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Project: The effects of oestradiol and progesterone on the expression of Focal Adhesion Pathway and proliferation markers in Ishikawa cell culture
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Introduction:
In women, in the second half of the menstrual cycle, oestradiol and progesterone secretion increases. During this phase the fertilised egg makes contact with the endometrium to begin implantation. Therefore, it is necessary that the concentrations of these reproductive hormones are suitable for development of a receptive endometrium. Inadequate proliferation of the endometrium will directly affect implantation and it may fail.

Aim:
This study aimed to determine if a range oestradiol and progesterone at the approximate concentrations and higher which are administered in clinical IVF cycles influenced endometrial proliferation markers in a cell culture model.

Impact:
Finding the concentration of these hormones which upregulates the expression of the proliferation markers will help to improve the success rate of IVF for women who have recurrent implantation failure.

Method:
To achieve this, Ishikawa cells, a low grade endometrial cancer cell line of luminal epithelial cells was cultured. The cells were exposed to a low and high concentrations of oestradiol and progesterone. The range of hormones chosen was based on approximate estimations of those used in an IVF setting and a higher concentration. The cells were grown in DMEM serum free media to synchronise the proliferation and enhance cellular activity. The cells were treated with oestradiol only, progesterone only and a combination of both hormones at both concentrations was added after 24-hours. The control contained media only. Cell count was performed to ascertain the number of viable cells in the control and treated cells giving an indication of the degree of proliferation for each treatment. Immunohistochemistry (IHC) tested for nine proliferative markers indicating the degree and location of the proteins. First the cultures grown on the coverslips were blocked using normal goat serum to minimise non-specific staining then incubated with primary antibody. Fluorescent labelled secondary antibody was then added and finally DAPI a fluorescent nuclear stain was incubated. The coverslips were inverted onto microscope slides to image under a fluorescent microscope using timed exposure. Western blotting was used to measure protein abundance. The cultured cells were lysed using RIPA buffer to extract the proteins. Sample buffer and DTT were then added prior to loading the samples onto an SDS gel. The proteins on the gel were then transferred onto a PDVF membrane which was placed in a blocker of either BSA or skim milk. Blocking reduced non-specific background before incubation with the primary antibody. The membrane was then cleaned and the secondary fluorescent labelled antibody, anti-rabbit or anti-mouse, was added for imaging.

Results:
IHC of the estrogen and progesterone treated cells gave evidence of the location of each marker as they appeared in the different cellular compartments; the plasma membrane, cytoplasm, nuclear membrane or nucleus. These locations represented the proteins role in proliferation. By measuring the degree of protein visible it was found that at high, concentrations of oestradiol; PCNA was negative in the nucleus with no MKI67 detected at all. At high concentrations of progesterone, the % positive

nuclei were reduced for PCNA and maintained for MKI67 compared to the control. The combination of oestradiol and progesterone at high concentration, the percent positive nuclei for PCNA and MKI67 was the same as the control. This finding suggests that high levels of oestradiol inhibit proliferation, whereas in combination with progesterone, this effect is reduced. Western blotting showed an overall increase of protein expression of PCNA at low concentrations of hormone treatment, PCNA is important in the metabolism of nucleic acids and the replication of DNA and appeared in the nucleus of actively replicating cells particularly in the progesterone treated samples. However, Cyclin A, a protein which drives cellular division by interaction with kinases appeared to be decreased. Whereas YWHAZ exhibited a reduction in band density. Progesterone receptor, an indicator of how cells directly respond to their hormonal environment exhibited no change in band intensity in any hormone treatments. In contrast to these Cyclin A exhibited stronger bands with higher concentrations of oestradiol and progesterone.

Conclusion:

In summary, it appears that higher amounts of oestradiol and progesterone may reduce proliferation adequacy of the endometrial cells tested. This finding could be an important factor when applied to IVF procedures where the cycle is completely controlled by the administration of these hormones with other compounds. To further improve the findings these tests will need to be repeated and also use other endometrial cell lines.