Biogenesis of the sperm head perinuclear theca during human spermiogenesis

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We analyzed the appearance and localization of the sub-acrosomal perinuclear theca (PT) during human spermiogenesis. The PT is tightly associated with acrosomal biogenesis. (Fertil Steril® 2009;■:■–■. ©2009 by American Society for Reproductive Medicine.)

Key Words: Perinuclear theca, acrosome, spermiogenesis

The perinuclear theca (PT) has been studied in animal models for more than two decades. Its composition and localization as well as its important function during oocyte activation and fertilization have been well described mostly in bovine spermatogenesis (1) or after sperm penetration in cattle (1), pigs (2), and rhesus monkeys (3). The biogenesis of PT in relation to acrosomal biogenesis in human spermatogenic cells has not been explored. For this purpose, we have used antiacrosin antibodies (C5F10) as an acrosomal marker together with antibodies against the subacrosomal PT (PT427) in spermatogenic cells of human testicular biopsies isolated by chemical digestion (4). The accurate determination of the developmental stages of the germ cells was based on the morphology of the developing acrosome and chromatin condensation revealed by phase contrast and epifluorescence microscopy.

We did not detect the perinuclear theca antigens in human spermatogenesis before the appearance of spermatids (data not shown). The immunofluorescent signal of the PT was first observed during spermiogenesis in association with the proacrosomic granule and extension of the acrosomal cap distally, over the spermatid nucleus (Fig.1, A–F). In animal models, the association among the acrosome, the PT, and the caudal manchette complex has been described as an event necessary for the shaping of the sperm head and the trafficking of proteins during spermiogenesis (5). Figure 1, I and L, shows the final modeling of the PT during nuclear elongation and in the fully differentiated sperm head. All along this process, images clearly show that the PT is located underneath the acrosome.

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Studies of this cytoskeletal structure are also important due to its participation in oocyte activation during fertilization. Intracytoplasmic sperm injection (ICSI) fertilization failures in the rhesus monkey were associated to the persistence of the PT that may impair the release of oocyte-activating factors (3). The role of the PT has become important during human ICSI, particularly in cases with severe teratozoospermia with cephalic abnormalities associated to acrosomal hypoplasia (6). Altogether, a better understanding of perinuclear theca biogenesis and function in humans will contribute to the advancement of andrology and infertility treatment.

Human testicular biopsy samples were donated for research by informed patients under written consent. Samples were selected from patients with obstructive azoospermia (with complete, normal spermatogenesis). Procedures were approved by the internal review board and ethics committee of the Centro de Estudios en Ginecología y Reproducción.

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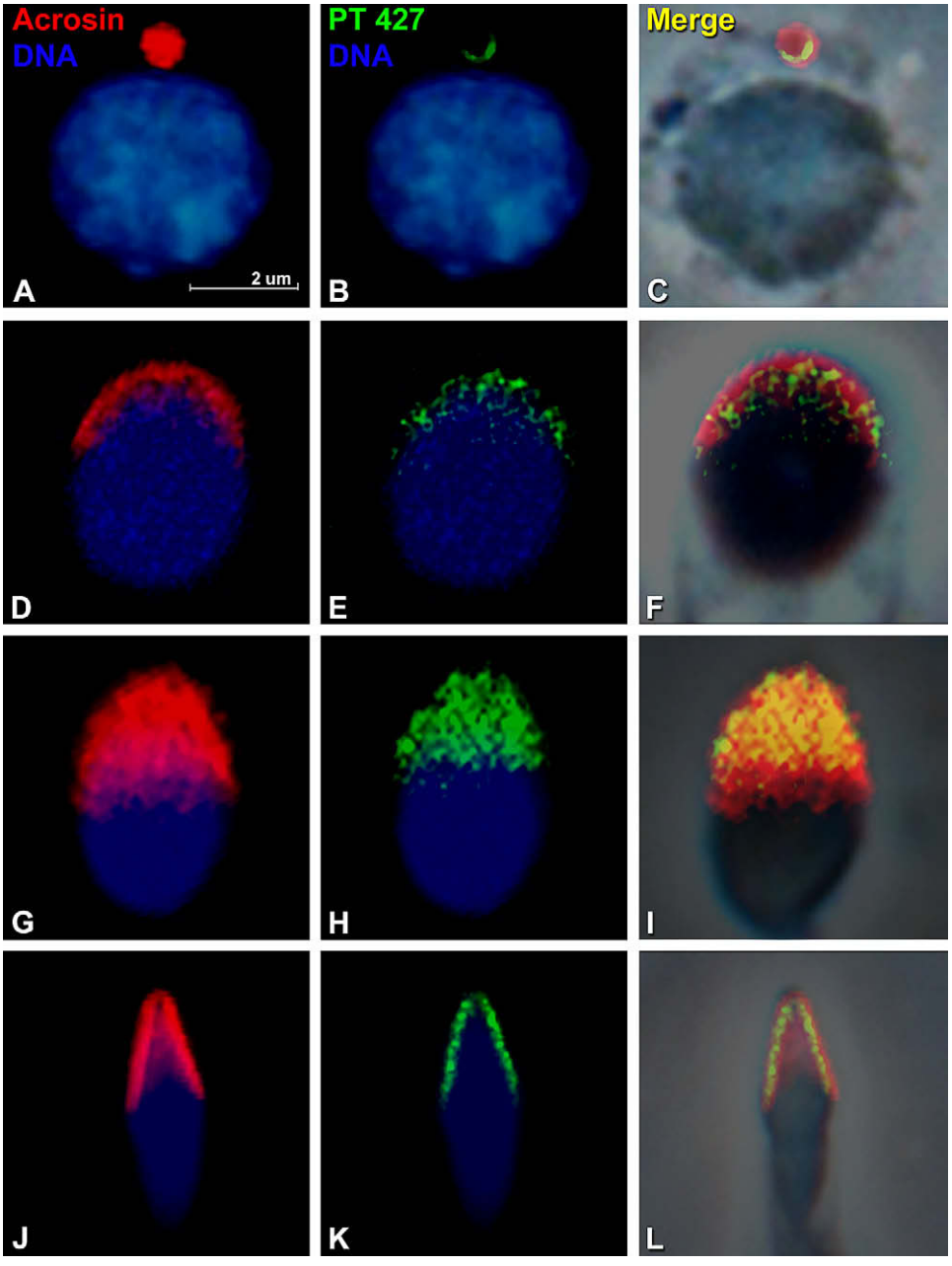
FIGURE 1

Immunofluorescence of different stages of human spermiogenesis showing acrosin (antibody C5F10, red, left panels), the PT (PT427 antiserum, green, central panels), and merged views (yellow) over contrast phase images of the same cells (right panels). (**A–C**) The biogenesis and initial distribution of the PT and its early association with the acrosomic vesicle and proacrosomic granule in round spermatids. (**D–F**) During cap phase and initial spermatid elongation, the acrosome extends distally on the apical pole of the spermatid nucleus with the PT associated underneath. In this stage, we can also clearly recognize the process of DNA condensation. (**G–I**) Front views and **(J–L)** side views of a completely elongated spermatid with a fully formed acrosome depict the PT in a subacrosomal location. Isolated cells of human spermatogenesis were examined using an epifluorescence microscope (Nikon BX40), under ultraviolet light with specific filters for the desired wavelengths. (Images edited using Adobe Photoshop 7.0.)



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1 Biogenesis of the sperm head perinuclear theca during human spermiogenesis

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To explore the biogenesis of the subacrosomal perinuclear theca during human spermiogenesis, we analyzed the presence and localization of this structure in relation to the development of the acrosome.