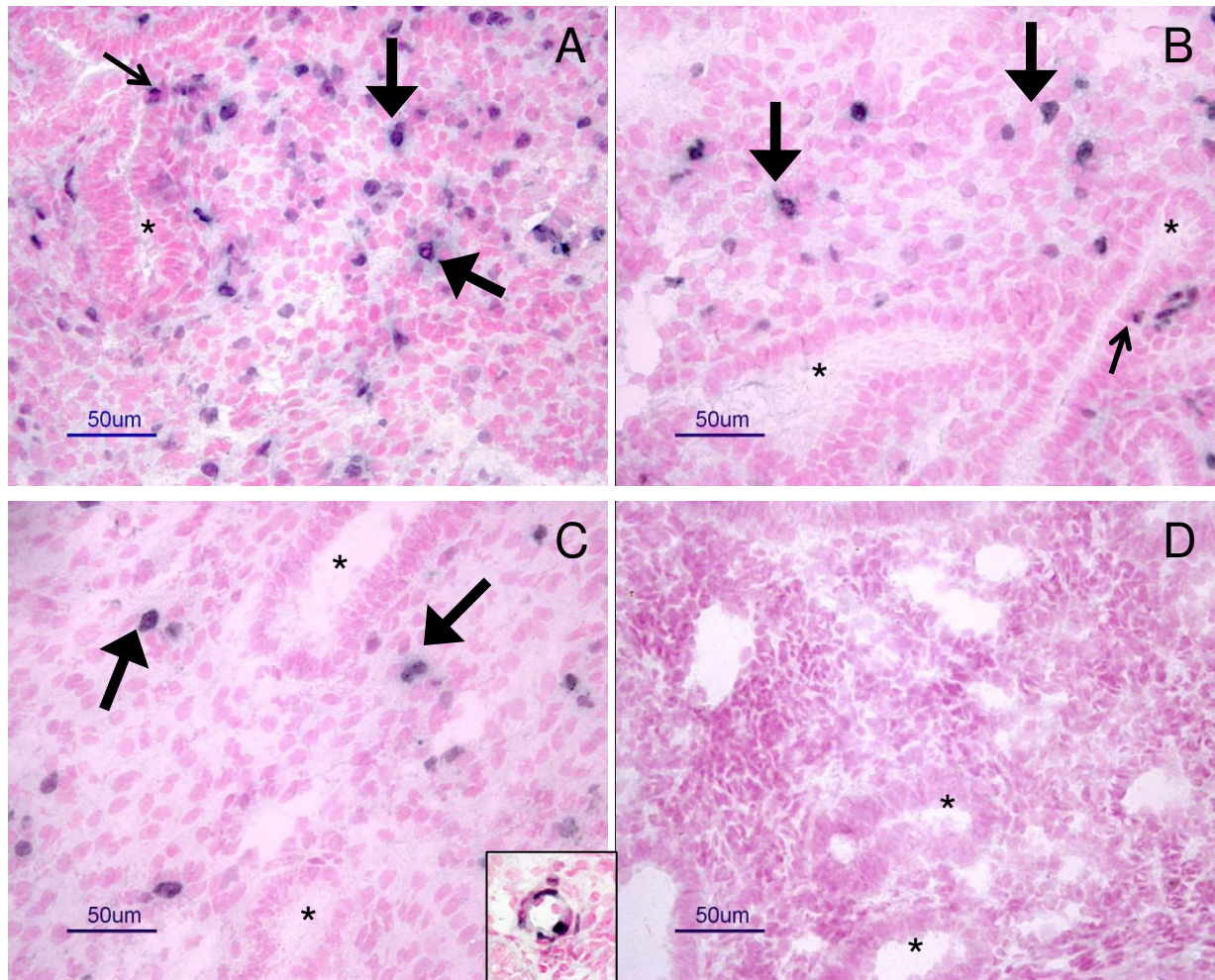


Figure 1 Immunohistochemical staining of Ki67. Representative micrographs of positive staining of Ki67 in human endometrium obtained from healthy women (A), and women with RSA (B) or RIF (C). Dark staining indicates proliferating cells, and pink staining indicates cell nuclei of non-proliferating cells. Note more proliferating cells in A than in B or C. Proliferating cells in endometrial glands are marked with small arrows in A and B, and in stromal tissue with large arrows in A, B and C. Insert in C demonstrated proliferating cells in a blood vessel. Endometrial glands are marked with stars (*). Control staining did not show any positive staining for Ki67 (D). Bar = 50 μm.

treatments applied before measurement of endometrial volume or thickness. Therefore, more detailed and uniformed studies should be performed to determine the role of endometrial cell proliferation, volume and thickness in reproductive failure.

In the present study, we demonstrated that vascularization of endometrium was similar for controls and women with reproductive failure, indicating a limited role of blood vessel function in implantation failure. In agreement to our study, Plaisier et al also showed no difference in vascularisation on the maternal side of implantation, namely decidualized endometrial stromal cells, in cases with missed abortions compared to controls, while they were able to demonstrated differences in receptors of growth factors in decidua parietalis and basalis at the

time of missed abortion [41]. Although the importance of angiogenesis for implantation, and normal uterine and placental function is well recognized [16,42,43], the role of inadequate vascularization or blood flow in implantation failure has not been investigated in detail. Inconsistent results of impact of uterine blood flow in implantation failure measured by ultrasound technologies were obtained [16,17]. For example, using ultrasound techniques [44], reduced endometrial and subendometrial 'perfusion' has been demonstrated which suggests a reduced vascularity in women with unexplained subfertility, irrespective of E2 and progesterone levels. In contrast, Ng et al. [45,46] did not find any association between infertility and blood flow measured via ultrasound techniques. These latest results are in agreement

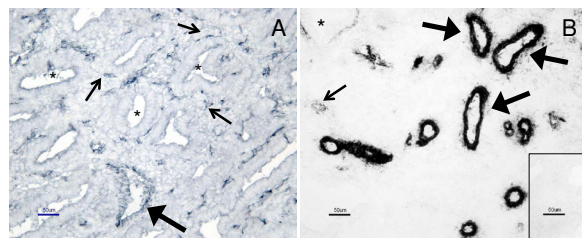


Figure 2 Immunohistochemical staining of factor VIII and SMCA. Representative micrographs of positive staining (dark color) of factor VIII (A) and smooth muscle cell actin (B) in human endometrium obtained from healthy women. Note positive staining in small (small arrows) and larger (large arrows) blood vessels. Endometrial glands are marked with stars (*). Control staining did not show any positive staining for factor VIII and SMCA (insert in B). Bar = 50 μm.

with our studies, since we did not observe changes in blood vessel density in reproductive failure subjects. The discrepancies of results in studies cited above are likely due to different techniques (ultrasound vs. immunohistochemistry and image analysis), different population and/or study design used in these experiments. Since human studies are restricted in their design, the studies performed in human tissue are limited in their character, therefore we were only able to look at the intrinsic endometrial regulation of women with reproductive failure at the protein level. Thus, additional studies should be performed to determine mRNA and protein expression especially for genes regulating cell proliferation in endometrium from women with and without reproductive failure.

Conclusion

In summary, we demonstrate a decrease in cell proliferation in endometrial tissues from the mid-secretory phase in women with reproductive failure compared to healthy

controls. However, vascularisation in endometrium was similar for the examined groups. We therefore hypothesize that adequate endometrial growth is a critical factor contributing to the success of implantation, as well as further continuation of an established pregnancy. Our results help to better understand the underlying pathomechanism of reproductive failure and may contribute to a discovery of marker(s) which can be used to predict successful vs. non-successful pregnancy.

List of abbreviations

ANA: antinuclear antibodies; ART: assisted reproduction technique; mRNA: messenger ribonucleic acid; C/EBP-beta: CCAAT/enhancer binding protein (C/EBP), beta; CRABP2: cellular retinoic acid binding protein 2; E2: estradiol-17β; hCG: human choriogonadotropin; IgG: Immunglobulin G; Ki67: proliferation marker; LH: luteinising hormone; PBS: phosphate buffered saline; RSA: recurrent spontaneous abortions; RIF: recurrent implantation failure; SG: silver grain; SMCA: smooth muscle cell actin.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AG participated in designing the study, coordinated tissue collection, evaluated results and participated in writing the manuscript and was responsible for intellectual content of the study; MvW participated in patient selection, consenting the patients and tissue collection; JJ participated in tissue collection and preparation for immunohistochemistry; TS participated in patient selection and in writing of the manuscript; TS performed image analysis and prepared data for statistical analysis; ATGB participated in designing of the study, evaluated results and participated in writing the manuscript, coordinated and supervised immunohistochemical staining and image analysis. All authors read and approved the final manuscript.

Acknowledgements

This study was supported in part by grant P20 RR016741 from the INBRE program of the National Center for Research Resources and ND Hatch Project ND01712. Presented in part at the 42nd Annual Meeting of the Society for the Study of Reproduction, Pittsburgh, PA, USA, New Orleans, LA, July 18-22, 2009. The authors would like to thank Mr. Kim C. Kraft, Mr. Robert Weigl and Mr. James D. Kirsch for their technical assistance.

Author Details

¹Department of Gynecological Endocrinology and Reproductive Medicine, University Hospital Heidelberg, Heidelberg, Germany, ²Department of Gynecological Endocrinology and Reproductive Medicine, University Hospital Berne, Berne, Switzerland and ³Department of Animal Sciences and Cell Biology Center, North Dakota State University, Fargo, North Dakota, USA

Received: 10 February 2010 Accepted: 21 June 2010

Published: 21 June 2010

References

- Farquharson RG, Jauniaux E, Exalto N: **Updated and revised nomenclature for description of early pregnancy events.** *Hum Reprod* 2005, **20**(11):3008-3011.
- Margalioth EJ, Ben-Chetrit A, Gal M, Eldar-Geva T: **Investigation and treatment of repeated implantation failure following IVF-ET.** *Hum Reprod* 2006, **21**(12):3036-3043.

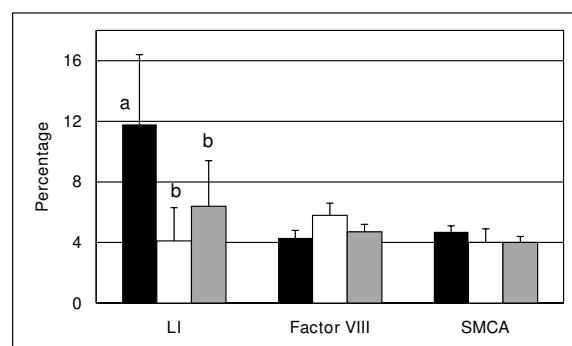


Figure 3 Relative Expression of assessed factors in the control and study groups. Labeling index (LI), expression of factor VIII and SMCA in endometrium of the control (black bars), RSA (open bars) and RIF (grey bars) groups. ^{a,b}P < 0.0004 for LI.

3. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S: **Recurrent miscarriage: aetiology, management and prognosis.** *Hum Reprod Update* 2002, **8**(5):463-481.
4. Nyboe Andersen A, Goossens V, Bhattacharya S, Ferraretti AP, Kupka MS, de Mouzon J, Nygren KG: **Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE.** *ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE).* *Hum Reprod* 2009, **24**(6):1267-1287.
5. Christiansen OB, Nielsen HS, Kolte AM: **Inflammation and miscarriage.** *Semin Fetal Neonatal Med* 2006, **11**(5):302-308.
6. Potdar N, Konje JC: **The endocrinological basis of recurrent miscarriages.** *Curr Opin Obstet Gynecol* 2005, **17**(4):424-428.
7. Tomasetti C, Meuleman C, Pexsters A, Mihalyi A, Kyama C, Simsa P, D'Hooghe TM: **Endometriosis, recurrent miscarriage and implantation failure: is there an immunological link?** *Reprod Biomed Online* 2006, **13**(1):58-64.
8. Savaris RF, Hamilton AE, Lessey BA, Giudice LC: **Endometrial gene expression in early pregnancy: lessons from human ectopic pregnancy.** *Reprod Sci* 2008, **15**(8):797-816.
9. Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, Giudice LC: **Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility.** *Endocrinology* 2003, **144**(7):2870-2881.
10. Koler M, Achache H, Tsafir A, Smith Y, Revel A, Reich R: **Disrupted gene pattern in patients with repeated in vitro fertilization (IVF) failure.** *Hum Reprod* 2009, **24**(10):2541-2548.
11. Lee J, Oh J, Choi E, Park I, Han C, Kim do H, Choi BC, Kim JW, Cho C: **Differentially expressed genes implicated in unexplained recurrent spontaneous abortion.** *Int J Biochem Cell Biol* 2007, **39**(12):2265-2277.
12. Tapia A, Gangi LM, Zegers-Hochschild F, Balmaceda J, Pommer R, Trejo L, Pacheco IM, Salvatierra AM, Henriquez S, Quezada M, Vargas M, Rios M, Munroe DJ, Croxatto HB, Velasquez L: **Differences in the endometrial transcript profile during the receptive period between women who were refractory to implantation and those who achieved pregnancy.** *Hum Reprod* 2008, **23**(2):340-351.
13. Bagchi MK, Mantena SR, Kannan A, Bagchi IC: **Control of uterine cell proliferation and differentiation by C/EBPbeta: functional implications for establishment of early pregnancy.** *Cell Cycle* 2006, **5**(9):922-925.
14. Barnea ER: **Embryo maternal dialogue: From pregnancy recognition to proliferation control.** *Early Pregnancy* 2001, **5**(1):65-66.
15. Maruyama T, Yoshimura Y: **Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium.** *Endocr J* 2008, **55**(5):795-810.
16. Alcazar JL: **Three-dimensional ultrasound assessment of endometrial receptivity: a review.** *Reprod Biol Endocrinol* 2006, **4**:56.
17. Fanchin R: **Assessing uterine receptivity in 2001: ultrasonographic glances at the new millennium.** *Ann NY Acad Sci* 2001, **943**:185-202.
18. Jirous J, Diejomaoh ME, Al-Abdulhadi F, Boland MH, Nazar M: **A comparison of the uterine and intraovarian arterial flows in nonpregnant women having a history of recurrent spontaneous miscarriage associated with antiphospholipid syndrome.** *Arch Gynecol Obstet* 2004, **270**(2):74-78.
19. Lisman BA, Boer K, Bleker OP, van Wely M, van Groningen K, Exalto N: **Abnormal development of the vasculosyncytial membrane in early pregnancy failure.** *Fertil Steril* 2004, **82**(3):654-660.
20. Murray MJ, Meyer WR, Zaino RJ, Lessey BA, Novotny DB, Ireland K, Zeng D, Fritz MA: **A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women.** *Fertil Steril* 2004, **81**(5):1333-1343.
21. Grazul-Bilska AT, Banerjee J, Yazici I, Borowczyk E, Bilski JJ, Sharma RK, Siemionov M, Falcone T: **Morphology and function of cryopreserved whole ovine ovaries after heterotopic autotransplantation.** *Reprod Biol Endocrinol* 2008, **6**:16.
22. Grazul-Bilska AT, Caton JS, Arndt W, Burchill K, Thorson C, Borowczyk E, Bilski JJ, Redmer DA, Reynolds LP, Vonnahme KA: **Cellular proliferation and vascularization in ovine fetal ovaries: effects of undernutrition and selenium in maternal diet.** *Reproduction* 2009, **137**(4):699-707.
23. Grazul-Bilska AT, Navanukraw C, Johnson ML, Vonnahme KA, Ford SP, Reynolds LP, Redmer DA: **Vascularity and expression of angiogenic factors in bovine dominant follicles of the first follicular wave.** *J Anim Sci* 2007, **85**(8):1914-1922.
24. Kirk R: **Experimental Design: Procedures for the Behavioral Sciences.** 2nd edition. Pacific Grove, CA, USA: Brooks/Cole; 1982.
25. Rai R, Regan L: **Recurrent miscarriage.** *Lancet* 2006, **368**(9535):601-611.
26. Jauniaux E, Burton GJ: **Pathophysiology of histological changes in early pregnancy loss.** *Placenta* 2005, **26**(23):114-123.
27. Strowitzki T, Germeyer A, Popovici R, von Wolff M: **The human endometrium as a fertility-determining factor.** *Hum Reprod Update* 2006, **12**(5):617-630.
28. Tantibirojn P, Crum CP, Parast MM: **Pathophysiology of placenta creta: the role of decidua and extravillous trophoblast.** *Placenta* 2008, **29**(7):639-645.
29. Mitselou A, Ioachim E, Kitsou E, Vougiouklakis T, Zagorianakou N, Makrydimas G, Stefanaki S, Agnantis NJ: **Immunohistochemical study of apoptosis-related Bcl-2 protein and its correlation with proliferation indices (Ki67, PCNA), tumor suppressor genes (p53, pRb), the oncogene c-erbB-2, sex steroid hormone receptors and other clinicopathological features, in normal, hyperplastic and neoplastic endometrium.** *In Vivo* 2003, **17**(5):469-477.
30. Niklaus AL, Aubuchon M, Zapantis G, Li P, Qian H, Isaac B, Kim MY, Adel G, Pollard JW, Santoro NF: **Assessment of the proliferative status of epithelial cell types in the endometrium of young and menopausal transition women.** *Hum Reprod* 2007, **22**(6):1778-1788.
31. Hapangama DK, Turner MA, Drury JA, Quenby S, Hart A, Maddick M, Martin-Ruiz C, von Zglinicki T: **Sustained replication in endometrium of women with endometriosis occurs without evoking a DNA damage response.** *Hum Reprod* 2009, **24**(3):687-696.
32. Mertens HJ, Heineman MJ, Evers JL: **The expression of apoptosis-related proteins Bcl-2 and Ki67 in endometrium of ovulatory menstrual cycles.** *Gynecol Obstet Invest* 2002, **53**(4):224-230.
33. Moller B, Rasmussen C, Lindblom B, Olovsson M: **Expression of the angiogenic growth factors VEGF, FGF-2, EGF and their receptors in normal human endometrium during the menstrual cycle.** *Mol Hum Reprod* 2001, **7**(1):65-72.
34. Smith SK: **Angiogenesis, vascular endothelial growth factor and the endometrium.** *Hum Reprod Update* 1998, **4**(5):509-519.
35. Punyadeera C, Verboost P, Groothuis P: **Oestrogen and progesterin responses in human endometrium.** *J Steroid Biochem Mol Biol* 2003, **84**(4):393-410.
36. Carranza-Lira S, Blanquet J, Tserotas K, Calzada L: **Endometrial progesterone and estradiol receptors in patients with recurrent early pregnancy loss of unknown etiology--preliminary report.** *Med Sci Monit* 2000, **6**(4):759-762.
37. Ilesanmi AO, Hawkins DA, Lessey BA: **Immunohistochemical markers of uterine receptivity in the human endometrium.** *Microsc Res Tech* 1993, **25**(3):208-222.
38. Raga F, Bonilla-Musoles F, Casan EM, Klein O, Bonilla F: **Assessment of endometrial volume by three-dimensional ultrasound prior to embryo transfer: clues to endometrial receptivity.** *Hum Reprod* 1999, **14**(11):2851-2854.
39. Zollner U, Zollner KP, Specketer MT, Blissung S, Muller T, Steck T, Dietl J: **Endometrial volume as assessed by three-dimensional ultrasound is a predictor of pregnancy outcome after in vitro fertilization and embryo transfer.** *Fertil Steril* 2003, **80**(6):1515-1517.
40. Zaidi J, Campbell S, Pittrof R, Tan SL: **Endometrial thickness, morphology, vascular penetration and velocimetry in predicting implantation in an in vitro fertilization program.** *Ultrasound Obstet Gynecol* 1995, **6**(3):191-198.
41. Plaisier M, Dennert I, Rost E, Koolwijk P, van Hinsbergh VW, Helmerhorst FM: **Decidual vascularization and the expression of angiogenic growth factors and proteases in first trimester spontaneous abortions.** *Hum Reprod* 2009, **24**(1):185-197.
42. Gargett CE, Rogers PA: **Human endometrial angiogenesis.** *Reproduction* 2001, **121**(2):181-186.
43. Reynolds LP, Grazul-Bilska AT, Redmer DA: **Angiogenesis in the female reproductive organs: pathological implications.** *Int J Exp Pathol* 2002, **83**(4):151-163.
44. Raine-Fenning NJ, Campbell BK, Kendall NR, Clewes JS, Johnson IR: **Endometrial and subendometrial perfusion are impaired in women with unexplained subfertility.** *Hum Reprod* 2004, **19**(11):2605-2614.
45. Ng EH, Chan CC, Tang OS, Yeung WS, Ho PC: **Factors affecting endometrial and subendometrial blood flow measured by three-**

dimensional power Doppler ultrasound during IVF treatment. *Hum Reprod* 2006, **21**(4):1062-1069.

46. Ng EH, Chan CC, Tang OS, Yeung WS, Ho PC: **The role of endometrial blood flow measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during in vitro fertilization treatment.** *Eur J Obstet Gynecol Reprod Biol* 2007, **135**(1):8-16.

doi: 10.1186/1477-7827-8-67

Cite this article as: Germeyer et al., Changes in cell proliferation, but not in vascularisation are characteristic for human endometrium in different reproductive failures - a pilot study *Reproductive Biology and Endocrinology* 2010, **8**:67

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

