using eight different projectors which enables the visualization of 3D datasets in real three dimensions. The V-Scope application is used to create a 'hologram' of the ultrasound image allowing depth perception and interaction with the rendered objects. Besides the visualisation aids, it is also possible to measure distances. For our study a newly implemented segmentation algorithm calculated the volume of the structure of interest semi-automatically. This algorithm is based on a region-growing approach. After placement of a seed point in the area of interest, starting from the seed point, the algorithm segments out the (fetal) region of interest. Differences in grey scale allow for measurement of different structures. Post-processing tools, e.g. the grow and shrink function or the brush function, allow the user to correct for incomplete segmentation. All fetal volume and CRL measurements were repeated 3 times and mean values were used in the analyses. The logarithmically transformed outcomes were analyzed using repeated measurements ANOVA (random coefficients in SAS PROC MIXED). Interobserver agreement was established by calculating the intraclass correlation coefficient (ICC).

**Results:** In all of the 91 included cases the total fetal body volume could be measured without encountering technical problems. The gestational ages ranged from 6+5 to 12+6 weeks (mean 9+3 weeks) and CRL ranged from 3 to 68 mm (mean 29 mm). The fetal body volumes ranged from 14 $mm^3$ to 29877 $mm^3$ (mean 4531 $mm^3$). Reference charts of the total fetal volume were constructed according to GA and CRL. Scatterplots of volumes versus GA and versus CRL showed that log-transformations of both axes resulted in approximate linear relationships. ANOVA calculations showed that when the CRL doubles, the mean fetal volume increases 6.5 fold ($p < 0.001$). When the GA doubles the mean fetal volume increases approximately 500 fold ($p < 0.001$).

The second examiner independently performed volume measurements of 20 fetuses, resulting in an ICC of 0.998, representing excellent agreement in all women.

**Conclusions:** We have constructed reference charts for growth of the fetal volume in the first trimester in pregnancies using a VR system. Volumetry of the fetus by using a VR application makes it possible to obtain information about the size of a fetus, which makes it possible to analyze growth, using all dimensions of the human fetus. This new information can be useful to differentiate between normal and abnormal growth in early pregnancies.

### O-020 Oral Comparative study of reproductive outcomes following ectopic and intrauterine pregnancies using Scottish national data

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**Introduction:** Previous research on fertility outcomes following ectopic pregnancy (EP) has focused on internal comparisons within EP registries. There are limited data on reproductive outcomes following an initial ectopic in comparison with other types of successful or failed intrauterine pregnancies making counselling of women regarding risks difficult.

**Objective:** To assess reproductive outcomes following ectopic pregnancy compared to those following livebirth, miscarriage and termination.

**Materials and Method:** National Scottish data on all women with a first pregnancy between 1981 and 2000 were linked to records of a subsequent pregnancy. A retrospective cohort study design was employed. The exposed cohort comprised women whose first pregnancy ended in an EP. There were three unexposed cohorts:

1. Women whose first pregnancy ended in a livebirth
2. Women who miscarried their first pregnancy
3. Women who terminated their first pregnancy

Outcomes assessed included any second pregnancy, livebirth, ectopic, miscarriage, termination or stillbirth in the second pregnancy. Relative risks (RR) with 95% confidence intervals (CI) were calculated for each outcome following an ectopic pregnancy compared to those following a livebirth, miscarriage or termination.

**Results:** In their first pregnancies, 3,151 women had an EP, 673,664 had a livebirth, 44,823 had a miscarriage and 83,571 underwent terminations. Following EP, 2,052 (65.1%) women conceived again. Of these, 1,590 (77.5%) pregnancies ended in a livebirth, 145 (7.1%) ended in another EP, 155 (7.6%) in miscarriage, 105 (5.1%) in termination and 14 (0.7%) in stillbirth. In contrast, a previous livebirth was followed by another livebirth in 305,318(81.7%) of women who conceived again {373,668 (55.5%)}. EP, miscarriage, termination and stillbirth occurred in 2,394 (0.6%), 23,103 (6.2%), 36,539 (9.8%) and 1,309(0.4%) respectively.

A second pregnancy occurred in 34,845 (77.7%) women following a miscarriage. Of these, 26,903 (77.2%) had livebirth, 384 (1.1%) had EP, 4,593 (13.2%) had another miscarriage, 1,743 (5.0%) had termination and 213 (0.6%) had stillbirth.

Conception occurred in 57,326 (68.6%) following termination. Of these, 40,297 (70.3%) ended in livebirth, 411 (0.7%) in EP, 3,612 (6.3%) in miscarriage, 10,926 (19.1%) in another termination and 277(0.5%) in stillbirth. Compared to a livebirth in the first pregnancy, an ectopic first pregnancy increased the chance of a second conception by 50%, but reduced the chance of a second livebirth by 23%. A first ectopic increased the risk of a second ectopic {R.R. 11.79 (95% C.I. 9.91, 14.03)}, miscarriage {R.R. 1.24 (95% C.I. 1.05, 1.46)} and stillbirth {R.R. 2.05 (95% C.I. 1.21, 3.49)} in the second pregnancy but not termination {R.R 0.50 (95% C.I. 0.41, 0.61)}.

Compared to an initial miscarriage, an ectopic pregnancy reduced the chances of having a further pregnancy by 50% {R.R. 0.54 (95% C.I. 0.50, 0.58)}. The chance of a further ectopic was more than 8 times higher in the group with a previous ectopic pregnancy {R.R. 8.31 (95% C.I. 8.24, 8.38)}, but the risk of a miscarriage was slightly reduced in this group {R.R. 0.98 (95% C.I. 0.97, 0.98)}.

Compared to women who had a termination of their first pregnancy, women with an ectopic pregnancy had a reduced chance of conceiving a second pregnancy {R.R. 0.86 (95% C.I. 0.79, 0.92)} and had an 11 times higher risk of having a further ectopic pregnancy {R.R. 11.31 (95% C.I. 11.25, 11.38)}. There was no difference detected in the risks of other outcomes.

**Conclusion:** Women with a previous EP suffered from reduced rates of conception compared to all groups of women who did not have a previous live birth. They also had much higher risk of a further EP compared to all groups of women.

SELECTED ORAL COMMUNICATION SESSION

## Session 5: Endometriosis: basic research

**Monday 29 June 2009** **10:00–11:30**

### O-021 Oral PPAR-gamma receptor ligand reduces the development of endometrial explants in baboons: A prospective, randomized, placebo-controlled study

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**Introduction:** Through immune modulation and anti-angiogenic properties, peroxisome proliferator-activated receptors (PPARs) ligands such as thiazolidinediones (TZDs) are posited to have beneficial effects on endometriotic lesions. A clinically readily available TZD, pioglitazone, was used in a preventative model of endometriosis in baboons.

**Materials & Methods:** This was a prospective, randomized, placebo-controlled study conducted at the Institute of Primate Research in Karen, Kenya. Endometriosis was induced using intrapelvic injection of eutopic menstrual endometrium in 12 female baboons with a normal pelvis that had undergone at least one menstrual cycle since the time of captivity. Endometrial tissue was extracted from each baboon by curettage and a standard amount of endometrium was then seeded onto several peritoneal sites as previously described.

At this point, the 12 baboons were randomized into 2 groups and treated from the day of induction. They received either PBS tablets (n = 6, placebo control, placebo tablets once a day by mouth) or pioglitazone (n = 6, test drug, 7.5 mg by mouth each day). A second and final laparoscopy was performed in the baboons to record the extent of endometriotic lesions between 24–42 days after induction (no difference in length of treatment between the two groups, $P = 0.38$). The type of lesion (typical, red, white and suspicious) was recorded. Biopsies were obtained to confirm the histological presence of endometriosis. A videolaparoscopy was performed to document the number and surface area of endometriotic lesions.

**Results:** The surface area and volume of endometriotic lesions were significantly lower in pioglitazone treated baboons than the placebo group (SA: 48.6 v. 159.0 $mm^2$ respectively, $P = 0.025$; Vol: 23.7 v. 131.8 $mm^3$ respectively, $P = 0.025$). The mean surface area (3.5 v. 17.8 $mm^2$, $P = 0.017$, pioglizatone v. placebo) and overall number (1.5 v. 9.5, $P = 0.007$, pioglizatone v. placebo) of red lesions were lower in the pioglitazone group.

**Conclusions:** A PPAR-gamma ligand, pioglitazone, effectively reduced the initiation of endometriotic disease in the baboon endometriosis model. Using this animal model, we have shown that TZD is a promising drug for preventive treatment of endometriosis.

## O-022 Oral The hyper-estrogenic environment in endometriosis is the result of impaired steroid metabolism

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**Context:** There is substantial evidence that the expression of steroid metabolizing enzymes in endometriotic lesions is altered, turning the ectopic endometrium into a source of 17ß-estradiol. However, whether these differences actually result in a net increase in local 17ß-estradiol production and/or activity has not yet been shown.

**Subjects and Methods:** The activities of the most important steroidogenic enzymes, involved in the synthesis of 17ß-estradiol, were determined by HPLC in matched eutopic and ectopic tissue from patients with endometriosis and in normal endometrium.

**Results:** In this study we demonstrate that the activity of steroid sulfatase is high, however the activities in eutopic and ectopic endometrium are not different. The activity of 17ß-estradiol synthesizing enzymes was higher in the ectopic endometrium of the patients and the activity of 17ß-estradiol metabolizing enzymes was significantly lower in ectopic endometrial tissue, resulting in a significantly higher ratio of the 17ß-estradiol synthesizing and metabolizing activities ($p < 0.01$) in the ectopic compared to the eutopic endometrium, which suggests increased production of 17ß-estradiol in the lesions. This is supported by the finding that mRNA levels of the estrogen-responsive gene TFF1 were elevated as well in all ectopic endometrial tissues.

**Conclusion:** We have shown that the environment in the endometriosis lesions is more estrogenic than that of eutopic endometrium of patients and controls, and that the elevated production of 17ß-estradiol is mostly the result of impaired metabolism.

## O-023 Oral Inhibition of cell proliferation, adhesion and invasion with anti L1CAM-mAb in an endometriosis model

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**Introduction:** Endometriosis is a benign and most frequently progressive disease. Especially the endometrioid subtype of ovarian cancer has a high probability for developing cancer on the basis of existing endometriosis. The cell adhesion molecule L1CAM (CD171) is overexpressed in ovarian and endometrial carcinomas and is associated with a bad prognosis. Relying on our previous study to find increased L1 expression in endometriosis, we were prompted to investigate L1-mediated proliferation, adhesion and invasion in an endometriosis in vitro model.

**Material & methods:** Cell proliferation and cell survivor was investigated using endometriosis cell-line Z12 in a cell proliferation assay. Cell adhesion and invasion assay with matrigel ("sandwich-assay") using the endometriosis cell-line Z12 was applied to investigate L1-mediated adhesion and invasion and inhibition of adhesion and invasion by anti-L1-antibodies.

**Results:** Cell proliferation of Z12 endometriosis epithelial cells was significantly decreased ($P < 0.001$) after preincubation with anti L1-mAb (10 μg/ml) ($0.95 \pm 0.34 \times 300.000$ cells) compared to preincubation with unspecific IgG-mAb ($3.88 \pm 1.16 \times 300.000$ cells) and not-treated cells ($3.63 \pm 1.12 \times 300.000$ cells). The relative invasion of Z12 endometriosis epithelial cells through matrigel was significantly inhibited ($P < 0.001$) after preincubation of the cells with anti L1-mAb ($26.40 \pm 13.50\%$) in comparition to preincubation with unspecific IgG-mAb ($76.04 \pm 24.06\%$) and not-treated cells ($76.30 \pm 26.84\%$). Anti L1-mAb could also inhibit the adhesion of Z12 cells on a silicate membrane in a sandwich assay ($P < 0.001$) ($68.69 \pm 16.71\%$) compared to unspecific IgG-mAb ($89.49 \pm 23.78\%$) and not-treated cells ($90.00 \pm 21.53\%$).

**Conclusions:** Based on our results, we propose that L1 could promote endometriosis development by increasing the cell-invasion, adhesion and aggravation. Further studies should evaluate the possible use of the anti L1-mAb in an animal endometriosis model.

## O-024 Oral Validation of endometriosis markers in the endometrial fluid aspirate

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**Background:** In a previous work, based in differential two-dimensional electrophoresis, we demonstrated that aspirated endometrial fluid is an adequate and reliable non-invasive biological sample for endometriosis related studies. We identified disease specific protein biomarker candidates with potential in early diagnosis. Here we describe the preliminary validation of several protein markers using quantitative immunoassays and western blot to measure their expression levels in women with endometriosis and healthy controls.

**Material & methods:** Endometrial fluid aspirates of women with laparoscopic evidence of endometriosis stages I and II (n = 17) and stages III and IV (n = 6) were collected, as well as aspirates from women without evidence of the disease after laparoscopic surgery (n = 12). For quantitative validation, antibody-based flow citometry analysis with fluorescent bead arrays has been performed (xMAP technology from Luminex). Moesin and 14-3-3 protein expression semiquantitative validation was performed by western blot and image analysis.

**Results:** Endometrial fluid aspirate processing protocol was optimised to be used in sandwich type bead-based immunoassays. These immunoassays allowed the detection and quantification of endometriosis protein markers in endometrial fluid, and different antibody pairs have been optimised for several endometriosis biomarkers. Moesin and 14-3-3 protein validation by western blot was statistically significant ($p = 0.031$ and $p = 0.0004$, respectively) to discriminate healthy women from those suffering from early and advanced endometriosis (Moesin fold change in early endometriosis versus controls = 0.75 and fold change in advanced endometriosis versus controls = 1.54; 14-3-3 protein fold change in early endometriosis versus controls = 1.95 and fold change in advanced endometriosis versus controls = 2.73).

**Conclusions:** Endometrial fluid is a disease specific, non-invasive sample for biomarker validation in endometriosis. Moesin and 14-3-3 proteins show potential as early diagnosis markers in endometriosis. Sandwich type bead-based immunoassays are suitable for endometriosis marker quantification in endometrial fluid aspirates.