Monozygotic twinning: is it related to apoptosis in the embryo?

Dear Sir,

The advent of prolonged in-vitro preimplantation embryo culture using sequential culture media (Gardner et al., 1998) has permitted many IVF laboratories to successfully adopt blastocyst transfer. Unfortunately, an unexpected side effect of this success has seen a small but significant appearance of monozygotic twinning. This has now been described by several authors (Peramo et al., 1999; Behr et al., 2000; Van Langendonckt et al., 2000; da Costa et al., 2001; Sheiner et al., 2001), in up to 5% of ongoing pregnancies. Curiously enough, no monozygotic twinning was reported during our 10 years of practice, including over 800 deliveries, when co-culture was used to obtain blastocysts. The phenomenon of monozygotic twinning is seemingly a result of an in-vitro artefact and leads us to presume that although culture techniques have been dramatically improved in recent years, we are still far from providing the best environment.

A number of hypotheses have been proposed in relation to this phenomenon. An association with in-vitro related zona hardening has been implicated, whereby embryos are pinched into two at the time of assisted hatching (Alikani et al., 1994). However, this aspect would not always lead to monozygotic twinning, as a small percentage would presumably form a fetal sac and an empty sac containing no fetus, if only trophodermal cells were pinched off. This may have been missed during diagnosis. In addition, the above reports linked to monozygotic twinning are not exclusively linked to the use of assisted hatching.

The appearance of monozygotic twinning leads us to suspect a possible anomaly in the distribution of inner cell mass (ICM) cells within the embryo. The question therefore arises as to whether the ICM is more sensitive to culture media. Interestingly, the cells within the ICM appear to be more sensitive to apoptosis and less resistant to disruption than trophoblastic cells (Pampfer, 2000). It is possible that some human embryos are hypersensitive and that there could be an over-stimulation of apoptosis in media containing an excessive glucose level. Moley et al. have shown that hyperglycaemia induces apoptosis in preimplantation mouse embryos (Moley et al., 1998). The pro-apoptotic effector Bax was found to be increased in murine blastocysts exposed to hyperglycaemia, through free radical formation (Pampfer, 2000). Pampfer et al. found that two intracellular effectors of apoptosis, caspase-3 and caspase-activated deoxyribonuclease (CAD), were involved in the embryotoxicity of high glucose (Pampfer et al., 2001). Subsequent data has suggested that Bcl-2 is involved in the protective response against the induction of chromatin degradation in blastocysts on their exposure to high concentrations of D-glucose in vitro, whereas nuclear fragmentation appears to result from the activation of an intracellular pathway that is independent of Bcl-2 (Pampfer et al., 2001). Although the same studies have not been performed in human embryos, the existence of apoptotic pathways impacting on embryo fragmentation has been shown (Hardy, 1999; Hardy et al., 2001).

The ability of preimplantation embryos to undergo an apoptotically-controlled remodelling indicates that anomalies in this process could occur. How could this relate to the phenomenon of monozygotic twinning? The following hypothesis could be envisaged. If a linear polarization of apoptotic cells occurs, as shown in Figure 1, the ICM could split during, or prior to, the hatching process, leading to two separate ICM and monozygotic twinning. A solution may be obtained from

![Figure 1. Hypothetical model of the relationship between apoptosis and monozygotic twinning. (a) Apoptotic cells (black dots) are apparent in the ICM; (b) a critical mass of apoptotic cells forms in a line and (c) cell fragmentation occurring in the line of apoptotic cells leads to a separation creating two ICM poles due to extra pressure during the hatching process.](image-url)
studies on rodents, indicating that reduced glucose levels in the culture medium and/or the presence of protective agents against free radicals within the blastocele might provide benefit. Co-culture using feeder cells had been shown to provide a continuous supply of free radical scavengers (Ouhibi et al., 1990). The free radical scavengers present in current blastocyst culture media may not be efficient or amenable to the embryo.

Monozygotic twinning is mainly, but not completely (Peramo et al., 1999; Schachter et al., 2001), a phenomenon linked to the prolonged culture of embryos. It is also an indication that we are still unable to confidently support culture of all embryos in vitro and that some embryos may still be more susceptible to the in-vitro environment. It also provides a warning that we always need to proceed carefully when adopting new techniques in IVF. Indications exist that in-vitro culture can alter preimplantation embryo function in a number of ways (Doherty et al., 2000), highlighting the need to continue research in the field of embryo culture so that we can provide the environment that best supports preimplantation embryo development in vitro.

**References**


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