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Saga of a sperm fertility biomarker☆,☆☆

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Abstract

A decade ago a novel sperm protein associated with the fertility of sperm was discovered by quantifying individual proteins in the sperm membrane proteome of cauda epididymal sperm from rats exposed to epididymal toxicants that compromised the fertility of these sperm. Upon identification, this protein (SP22) was found to a ubiquitous, highly conserved protein never before observed in the male reproductive tract. The expression of SP22 in sperm appears driven by a testis specific mRNA transcript, and the molecule is translocated from the cytoplasmic droplet of rete testis sperm to the equatorial segment of epididymal and ejaculated sperm. The appearance of SP22 mRNA and protein coincide with the formation of pachytene spermatocytes and round spermatids, respectively, and given this testis ontogeny of SP22, we validated its use as a biomarker of fertility by extending our studies to toxicants that target spermiogenesis. Studies of both epididymal and testicular toxicants now have demonstrated that compromised SP22 gene expression is sensitive and correlated with fertility. Importantly, this applies to ejaculated sperm as well as epididymal sperm. With the goal of developing a user-friendly diagnostic assay for SP22 on epididymal and ejaculated sperm, we are attempting to identify exposed, functional domains of the protein. For this, we have generated antibodies to both full length and truncated SP22 recombinants, as well as antibodies to synthetic SP22 peptides. Each antibody has been characterized for its ability to inhibit fertilization both *in utero* and *in vitro*. Linear epitope mapping has been done for each antibody, and synthetic peptides corresponding to each epitope have been used in competition experiments designed to elucidate exposure on the sperm surface and function. Most of the linear epitopes identified appear to be exposed although there are relative differences in the degree of their exposure. Interestingly, one of the exposed epitopes does not appear to be functional, at least by itself. Many more domains of the molecule need to be studied, but based on our findings with the

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epitopes already identified, it seems a combinatorial targeting strategy may be beneficial. If one assumes that the protein's role in fertility resides in a single exposed epitope, or some combination of exposed epitopes, such targeting may also ultimately lead to successful modulation of the fertilizing potential of sperm.
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1. Discovery and identification

Over the past decade numerous studies have reported a decline in semen quality in men in various parts of the world. Carlsen et al. (1992) reported that semen quality declined 50% between 1930 and 1991. While these data have been quite controversial, recent data from prospective cohort studies tend to support the notion that ejaculated sperm numbers and sperm quality are indeed declining. Jorgensen et al. (2001) reported that median sperm concentrations were 61 million/ml in Denmark compared to 82 million/ml in Finland using standardized methodology across the regions. Differences in semen quality also have recently been reported in the United States. Swan et al. (2003) found that sperm concentrations in Missouri men were 59 million/ml compared to 102 million/ml for men in New York. This difference seemed to be associated with pesticide residues that prevailed in the agricultural cohort. While sperm concentration is certainly an indicator of semen quality, more attention recently has focused on the quality of ejaculated sperm. Hauser et al. (2002) reported a trend between organochloride levels in serum and compromised sperm motility and morphology. Subsequently, Toft et al. (2005, 2006) observed trends for increased time to pregnancy and decreased sperm motility associated with increased organochlorine concentrations in blood.

Spermatogenesis in humans is much less efficient than in other mammals and laboratory rodents with respect to the number of sperm produced per gram of testis (Amann, 1986). Coupled with the fact that up to 50% of the sperm in a normal man's ejaculate can be morphologically abnormal (Wyrobek et al., 1982), one can easily imagine that environmental insults could further compromise the process of spermatogenesis and sperm maturation. The net result of such additional compromise would be a reduction in sperm production and sperm quality sufficient to render a man infertile. Thus, we began the task of identifying a sperm protein that could serve as a more sensitive biomarker of fertilizing ability than sperm number, motility, or morphology.

Because the rat is a robust breeder that tends to be refractory to toxic insult (Klinefelter and Gray, 1993) we used *in utero* insemination to increase the sensitivity of fertility as an endpoint (Klinefelter et al., 1997). By inseminating a fixed, optimal number of proximal cauda epididymal sperm into receptive females, we were able to identify subtle alterations in fertility. Four distinct epididymal toxicants (i.e. ethane dimethanesulphonate, chloroethylmethanesulphonate, epichlorohydrin, and hydroxflutamide) were tested at each of two doses; fertility was related to multiple other endpoints including sperm motility, sperm morphology, cauda reserves, epididymal androgen status, and individual proteins in the sperm membrane proteome. Fertility decreased significantly in a dose-related fashion with each toxicant. When proteins in detergent extracts of proximal cauda sperm were quantified following two-dimensional gel electrophoresis, numerous proteins were found to be altered by treatment. However, only one protein was diminished significantly in a dose-related fashion by each treatment. Moreover, the concentration of this protein, hereafter referred to as SP22, was highly correlated ($r^2 = 0.83$) with the fertility of the inseminated sperm.