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Comment on G. Herranz: The timing of monozygotic twinning: a criticism of the common model. *Zygote* (2013)

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Comment

The topic of monozygotic twinning (MT) on which the recent paper by Herranz (Herranz, 2013) focuses is indeed a fascinating one, not only for the embryologist. And, as pointed out by the author, it may on principle also be relevant for ethical discussions in the context of embryo research and stem cell work in humans. Obviously, this is the reason why the paper was published in *Zygote*, in spite of the fact that it concentrates on history and speculation. When reading through the paper, however, I have ended up disappointed.

The paper does have one merit: it is based on very careful reading of old, in part classical, descriptive literature, including original papers, reviews and textbooks, and it depicts meticulously the history of the emergence of the generally accepted model of MT in the human. It is indeed fun reading that first, historical part of the paper. The text correctly states that the universally accepted idea about the various modes of MT (addressed as 'the model') is based on few experimental data, which is a known fact and not surprising because embryo experimentation meets (and must meet) restrictions in humans. Those passages of the paper that come after this main part, however, are very disappointing: The Conclusions are very poor, and so is the Addendum – presenting an alternative hypothesis proposed by that author. His hypothesis consists, in substance, of nothing more than the assumption that the mechanism of MT is based solely on separation of (the first two) blastomeres [i.e. just one of the mechanisms which the author addresses in the main part of the paper, specifically under the heading 'Part (a)'], while suggesting that the other mechanisms depicted in nearly all textbooks [splitting of the inner cell mass or of the embryonic shield, his 'Part (b)' and 'Part (c)', and double gastrulation ('Part (d)'] do not play any role in practice, and that the various different arrangements of the fetal membranes as found in twins are the results of differences in the degree of fusion of extra-embryonic membranes. The latter possibility had already been suggested by other authors, as also stated in the present paper.

One aspect of this paper is of particular concern: the mechanism that the author proposes to be the only one at work in natural MT, i.e. the separation of the first two blastomeres, is addressed as a variant of *fertilization* rather than of *development*. And the author makes the point that this attribution should be relevant for discussions on the ethics of embryo and stem cell research. Although he does not specify what ethical conclusions could be drawn from his suggestions, it is obviously warranted to take a closer look at the question how stringent is this argument. Unfortunately, it turns out that this proposal on terminology is confusing: it may invite the mistake that it is based on biological facts. However, no compelling biological evidence is presented for the correctness of extending the time frame for the fertilization cascade until the end of the first cleavage. To address the first cleavage division and the resulting positioning of the two first blastomeres as part of the fertilization cascade remains just a proposal on terminology, no more, as long as no biological data are presented that may support this proposal. Yes, the initiation of cleavage (not only of the first but likewise of subsequent cleavage events) is indeed normally connected with signalling initiated by sperm penetration. But cleavage can likewise be initiated by artificial egg activation, without sperm. It appears much more reasonable to stick to the classical view that the first cleavage already marks the beginning of embryonic development (after fertilization as well as in parthenogenesis). Studies on gene expression patterns have recently revealed that activation of certain genes that are relevant for

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development occurs much earlier than previously thought.

Why is the author's proposal misleading when being put in a context of bioethical considerations? It could be mistaken by some to argue against considering the *developmental potentiality* of cells as an important ethical topic: it could invite the shorthand conclusion that cloning by splitting or by isolation of cells cannot be considered possible after (what the author defines as) fertilization. A serious deficiency of this publication is that it fails to address relevant data from *experimental embryology* and *developmental biology* but rather restricts its discussion to descriptive and speculative publications. As a result, the author completely misses discussing aspects of potentiality. For example, the reader should have been reminded of literature on successful embryo splitting in mammals (a method of cloning), particularly effective in cleavage stages (for example, discussed together with the technical problems met with in primates in Schramm & Paprocki, 2004). With regard to the ethical aspects that the author mentions, the potentiality of blastomeres is of utmost relevance for discussions on blastomere biopsy as part of preimplantation genetic diagnosis (PID), a topic that is of great interest in recent ethical discourses. The omission of data from developmental biology is of particular concern in those chapters that deal with the MT modes by splitting of the inner cell mass and of the embryonic disc and by duplication of the primitive streak. Here a wealth of data is available from experiments that have been carried out in various species of vertebrates including mammals (mouse and rabbit; reviewed before by Denker, 2004). This literature shows that developmental potential (in the sense of basic body plan formation, including individuation capacity) is maintained well beyond the cleavage stages. If the author had addressed such experimental data it would have become clear to the reader why and how potentiality is relevant for the discussion of topics such as cloning and stem cell ethics [embryonic stem (ES) as well as induced pluripotent stem (iPS) cells; Denker, 2009].

Minor points

The arguments presented by the author are not always stringent, often due to a failure to consider recent literature. So, with regard to MT by blastomere separation/splitting of cleavage stages, he criticises other authors for not taking into account that the zona pellucida (z.p.) normally encases the blastomeres relatively tightly so that separation to form two individual entities rather than sticking together appears improbable, given the fact that blastomeres indeed show a tendency to stick together in chimera formation experiments. What he omits, however, is that authors usually assume that in MT a precocious action of proteinases may play a role *in vivo*, causing early weakening (or even partial dissolution) of the z.p., thus facilitating the separation of blastomeres. It has been speculated in the literature that genetically determined differences in the levels and timing of appearance of proteinase activities may be a reason for the higher incidence of MT seen in certain families (although no direct proof for this is available). Conversely, the author stresses the lack of any published *in vitro* observations about twinning during cleavage and blastocyst formation, in *in vitro* fertilization and embryo transfer (IVF-ET) programmes. He could have offered an explanation, however, when keeping in mind the fact that the physical properties of the z.p. tend to differ *in vivo* versus *in vitro*. A well known problem in IVF is z.p. hardening (possibly by lack of normal proteinase action *in vitro*), causing problems that these *in vitro* cultured blastocysts have with hatching (so that even assisted hatching by e.g. zona drilling is performed; Hammadeh *et al*., 2011). So we have to consider the possibility that *in vitro* the blastomeres are encased by the z.p. more strongly and over a longer time period than *in vivo*, so that, in the context of the author's reasoning, it should not appear surprising that in IVF-ET spontaneous splitting of cleavage stages and a formation of twin blastocysts usually is not observed. In contrast, evidence for a splitting of inner cell masses has indeed been observed during blastocyst hatching *in vitro* (whenever that event is allowed to occur), and such literature is available and should have been cited in the present paper.

When discussing how the embryonic disc may split at later stages ('Part (c)') it would have been reasonable for the author to address at least some of the information available about cellular details found in the polyembryonic armadillo (Enders, 2002). The author mentions this species but cites only older literature and gives the impression that details are unknown.

In continuing the discussion of splitting at late stages, the author refers in 'Part (d)' to the complexity of the structured egg cylinder of the mouse, citing the review by Tam & Gad (2004). But he does not point out that all this refers specifically to the mouse, not the human, which is in a sense unfair to readers who might not be aware of the fact that the mouse egg cylinder (which does not exist in a comparable form in the human nor in many other species) is a very special and complex structure. It would have been fair to add here that MT is NOT found in the mouse *in vivo* (maybe in part due to the complexity of the egg cylinder), so these data cannot be used as a reasonable argument in

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a discussion of twinning mechanisms in the human. On the contrary, the species difference could be seen as an argument for considering such later stage splitting as possible in the human.

Conclusion

In conclusion, the article by Herranz (2013) can be read as a detailed historical review of older literature, but its complete omission of relevant functional data from developmental biology must lead to the warning that care should be taken when consulting this publication in the context of any ethical considerations with regard to embryo experimentation and stem cell derivation/use in the human.

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