Reviewer's report

**Title:** Assessment of leukemia inhibitory factor and glycoprotein 130 expression in endometrium and uterine flushing: a possible diagnostic tool for impaired fertility

**Version:** 3 **Date:** 15 July 2011

**Reviewer:** Evdokia Dimitriadis

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This is a descriptive paper and investigates the mRNA and protein levels of the cytokine, leukemia inhibitory factor (LIF) and its signaling co-receptor, gp130 in endometrium of normal fertile and subfertile women during the mid-secretory phase of the menstrual cycle (the time when embryo implantation occurs). The authors compare the levels of LIF and the soluble gp130 receptor in uterine washings between the groups of women.

There are a number of studies that have investigated the levels of LIF in endometrium and uterine washings of women with subfertility, while none have previously examined the levels of soluble gp130 in uterine washings. This aspect of the work is exciting as it may be useful as a non-invasive diagnostic as there is currently no marker of women with endometrial related infertility.

**Major revisions:**

1. In the introduction, the authors can add more references. For example: A reference for LIF expression in the fallopian tube and cervical mucus. Also, references for LIF in uterine flushings.
2. Also, information on whether soluble gp130 competes antagonises LIF signalling so that once LIF binds to soluble gp130 does it then inhibit downstream LIF signalling. If this is the case it may be useful to look at the ration of LIF:soluble gp130.

**Material and methods:**

3. Add more details on how the flushings were processed before storage. Were any haemolysed?

   How much fluid was recovered after flushing. Were the protein levels measured in the washings? How do you standardize this between samples? Would expressing the levels differ if you express the data per protein in washings or per ml of washings?

**Results:**

4. RT-PCR is not quantitative so that you can only comment on whether the gene is there or not. You can not comment on the mRNA levels detected.

   Could label the Figure better.
Discussion.

5. ‘These results are not conflicting with the observation by Ledee-Bataille et al., [19] who showed an inverse correlation between uterine flushing LIF concentration and the likelihood of successful implantation. He might use different ELISA kit.’

I think that the above statement in the discussion suggests that it is critical to perform a Western analysis on the tissues or washings to see what LIF forms the ELISA may be measuring. I don’t think it’s plausible to state that the variability between studies is due to different ELISA kits. What kits were used in the studies? What forms of LIF do they measure? There may be other differences between the studies.

6. ‘The issue that fertile women secreted higher soluble gp130 in uterine flushing in presence of gp130 mRNA expression as low as in infertile women is not conflicting because soluble gp130 can be produced also by proteolytic cleavage of the membrane bound receptor’ This is confusing. Did the previous publication show high or low soluble gp130 secreted from the endometrial biopsies in vitro from women with infertility compared to fertile women? If so, how does this relate to the present study where soluble gp130 was significantly decreased in the infertile compared to fertile women?

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'