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Commentary

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New experimental data refuting the idea of a fast electrical block to polyspermy in sea urchin eggs

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Editorial commentary

Scientists have been fascinated for over 100 years why only one spermatozoon enters the sea urchin egg when inseminated with many spermatozoa under laboratory conditions. Rothschild, in a mathematical analysis of fertilization, erroneously treated spermatozoa as gas molecules and sperm–egg interaction as a first order chemical reaction, suggesting that the fertilizing spermatozoon reduced the receptivity of the sea urchin egg surface to other spermatozoa by 1/20th (Rothschild and Swann, 1951, 1952). In the 1970s it was suggested that one of the activation events, the depolarization of the egg plasma membrane, served to block the entry of excess spermatozoa and this mechanism was termed 'the fast electrical block' to polyspermy (Jaffe, 1976). However, an equally plausible explanation for monospermy is that the majority of spermatozoa, although motile and capable of attaching to the egg surface, are either physiologically incompetent or are attached to areas of the egg surface that do not support entry (Dale, 2018). To date there is no direct experimental evidence to support either hypothesis.

In this edition of *Zygote*, new data from the Laboratory of Luigia Santella at the Stazione Zoologica, Naples has re-ignited the debate, both by raising doubts on the existence of a fast electrical block to polyspermy and by advancing new thoughts on the process of fertilization itself (Limatola *et al.*, 2019). Sea urchins are marine animals and fertilization occurs externally. The authors reduced the concentration of Na^+ in the sea water surrounding the eggs to reduce the amplitude of the electrical depolarization at fertilization and observed several surprising effects. First, although exposed to high densities of spermatozoa, most of the eggs were monospermic. This finding contradicts the expected result if electrical depolarization serves as a block to prevent the entry of supernumerary spermatozoa; we would have expected polyspermy when the depolarization was reduced in amplitude. Shortly after the depolarization event, a membrane is elevated around the egg surface, called the fertilization membrane, which is thought by many to constitute a slow mechanical block to polyspermy. Santella's group showed that although this mechanical barrier was impaired in eggs pre-incubated in low Na^+ sea water, the eggs were again predominantly monospermic. Many past papers on polyspermy are open to criticism as the authors did not directly observe sperm entry into eggs, but instead inferred polyspermy from abnormal cleavage patterns of the ensuing embryos. The present authors not only quantified sperm entry in sea urchin eggs using the fluorescent dye Hoechst 33422, but went on to show that the abnormal cleavage patterns displayed by the eggs pre-incubated in low Na^+ were due to alterations in the dynamics of the cortical actin filaments following fertilization and not to the formation of multipolar spindles associated with supernumerary sperm centrosomes.

Let us consider what happens in nature. Data collected from natural spawnings show a low fertilization rate, with fertilization success in free-spawning benthic organisms often less than 1% (Levitan, 1993). Therefore, in the environment, sperm–egg collisions in sea urchins may be rare, and the availability of sperm may affect female reproductive success (see review on sperm limitation; Levitan and Peterson, 1995). If indeed, under natural conditions, sperm–egg ratios are low then selective pressures may have favoured the achievement of monospermy, rather than the evolution of mechanisms to prevent polyspermy. Therefore, fertilization in sea urchins is not a haphazard frenzy with the eggs being bombarded by hordes of competent spermatozoa, but a fine-tuned, gradual, and controlled encounter of gametes. Only competent spermatozoa that respond to a correct sequence of triggering events as they progress through the egg investments will be successful. Unsuccessful spermatozoa fall by the wayside.

By designing experiments in the laboratory in which eggs, deprived of their extracellular coats, are inseminated with unnaturally high sperm densities we may have created a biological artefact. Santella's group, careful not to adulterate the eggs before experimentation, either by removing the jelly layer or stressing the eggs mechanically, confirmed what has been already known and was pointed out over 80 years ago by E.E. Just (1939): sea urchin eggs in optimal condition are difficult to render polyspermic in the laboratory. Nevertheless, it is a fact that sea urchin eggs may be exposed to relatively high numbers of spermatozoa in the laboratory without

becoming polyspermic. The paper by Limatola *et al.* (2019) has cast serious doubt on the hypotheses of a fast electrical block to polyspermy. Proponents of the fast electrical block hypothesis should now reply to these objections and, more importantly, identify and characterize a membrane mechanism that is both voltage sensitive and has the capability to regulate sperm entry.

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