

## Introduction

- Implantation failure affects approximately 50% of assisted reproductive conceptions (1).
- Repeated implantation failure (RIF) is diagnosed after the failure of three or more IVF cycles in which one or two high quality embryos are replaced.
- Successful implantation requires a viable embryo to initiate interaction with a receptive endometrium.
- Defects in endometrial receptivity are thought to contribute to the pathology of RIF but the molecular mechanisms remain unclear.
- Recently altered expression of microRNA (miR)-145 was observed in endometrial samples taken from women suffering from RIF (2).
- microRNAs are small endogenous non-coding regions of the genome which post-transcriptionally regulate gene expression.
- In other systems, miR-145 exerts its action by regulation expression of the type-1 IGF receptor (IGF1R) expression (3).

We hypothesised that miR-145 contributes to the underlying pathology of RIF, through its interaction with IGF1R.

## Aims

1. To determine whether miR-145 overexpression in endometrial cells, affects embryo attachment.
2. To ascertain whether miR-145 regulates IGF1R expression in the endometrium.
3. To examine the contribution of IGF1R in mediating embryo attachment.

## Methods

- To model early implantation *in vitro* we utilised the human endometrial cell line (Ishikawa) and ligand-coated bead model as previously described (4).
- Ishikawa cells were transfected with control (pre-C) or miR-145 specific mimetics (Ambion) to replicate the miR-145 overexpression seen in RIF patients (2).
- Overexpression was confirmed by QPCR.
- Embryo sized beads were coated in IGF-I or BSA (control) and co-cultured with Ishikawa cells (Figure 1) for 24-48 hours. Stability of bead attachment was assessed using our previously published stability scale (4).
- The effect of miR-145 overexpression on IGF1R expression was explored at both the mRNA (QPCR) and protein (western blotting) level.
- To assess whether the interaction between miR-145 and IGF1R was direct, predicted miR-145 binding sites in IGF1R were cloned into the pmir-GLO vector and luciferase activity was assessed using the Dual-Glo luciferase system (Promega).
- To determine whether the effects of miR-145 overexpression on embryo attachment were likely to be attributed to IGF1R, we compared the effect of miR-145 overexpression to IGF1R knockdown using IGF1R specific siRNA.
- For direct confirmation that the effects of miR-145 on embryo attachment were attributed to miR-145 regulation of IGF1R expression, we blocked the binding of miR-145 to IGF1R using site specific target blockers (Qiagen), and observed attachment using the implantation model.

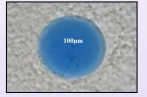
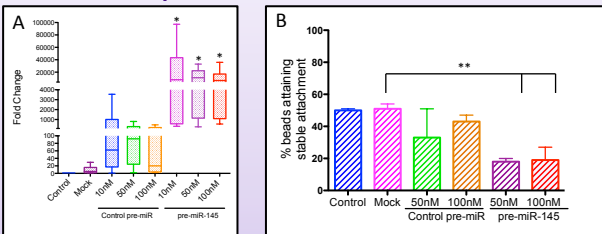


Figure 1. IGF-I coated beads (100µm; visible in blue) were co-cultured with human endometrial (Ishikawa) cells under beads in this image) for up to 48 hours.

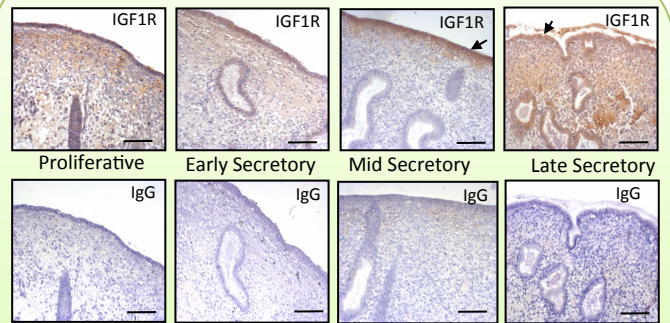
## Results

### 1. miR-145 overexpression decreases IGF-I bead attachment



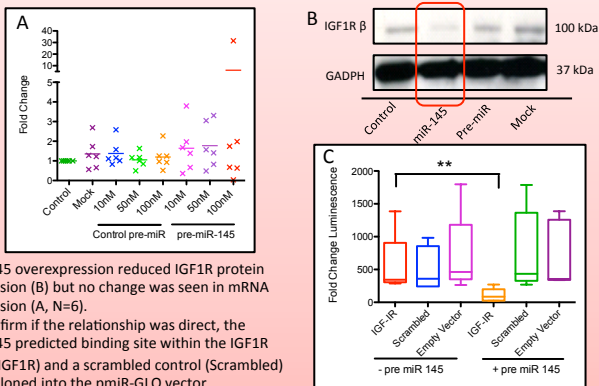
45% of IGF-I coated beads attached to Ishikawa cells after 48 hours, in comparison to lower levels of attachment by BSA coated, control beads (data not shown). Following miR-145 overexpression (pre-miR-145) (quantified by QPCR; A, N=6), IGF-I bead attachment was significantly reduced (B, N=3). No change in IGF-I bead attachment was observed with a non-targeting miR mimetic (PreC). \*p<0.0001 \*\*p=0.0021. Median and Range.

### 2. IGF1R is expressed in endometrial luminal epithelium



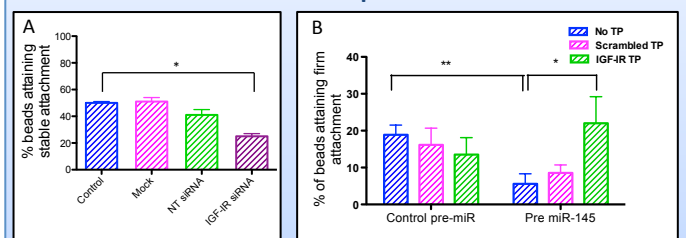
miR-145 regulates IGF1R expression in other cell types. During implantation the embryo must interact with a receptive endometrium, endometrial receptivity is restricted to days 19-24 (mid-late secretory phase) of the menstrual cycle. We examined whether IGF1R was present on the endometrial epithelium at different stages of the menstrual cycle. IGF1R was detected in the endometrium throughout the menstrual cycle but was more abundant at the epithelial surface (indicated by arrows) during mid-late secretory phase, consistent with a potential role in implantation. Scale bars represent 50µm.

### 3. miR-145 directly regulates IGF1R expression



- miR-145 overexpression reduced IGF1R protein expression (B) but no change was seen in mRNA expression (A, N=6).
- To confirm if the relationship was direct, the miR-145 predicted binding site within the IGF1R gene (IGF1R) and a scrambled control (Scrambled) were cloned into the pmir-GLO vector.
- As expected high levels of luciferase were observed in the scrambled and empty vector with and without co-transfection with the miR-145 mimetic (+/- pre miR 145, C).
- Co-transfection of the IGF1R vector with the miR-145 mimetic (pre miR 145) resulted in significant decrease in luciferase activity when compared to the IGF1R vector without the miR-145 mimetic (C, N=5). Demonstrating that miR-145 directly regulates IGF1R expression. \*\* p=0.0079

### 4. The functional effect of miR-145 on bead attachment is attributed to altered IGF1R expression



siRNA knockdown of IGF1R resulted in a similar reduction in attachment as miR-145 overexpression, non targeting (NT) siRNA was included as a control (A, N=3, median and range)(\*p=0.0308). Target protectors (TP) preventing the miR-145, IGF1R interaction (IGF1R TP) reversed the effect of miR-145 overexpression on attachment (B, mean and SEM) (N=5). No change was seen with controls (Scr TP). Showing that IGF1R mediates the change in attachment seen following miR-145 overexpression. \* P value <0.05; \*\* P<0.01

## Conclusions

- IGF1R is localised primarily to the luminal endometrial epithelium, which supports its role in mediating the initial stages of embryo attachment
- Both miR-145 overexpression and IGF1R knockdown in endometrium cause a reduction in attachment demonstrating their importance in regulating embryo attachment.
- This study suggests that by altering miR-145 and therefore IGF1R expression, it may be possible to improve implantation rates in women with RIF.

## References

1. Boomsma et al. 2009. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF.
2. Revel et al. 2011. MicroRNAs are associated with human embryo defects.
3. Law et al. 2012. MiR-145 modulates multiple components of the insulin-like growth factor pathway in hepatocellular carcinoma.
4. Kang et al. 2014. The role of the osteopontin – integrin αvβ3 interaction at implantation: functional analysis using three different *in vitro* models.