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# *In vitro* embryo outgrowth is a bioassay of *in vivo* embryo implantation and development

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## ABSTRACT

**Objective:** To determine the efficacy of embryo outgrowth on fibronectin as a low cost, high throughput alternative to embryo transfer to model embryo attachment and the initial stages of implantation. **Methods:** Following *in vitro* embryo culture, embryo quality was assessed *via* embryo transfer or embryo outgrowth with metabolic assessment. **Results:** This study shows that blastocysts attach to fibronectin at the same rate that they implant *in vivo*, and that the carbohydrate utilisation of embryos that successfully outgrow is comparable to those that are able to develop into a fetus. **Conclusions:** Embryo outgrowth is a suitable alternative endpoint to embryo transfer.

## 1. Introduction

Fetal development following embryo transfer remains the definitive means of determining embryo viability. In the mouse model, embryo transfer to a pseudopregnant recipient female is used to quantitate embryo quality, where compromised embryos have aberrant implantation and fetal/placental development. However this requires time, surgical skills, funding, animal husbandry and ethical consideration. Consequently, this technique is prohibitive for large scale screening and can be beyond the expertise of a research laboratory. A potential alternative method of assessing embryo viability is an *in vitro* model of the initial stages of embryo attachment and implantation attained through embryo outgrowth on fibronectin coated plates.

Being able to distinguish between experimental treatments that can either improve or diminish embryo quality is of significance in a research environment. Morphological grading schemes are commonly employed to classify embryo quality, however many embryos that morphologically appear competent fail to implant [1]. Embryo metabolism has been related to embryo

viability in a number of species, and of significance, has been used to prospectively select embryos for transfer[2]. Mouse blastocysts developed in culture that exhibit a high glucose utilisation and a low glycolytic rate, similar to that of embryos developed *in vivo*, have been shown to be more likely to result in a fetus after transfer than an embryo selected based on morphology alone.

The aim of this study was, therefore, to determine the suitability of *in vitro* embryo outgrowth as a bioassay of embryo implantation and development, and validate a low cost, high throughput alternative to embryo transfer experiments.

## 2. Materials and methods

### 2.1. Experimental outline

Following *in vitro* embryo culture, embryo quality was assessed via embryo transfer or embryo outgrowth with metabolic assessment[3].

### 2.2. Metabolic assessment

Real-time analysis of glucose uptake and lactate output of individual embryos was performed using ultramicrofluorescence[2] prior to embryo outgrowth.

### 2.3. Embryo outgrowth

Flat-bottomed 96-well tissue culture plates were rinsed with

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PBS and coated with 10  $\mu\text{g/mL}$  fibronectin overnight at 4  $^{\circ}\text{C}$  in a humidified chamber. Coated wells were rinsed with PBS and incubated with 4 mg/mL BSA in PBS for 2h, before being rinsed and filled with 150  $\mu\text{L}$  of G2 medium with 5% fetal calf serum, and equilibrated at 37  $^{\circ}\text{C}$ , 6%  $\text{CO}_2$  and 5%  $\text{O}_2$  under paraffin oil for 4 h. Thirty seven blastocysts were placed individually into the coated wells following metabolic assessment and attachment recorded at 48h and total area outgrown measured at 72 h.

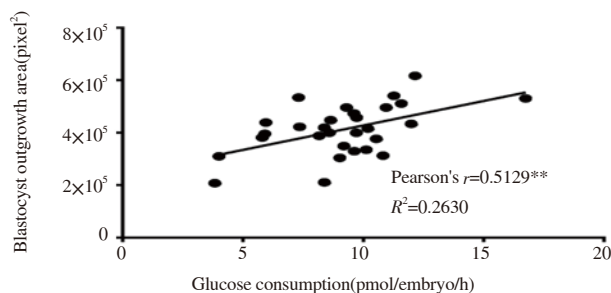
#### 2.4. Embryo transfer

12 week old female mice mated with vasectomised males to induce pseudopregnancy. Ninety seven embryos were transferred on day 4 of development into the reproductive tracts of 20 recipient females staged at day 3.5 of pregnancy. Recipient female mice were anaesthetised with an intraperitoneal injection of ketamine (75 mg/kg) and medetomidate (1 mg/kg). Five embryos were transferred (with a glass pipette) through a small dorsal incision into the lumen of each uterine horn. The skin wound was sealed with sutures and atipamezole (1 mg/kg) was administered for postoperative recovery. Pregnant females were sacrificed 10 days later to determine implantation rates.

Statistical analysis was performed with PRISM version 3.00 for Windows (GraphPad, San Diego, CA).

### 3. Results

Twenty nine blastocysts successfully attached and outgrew, and 8 blastocysts failed to attach and grow *in vitro*. Seventy six blastocysts successfully implanted into the uterine wall, and 21 failed to do so. As such, blastocysts attached to fibronectin *in vitro* at the same rate as they did to the uterine wall *in vivo* (78.38% vs. 78.35% respectively). Blastocysts that failed to attach to the fibronectin matrix and outgrow had significantly higher glycolytic rates than those that successfully outgrew [(48.14  $\pm$  5.76)% vs. (36.22  $\pm$  2.03)% respectively;  $P < 0.05$ ]. Of the 29 blastocysts that did outgrow, there was a significant positive correlation between blastocyst glucose uptake and total area outgrown (Figure 1).



**Figure 1.** Blastocysts that consume more glucose outgrow to a greater extent. The relationship between blastocyst glucose consumption and subsequent total outgrowth area after 72 h. Pearson's  $r = 0.5129$ ,  $R^2 = 0.2630$ .  $**P < 0.01$ ,  $n = 29$  blastocysts.

### 4. Discussion

Whether optimising clinical IVF culture conditions, examining the effects of parental nutrition prior to conception, or investigating reproductive consequences of exposure to environmental toxins, there is a need for a functional measurement to distinguish

viable embryos. Common measures of embryo quality including morphological grading and blastocyst cell count, while providing an indication, are far from definitive. Here we show that embryo outgrowth may be used as an *in vitro* bioassay of implantation and development.

Importantly, embryos attached to the fibronectin matrix at the same rate that they implanted *in vivo*. Of note, the glycolytic rate of embryos that failed to attach *in vitro* was significantly higher than those that successfully attached. Previously, it has been shown that blastocysts with low glycolytic rates have increased viability and a four-fold higher pregnancy rate than those with high glycolytic rates[2]. The range of glycolytic rates observed by Lane and Gardner was much greater than in this study; however the optimised embryo culture conditions currently used could account for this difference. Also, the positive correlation between embryo glucose uptake and area outgrown *in vitro* is consistent with previous studies showing that blastocysts that take up more glucose have increased pregnancy rates compared to those with lower glucose utilisation[4, 5].

In conclusion, mouse blastocysts attached to a fibronectin matrix *in vitro* at the same rate that embryos implanted into the uterine wall *in vivo*. Furthermore, similar to *in vivo* transfer experiments, embryos with higher glycolytic rates failed to attach to the *in vitro* matrix, and those embryos taking up higher amounts of glucose outgrew to the greatest extent. Embryos in this *in vitro* attachment model show a similar metabolic profile and implantation development potential to those undergoing *in vivo* implantation. We therefore propose that embryo outgrowth is a suitable, cost and time effective alternative endpoint to embryo transfers to facilitate the rapid screening of culture conditions or treatments. Embryo transfer, to assess both fetal and placental development will still be required as the ultimate assessment of embryo normality, but the ability to pre-screen using the *in vitro* model described here will greatly reduce time, cost and the need for surgeries.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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