Point of VIEW

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Egg Integrins: Back in the Game of Mammalian Fertilization

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ABSTRACT Recent data provide insights into the function of egg integrins in mammalian fertilization and address some of the controversies regarding the involvement of these molecules in sperm−egg interaction.

Mammalian sperm interact with eggs on three levels, first with the two extracellular coats around the egg and ultimately with the egg plasma membrane. Embryo creation is the result of gamete membrane binding (or adhesion) and subsequent fusion. Work published in the mid-1990s raised the exciting possibility that the integrin family of cell adhesion molecules (*1*) could be involved in this gamete membrane interaction step of mammalian fertilization. Integrins are heterodimers, with 18 α subunits and eight β subunits making 24 different combinations (*1*). A β_1 integrin(s) on eggs was thought to be a receptor for a sperm ligand(s), with the main candidates on sperm being members of the A Disintegrin and A Metalloprotease domain (ADAM) family (*2, 3*). However, a 2003 report raised questions about this model, with the demonstration that mice with eggs lacking the β_1 integrin subunit are fertile and these β_1-deficient eggs are capable of being fertilized (*4*). This work demonstrated that expression of β_1 in eggs was clearly not essential for fertilization to occur. But is there still a role for β_1 present on wild-type eggs? A study in this issue by Baessler *et al.* (DOI 10.1021/cb900013d) (*5*) from the lab of Nicole Sampson sheds light on this matter, conjuring up memories of the famous Mark Twain quote, "The reports of my death have been greatly exaggerated." It seems that now the same can be said about the role of β_1 integrins on eggs in fertilization.

The Sampson lab has taken the approach of utilizing various peptide mimetics of the ADAM2 (previously known as fertilin β) disintegrin domain containing the tripeptide sequence ECD. They and other groups have shown that peptides based on the disintegrin sequence of ADAM2 inhibit fertilization (*6–8*), and the Sampson lab has extended this work by using more complex ADAM2-based peptides. They have designed peptides of different valencies, with their main tool in their recent work (*5*) being a multivalent polymer with an average of 10 ECD peptides, called I_{10}. A low valency peptide, dubbed $1_2 2_{13}$, with only two ECD peptides and 13 copies of a control sequence (ESA), was used as a control, as was a multivalent polymer of the ESA sequence (2_{10}). Past work with ECD-containing peptides was not without controversy, with speculation that these peptides could nonspecifically block other, non-integrin molecules on the egg membrane and/or could alter the egg membrane, rendering it less capable of supporting sperm interactions (*4*). Experiments in the current paper by the Sampson group address these questions (*5*).

Using the same model as the previous study of β_1-deficient eggs (*4*), Baessler *et al.* show that the ECD 10-mer peptide 1_{10} binds to wild-type eggs but not to β_1-deficient eggs, demonstrating that β_1 integrins on the egg are required for ECD binding. Since other ADAM disintegrin domain sequences are similar and also have inhibitory effects

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on fertilization (*9, 10*), this discovery is likely applicable to multiple ADAMs and not just ADAM2. Moreover, the 1_{10} peptide has little to no inhibitory effect on fertilization of β_1-deficient eggs. The low valency ECD peptide $1_2 2_{13}$ had inhibitory effects on fertilization only at much higher concentrations, but this too was only observed with wild-type eggs and not with β_1-deficient eggs. They also went beyond their ECD-containing peptides and examined the interaction of sperm with these β_1-deficient eggs. Although basic *in vitro* fertilization (IVF) assays had been performed with these eggs (*4*), Baessler *et al.* took this analysis further and examined the inseminated eggs by video microscopy and observed that there was a slight delay in sperm binding to β_1-deficient eggs.

An additional question about these peptides concerned what effects they might have on the eggs, namely, whether they could induce a change in the egg membrane so that sperm could not bind, instead of acting by blocking egg receptors for sperm ligands. Fertilization by sperm triggers a change in egg membrane function, from a state that is receptive to sperm to a state that is unreceptive to sperm (also known as the membrane block to polyspermy); however, the signaling pathway leading to this event associated with the egg-to-embryo transition appears to be complex. Unlike many of the changes occurring in the egg upon fertilization, the establishment of the membrane block to polyspermy is not triggered solely by increases in intracellular Ca^{2+} and likely also requires as-yet unidentified signals associated with sperm entry (*11*). Baessler *et al.* show that the ECD 10-mer 1_{10} induces transient increases in intracellular Ca^{2+}, but this response was observed in both wild-type and β_1-deficient eggs, showing that these activation-like responses to the application of peptide 1_{10} are not dependent on the presence of the β_1 integrin on the egg surface. Interestingly (and somewhat paradoxically), Ca^{2+} signals induced in eggs by these sorts of peptides seem to be β_1 integrin-independent but ECD-dependent, based on the finding that the ESA 10-mer peptide 2_{10} does not induce eggs to undergo egg activation-like responses, which may be an interesting area of future investigation. To address the issue of whether the ECD peptides act by blocking egg receptors, the authors show that extensive washing of eggs incubated in peptide results in the eggs being able to be penetrated by sperm. This indicates that the blocking of sperm–egg interaction is lost once the peptide is removed from the egg surface.

The data of Baessler *et al.* (*5*) together with a recent complementary study by my lab (*12*) provide a convincing case that egg integrins do play a role in fertilization, despite the fact that expression of the β_1 integrin subunit by eggs is not essential for female mice to be fertile (*4*). The β_1 integrin gene product may not be required for fertilization but could convey some reproductive advantage; thus while it is not essential, it is beneficial and maintained by positive selection pressures. In our work, RNAi-mediated knockdown in eggs was attempted for two integrin subunits of interest, β_1 and an α subunit. Acute RNAi-mediated knockdown of β_1 protein on the egg surface was unfortunately not successful, but results from IVF studies with a function-blocking anti-β_1 antibody (*12*) are consistent with recent data noted above. IVF studies showed that reduction of sperm–egg binding and fusion was not achieved when the eggs were challenged with a high number of sperm (500 sperm per egg in the insemination drop) but could be achieved with lower numbers of sperm (*12*), which could be explained by the finding that sperm binding to β_1-deficient eggs is delayed (*5*).

The insights from these two papers (*5, 12*) represent a new starting point for revisiting egg integrins. First is the question of which sperm ligands bind to egg integrins. With the work on ADAM-based peptides (*6–10*), ADAMs are key candidates, although this presents an interesting challenge as several ADAMs can bind different integrins and multiple ADAMs are present on sperm (*e.g.*, ref 13), nearly all of which have disintegrin domain sequences identical or similar to the ECD peptides. Second, the α subunit(s) that is paired with β_1 and that functions as a receptor for sperm is an area of interest. Mammalian eggs express multiple α subunits (*14*). The Sampson group used chemical cross-linking with their ECD peptide to identify the integrin $\alpha_6\beta_1$ as a cross-linked partner on the egg surface (*15*). Eggs lacking α_6 can be fertilized (*16*), but it is possible that α_6, like β_1, is involved in but not required for fertilization. On the other hand, an anti-α_6 function-blocking antibody has moderate (*2*) or little (*3, 16*) effect on mouse sperm–egg interactions, while it has modest (*17*) to significant (*18*) inhibitory effects on human sperm–egg interactions, which raises interesting questions regarding possible differences in the molecular basis of sperm–egg interaction between species, including the involvement of which types of integrins on eggs are involved (discussed in ref 14). More recent work shows that eggs with reduced amounts of α_9 support sperm binding and fusion less well than do control eggs (*12*), in agreement with the finding that several ADAMs can interact with $\alpha_9\beta_1$ (*10*).

In summary, it appears that egg integrins do have a role in sperm–egg interaction. There clearly are other, more critical egg molecules; for example, CD9 in mouse eggs is nearly essential for sperm–egg fusion (reviewed in ref 14). But going beyond the straightforward phenotype of completely failed fertilization with careful analyses such as those discussed here can be the foundation for greater understanding of integrin functions, cell–cell and integrin–ligand interactions, and of course, the process of fertilization itself.

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