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RESEARCH HIGHLIGHT

Green spermatozoa illuminate a 30-year-old model: sperm–egg adhesion involves intra-acrosomal proteins

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Fertilisation in mammals involves many synchronized steps including sperm–egg adhesion. Prior to sperm–oolemma fusion, spermatozoa need to undergo the acrosome reaction (AR) or exocytosis. The universal belief, for many years, has been that the AR was initiated upon binding to the zona pellucida (ZP). As such acrosomal proteins were not thought to be involved in the primary contact with the ZP. These proteins were only suggested to be biologically relevant once the sperm were attached to the ZP and during subsequent events. However, recent data in the mouse have unequivocally demonstrated that spermatozoa can begin exocytosis before contact with ZP.[1] It is a remarkable finding as not only will the interpretation of the interaction between sperm and cumulus cells need to be revised, but the processes of capacitation, vesiculation and exposure of acrosomal content need reexamination.

Jin and colleagues[1] used a novel approach to demonstrate the site of AR: video microscopy of *in vitro* fertilisation using a double transgenic mouse model, where sperm cells expressed green fluorescence in their acrosomes (*EGFP* gene driven by acrosin promoter) and red fluorescence in their mitochondria (mid-piece region; *DsRed2*: red fluorescent protein gene driven by CAG promoter (ubiquitously) with a mitochondrial import sequence signal). This powerful and innovative tool demonstrated that the AR begins before reaching the ZP and very importantly that these spermatozoa were able to fertilize. Moreover, approximately half of the acrosome-intact cells remained on the ZP without undergoing the AR and, after a short period, they swam away! Interestingly, the rate of *in vitro* fertilisation using cumulus-free oocytes was increased when supplemented with cumulus cells, suggesting an important role of this natural matrix surrounding the egg during fertilisation.

To put this in context, sperm physiologists studying the AR in mammals have been involved in a long-standing debate about the location and timing of the AR. For example, where the AR occurs and when the acrosomal content starts to be exposed on/in the plasma membrane? The prevailing data from the mouse model supported the hypothesis that AR is a binary event (acrosomal intact/acrosomal reacted) and AR is induced by the contact with ZP. However, there have been data to suggest exposure of acrosomal contents prior to ZP adhesion. As such a refined model is a more dynamic system, where the acrosomal content is exposed before sperm–ZP adhesion and the AR is a more progressive symphony of events (**Figure 1a and b**).

The AR has been previously suggested to occur before any contact with the extracellar matrix of the egg in a number of different mammalian species. For example, it was suggested as early as 1987 that human exocytosis might involve intermediate stages, such that cells undergoing the AR bind to the ZP.[2] However, despite this, the model shown in **Figure 1a** has continued to prevail in mouse, the most common model for mammalian fertilisation.[3] Interestingly, most of the mouse reports suggesting that AR occurs at the zona surface, were performed using zona where the cumulus cells were removed by hyaluronidase treatment.[4] Although the concept of the model shown in **Figure 1b** was hypothesized previously,[5] only recently has direct cellular evidence in the mouse been provided.[6,7]

On reflection, complementary data obtained in mouse reported that an acrosomal protein involved in sperm–ZP adhesion and associated with species specificity, named zonadhesin, was exposed at the sperm surface depending on the capacitation state.[7] This last report and Jin's study are consistent with the new model proposed in **Figure 1b** (intermittent dynamic model) to explain sperm–egg interaction and AR. Interestingly, using a mouse model in which *EGFP* is expressed in the acrosome, similar results were observed, exposure of the acrosomal protein zonadhesin at the sperm surface clearly occurring in acrosome-intact cells (**Figure 1c**). According to Nakanishi and collaborators,[8] *EGFP* in this mouse model is quickly lost with the beginning of exocytosis (3 s). Under capacitating conditions, 35% of the total sperm population showed exposed zonadhesin while maintaining acrosomal integrity (*EGFP*-positive acrosome—**Figure 1c**). However, the molecular mechanism of zonadhesin exposure during capacitation is not yet fully understood.[9]

In summary, research on gamete interaction and AR is entering a fascinating new era. The study performed by Jin *et al.*[1] and supported by other[7] represents a major advance in sperm physiology. Following this, more questions will appear potentially leading to new models of the molecular and cellular interactions between the cumulus oophorus and spermatozoa. In the near future, we may be able better to characterize the roles of molecules secreted by cumulus cells, like progesterone, in triggering or promotion of sperm fertilizing ability. Moreover, a better comprehension of sperm physiology at the molecular level will permit a more sophisticated approach to the diagnosis of sperm dysfunction.[10]

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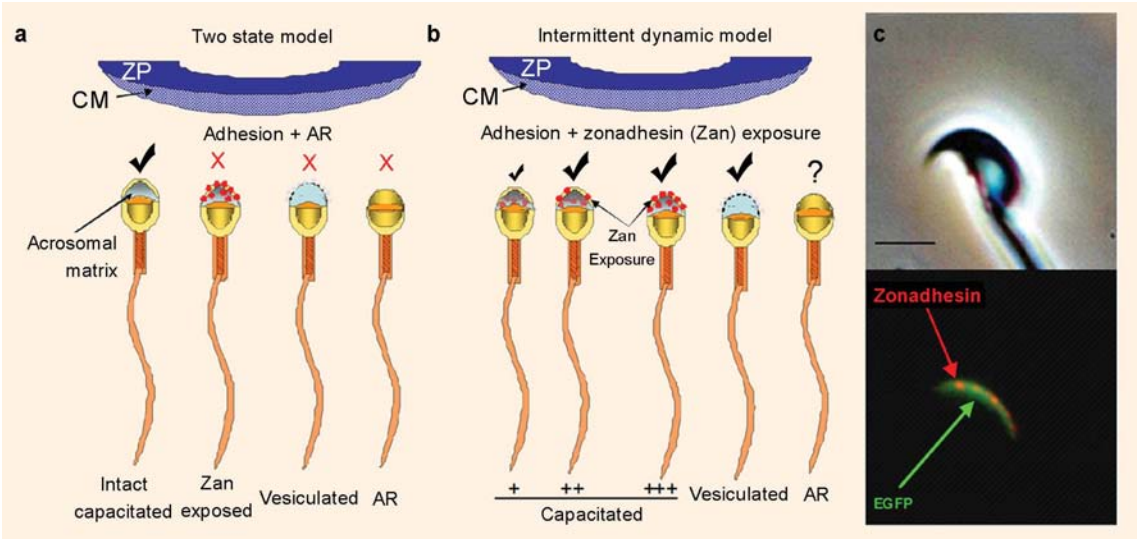

Figure 1 Sperm–egg interaction model. (**a**) Model previously established to explain sperm–egg interaction, where only intact spermatozoa are competent to interact with zona pellucida (ZP) glycoproteins, tick symbol represents the potential adhesion and X represents no possible adhesion (two state model: previous), the structure in blue represents the acrosomal matrix. (**b**) More recent view to explain sperm–egg interaction involving a series of transition states exposing acrosomal content such as zonadhesin molecules (in red) at the sperm surface before sperm–ZP contact (intermittent dynamic model). (**c**) Spermatozoon (intact acrosome) from an *EGFP* (green) mouse exposing zonadhesin (red) at its surface in conditions supporting capacitation. + represents the degree of capacitation. AR: acrosomal reaction; CM: cumulus mass; Zan: zonadhesin.

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