Why do penetrating sperm create an oblique path in the zona pellucida?

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Sperm penetration of the zona pellucida

In most invertebrates and vertebrates, sperm penetration of the egg coat depends on acrosomal proteases acting enzymatically, or occasionally non-enzymatically (Lewis *et al.* 1982), to create a hole in the usually thin (0.5–2.0 μm) coat. However, the egg coat in eutherian mammals – the zona pellucida – stands out by virtue of its elastic resilience (Green 1998) and thickness (ca. 7–15 μm according to species), particularly so given an oocyte diameter of only $\pm 100 \mu\text{m}$. The available evidence suggests that these characteristics of the egg coat have had a profound effect on the mechanism of its penetration. The novel design of the sperm head and its behaviour during fertilization imply that, after binding, zona penetration in eutherian mammals depends largely and perhaps only on the oscillating thrust of the sperm (Bedford 2004).

Regardless of the means spermatozoa use to pass through the eutherian zona, it has often been noted that the sharply defined penetration slit has a distinctly tangential trajectory. Observed over a century ago for rats and mice by Sobotta (Austin 1961), an oblique angle of zona penetration has since been observed in the guinea pig and Libyan jird (Austin & Bishop 1958), the rabbit (Dickmann 1964), pig (Dickmann & Dziuk 1964), sheep (Dziuk & Dickmann 1965), and subsequently in the cow, bat, dog, gerbil, deer mouse and man. What first appeared in the phase contrast microscope to be a filament sometimes projecting from the penetrating sperm head (Dickmann 1964), proved to be a cleavage line or split in the zona substance (Bedford 1968, Szollosi & Hunter 1973). Austin's comment that "no adequate reason has yet been advanced to account for this direction of penetration" still holds true, having both mechanistic and functional implications.

In considering the mechanistic aspect, even now one cannot be certain as to what determines the oblique angle

of penetration. Multiple penetrations of the rabbit zona reveal that the angle of penetration trajectory can vary even within one zona, and this path can be almost orthogonal or direct in some muroid rodent eggs mounted beneath a cover glass (Gaddum-Rosse 1985). This makes it seem doubtful that the penetration angle is ordained by some intrinsic characteristic of the zona matrix. On the other hand, the eutherian sperm head is dorso-ventrally flattened, allowing it to oscillate laterally during zona penetration, and it is possible to see that the way in which the sperm head lies flat as it binds to the zona may pre-ordain the oblique angle of its path through this coat. In several ungulates and the rabbit at least, it appears that one side of the sperm head is slightly curved, and that the flat or slightly concave side faces the zona as penetration begins. Thus, the curved form of the slit seen in some species may be determined by the slight dorso-ventral asymmetry in the sagittal profile of the sperm head (Dziuk & Dickmann 1965, Green 1988, PJ Dziuk, University of Illinois, USA – personal communication).

A likely functional consequence of the oblique mode of penetration is indicated in considering the zona pellucida's behaviour during growth of the blastocyst. In most eutherian mammals, including ungulates, carnivores, lagomorphs, insectivores and primates, blastocyst expansion produces considerable stretching and progressive thinning of the zona, allowing this coat to persist for much of that growth period, often until implantation. This pattern appears to represent the primitive eutherian condition usually in conjunction with the relatively superficial epithelio- and endothelio-chorial types of placentation (Mossman 1987). Occasionally, expanded blastocysts establish a haemochorial placenta (Rasweiler 1990), but in the familiar caviid and muroid rodents this type tends to be associated with a relatively small conceptus. Thus, the guinea pig zona shows little change in its dimensions by the time of hatching (Blandau 1971), while the 100 μm 2-cell mouse conceptus finally expands to only about

Figure 1 This depicts the situation in which a small radially directed hole (a) enlarges significantly as the zona stretches and thins during expansion of the trophoblast (b), which then tends to protrude or herniate. By contrast, the typically oblique direction of zona penetration in eutherian mammals (c) acts, during stretching of the zona (d) by the expanding trophoblast, to obscure and perhaps even eliminate that penetration path (represented as a dotted line) in the blastocyst growth phase.

130 μm , with its thickness then reduced to hardly a third, from 8 μm to just 2.8 μm . Though smaller than that in macaques, the hatching human blastocyst is nevertheless at least 2.5 times larger than the egg, and the zona is stretched then to less than one fifth of its original thickness.

The ability to stretch around the expanding blastocyst would appear to derive from the zona's unusual thickness and relative elasticity. However, as illustrated in Fig. 1a and b, it seems that such tension and consequent thinning would tend to enlarge a direct or radial hole in the zona. By contrast, where the penetration slit is oblique (Fig. 1c) zona stretching must bring into close apposition the inner and outer borders of that slit, in the manner of a leaf valve (Fig. 1d), so avoiding the development of such a hole. Although a distinct slit persists in some newly-fertilized eggs (Fig. 1c), it is unclear whether the blastocyst growth phase not only closes but may even obliterate this line of cleavage in the zona. Since preovulatory withdrawal of the trans-zonal corona cell processes does not seem to leave tracks that are visible in the transmission electron microscope, such a complete elimination of the fertilizing sperm's path might indeed be possible.

Not only could a direct hole in the zona compromise some of its early functions suggested by Denker (2000), such a hole can result subsequently in partial herniation and trapping of a trophoblast segment, or a failure of hatching (Malter & Cohen 1989, Cohen & Feldburg 1991). Blebbing or herniation of the expanding trophoblast may occur also after micro-needle puncture of the human zona during intracytoplasmic sperm injection (ICSI), as well as after the breach in the zona made during embryo biopsy (Veeck & Zaninovic 2003). An oblique entry would appear to be of particular benefit for such as the rabbit whose zona has no block to polyspermy but, interestingly, is invested in the oviduct by a thick mucoprotein coat. As the rabbit zona becomes obliterated in the expanded state, this mucoprotein and other tract secretions seem to act as a substitute coat, as happens also in several other

mammals including the horse, some seals and other carnivores (Denker 2000).

Conclusion

Binding of the sperm head by its flat surface could initiate the oblique angle of its subsequent penetration through the eutherian zona. The tangential trajectory of that path ensures that it then closes as the zona stretches and thins during expansion of the blastocyst, so preventing the development of a hole(s) that could lead to trophoblast herniation and or sometimes compromise normal hatching. If micro-needles can be pushed through the zona at such a tangent, this might perhaps reduce the incidence of trophoblast blebbing in manipulated embryos during culture to the blastocyst stage.

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