Electrical potential difference between exocelomic fluid and maternal blood in early pregnancy

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The mechanisms and driving forces for exchange of solute and water between mother and embryo in early human pregnancy are poorly understood. The embryonic compartments that might be involved in exchange are quite different at this stage from those that exist later than 8–10 wk of gestation (9). Until the 10th wk of pregnancy, placental villi cover the entire surface of the gestational sac (Fig. 1); two-thirds of these then progressively degenerate, leaving the definitive placenta. The outer cell layer of each villus is the multinucleated syncytiotrophoblast, the major transporting epithelium of the placenta. Underlying this is a layer of stem cytotrophoblast cells that divide, differentiate, and fuse throughout gestation to enlarge the syncytiotrophoblast. For much of the first trimester the cytotrophoblast cells form a continuous cell layer, but by the 10th wk this has become discontinuous. The core of the villi is made up of chorionic mesenchyme, in which the embryonic capillaries form. The chorionic mesenchyme is in direct contact with, and forms, the maternal facing boundary of the exocelomic cavity, a fluid-filled space only present up until the 12th wk of pregnancy (9). The secondary yolk sac, which also disappears by the end of the first trimester, lies within the exocelomic cavity. The exocelomic cavity is separated by the amnion from the amniotic cavity that encloses the embryo. Therefore exchange might occur at this stage of pregnancy either between maternal extracellular fluid in the intervillous space of the placenta and the blood in the embryonic capillaries, after the 5th wk when vascularization occurs (10), or between the maternal extracellular fluid and the fluid in the exocelomic cavity (8). Both routes involve transfer across the trophoblast. Solute and water in the exocelomic cavity might gain access to the embryo either via the secondary yolk sac or by passing across the amnion, which seems unlikely to present a significant barrier, at least for small molecules (8, 9).

Diffusion is, quantitatively, a very important mechanism of transfer across the term human placenta (4, 15). The predominant driving forces for the net diffusion transfer of ions will be any concentration differences and any electrical potential differences (PDs) across the placenta (13). No previous study has determined whether such a PD does exist across the placenta in the first trimester, and this was the aim of the investigation reported here. We were able to address this question in vivo because the exocelomic fluid is in direct contact with the mesenchyme of the placental villi and because the exocelomic cavity is accessible to catheters by the use of ultrasound guidance (7). We therefore measured the PD between a saline-filled catheter in the exocelomic cavity and another catheter in a maternal vein in women undergoing termination of pregnancy. For comparison purposes we also similarly measured, where possible, the PD between the amniotic cavity and maternal blood.

METHODS

Patients. Healthy women with apparently normal pregnancies who were undergoing termination, at between 6 and 12 wk of gestation, for psychological reasons (clause B of the United Kingdom Abortion Act, 1967) entered the study. Approval for the study was given by the Ethical Committee of University College London, and written informed consent was obtained from each patient before surgery. In all cases gestational age (from the first day of the last menstrual period) was determined from the crown-rump length measured during ultrasound scanning.

All patients were unpremedicated. Anesthesia was induced with propofol supplemented with fentanyl and was maintained with 1% isoflurane with 70% nitrous oxide in oxygen. No intravenous fluid was given pre- or intraoperatively. All patients were spontaneously ventilating.
PD measurements. The technique described here for measurement of PD was similar to that used to measure between-compartment electrical potentials in the sheep and pig conceptus (1, 17). In the sheep studies it was shown that catheters filled with 150 mM NaCl gave the same value for PD as KCl-agar bridges.

In the present study a sterile bubble-free and free-flowing saline (150 mM NaCl)-filled nylon catheter (ref 800/200/100/200 1D 0.50 mm, OD 0.63 mm; Portex Limited, Hythe, UK) attached to a three-way stopcock and supplied from a reservoir of saline in a 50-ml syringe was inserted 4–6 cm into a forearm vein through a 17.5-gauge cannula that had been inserted into the vessel. A second similar nylon catheter attached to a three-way stopcock with its own reservoir of saline was inserted transvaginally into the exocelomic cavity (see Fig. 1), under ultrasonic guidance using a 5-MHz convex transvaginal probe (Aloka SSD-500; Aloka, Tokyo, Japan) through an 18-gauge syringe needle in a 16-gauge guide attached to the shaft of the probe. The catheter was advanced until it was visible on the ultrasound scan, 2–3 mm beyond the tip of the needle. The stopcocks were connected via bubble-free polyvinyl sterile saline-filled extension tubing to a matched pair of calomel half-cell electrodes (type 33 1370 210; Kent-Taylor Limited, Stonehouse, UK), which, in turn, were connected to a high input impedance (>10^10 Ω) direct-current digital voltmeter (model 1055, Datron Electronics, Norwich, UK). The voltmeter was connected to the mains via an isolating transformer (MIT) to ensure patient safety.

A positive reference voltage offset was added to the output of the voltmeter to overcome the inability of the computer system to record negative voltages. The recording system was calibrated using the precision millivolt source. Before the start of PD measurements, the offset PD between the two calomel electrodes was recorded while the ends of the catheters were in the same container of saline and an appropriate correction was made (always <1.3 mV).

Once catheters were in position in a maternal vein and the exocelomic cavity, saline was flushed through so as to create a bubble-free circuit and PD was monitored continuously by visual inspection of the voltmeter and recorded at 3-s intervals by the data logger. A satisfactory circuit for measurement was taken to have been established if the PD was stable (in the presence of bubbles in the circuit-wide swings in voltage that were seen) and if a known voltage from the precision millivolt source added to the circuit altered the recorded value appropriately. If necessary, the flushing and voltage addition procedure was repeated until a circuit was established. Once this occurred, the voltage addition was removed and the measurement of PD between maternal blood and the exocelomic cavity was allowed to stabilize. PD was then recorded for 15–27 s, after which 100 mV from the precision millivolt source was added to check the circuit again; finally PD was recorded once more for 9–27 s. The two catheter connections were then reversed at the stopcocks to confirm symmetry about the calomel electrode offset potential. After reversal, the procedure of flushing through the catheters and voltage addition was repeated to ensure a bubble-free circuit. A stable recording of PD was again obtained, over 12–60 s, before and after addition of 100 mV into the circuit, this time to confirm appropriate addition to a PD of opposite polarity to that recorded before reversal. The mean of these four sets of measurements was taken as the mean PD for that conceptus. The following further criteria were applied for a PD measurement to be accepted. 1) The tip of the catheter was seen ultrasonographically to be surrounded by fluid in the cavity. 2) No placental separation or bleeding was visualized after insertion of the needle. 3) PD measurements were from a conceptus with an ultrasonographically normal single fetus. Measurements from eight patients of the 14 in whom recordings from the exocelomic cavity were attempted met these criteria.

Measurements from the amniotic cavity were attempted in some patients after a recording from the exocelomic cavity had been made. We also attempted to make measurements solely from the amniotic cavity in other patients in whom, because they were at a later stage of gestation, the exocelomic cavity was not accessible; in these instances the amniotic cavity was accessed by transabdominal ultrasound-guided puncture. The needle guide was inserted into the amniotic cavity and the catheter was introduced, its tip being positioned, as before, 2–3 mm beyond the end of the guide. Measurement of the PD between the maternal vein and amniotic cavity was then made. The same criteria were applied as for the exocelomic cavity measurements, except that in only one patient (II in Table I, in whom a measurement was made by transabdominal ultrasound-guided puncture and in whom no measurement from the exocelomic cavity was
the maternal blood/amniotic cavity PD (equation for between the maternal blood/exocelomic cavity PD and correlation between the maternal blood/exocelomic cavity and maternal blood in early human pregnancy. Such a PD indicates that there is a site of inclusion criteria, are shown in Table 1. In every case a PD was recorded with the cavity negative with respect to maternal blood. The PD in each patient was very stable; the range of times over which it was recorded in the eight patients was 3.6–5.2 min and overall there was no significant difference between the mean of the first recording of PD and that of the last. The mean (±SE) PD was −8.7 ± 1.0 mV. There was no significant correlation between the maternal blood/exocelomic cavity PD and gestational age (r² = 0.36, P > 0.1).

The amniotic cavity was also negative with respect to maternal blood in all four measurements between these two compartments (Table 1). The mean of the four measurements was −6.7 ± 1.3 mV. Evaluating the three patients in whom measurements were made between both cavities and maternal blood, we found there was a linear correlation (r² = 1.0; P < 0.001) between the maternal blood/exocelomic cavity PD and the maternal blood/amniotic cavity PD (equation for the line is given by y = 0.92x + 0.54, where x is the maternal blood/exocelomic cavity PD and y is the maternal blood/amniotic cavity PD).

DISCUSSION

The data reported here provide the first evidence of which we are aware that there is a PD between the amniotic cavity and maternal blood and between the amniotic cavity and maternal blood in early human pregnancy. Such a PD indicates that there is a site of charge separation between these maternal and embryonic extracellular fluids. Five possibilities as to the genesis of the PD occur to us.

First, there might be a diffusion potential at the tips of the catheters, different in the maternal and embryonic compartments, that leads to an apparent PD between them. However, this seems unlikely as the ion composition of exocelomic fluid and of amniotic fluid is very similar to that of maternal plasma (6, 7).

Second, the possibly electrogenic fetal epithelia of, e.g., lung, stomach, or intestine might be responsible, although they are only at the earliest stages of development at the gestational ages studied here.

Third, the amnion might be the site of charge separation, but this has a thin epithelium, poorly complemented with organelles such as mitochondria and endoplasmic reticulum (9), which seems unlikely to generate polarized ion fluxes.

Fourth, the secondary yolk sac within the exocelomic cavity might contribute to the PD, especially as the yolk sac of rodents certainly does seem to generate a PD in vitro (2). Unfortunately there have been no functional studies of the human secondary yolk sac.

Finally, and in our view most probably, the PD might be generated within the placental villi. In an in vitro microelectrode study on term placental villi a transsyncytiotrophoblast PD was found with a range in values from 0 to −15 mV (5), which is similar in magnitude and of the same polarity to the PD measured in vivo in the present study. It therefore seems likely that it is the syncytiotrophoblast that generates the maternal blood/exocelomic cavity PD.

There have been two previous measurements of PD between maternal and fetal extracellular fluid in humans, both at later stages of gestation than the present study. Mellor and colleagues (12), studying pregnant women at term, found the PD between an electrode in the maternal peritoneal cavity and a salt bridge in the umbilical vein to be 0 ± 3 mV (n = 7) and between the former electrode and a salt bridge in the amniotic cavity to be 2 ± 4 mV (n = 10); i.e., both measurements were insignificantly different from zero. Stulc and colleagues (16), making measurements on women undergoing legal termination by hysterotomy at between 15 and 22 wk gestation, found the PD between a salt bridge in a maternal cubital vein and one in an umbilical artery to be −2.7 ± 0.4 mV (n = 7). However, both these reports have to be treated with some caution, because in neither was there any demonstration that there was an intact electrical circuit present during recordings, unlike the present study. Furthermore, the measurement between maternal extracellular fluid and umbilical blood in the study of Mellor et al. (12) was made after delivery of the fetus.

Maternal/fetal PDs have been recorded in a number of other species besides humans, and there has been controversy regarding the site of electro genesis (11). It is now clear that there are species differences; in the guinea pig, for example, the maternal/fetal PD is reported to be generated by the endometrial epithelium (3), whereas in the rat it is probably generated by the placenta (14). As already mentioned, the present study and the previous in vitro study (5) suggest that the placenta generates a PD in women.

Normal growth and development of the embryo and fetus depends on transfer of solute and water from...
maternal plasma in appropriate amounts. Regardless of the site of its generation, the existence of a maternal blood/exocelomic cavity PD will drive net diffusion of charged solutes between mother and embryo. For example, there are only small differences in the concentrations of sodium, potassium, and chloride in maternal plasma compared with those in exocelomic fluid (6, 7) and, based on this alone, the driving force for net diffusion from mother to embryo or vice versa is very small. However, the presence of an 8.7-mV PD, embryo side negative, means that there is an electrical driving force for net diffusion of sodium and potassium to the embryo; such diffusion may not, alone, be sufficient for embryonic and fetal growth but it will certainly contribute. By contrast, for chloride, the effect of the PD is to drive net diffusion from embryo to mother. Therefore, as the developing and growing embryo has to accumulate chloride, there must be transport mechanisms operating in the placenta that, from the earliest stages of pregnancy, drive net transfer in the maternal to embryo direction against the electrochemical gradient. Similar analyses can, as a result of this study, be applied to the consideration of the forces affecting maternal/embryo exchange of other cations and anions. The demonstration that this PD does exist in vivo therefore provides one of the pieces of information that is fundamental to the understanding of the mechanisms of maternal/embryo exchange in early human pregnancy.

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