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LETTER TO THE EDITOR

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Implantation: Cell biology of embryo penetration route revisited

In their article "How to create an embryo penetration route," Uchida et al.[1] discuss cell biological views of embryo implantation based on recent experimental data[2] they obtained with an in vitro model employing human endometrial and trophoblast cell lines. The authors conclude that they can define a precondition which must be met by uterine epithelial cells in order to allow trophoblast attachment to occur at their apical cell pole, that is that the cells must undergo certain changes in specific epithelial properties: a shift in adhesion protein expression (from E-cadherin to N-cadherin) as well as of intermediate filaments (cytokeratin to vimentin), combined with a change in cell behaviour. Uchida et al. observed that those uterine epithelial cells which are receptive for trophoblast attachment show increased motility, and they conclude that this property may be instrumental in giving way to the advancing trophoblast. By extrapolation to the situation in vivo, they postulate that this change in properties of the uterine epithelium may permit the blastocyst to start implanting, and that the crucial event here is not apoptosis of the uterine epithelium as a number of authors are assuming (at least not in the human). Uchida et al. interpret their observations as being indicative of epithelial–mesenchymal transition (EMT) of the uterine epithelium occurring at endometrial receptivity (which is hormonally triggered and becomes completed at trophoblast contact), and they present this hypothesis as a new theory of endometrial receptivity and implantation initiation.

Uchida et al. deserve to be congratulated for their careful investigation and their findings. In addition, it is also a merit of their article to draw attention to the EMT concept as applied to embryo implantation. However, it should be of interest to readers that this theory is not new, in contrast to the impression these authors give. This hypothesis was originally developed years ago on the basis of a comparison with certain processes in embryology, that is the so-called embryonic fusion processes.[3–5] Its validity was subsequently explored in a large series of investigations (to be addressed in more detail further below).

Indeed contact formation between two epithelia via their apical pole, in this case the trophoblast of the blastocyst and the uterine epithelium, is a remarkable process which has puzzled developmental biologists. In fact, it appears to disobey basic principles of cell biology because the apical cell pole of epithelia is normally non-adhesive. For this reason, embryo implantation has been addressed as a cell biological paradox, to stimulate research into these phenomena.[3,4,6] EMT was known from studies performed in other cell systems as a process of reprogramming resulting in changes in phenotype and cell behaviour of epithelial cells, but this concept had not been applied to embryo implantation up to that time point. We proposed, therefore, that the EMT concept may provide valuable insights for implantation research when looking over the fence and comparing with studies on other EMT processes (e.g. fusion of palatal shelves during development, or tumour cell invasion).[3,4,6] It may be worth noting that this hypothesis (comparability of embryo implantation with other EMT-like processes) indeed still appears to be the only existing cell biological theory on embryo implantation that focuses on global aspects of cytoplasmic organization and of cell behaviour of trophoblast and the uterine epithelium. It specifically postulates that parts of an EMT programme are used as the machinery to set hormone signalling into action at the level of cell behaviour, without, however, involving a complete switch from the epithelial into the mesenchymal cell programme, in the case of uterine epithelium. In the trophoblast, these changes are more extensive and obvious.[4–6]

To apply the EMT concept to embryo implantation research has proven very useful at least as a heuristic approach, allowing our laboratory to plan and perform a large series of experimental investigations into cell biological details behind the changes in epithelial cell behaviour at receptivity. These studies have originally used ex-vivo material from laboratory animals and human endometria. Later on, we developed an in vitro system employing human choriocarcinoma cell spheroids[7] attaching to uterine epithelial monolayers,[8] that is the system Uchida et al. are now also using.[1] Other groups have not focussed on complex changes in the epithelial programme and cell behaviour (as in EMT) but on partial aspects such as apicobasal polarity,[9] or properties of the apical plasma membrane and junctions.[10,11]

Previous data from our group that may be of interest but have not been mentioned by Uchida et al. include with regard to the *uterine epithelium* (on which Uchida et al. are focussing) information on:

- Reorganization of the actin cytoskeleton indicating changes in the motility apparatus; Rho regulation [12–15] (in vitro model).
- Monitoring of adhesive forces during attachment of trophoblast to uterine epithelium, and of the time course of this attachment [16] (in vitro model).
- Calcium signalling as a typical response to mechanical irritation of the apical cell pole in uterine epithelial cells that are competent for trophoblast adhesion [17] (in vitro model).
- Role of junctional complex organization [18] (in vitro model).
- Redistribution of integrins,[19,20] E-cadherin,[4,20] marker proteins of apical vs. basolateral membrane domains [21,22] and glycocalyx

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glycoconjugates [23] (in vitro model; ex-vivo endometria and implantation chamber)
- Vimentin upregulation (in particular in the vicinity of the implanting blastocyst)[24] (rabbit model, ex-vivo endometria and implantation chamber).
- Changes in cell membrane lipid organization[25] (rabbit model, ex-vivo endometria and implantation chamber).
- Penetration of basal processes of uterine epithelial cells through their own basement membrane, being indicative of changed apico-basal polarity at receptivity[26] (rabbit model, ex-vivo endometria and implantation chamber).
- The data have led to a change of traditional views about the role of the host tissue in embryo implantation: the uterine epithelium appears not to be passive but to participate actively in trophoblast adhesion and penetration and may thus control it in a subtle way from the start.[14]

When the *trophoblast* acquires invasiveness, phenotypic changes are more obvious than in the uterine epithelium at receptivity (and as a response to trophoblast contact) [6] (the idea of EMT-like transformation in the trophoblast has later been taken up by other authors:[27,28]). Using various in vitro adhesion and invasion models (including the choriocarcinoma spheroid/endometrial monolayer system), the role of aspects of trophoblast differentiation has been studied in detail:[29,30]

- Differing adhesion and invasion capabilities of the different choriocarcinoma cell lines (JAR, BeWo, Jeg-3) in the in vitro model (confrontation with uterine epithelial monolayers or with complex endometrial explants).[8,31]
- Role of various modulated states of invasiveness of trophoblast cells.[30]

Taking a side view, Uchida et al.[1] furthermore compare the penetration of the blastocyst through the uterine epithelium at embryo implantation with leukocyte transmigration through the vascular endothelium at inflammation. The authors correctly note that there are, however, a number of differences between these two processes, including the fact that leukocytes transmigrate as single cells between individual endothelial cells, while implantation of the blastocyst requires interaction with a larger number of uterine epithelial cells in a relatively broad area. In the search for a cell biological understanding of implantation, the findings by Uchida et al. emphasize that uterine epithelial cells move apart and give space to the advancing front of the trophoblast, that is that they show a remarkable change in cell behaviour, while leukocyte transmigration through vascular endothelium is a process that is much more limited in space, involving single (leukocyte) cells penetrating through minor dehiscences between neighbouring endothelial cells. Comparison with leukocyte transmigration through vascular endothelium may then indeed have some heuristic value pointing to certain peculiarities of the cell biology of embryo implantation. However, even earlier than the two papers which Uchida et al. are citing in this context,[32,33] these two systems had been compared and the differences had been discussed, with a focus, for example, on junctional proteins and their association with the cytoskeleton, and on cell behaviour, as differing between vascular endothelial and uterine epithelial cells.[34,35]

In conclusion, it should be of value, for any investigator planning to continue exploring the cell biology of the initial events at embryo implantation, to be aware of the data which have been collected before, using this in vitro system employing human cells, as well as ex-vivo materials. As all in vitro models have (varying) degrees of artificiality, extrapolation to the situation in vivo must of course be performed with caution. When planning for extending such investigations, for example when using more complex living or ex-vivo materials, it should be important to remember ethical constraints with regard to the use of human embryos for experimental investigation. This consideration has been and still is an important argument for choosing in vitro models like this one, that is for using human cell lines rather than human blastocysts in such experimental studies. In this respect, ethical considerations must always be seen as more important than aspects of practicability.

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