Development of the bovine acrosome
An ultrastructural and cytochemical study*

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Summary. In the present study the development of the bovine acrosome was investigated using conventional electron-microscopical techniques as well as the phosphotungstic-acid (PTA) technique (Rambourg 1967) including enzymatic digestion experiments. As in other species and in accordance with previous light-microscopical studies (Clermont and Leblond 1955) four phases of acrosomal differentiation can be discerned: the Golgi-phase, cap-phase, acrosome-phase, and maturation-phase.

In the bull no internal pattern of the acrosomal content can be observed, either with conventional uranyl acetate-lead citrate staining or with the PTA-techniques. Our results support the observation in other species (Fawcett et al. 1971) that no intrinsic polymerization or crystallization process of the acrosomal content is responsible for acrosomal shaping. Some of our results suggest the influence of external forces on acrosomal development in the bull. During the cap-phase and the acrosome-phase accumulations of smooth endoplasmic reticulum and a layer of fine filaments can be observed in the Sertoli-cell cytoplasm, immediately adjacent to the developing acrosome. A temporary influence of these structures on acrosomal development seems possible. The PTA-positive staining of the developing bovine acrosome is probably due to the presence of acrosomal glycoproteins; however, our results do not exclude the possibility that molecules other than glycoproteins contribute to the positive PTA-staining of the developing acrosome.

Key words: Acrosome, bovine – Acrosomal development – Acrosomal glycoproteins – Ultrastructure – Cytochemistry

During spermiogenesis the small, round spermatids resulting from the second meiotic division are transformed into the complex spermatozoa, which are released

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* Supported by a grant from the Deutsche Forschungsgemeinschaft
in the lumina of the tubuli seminiferi. In the process of spermatid differentiation the most striking morphological events are the nuclear condensation, the establishment of a flagellar structure, the elimination of excess cytoplasm and the formation of the acrosome. The form of the acrosome in mature spermatozoa is quite variable among the species (Fawcett et al. 1971; Nicander and Bane 1966). In the bull it covers as a cap-shaped structure (Nicander and Bane 1966) the proximal two-thirds of the sperm head.

With biochemical techniques, Hartree (1975) could demonstrate that the acrosomes of the bovine spermatozoa contain several glycoproteins that are associated with enzymatic activities. Sperm acrosomal enzymes, especially hyaluronidase and acrosin, have been implicated to play a significant role during the fertilization process (Hartree 1977). Hyaluronidase has been shown to disperse the cumulus oophorus in rabbit, hamster and other animals, allowing spermatozoa to penetrate to the surface of the zona pellucida. Penetration of the zona pellucida is then achieved by the action of acrosin (McRorie and William 1974).

The function of bovine hyaluronidase in fertilization has been questioned recently (Lorton and First 1979). Ova collected from bovine oviducts 2 to 3 h after ovulation were completely devoid of surrounding follicular cells in the absence of exposure to sperm hyaluronidase (Lorton and First 1979).

In the bull as in other species (man: Holstein 1976; Holstein and Schirren 1979), malformation of the acrosome is thought to be an important factor for male infertility. Several studies have been performed on the ultrastructure and cytochemistry of ejaculated and epididymal bovine spermatozoa (Blom and Birch-Andersen 1965; Nicander and Bane 1966; Saacke and Almquist 1964; O'Donnell et al. 1970; Wooding and O'Donnell 1971), but comparatively little information is available on the process of acrosomal development during spermiogenesis in the bovine testis (Holt 1979; Lengfelder 1978). Using conventional electron-microscopical techniques and the cytochemical phosphotungstic acid stain, which is regarded to be a specific stain for glycoproteins when used at low pH (Rambourg 1967), the present paper reports on the development of the bovine acrosome.

Materials and methods

Fixation and embedding procedures

For the present study testes of 15 sexually mature bulls (Deutsches Fleckvieh) were fixed by vascular perfusion as previously described (Wrobel et al. 1979). The fixation solution consisted of 1% formaldehyde (prepared from paraformaldehyde, Serva) and 1.25 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Following fixation small pieces (1 mm³) were cut from different sites of the testis and postfixed for 2 h in fixative solution of the same composition. The specimens were washed overnight in 0.1 M cacodylate buffer (pH 7.4) and embedded in glycol-methacrylate (GMA) according to Leduc and Bernhard (1967) or postfixed in 1% cacodylate-buffered osmium tetroxide (pH 7.4), dehydrated in a graded series of ethanol and embedded in Araldite.

Staining of the sections

Ultrathin sections of testis embedded in Araldite were routinely stained with uranyl acetate and lead citrate (Reynolds 1963).

Rambourg technique for glycoproteins (Rambourg 1967): Silver sections from glycol-methacrylate embedded testicular tissue were collected on nickel or gold grids and floated on a solution of 1%