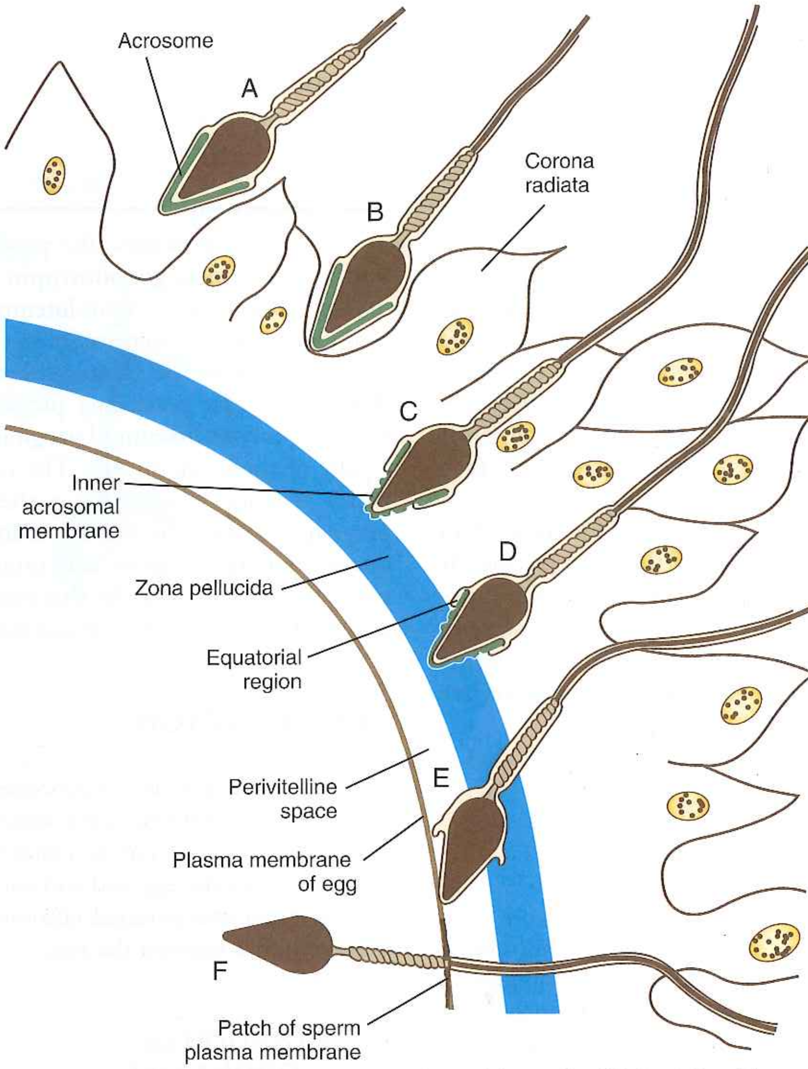

FIGURE 2-4. The sequence of events in penetration of the coverings and plasma membrane of the egg. **A** and **B,** Penetration of the corona radiata. **C** and **D,** Attachment to the zona pellucida and acrosomal reaction. **E** and **F,** Binding to plasma membrane and entry into the egg.

(Fig. 2-5). The zona pellucida of an unfertilized mouse egg is estimated to contain more than 1 billion copies of the ZP_3 protein.

After they have penetrated the corona radiata, spermatozoa bind tightly to the zona pellucida by means of the plasma membrane of the sperm head (see Fig. 2-4). The ZP_3 molecule, specifically the O-linked oligosaccharides attached to the polypeptide core, acts as the sperm receptor in the zona in the mouse. Molecules on the surface of the sperm head are specific binding sites for the ZP_3 sperm receptors on the zona pellucida. More than 24 molecules have been proposed, but the identity of the zona-binding molecule remains unknown. Interspecies molecular differences in the sperm-binding regions of the ZP_3 molecule may serve as the basis for the inability of spermatozoa of one species to fertilize an egg of another species. In mammals, there is less species variation in the composition of ZP_3; this may explain why penetration of the zona pellucida by spermatozoa of closely related mammalian species is sometimes possible, whereas it is rare among lower animals.

On binding to the zona pellucida, mammalian spermatozoa undergo the **acrosomal reaction**. The essence of the acrosomal reaction is the fusion of parts of the outer acrosomal membrane with the overlying plasma membrane and the pinching off of fused parts as small vesicles. This results in the liberation of the multitude of enzymes that are stored in the acrosome (Box 2-1).

The acrosomal reaction in mammals seems to be stimulated by the ZP_3 molecule acting through G proteins in the plasma membrane on the sperm head. In contrast to the sperm receptor function of ZP_3, a large segment of the polypeptide chain of the ZP_3 molecule must be present to induce the acrosomal reaction. An initiating event of the acrosomal reaction is a massive influx of Ca^{++} through the plasma membrane of the sperm head. This process, accompanied by an influx of Na^+ and an efflux of H^+, increases the intracellular pH. Fusion of the outer

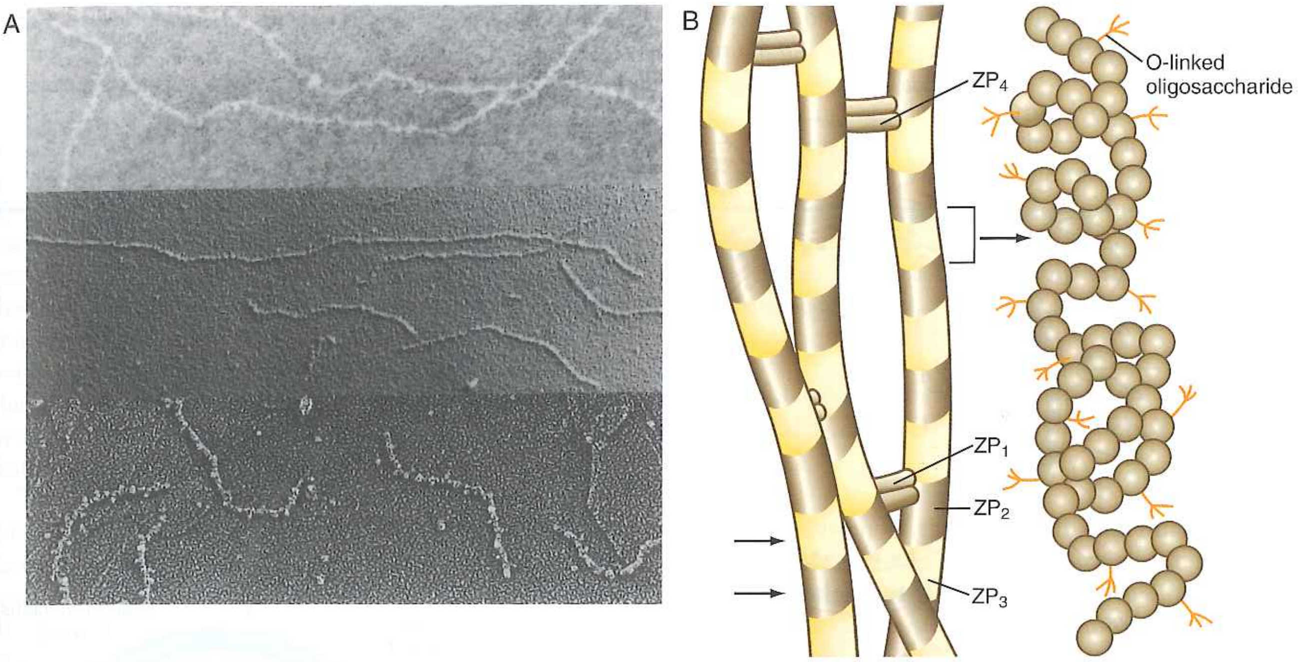

FIGURE 2-5. A, Filamentous components of the mammalian (mouse) zona pellucida. **B,** Molecular organization of the filaments in the zona pellucida. *Far right,* Structure of the ZP_3 glycoprotein.(From Wassarman PM: Mammalian fertilization, *Sci Am* 259(6):82, 1988. Copyright Neil O. Hardy.)

Box 2-1. **Some Major Mammalian Acrosomal Enzymes**

- Acid proteinase
- Acrosin
- Arylaminidase
- Arylsulfatase
- Collagenase
- Esterase
- β-Galactosidase
- β-Glucuronidase
- Hyaluronidase
- Neuraminidase
- Phospholipase C
- Proacrosin

acrosomal membrane with the overlying plasma membrane soon follows. As the vesicles of the fused membranes are shed, the enzymatic contents of the acrosome are freed and can assist the spermatozoa in making their way through the zona pellucida.

After the acrosomal reaction, the inner acrosomal membrane forms the outer surface covering of most of the sperm head (see Fig. 2-4D). Toward the base of the sperm head (in the equatorial region), the inner acrosomal membrane fuses with the remaining **postacrosomal plasma membrane** to maintain membrane continuity around the sperm head.

Only after completing the acrosomal reaction can the spermatozoon successfully begin to penetrate the zona pellucida. Penetration of the zona is accomplished by a combination of mechanical propulsion by movements of the sperm's tail and digestion of a pathway through the action of acrosomal enzymes. The most important enzyme is **acrosin**, a serine proteinase that is bound to the inner acrosomal membrane. When the sperm has made its way through the zona and into the **perivitelline space** (the space between the egg's plasma membrane and the zona pellucida), it can make direct contact with the plasma membrane of the egg.

BINDING AND FUSION OF SPERMATOZOON AND EGG

After a brief transit period through the perivitelline space, the spermatozoon makes contact with the egg. In two distinct steps, the spermatozoon first binds to and then fuses with the plasma membrane of the egg. Binding between the spermatozoon and egg occurs when the **equatorial region** of the sperm head contacts the microvilli surrounding the egg. Molecules on the plasma membrane of the sperm head, principally sperm proteins called **fertilins** and **cyritestin**, bind to **α_6 integrin** and **CD9 protein** molecules on the surface of the egg. The acrosomal reaction causes a change in the membrane properties of the spermatozoon because, if the acrosomal reaction has not occurred, the spermatozoon is unable to fuse with the egg. Actual fusion between spermatozoon and egg brings their plasma membranes into continuity.

After initial fusion, the contents of the spermatozoon (the head, midpiece, and usually the tail) sink into the egg (Fig. 2-6), whereas the sperm's plasma membrane, which is antigenically distinct from that of the egg,

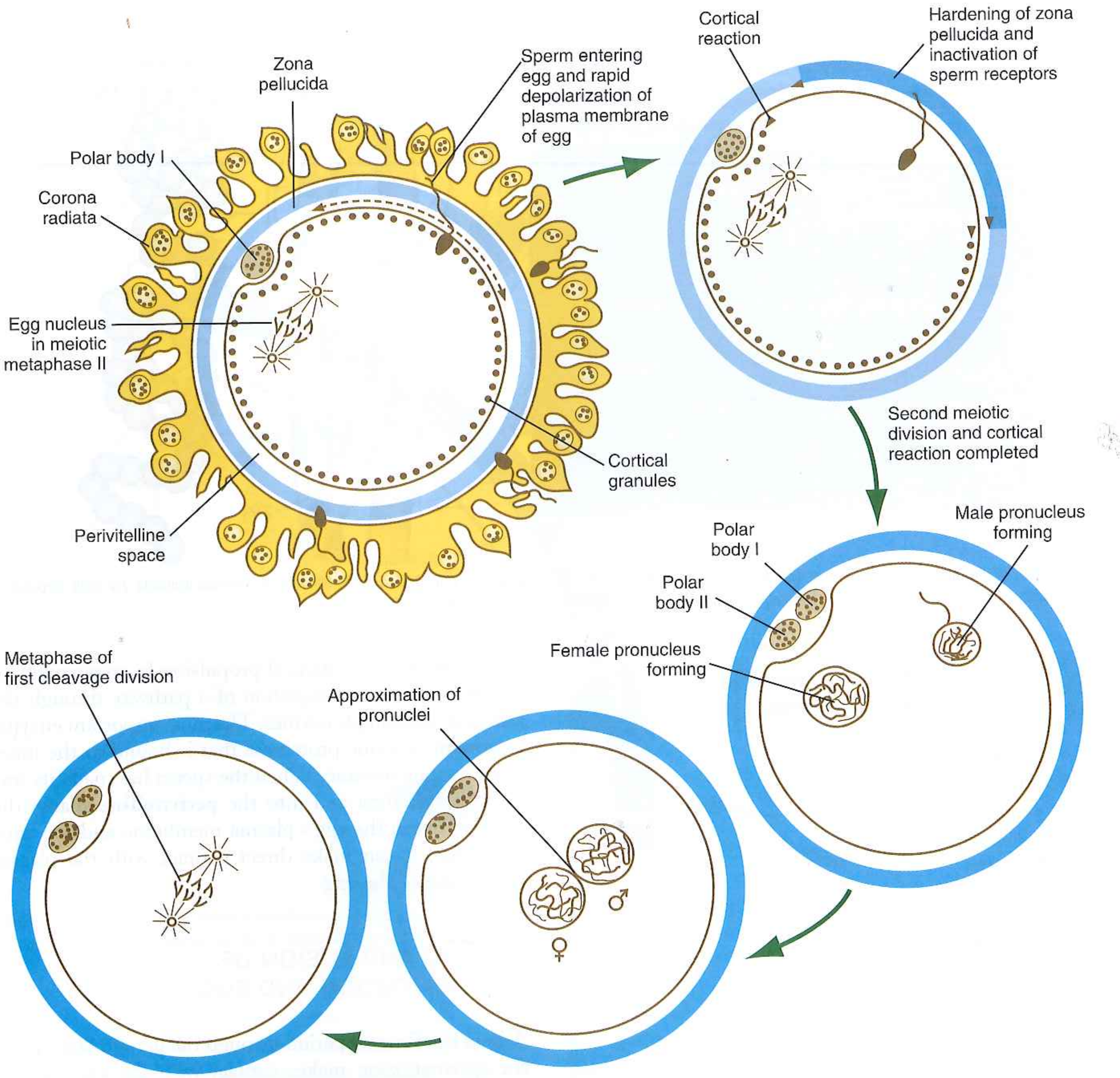

FIGURE 2-6. Summary of the main events involved in fertilization.

becomes incorporated into the egg's plasma membrane and remains recognizable at least until the start of cleavage. Although mitochondria located in the sperm neck enter the egg, they do not contribute to the functional mitochondrial complement of the zygote. In contrast, in humans, the sperm contributes to the zygote the centrosome, which is required for cell cleavage.

PREVENTION OF POLYSPERMY

When a spermatozoon has fused with an egg, the entry of other spermatozoa into the egg (**polyspermy**) must be prevented, or abnormal development is likely to result. Two blocks to polyspermy, fast and slow, are typically present in vertebrate fertilization.

The **fast block to polyspermy**, which has been best studied in sea urchins, consists of a rapid electrical depolarization of the plasma membrane of the egg. The resting membrane potential of the egg changes from about −70 mV to +10 mV within 2 to 3 seconds after fusion of the spermatozoon with the egg. This change in membrane potential prevents other spermatozoa from adhering to the egg's plasma membrane. The fast block in mammals is short-lived, lasting only several minutes, and may not be as heavily based on membrane depolarization as that in sea urchins. This time is sufficient for the egg to mount a permanent slow block. The exact