Commentary

Soluble HLA-G release by the human embryo: an interesting artefact?

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Abstract

Several publications have recently claimed that early human embryos release soluble human leukocyte antigen-G (HLA-G), and that there is a direct positive relationship between the quantity of this protein secreted and embryo quality and viability. These publications report a detection limit of 1 or 10 ng/ml of medium, and the quantity of material released was of the order of magnitude from 3 to 80 ng per day and per embryo. The protein content of a human preimplantation embryo can be estimated at 45–50 ng. This means that the HLA-G release reported could be from 10 to 100% and above the total protein content of the embryo. In recent decades, bio-industry has intensively engineered Chinese hamster ovary cells and optimized their gene expression in order to achieve extremely high yields of recombinant protein secretions reaching 50 pg per cell and per day. This is 100 times lower than what is proposed for soluble HLA-G release by the embryo. It is obvious that the embryo releases signals, but these are unlikely to be secreted at the order of magnitude of the concentrations that have been so far estimated.

Keywords: *embryo selection, HLA-G, human embryo protein synthesis, implantation*

Early preimplantation embryos release factors that regulate ovum transport and prepare the female genital tract environment for implantation (Betteridge *et al*., 1979; Villalon *et al*., 1982). Identification of these messages has always been difficult due to the scarcity of the material: an embryo's dry mass can be estimated at 30 ng in the mouse and 70 ng in the human (Lowenstein and Cohen, 1964; Chi *et al*., 1988). Moreover, these signals can be trapped and transported by albumin (Ménézo and Khatchadourian, 1986). An embryo signalling molecule has been identified in the hamster: kentsin (Kent, 1975), a tetrapeptide with opiate properties (Bueno *et al*., 1986).

Several papers have recently claimed that the early preimplantation human embryo releases soluble HLA-G, and that this release could be considered as a marker of embryo viability (Sher *et al*., 2004). In the work of Noci *et al*. (2004), the embryos were cultured in around 500 µl of medium. The positive detection threshold was found to be 1 ng/ml, corresponding to a detectable release of 0.5 ng per embryo. In the group of transferred embryos, a mean release of 3–6 ng was observed with a maximum of 36 ng/ml. This means that 3–15 ng of HLA-G was released by each embryo. This corresponds to 3–30% of the total protein content of the embryo. In the paper of Yie *et al*. (2005), the embryos (1–4) were cultured in 400 µl of culture medium. The detection limit of the test was 10 ng/ml, indicating a higher order of magnitude. The mean release observed was 0.165 µg/ml, i.e. 66 ng for the 400 µl of culture medium. This leads to 66 ng for 1–4 embryos: between 16 and 66 ng per embryo, a relatively large amount compared with the total embryo protein content (**Table 1**).

All of the estimated values for dry mass and protein content of the mouse embryo are very similar. On the basis of the work of Brinster *et al*. (1967), and Lowenstein and Cohen (1964), the protein content of the zona-free mouse embryo can be estimated at 18–20 ng, with a slight decrease during the first few days of preimplantation development. The weight of dry matter increases after maternal to zygotic transition (Turner *et al*., 1992). The protein content of cattle oocytes is around 130 ng (Grealy *et al*., 1996). According to the work of Chi *et al*. (1988), the protein content of the human embryo can be estimated to be 2.3 times greater than that of the mouse (**Table 2**). The amount of protein can thus be estimated on the basis of the following calculation: $V = 4/3\ \pi R^3$, where R is the radius of the sphere (Euclid, 325–265 BC). Therefore the difference in volume between the mouse and the human embryo is based upon the difference in their radii. The value of 2.3 as a volume ratio is based on the fact that the radius of the human embryo is one-third larger than the mouse embryo radius, and the ratio of human/mouse radii is 1.33. This is the value proposed by Chi *et al*., which is acceptable. If the radius of the human embryo is considered instead to be 50% larger than that of the mouse, then the calculation becomes $(1.5)^3 = 3.37$, not dramatically different from 2.3. Using 2.3 as the volume ratio, the estimated protein content of the human embryo, without its zona pellucida is calculated as 45 ng. If the calculation is re-done to consider a 50% increase in diameter for the human embryo, the protein content would be $(45/2.3) \times 3.37 = 66$ ng. This figure still means that in some cases the calculated release of HLA-G is 25%, and greater than the total protein content of the embryo.

Over the past two decades, bio-industry has extensively engineered CHO cells to achieve extremely high yields of recombinant proteins, in particular monoclonal antibodies: the yield can reach 50 pg per cell and per day. The papers of Yie *et al*. (2005) and Noci *et al*. (2004) claim an HLA-G production rate of 5.5 and 7.3 ng HLA-G per blastomere and per day. This production rate is more than 100-fold higher than the best production rates achieved with CHO cells, after decades of optimizing their gene expression. The mRNA content of an embryo is roughly 20 times greater than that of a normal diploid cell such as the CHO cell, but in the embryo there is a high

Table 1. Proposed production of human leukocyte antigen-G by human embryos, ng/embryo (% of total embryo protein content).

	Noci et al. *2004*	*Yie* et al. *2005*
Mean production	0.76 (1.6)	22 (49)
Maximum production	7.3 (16)	66 (147)

Table 2. Protein content of mouse embryo (ng, *zona free).

Reference	*1-cell*	*2-cell*	*8-cell*	*Morula*
Brinster (1967)	27.8	26.1	23.4	20.6
Lowenstein and Cohen (1964)	20.2 (18.4*)			

For human embryos, multiply by 2.3 (according to Chi *et al*., 1988); estimated protein concentration in zona-free early embryos = 40–50 ng.

level of expression of the enzymes involved in metabolism, protein synthesis and DNA repair: the embryo has to deal with the synthesis of a wide variety of compounds, not just a single entity. Roudebush *et al*. (2002) indicate that a human embryo secretes 50–100 pmol of platelet activating factor (PAF); on the basis of a molecular weight of 500 for PAF, this corresponds to 15–50 ng PAF. If the proposed level of secretion for HLA-G is added, an embryo with 75 ng of total dry matter would have to invest the majority of its energy in synthesizing and releasing these two molecules alone. The level of production described by Desai *et al*. (2006) seems more reasonable (around 1 ng per D3 embryo), but although this author reports that HLA-G production increased with cell stage, no correlation was found between the highest secretion of HLA-G and pregnancy.

Sallam *et al* (2006) recently provided further confirmation regarding relative orders of magnitude with respect to protein release: they estimated the mean total quantity of protein released by human embryos into culture medium from the time of in-vitro fertilization to embryo transfer to be 6.10 pg/embryo per hour, i.e. approximately 150 pg (0.15 ng) of protein released per day, independent of embryo quality (as defined by implantation). Using an estimate of 50 ng as the total protein content of a zona-free embryo, this study suggests that an embryo secretes only 0.3% (0.15/50) of its total protein content into the medium during in-vitro culture.

It is clear that mRNAs for HLA-G have been detected in the human blastocyst by reverse transcription-polymerase chain reaction, a stage significantly later than that of maternal to zygotic transition (Jurisicova *et al*., 1996). It is also common knowledge that it is a great deal easier to detect specific mRNAs than it is to detect specific proteins.

Certainly, it is obvious that the embryo may release signals into the culture medium. Whether or not these include the secretion of soluble HLA-G at the concentration described by the authors is questionable. The most interesting part of this proposal is whether the amount of HLA-G secreted is quantitatively related to the quality of the embryo; if this is the case, it is a significant finding. It could be due to translation of one of the messages for factors that regulate ovum transport and prepare the female genital tract environment for implantation, and if this is the case, real identification of such a message is mandatory.

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