

Trophoblastic vesicles of preimplantation blastocysts can enter into quiescence in the absence of inner cell mass

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SUMMARY

Preimplantation mouse blastocysts were dissected into inner cell mass (ICM) and trophoblast cells. These fragments were transferred to pseudo-pregnant mice which were left intact or ovariectomized. The latter group received progesterone to permit blastocysts and the dissected fragments to enter into quiescence, prior to injection of oestradiol to induce implantation. Trophoblastic vesicles, without ICM, entered into quiescence and implanted whereas the ICM did not. The entry of trophoblast into quiescence does not appear therefore to be governed by the ICM.

INTRODUCTION

Preimplantation blastocysts are composed of at least two cell types, inner cell mass (ICM) and trophoblast, which show complex intercellular interactions (Gardner & Papaioannou, 1975). These could be governed by surface-to-surface interactions, junctional channels, formation of chemical gradients within the embryo, or extracellular environmental components such as uterine proteins and a selection of serum macromolecules (Surani, 1977). *In vitro* studies have shown that the polyploidization of trophoblast giant cells may occur more rapidly in the absence of ICM (Ansell & Snow, 1975).

Preimplantation blastocysts enter into metabolic quiescence in ovariectomized females maintained on progesterone alone (Weitlauf, 1974); implantation ensues when such females are injected with oestradiol (McLaren, 1973). Trophoblastic vesicles in the absence of ICM might prove unable to enter into metabolic quiescence and subsequently implant if the ICM controls the timing of implantation. Gardner (1971) demonstrated that microsurgically isolated fragments of trophoblast, on transfer to pseudopregnant recipients, implanted and gave rise only to primary giant cells, whereas the isolated ICM did not implant. The time interval between transfer of the fragments and implantation was short and any persisting influence of the ICM on trophoblast and vice versa may have been overlooked. There was also little time for these fragments to adapt and

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respond to the uterine environment before implantation. Cell number approximately doubles as blastocysts enter into quiescence (McLaren, 1968; Surani, 1975); the cells then arrest in the G₁ phase of the cell cycle (Sherman & Barlow, 1972).

In this study, we have transferred trophoblast and ICM to recipients under endocrinological conditions which permit entry of blastocysts into metabolic quiescence, to determine whether these fragments can enter into quiescence and subsequently undergo implantation.

MATERIALS AND METHODS

Randomly bred CFLP mice (Anglia Laboratories) were mated at oestrus and blastocysts were flushed out from the uterine lumen on day 4 of pregnancy (day 1 = day of copulation plug). The zona pellucida was removed mechanically and the blastocyst dissected by microsurgery to ensure complete separation of the ICM from trophoblast, as previously described (Gardner, 1971). Some quiescent blastocysts were obtained on the 10th day following ovariectomy of pregnant females on day 2 of pregnancy and similarly dissected.

ICM and trophoblastic fragments were cultured for 3–4 h in PBI medium with 10% foetal calf serum (Gardner, 1971), during which time the trophoblastic fragments formed fluid-filled vesicles. The vesicles obtained from day-4 blastocysts were subjectively divided into 'small' and 'large'; cell counts on some of the vesicles showed that the former consisted of 10–20 cells and the latter were usually slightly in excess of 20 cells. The ICM consisted of 10–15 cells. The dissected fragments were transferred on day 3 of pseudopregnancy to the right uterine horn of recipients which had been mated to vasectomized males. If available, intact blastocysts were transferred to the left horn. One group of recipients was left intact and examined for implants 4 days after transfer. A second group was ovariectomized immediately after transfer and, 2 days later, given a daily subcutaneous injection of 1.0 mg progesterone in 0.1 ml arachis oil for 4 days, after which embryos entered into quiescence. Some of the females were killed at this stage and their uterine lumina examined for blastocysts and dissected fragments. The rest of the ovariectomized females were given 20 ng oestradiol together with 1.0 mg progesterone for a further 3 days and the uterine horns were examined for implantation sites. All implantation sites were fixed for histological examination by light microscopy.

RESULTS

Forty-two out of 127 trophoblastic vesicles implanted (33%), compared with 73 out of 90 blastocysts (81%), transferred to both intact and ovariectomized recipients (Table 1). A few ICM fragments were also transferred but none evoked a decidual response and no remains of these fragments could be detected in the uterine flushings. This observation is in line with previous studies

Table 1. *Implantation rates of trophoblastic vesicles and blastocysts transferred to ovariectomized and intact pseudopregnant recipients*

Source of vesicles and blastocysts	Recipients	Trophoblastic vesicles				Blastocysts				
		No. recipients	No. transferred	No. implants (%)	No. recipients	No. transferred	No. implants (%)	No. recipients	No. transferred	No. implants (%)
Day 4 pregnant females	Ovariectomized	9	66 (small)	7 (10.6)	13	61	51 (83.6)			
	Intact	6	32 (large)	18 (59.4)	2	10	9 (90.0)			
10th day of delay	Ovariectomized	2	10	7 (70.0)	2	4	4 (100)			
	Intact	2	13	6 (46.2)	2	15	9 (60.0)			

showing that trophoblastic vesicles, but not the ICM, can evoke a decidual response (Gardner & Johnson, 1972; Snow, 1973). When quiescent blastocysts and trophoblastic vesicles were flushed from the ovariectomized females prior to oestradiol administration, the number of cells in the blastocysts was found to have increased from about 60 to 120, whereas the number of cells in the vesicles remained at about 20. Histological examination of all implantation sites showed that where trophoblastic vesicles had implanted, no embryonic cell derivatives were observed and only primary giant cells could be detected, whereas intact blastocysts always developed at least to the early egg-cylinder stage.

DISCUSSION

We conclude that although there is no detectable increase in cell number in trophoblastic vesicles, they can enter into quiescence and implant in the absence of ICM. Fewer trophoblastic vesicles implanted compared with blastocysts, perhaps because the smaller vesicles made inadequate contact with the uterine epithelium. Vesicles implanted in greater numbers when their initial cell number was larger, and when the interval before implantation was short as in transfers to intact recipients. Trophoblastic vesicles prepared from quiescent blastocysts, which have larger numbers of cells (about 30 cells), also showed better survival and implantation rates. A few quiescent blastocysts were found by histological examination to possess delaminating endoderm; it is difficult to ensure that trophoblastic vesicles from these blastocysts were free of ICM derivatives, but none displayed any embryonic cells after implantation.

Since the completion of this study, trophoblastic vesicles prepared *in vitro* by culturing 2-cell embryos with [³H]thymidine (Snow, 1973) have been reported to give essentially similar results to those described here (Snow, Aitken & Ansell, 1976).

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