# Increased levels of testosterone associated with polycystic ovary syndrome negatively affect *HOXA10* and integrin *β3* expression in endometrial cells and embryo attachment using a trophoblast cell spheroid model

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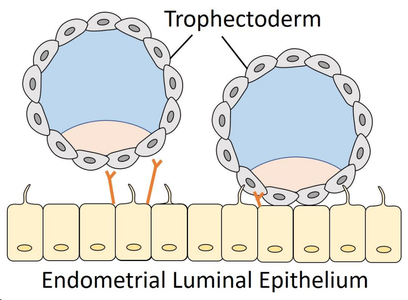
## Introduction

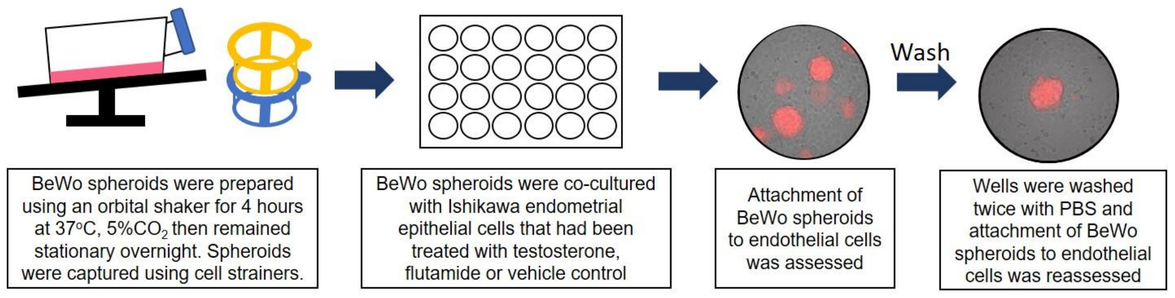
Polycystic ovary syndrome (PCOS) is a reproductive and endocrinological disorder that affects up to 10% of women of a reproductive age. 70-80% of patients with PCOS are determined to be infertile. Symptoms include anovulation, hyperandrogenism, insulin resistance and elevated circulating testosterone (TS) concentrations. High concentrations of TS have been shown to reduce the expression of *HOXA10*, a transcription factors involved in uterine receptivity embryo implantation, in an endometrial cell model[1] and in PCOS sufferers[2]. *HOXA10* regulates αVβ3, an integrin necessary for successful embryo implantation[2].




Here we assess the functional effects of physiological and pathophysiological levels of TS in an *in vitro* model of embryo attachment (**Figure 1**), as well as gene expression and protein production of key regulators of this process.

## Methods

An *in vitro* model of human embryo attachment to an endometrial monolayer was performed (**Figure 2**. modified from Ho et al. 2012[3]). Ishikawa endometrial cells were treated with TS (1nM, 10nM, 100nM) and/or Flutamide (1μM) for 24h to measure the effect on spheroid attachment compared to vehicle control.




The effect of TS and/or flutamide on the gene expression of *HOXA10* and *β3*, and protein expression of αvβ3 on Ishikawa cells were assessed using qRT-PCR and immunocytochemistry respectively.

## Results

### 1. Effect of TS and Flutamide on Ishikawa cells

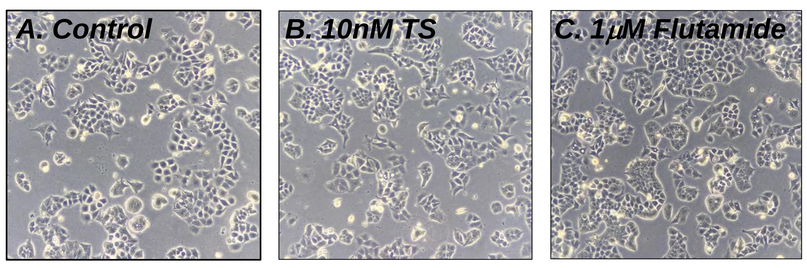



**Figure 3** – Testosterone (TS) and flutamide (androgen receptor antagonist) treatments (24h) do not effect the morphology or growth of Ishikawa cells.

### 2. Effect of TS on BeWo spheroid attachment assay

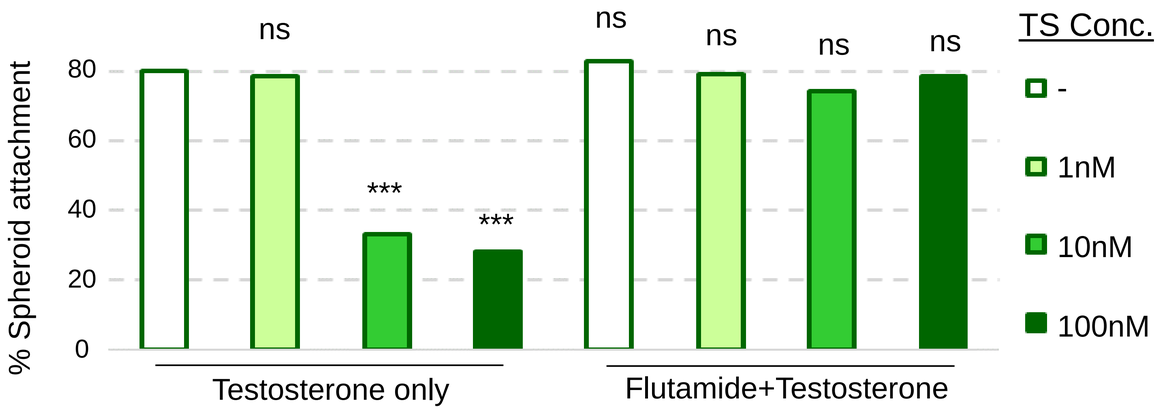



**Figure 4** - The effect of TS on embryo-endometrial cell attachment rate was measured using the BeWo Spheroid assay and an Ishikawa cell monolayer. Attachment of BeWo spheroids to Ishikawa cells pretreated with TS (1nM, 10nM and 100nM) or vehicle control (-) for 24h. % spheroid attachment for each condition was calculated and compared to control (*** =P<0.001, T-test, n=3). Co-treatment with 1μM flutamide rescued the TS-induced decrease in attachment (not significant when compared to vehicle control; p>0.05, n=3). TS doses were selected based on average TS concentrations in women suffering with PCOS (1-5nM[4,5]) and supraphysiological levels (100nM).

### 3. TS inhibits expression of *HOXA10* and *β3*

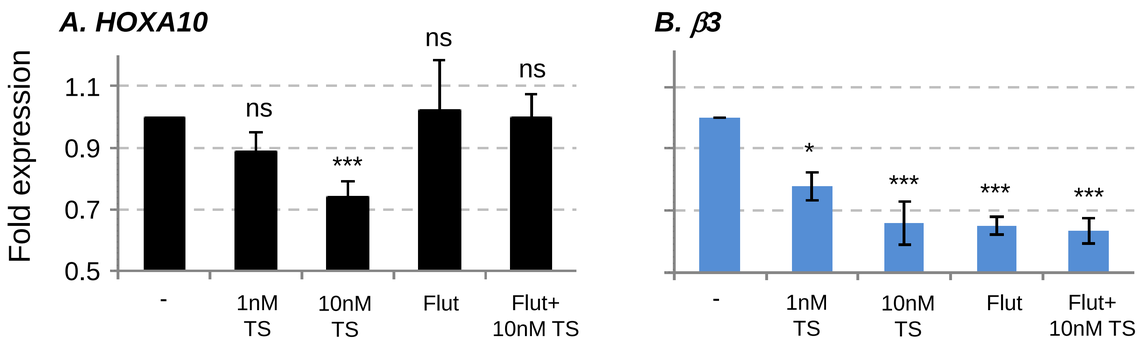



**Figure 5** – Gene expression of (A) *HOXA10* and (B) *β3* was quantified using qRT-PCR in Ishikawa cells treated with TS (1nM, 10nM) and/or flutamide (Flut; 1μM) for 24h. Data shows mean +/- SEM (n=4; T-tests compared to vehicle control. ns = not significant). TS significantly reduced the expression of both genes. Combined treatment with flutamide rescued TS-reduced expression of *HOXA10*.

### 4. Expression of αvβ3 is reduced following TS treatment

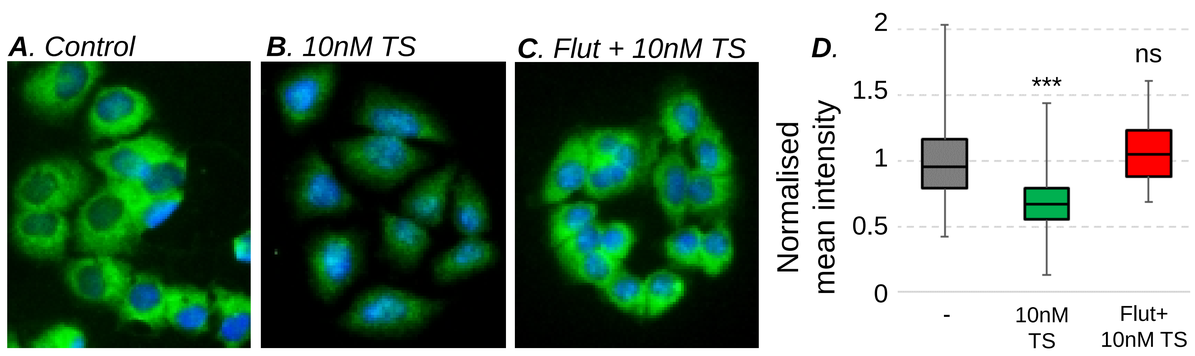



**Figure 6** – Expression of αvβ3 protein in Ishikawa cells was analysed using immunocytochemistry following the treatments described (A-C). Quantification of mean fluorescence intensity was performed using ImageJ (D). A significant decrease in expression was detected following treatment with 10nM TS. Protein expression was rescued with flutamide. (n = 2, >150 cells per treatment. Boxes show median and interquartile range. Bars show minimum to maximum).

## Conclusions

- Hyperandrogenism observed in women with PCOS results in reduced endometrial receptivity through the decreased expression of *HOXA10* gene and αvβ3 protein and this may in part be responsible for infertility in these individuals.
- The effects of excess testosterone on embryo attachment to endometrial cells may be reversed by the androgen inhibitor flutamide, though it remains unclear as to why the functionally important *β3* subunit is unaffected by this treatment despite functional restoration. This will be the focus of further investigations into the signalling pathways involved.
- Highlighting other potential causes of infertility in PCOS may help reproductive success in women with PCOS who are primarily treated for anovulation, often by IVF, but may still experience problems with endometrial receptivity.

## References

[1]Cermik et al (2003) *J Clin Endo Metab*. 88: 238, [2]Daftary et al (2002) *Mol Endo*. 16: 571, [3]Ho et al (2012) Fert Ster, 97: 974, [4]Sheehan (2004) Clin Med Res. 2: 13, [5]Handelsman et al (2018) *Endo. Revs.* 39: 803