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Soluble P-Cadherin Found in Human Semen

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ABSTRACT: Cadherins constitute a family of calcium-dependent cell-cell adhesion molecules. P (placental)-cadherin is a 118-kd protein expressed by basal cells in epithelial tissues. P-cadherin also has been described as a soluble protein in certain biological fluids, including human serum and breast milk. Here, we report the presence of an 80-kD fragment of P-cadherin in human semen. No significant differences were found in semen samples from fertile and nonfertile patients. Our results add evidence to previous data indicating that soluble fragments of P-cadherin have a widespread distribution in bodily fluids and suggest that soluble P-cadherin might have functions other than basal epithelial cell-cell adhesion.

Key words: Cadherins, seminal fluid, cell-cell adhesion.

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Cadherins constitute a family of homotypic calcium-dependent cell-cell adhesion proteins that play a fundamental role in cell sorting and morphogenesis during development and maintenance of the differentiated phenotype in adult tissues (Takeichi, 1991). The best characterized members of the family are called classical cadherins and include E (epithelial)-, P (placental)-, and N (nerve)-cadherin, named after the tissues in which they were originally found. Cadherins are important transducers of positional information signals to the cytoplasm. They initiate and contribute to the architectural changes that occur during cellular differentiation (Edelman, 1989) and cytoskeletal organization (McNeill et al, 1990). The interplay between cadherin-mediated signals and the cytoskeleton regulates cell-cell adhesion as well as developmental processes, including migration, proliferation, apoptosis, and differentiation (Huber et al, 1996).

P-cadherin is a 118-kd transmembrane glycoprotein first described in the mouse placenta (Nose and Takeichi, 1986). In epithelial tissues, P-cadherin expression appears limited to basal and myoepithelial cells (Daniel et al, 1995). In the mammary gland, P-cadherin gene knockout causes precocious development and lactation in virgin females (Radice et al, 1997). Aberrant P-cadherin expression in breast cancers is associated with aggressive tumor behavior (Peralta Soler et al, 1999). However, P-cadherin overexpression in the mouse mammary epithelium does not cause significant change in mammary phenotype or development (Radice et al, 2003).

The proteolytic cleavage of the extracellular domain of cadherins results in soluble fragments that can be released into fluids, including serum (Knudsen et al, 2000). In the lactating breast, P-cadherin is secreted into the milk as a soluble 80-kd fragment (Peralta Soler et al, 2002). In this study, we present the first report of the presence of readily detectable levels of an 80-kd, soluble P-cadherin fragment in human semen.

Materials and Methods

Human semen was obtained from men undergoing analysis for infertility (n = 21). Semen samples were collected by masturbation after 3 to 4 days of sexual abstinence following World Health Organization (WHO)–recommended procedures (1999), and by approved protocols by the Ethics Committee. Semen was analyzed according to WHO guidelines (1999). Of the 21 patients, 8 were classified as fertile on the basis of their capacity to promote pregnancy, and 13 patients were classified as infertile because they could not promote pregnancy 1 year from the first visit.

Semen was centrifuged at 1000 rpm for 20 minutes, seminal fluid was separated from sperm, and total protein concentration was measured with a BioRad kit (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, Calif). The expression of soluble P-cadherin in seminal fluid was determined by Western blot analysis. Thirty micrograms of sperm-free seminal fluid protein were run in a 15% acrylamide gel (Sigma Chemical Co, St Louis, Mo). A cell lysate from a human epidermoid carcinoma cell line (BD Transduction Laboratories, San Jose, Calif) was used as positive control. Molecular mass was assessed with the use of a

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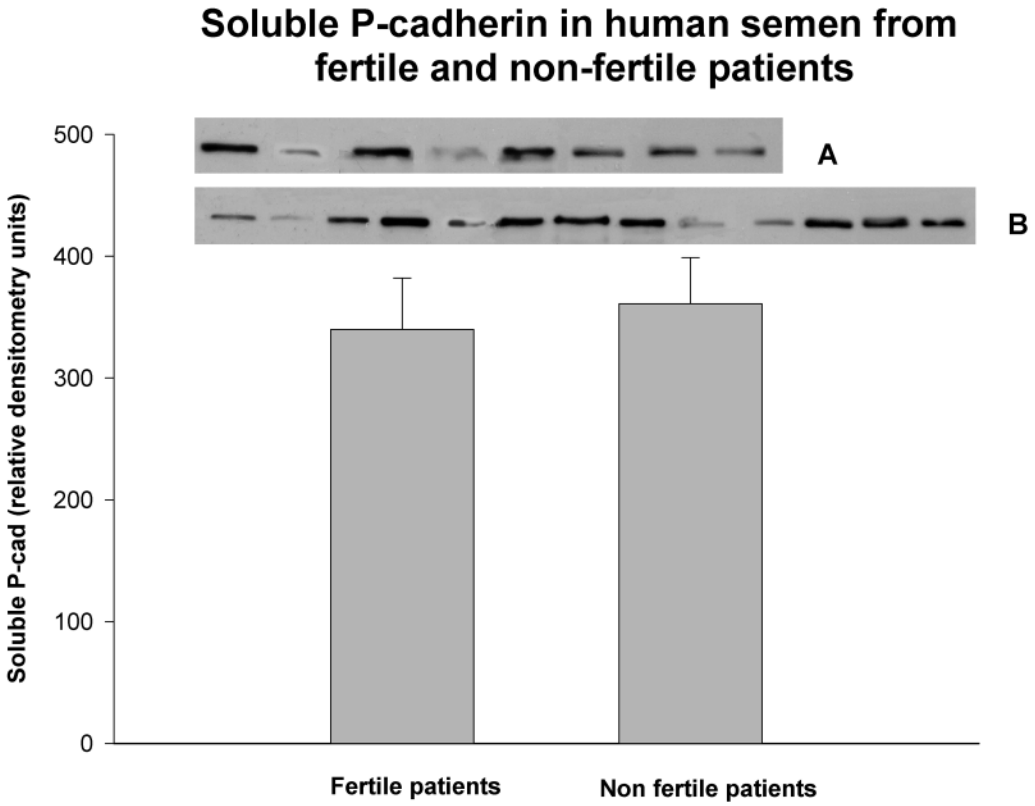

Immunoblot analysis showing a single 80-kd band representing soluble P-cadherin from human semen from 8 fertile patients **(A)** and 13 nonfertile patients **(B).** Densitometric analysis of P-cadherin expression is expressed as the mean ± SEM. The 2 groups are not significantly different (unpaired *t* test).

Full Range Rainbow Molecular Weight Marker (Amersham-Life Science, Bucks, United Kingdom). The proteins were transferred to a nitrocellulose membrane (Hybond ECL, Amersham-Pharmacia-Biotech, Bucks, United Kingdom). Nonspecific sites were blocked with phosphate-buffered saline (PBS) containing 5% low-fat milk powder and 0.1% Tween-20 at room temperature. The membranes were incubated for 1 hour at 37°C with anti–P-cadherin mouse monoclonal antibody (Transduction Laboratories) diluted 1:100 in blocking buffer. After extensive washing with PBS and 0.1% Tween-20, the samples were incubated with a peroxidase-conjugated (HRP) goat anti-mouse secondary antibody (Pierce, Rockford, Ill), diluted 1:5000 in blocking buffer. The blots were thoroughly rinsed in PBS and 0.1% Tween-20 and detected with an ECL Western blotting detection system (Amersham Biosciences, Bucks, United Kingdom) on Hyperfilm (Amersham-Pharmacia-Biotech) following the manufacturer's instructions. Scanning and quantification was performed with Scion Image software (V.4.0.2, Scion Image Corp, Frederick, Md) at 3 different exposure times. P-cadherin levels were statistically analyzed with unpaired *t* tests, and the results were expressed as means ± SEM. Significance was reported at $P < .05$.

Results

The expression of P-cadherin was determined in 21 patients undergoing analysis for male infertility. Of the 21 patients, 8 were classified as fertile and 13 patients were classified as infertile because they could not promote pregnancy 1 year from the first visit.

Western blots revealed a single 80-kd band corresponding to soluble P-cadherin. Densitometric analysis showed no significant differences between infertile and fertile patients (Figure).

Parameters evaluated in the infertile group included count, motility, morphology, bacterial culture, and leukocyte count (Table). The limited number of cases analyzed in this study did not allow us to correlate P-cadherin levels with these parameters.

Discussion

P-cadherin is found in the reproductive organs in both sexes in an early, undifferentiated stage of development. After sexual differentiation, P-cadherin becomes undetectable in the ovary but remains expressed in the male reproductive organs, including testis, epididymis, prostate, and seminal vesicles. In the testis, P-cadherin is localized in the developing Sertoli cells together with Müllerian-inhibiting substance (Johnson et al, 2000). However, within the adult testis, Sertoli cells express mostly

*Analysis of semen parameters from the group of nonfertile patients**

Infertile Patient	P-Cadherin Levels, Relative Area Units	Sperm Concentration M/mL	Bacteriological Analysis	Sperm Morphology†	Motility	WBC (as Neutrophils), M/mL
1	234	97	*Chlamydia trachomatis* +	Normal	Altered	0
2	143	27	*C trachomatis* +	Altered	Normal	0.58
3	326	2	Negative	Altered	Altered	0
4	213	140	*Staphylococcus* +	Altered	Normal	0.92
5	435	12	Negative	Altered	Normal	1.5
6	591	10	Negative	Altered	Altered	0
7	455	2.1	Negative	Altered	Altered	2
8	285	18	Negative	Altered	Normal	1
9	190	0.8	Negative	Altered	Altered	0
10	438	82	*C trachomatis* +	Normal	Normal	0
11	434	1.4	*C trachomatis* +/gram(−) bacilli	Altered	Altered	0.16
12	483	6	Negative	Altered	Altered	0.4
13	482	2.7	Negative	Altered	Altered	0

* M/mL indicates millions per milliliter; WBC, white blood cells.
† Comparative normal values were from World Health Organization guidelines. Morphology was evaluated on the basis of spermatozoa head, midpiece, and tail characteristics.

E-cadherin and N-cadherin (Lee et al, 2003), whereas P-cadherin expression is limited to peritubular myoid cells (Lin and DePhilip, 1996), making them an unlikely source of P-cadherin in semen.

The size of soluble P-cadherin (80 kd) in semen is identical to the fragment found in milk, suggesting a common proteolytic cleavage mechanism. Soluble P-cadherin in ejaculated semen might result from release of the extracellular domain of P-cadherin from spermatozoa because previous data showed P-cadherin in ejaculated spermatozoa as a 90-kd protein (Rufas et al, 2000). However, the lack of correlation between P-cadherin expression in semen and the fertility status of the patients suggests that sperm might not be the source of P-cadherin in ejaculated seminal fluid. In the prostate and seminal vesicles, P-cadherin expression is largely limited to prostate-specific antigen–negative cells, including the basal cells of the prostate and seminal vesicle epithelium (Peralta Soler et al, 1997). Basal cells in the prostate are secluded from contact with the prostatic luminal secretion. However, P-cadherin was occasionally seen as a secretory protein in prostatic glands and in seminal vesicles (Peralta Soler et al, 1997), similar to the expression pattern observed in lactating breast (Peralta Soler et al, 2002), suggesting that those tissues could be the source of P-cadherin in seminal fluid.

Although the density of the bands was heterogeneous, P-cadherin expression and the various morphological parameters analyzed in the infertile patients were not correlated. However, the small number of samples limited the statistical value of the data.

The function of released soluble cadherin fragments in fluids is unknown. Interestingly, the extracellular domain of P-cadherin contains sequences identical to the *Listeria*-binding site of E-cadherin, which can act as decoy receptors for the bacteria (Cossart et al, 2003; da Silva et al, 2003; Pizarro-Cerda et al, 2004). But in contrast to E-cadherin–soluble fragments, which can disassemble epithelial tissues by binding to the cellular E-cadherin, soluble P-cadherin fragments cannot bind to cellular P-cadherin because of its secluded location in basal cells. Perhaps the soluble P-cadherin fragments in fluids serve as an alternative bacterial binding peptide without the deleterious effects that E-cadherin soluble fragments could have on the integrity of epithelial tissues. Another possibility is that the soluble seminal P-cadherin has an effect on P-cadherin–expressing female genital tract tissues. This possibility is supported by previous data showing expression of P-cadherin in normal and neoplastic uterine tissues, including normal myometrium and uterine leiomyomas (Tai et al, 2003), glandular tumors of the cervix (Han et al, 2000), and small cell carcinomas of the cervix (Zarka et al, 2003). Another possible role for P-cadherin could be the heterotypic interaction between the P-cadherin ectodomain and the E-cadherin extracellular domain within the male or female genitourinary tract. A recent report showed differences in E-cadherin expression in spermatozoa from fertile and infertile males (Purohit et al, 2004), suggesting a role for heterotypic cadherin interaction between spermatozoa and oocyte during fertilization.

Previous studies on the proteolytic processing of N-cadherin indicated critical specific cleavage sites for cell-cell interaction and for the assembly of the cadherin-catenin complex (Wahl et al, 2003). Interestingly, an 80-kd proteolytic fragment of E-cadherin resulting from metal-

loprotease-dependent cleavage was found as a potential biomarker for prostate cancer progression (Kuefer et al, 2003). Thus, identification of the cleavage site within P-cadherin will certainly be valuable in elucidating the mechanisms of P-cadherin ectodomain formation and its potential role in human reproduction and cancer.

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