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SEMINAL PLASMA BIOCHEMICAL MARKERS AND THEIR ASSOCIATION WITH SEMEN ANALYSIS FINDINGS

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ABSTRACT

Objectives. To examine the clinical value of six seminal plasma components in the evaluation of sperm quality and in the differential diagnosis of men with infertility.

Methods. We analyzed 202 seminal plasmas for prostate-specific antigen, glucose, pepsinogen C, insulin-like growth factor binding protein-3, prostaglandin D synthase (PGDS), and BRCA1-like immunoreactive protein (BRCA1-LIP) using quantitative immunofluorometric procedures. The semen donors were categorized in four clinical groups: normal, oligospermic, azoospermic, and vasectomy patients. We then evaluated whether any of these biochemical markers were associated with other parameters of sperm quality, including patient age, total cell concentration, percentage of motility, and percentage of normal morphology.

Results. We found that only PGDS concentration was significantly associated with other parameters of sperm quality. PGDS concentration correlated positively with total cell concentration ($r = 0.55$), percentage of motility ($r = 0.31$), and percentage of normal morphology ($r = 0.31$). Median PGDS concentration in seminal plasma decreased progressively from normal to oligospermic to azoospermic to vasectomy patients ($P < 0.001$). There was no overlap between seminal plasma PGDS concentration of normal subjects versus vasectomy patients. The only other parameter that was moderately decreased in vasectomy patients was BRCA1-LIP. The source of PGDS in seminal plasma was determined with various techniques, including immunohistochemistry. This protein is produced and secreted by the Sertoli cells.

Conclusions. Our findings suggest that PGDS concentration in seminal plasma correlates with other known indicators of semen quality and is a new marker of post-testicular obstruction. This biochemical parameter could be used to aid in the differential diagnosis of obstructive and nonobstructive azoospermia in men with infertility. UROLOGY **53:** 596–603, 1999. © 1999, Elsevier Science Inc. All rights reserved.

Diagnosis of male infertility is a rapidly developing field of investigation.[1] Systems such as computer-assisted semen analysis (CASA) have aided in advancing the standardization of procedures to characterize the quality of sperm. For an accurate diagnosis of defective sperm function, the physical criteria of CASA should be supplemented with biochemical assays that may reveal the functional competence of the sperm. Indeed, evidence does suggest that mammalian seminal plasma contains factors that influence male fertility.[2]

Prostate-specific antigen (PSA) is a 33-kDa single chain glycoprotein belonging to a large group of extracellular serine proteases.[3,4] PSA is produced and secreted by the prostatic epithelium.[5] The enzymatic activity of PSA has been termed restricted chymotrypsin-like,[6,7] because it slowly hydrolyzes peptide bonds behind certain tyrosine and leucine residues. The main physiologic substrates identified for PSA are the gel-forming proteins produced and secreted from the seminal vesicles, semenogelin I and II.[7–10] The coagulum at ejaculation is liquefied within minutes as the semenogelins are degraded into smaller soluble fragments by the proteolytic activity of PSA.[8] Many tissues, fluids, and cells have been associated with PSA immu-

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Submitted: June 18, 1998, accepted (with revisions): September 10, 1998

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0090-4295/99/$20.00
PII S0090-4295(98)00550-0

noreactivity, but PSA has been found to be most concentrated in the seminal plasma[11] and is one of the three most predominant prostate-derived proteins in the seminal fluid.[12]

Prostaglandin D synthase (PGDS) belongs to the lipocalin superfamily,[13] which includes an array of extracellular transport proteins (including retinol-binding protein) showing high binding affinity for specific cell receptors and small hydrophobic ligands. First identified by Clausen[14] in 1961, PGDS was characterized by Killian *et al.*[15] as a 26-kDa fertility-associated protein in bull sperm and is unique among the lipocalins in that it is the only enzyme in the family.[16] This enzyme catalyzes conversion of prostaglandin H_2 to prostaglandin D_2,[17] which is involved in a variety of biologic functions such as inhibition of platelet aggregation in vitro, smooth muscle contraction and relaxation, synaptic transmission, sleep induction, and hypothalamic control of temperature.[17] PGDS can now be measured with high sensitivity by immunofluorometry.[18] PGDS was found in large quantities in seminal plasma, but its role in this human fluid has not been investigated in detail.[18]

Human seminal plasma was found to contain a zymogen with properties related to a gastric pepsinogen.[19] This was later identified as pepsinogen C,[20] a 35-kDa molecule. Pepsinogen C is a member of the aspartic proteinase family and is the inactive precursor of pepsin C. It is thought that this molecule may be involved in the pathogenesis of breast disease,[21–23] but it is of interest in our study as a component of seminal fluid.[24–26] Assays for pepsinogen C have recently been developed.[27]

Insulin-like growth factor (IGF)-binding protein 3 (IGFBP-3) is the main circulating IGF-binding protein in humans.[28,29] This protein is a member of a family of homologous but distinct proteins ubiquitous in biologic fluids, tissues, and extracellular matrix. Greater than 95% of the growth-promoting peptides IGF-I and IGF-II bind to IGFBP-3.[29] Human IGFBP-3 exists in serum as a 140-kDa complex, comprising a glycosylated IGF-binding subunit and an acid-labile subunit.[28] This complex has not been completely characterized in seminal plasma. It is known that IGFBP-3 is able to inhibit the stimulatory effect of follicle stimulating hormone,[30] an important hormone in spermatogenesis and thus sperm quality. Commercially available methods can now quantify IGFBP-3 with high sensitivity and specificity.

The BRCA1 gene encodes a 1863 amino acid tumor suppressor protein[31] that is believed by some to belong to the granin family of secreted proteins.[32] Granins exert their influence by the release of peptides that have biologic activities. As previously reported by Lianidou *et al.*,[33] a BRCA1-like immunoreactive protein, known in this work as BRCA1-LIP, has been found in seminal plasma. The present study examines the correlation between this protein and sperm quality by using BRCA1-LIP assays described elsewhere.[33]

More than half the sugar consumed by the spermatozoa is glucose.[34] Although probably all sugars provide energy at the time of ejaculation, as time progresses, it has been found that glucose is generally used more. Thus, the possibility arises that the quality of sperm may be enhanced by the presence of glucose.

In this work, six different biochemical assays were carried out on 202 seminal plasma samples. Using the enzyme-linked immunosorbent assay technique, this study attempts to establish a relationship not only between sperm quality and the biochemical markers but also among the biochemical markers themselves. Biochemical markers that were tested include pepsinogen C, BRCA1-LIP, IGFBP-3, PGDS, PSA, and glucose. Of these, the first four are examined as potential biochemical indicators of human sperm quality for the first time.

MATERIAL AND METHODS

Clinical Samples

Two hundred two semen samples were obtained from the diagnostic semen laboratories at Mount Sinai Hospital, Toronto, Canada and Etobicoke General Hospital, Etobicoke, Canada. Provided with the samples were the results of CASA, including the total cell concentration, percentage of motility, and percentage of normal morphology. The samples were kept frozen at −20°C. Before use, the samples were thawed overnight at 4°C and centrifuged at 7000*g* for 10 minutes to separate the spermatozoa from the seminal plasma. Initial studies indicated that the biochemical analytes measured are not affected if the seminal plasma samples are frozen and thawed less than three times.

For the measurement of analyte concentrations, the seminal plasma samples were diluted as follows: 10^6-fold for PSA; 10^4-fold for pepsinogen C; 200-fold for PGDS; 5×10^4-fold for BRCA1-LIP; 100-fold for IGFBP-3, and twofold for glucose. The diluent was a 60 g/L solution of bovine serum albumin in a 50 mmol/L Tris buffer, pH 7.80.

The semen samples were divided into four clinical groups on the basis of their total cell concentration and percentage of motility. The normal group (normozoospermia) consisted of samples with a total cell concentration greater than 20×10^6/mL and percentage of motility greater than 50%. The azoospermic group consisted of samples with a total cell concentration of zero. The oligospermic group consisted of the remaining samples with sperm characteristics between those defined for the normal and azoospermic groups. The classification criteria are according to the World Health Organization.[35] Furthermore, we identified 6 patients who underwent vasectomy, and we treated them as a separate group. Statistical analysis was performed on the four clinical groups defined.

Measurement of Analyte Concentrations

The concentration of seminal plasma PSA was measured with an immunofluorometric assay described by Ferguson *et al.*[11] This one-step procedure uses a mouse monoclonal capture antibody, a biotinylated mouse monoclonal detection

TABLE I. *Description of the variables studied in seminal plasma*

Variable	No. of Samples	Mean (SD)	Median	Range
Patient age (yr)	178	36 (6)	34	20–53
Total cell concentration ($\times 10^6$/mL)	181	55 (75)	28	0–364
% Motility	181	30 (30)	21	0–90
% Normal morphology	164	49 (29)	60	0–95
PSA ($\times 10^6$; μg/L)	202	0.91 (0.78)	0.71	0–6.5
Pepsinogen C ($\times 10^4$; μg/L)	202	2.6 (2.7)	1.8	0.02–15
PGDS ($\times 10^4$; μg/L)	202	0.75 (1.0)	0.40	0.003–6
BRCA1-LIP ($\times 10^5$; U/L)*	200	12 (25)	4.3	0.04–250
IGFBP-3 ($\times 10^2$; μg/L)	201	1.8 (1.1)	1.6	0–7
Glucose (mmol/L)	201	6.6 (2.9)	6.8	0.2–15

KEY: No. = number; SD = standard deviation; PSA = prostate-specific antigen; PGDS = prostaglandin D synthase; BRCA1-LIP = BRCA1-like immunoreactive protein; IGFBP-3 = insulin-like growth factor binding protein 3.
** Since there is no standard preparation for BRCA1-LIP, the concentration is in arbitrary units per liter, based on an in-house seminal plasma sample used as standard.*

antibody, alkaline phosphatase-conjugated streptavidin (SA-ALP), and the fluorogenic substrate diflusinal phosphate (DFP).

Seminal plasma pepsinogen C concentration was measured with an immunofluorometric procedure developed by Diamandis *et al.*[27] This was also a one-step assay using a mouse monoclonal capture antibody, a biotinylated mouse monoclonal detection antibody, SA-ALP, and DFP.

PGDS concentration in seminal plasma was measured by an immunofluorometric assay developed by Melegos *et al.*[18] The procedure uses a one-step incubation period, a mouse monoclonal capture antibody, a biotinylated mouse monoclonal detection antibody, SA-ALP, and the substrate DFP.

The concentration of BRCA1-LIP in seminal plasma was measured using an immunofluorometric assay developed by Lianidou *et al.*[33] This multistep assay employs goat-antimouse IgG-coated polystyrene microtiter plates, a mouse monoclonal capture antibody, a rabbit antiserum, goat-antirabbit IgG conjugated to alkaline phosphatase, and DFP as the fluorogenic substrate.

The Tb^{3+} fluorescence for the above mentioned assays was measured by time-resolved fluorometry, as described elsewhere.[11] The calibration curves and data reduction were performed automatically by the CyberFluor 615 Immunoanalyzer (MDS Nordion, Kanata, Ontario, Canada).

Analytical data regarding these research assays, including calibration method, precision, cross-reactivity, recovery, and dynamic range, can be found in the cited publications.[11,18,27,33]

The seminal plasma concentration of IGFBP-3 was measured with a commercial assay from Diagnostic Systems Laboratories, Webster, Texas. Glucose was measured with an automated procedure on the Roche Integra analyzer (Roche, Basel, Switzerland).

Immunohistochemistry for PGDS

PGDS staining was performed on a variety of tissues, including testis, seminal vesicle, prostate, and spleen, using a monoclonal antibody developed as described elsewhere.[36] Four micron-thick paraffin sections were dewaxed in xylene, rehydrated through graded alcohols, and then immersed in 3% aqueous hydrogen peroxide solution to block endogenous peroxidase activity. Sections were washed in Tris-buffered saline, pH 7.6, and blocking reagent (Signet Laboratories, Dedham, Mass) was applied for 20 minutes to reduce nonspecific staining. The antibody (approximately 1 mg/mL) was used at 1 in 300 dilution in a diluent containing 1% bovine serum albumin for 1 hour at room temperature. Biotinylated multispecies anti-immunoglobulin serum (Signet) at a dilution of 1 in 3 was used as the linking reagent. After washing, peroxidase-labeled ultra streptavidin (Signet) at a dilution of 1 in 3 was applied and diaminobenzidine (Sigma Chemical, St. Louis, Mo) was used as the substrate chromogen for visualization. Counterstaining was with Mayer's hematoxylin. Sections were dehydrated, cleared, and mounted in Permount.

Tissue Extraction

Testis, epididymis, vas deferens, seminal vesicle, prostate, and spleen tissue were obtained at autopsy from 1 male patient. These tissues were pulverized and then extracted as described elsewhere.[37] Extracts were analyzed with the procedures described.

Statistical Analysis

Statistical calculations were performed using SAS computer software (SAS Institute, Cary, NC) and the *P* values were derived from two-side statistical tests.

RESULTS

A description of the variables studied, including sample size, mean, standard deviation, median, and range, are shown in Table I. Analysis of variance (ANOVA) was performed on the means of the four clinical groups for each variable, as was the Wilcoxon test on the medians of the clinical groups. These results are listed in Table II. Furthermore, the chi-square test or Fisher's exact test was carried out to determine the associations between the variables studied and the clinical groups (data not shown).

From Table II, as well as the chi-square analysis, we found that there is a statistically significant association between the four clinical groups and PGDS concentration ($P = 0.02$ for ANOVA, $P < 0.001$ for Wilcoxon, and $P < 0.001$ for chi-square) but not any of the other analytes.

The Spearman correlation coefficients were also calculated to examine correlations between param-

TABLE II. *Comparisons of means and medians among the four clinical groups*

Variable	Patients (n)	Mean (SD)	Median	Range
Age (yr)				
Normal*	29	33.4 (5.5)	33	20–48
Oligospermic†	123	35.6 (6.1)	34	24–53
Azoospermic‡	20	35.9 (6.3)	35	24–50
Vasectomy	6	42.2 (5.3)	45	34–46
P value		0.014§	0.022‖	
% Normal morphology				
Normal	29	77.2 (8.5)	75	60–95
Oligospermic	110	53.1 (21.3)	53	0–95
Azoospermic	19	NA	0	
Vasectomy	6	NA	0	
PSA ($\times 10^6$; μg/L)				
Normal	29	0.76 (0.54)	0.67	0.00–2.16
Oligospermic	126	0.96 (0.82)	0.75	0.00–6.48
Azoospermic	20	0.68 (0.40)	0.64	0.16–1.98
Vasectomy	6	0.41 (0.21)	0.34	0.23–0.75
P value		0.118	0.050	
Pep C ($\times 10^4$; μg/L)				
Normal	29	3.72 (3.36)	2.20	0.03–14.45
Oligospermic	126	2.44 (2.26)	1.74	0.02–14.60
Azoospermic	20	2.12 (1.43)	1.98	0.12–4.75
Vasectomy	6	3.19 (1.58)	3.42	1.11–5.56
P value		0.047	0.148	
PGDS ($\times 10^4$; μg/L)				
Normal	29	1.01 (1.18)	0.75	0.03–5.96
Oligospermic	126	0.79 (1.04)	0.39	0.01–5.78
Azoospermic	20	0.28 (0.55)	0.04	0.01–2.06
Vasectomy	6	0.005 (0.005)	0.005	0.003–0.01
P value		0.024	<0.001	
BRCA1-LIP ($\times 10^5$; U/L)				
Normal	29	7.68 (8.64)	3.51	0.24–37.41
Oligospermic	125	14.75 (30.22)	4.58	0.04–249.84
Azoospermic	20	5.83 (5.92)	4.14	0.39–20.98
Vasectomy	6	1.07 (0.76)	1.01	0.16–1.94
P value		0.217	0.012	
IGFBP-3 ($\times 10^2$; μg/L)				
Normal	29	1.80 (0.97)	1.59	0.60–4.49
Oligospermic	125	1.84 (1.14)	1.60	0.00–6.06
Azoospermic	20	1.84 (1.46)	1.30	0.50–7.04
Vasectomy	6	2.36 (1.21)	2.36	0.85–3.78
P value		0.743	0.670	
Glucose (mmol/L)				
Normal	29	7.37 (2.92)	7.08	0.84–12.18
Oligospermic	125	6.65 (2.91)	7.12	0.20–15.00
Azoospermic	20	6.08 (2.10)	6.14	2.74–9.96
Vasectomy	6	7.23 (3.46)	7.25	2.66–11.70
P value		0.429	0.437	

KEY: Pep C = pepsinogen C; NA = not applicable; ANOVA = analysis of variance; other abbreviations as in Table I.
* *Total cell concentration >20 × 10^6 cells/mL and percentage of motility >50%.*
† *Any condition between normal and azoospermic.*
‡ *Total cell concentration = 0.*
§ *ANOVA test.*
‖ *Wilcoxon test.*

eters. We report correlation coefficients that were significant at the level of $P < 0.01$. Patient age was negatively associated with both percentage of motility ($r = -0.20$) and percentage of morphology ($r = -0.20$). Total cell concentration was positively associated with percentage of motility ($r = 0.63$), percentage of normal morphology ($r = 0.62$), and PGDS concentration ($r = 0.55$). Percentage of motility was further associated with percentage of morphology ($r = 0.75$), pepsinogen C

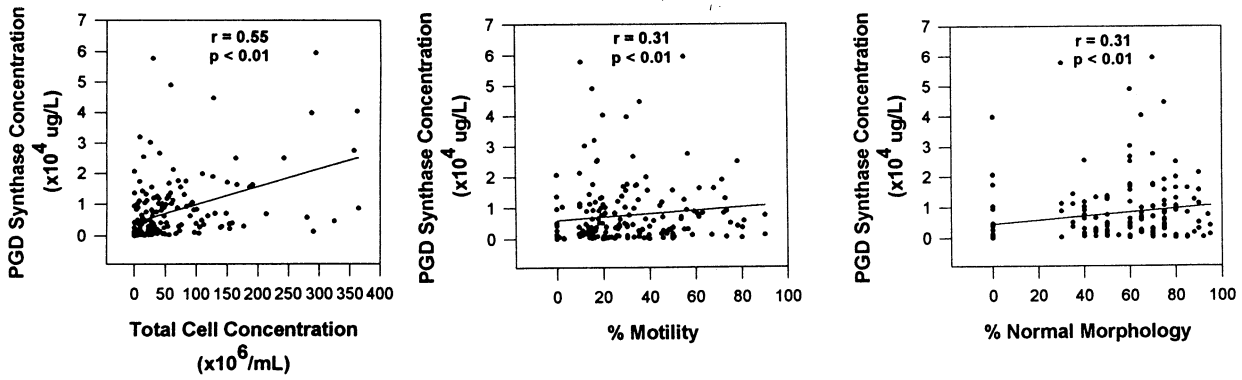

FIGURE 1. *Correlation between PGDS concentration in seminal plasma and total cell concentration, percentage of motility, and percentage of normal morphology of spermatozoa. The Spearman correlation coefficients are indicated.*

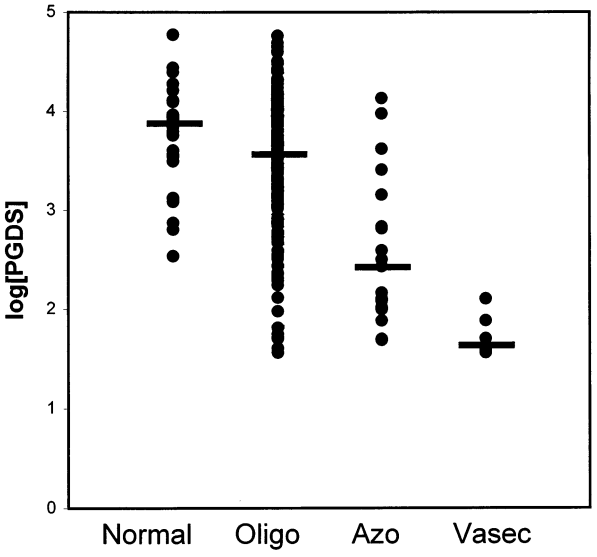

FIGURE 2. *Distribution of seminal plasma PGDS concentration in normal, oligospermic (Oligo), azoospermic (Azo), and vasectomy (Vasec) patients. The medians are indicated by the horizontal lines. [PGDS] = $\times 10^4$ μg/L.*

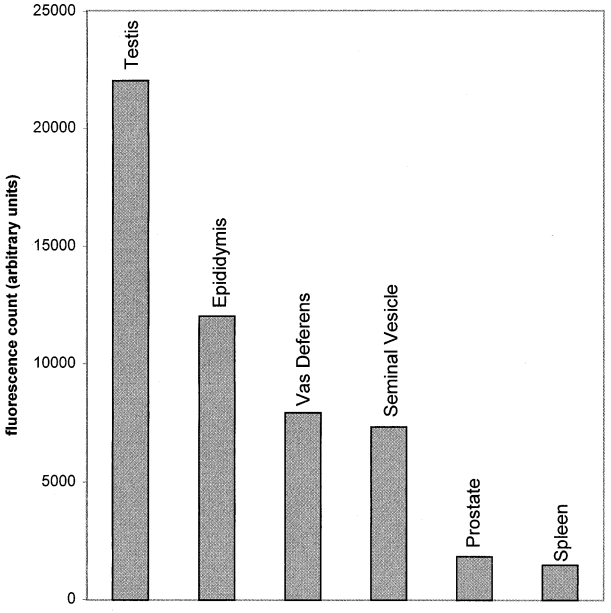

FIGURE 3. *Assay of PGDS in extracts from the human tissues shown. Extracts were diluted 100-fold and the response is plotted as arbitrary fluorescence units. Highest PGDS concentration is found in testicular extracts.*

($r = 0.19$), and PGDS concentration ($r = 0.31$). Percentage of normal morphology was associated with PGDS concentration ($r = 0.31$). PGDS concentration was positively correlated with PSA ($r = 0.24$). Pepsinogen C concentration was associated with glucose concentration ($r = 0.34$). BRCA1-LIP was positively associated with glucose concentration ($r = 0.21$).

The identified correlations between PGDS concentration and total cell concentration, percentage of motility, and percentage of normal morphology are shown in Figure 1.

In this series of patients, we identified 6 who underwent vasectomy. In these patients, the seminal plasma concentration of PGDS and BRCA1-LIP were significantly reduced (Table II). In Figure 2, we present the distribution of PGDS concentration in the four patient groups. The median of the PGDS concentration decreased progressively from the normal to oligospermic to azoospermic to vasectomy patients. There was no overlap in PGDS concentration between the normal and the vasectomy groups. The BRCA1-LIP concentration was reduced only in the vasectomy patients and to an extent that significant overlap still existed between all groups.

Among the azoospermic group (total of 20 patients), we identified 4 who had proven epididymal obstruction and 7 who had primary testicular failure; the other 9 patients did not have a known diagnosis. All 4 patients with epididymal obstruction had seminal plasma PGDS levels similar to

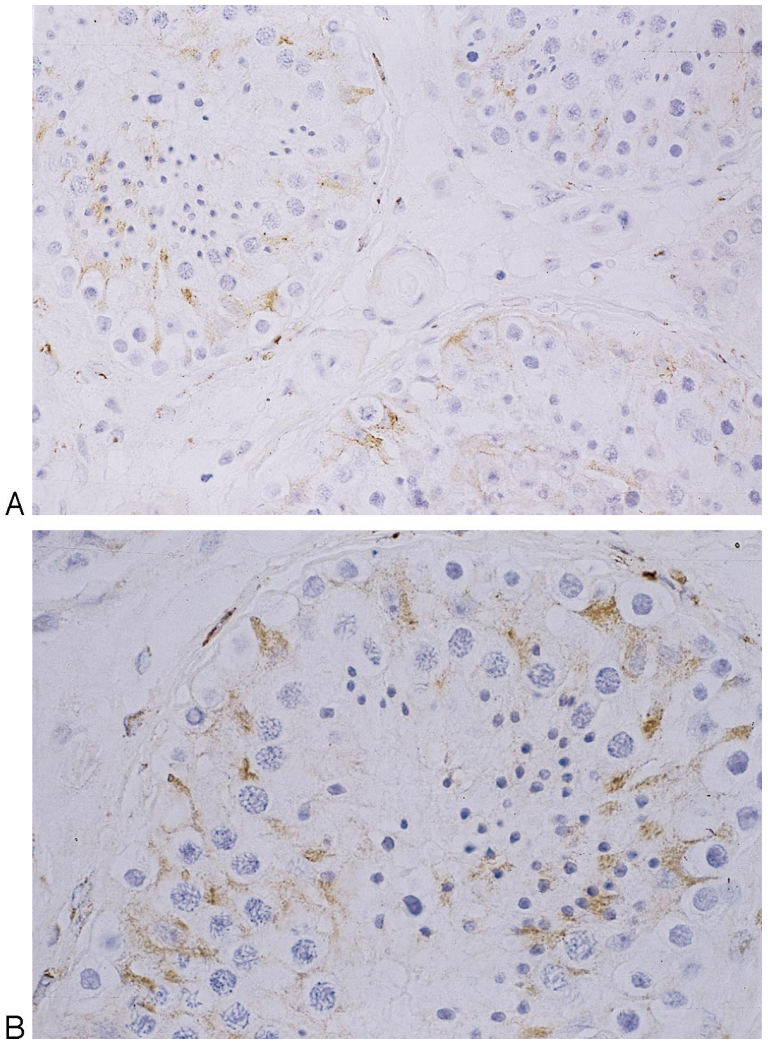

FIGURE 4. *(A) Photomicrograph showing three seminiferous tubules with intervening interstitium. Note positive staining with antibody against PGDS localized within the sustentacular (Sertoli) cells of the seminiferous tubules. No germ cell staining is apparent. (B) High-power photomicrograph of a single seminiferous tubule showing Sertoli cell localization of PGDS.*

those of vasectomy patients (0.02×10^4 μg/L or less). The 7 patients with primary testicular failure had PGDS levels of 1.36×10^4, 0.95×10^4, 0.42×10^4, 0.07×10^4, 0.03×10^4, 0.03×10^4, and 0.01×10^4 μg/L, respectively.

Since the source of PGDS in seminal plasma is not known, we attempted to identify it using various methods. We compared the PGDS concentration in vasectomy and nonvasectomy patients. The almost complete absence of PGDS in seminal plasma of vasectomy patients indicates that this protein is probably produced by either epididymal or testicular tissue. We obtained fresh tissue during autopsy from spleen (control), testis, epididymis, seminal vesicle, and prostate and homogenized. We then analyzed the homogenates for PGDS at various dilutions. Some of the data are shown in Figure 3. PGDS is present at high levels in epididymal and testicular tissue. We have also precisely localized the source of PGDS by immunohistochemistry (Fig. 4). This protein is produced by the Sertoli cells and secreted into the seminal plasma. When the monoclonal anti-PGDS antibody was blocked with recombinant PGDS before use, the staining completely disappeared.

COMMENT

This study was designed to identify seminal plasma components that may have value in the differential diagnosis of male patients with infertility problems. The biochemical analytes under investigation were selected on the basis of two criteria: (a) their possible diagnostic value in seminal plasma had not been previously studied; and (b) a method for the accurate measurement of their concentration in seminal plasma was available. Four of the six analytes investigated (pepsinogen C, PGDS, BRCA1-LIP, and IGFBP-3) met these criteria. The other two analytes (PSA and glucose) were included for comparison. We recently developed new quantitative assays that allow the measurement of these analytes in seminal plasma and other fluids.[11,18,27,33]

Examination of the data of Table II reveals that only PGDS concentration has potential as a semi-

nal plasma fertility marker. This marker concentration is reduced progressively from normal to oligospermic to azoospermic patients and is almost absent from the seminal plasma of vasectomy patients. There was no overlap between seminal plasma PGDS concentrations of normal and vasectomy patients (Fig. 2). The lowest PGDS concentration of normal individuals (0.03×10^4 μg/L) was three times higher than the highest PGDS concentration of vasectomy patients (0.01×10^4 μg/L). These data, and the indication that Sertoli cells are the major source of seminal plasma PGDS, prompt us to speculate that PGDS concentration in seminal plasma may be a marker of (a) Sertoli cell sufficiency and/or (b) post-testicular obstruction. We speculate that in men with infertility, seminal plasma PGDS levels similar to those found in vasectomy patients may indicate post-testicular obstruction. We identified a significant fraction of patients with azoospermia and a few with oligospermia who had such low levels. We also identified other patients who were either oligospermic or azoospermic and had seminal plasma PGDS levels within the range of normal men (Fig. 2). We believe that these patients had a patent vas deferens but suffered from other causes of male infertility. This suggestion is supported by our finding that in 6 of 7 patients with azoospermia and testicular failure but not obstruction, the PGDS levels were similar to those of normal subjects, and 4 of 4 patients with azoospermia and obstruction had PGDS levels below those of normal subjects and similar to those with vasectomy. More data with a larger and a better defined series of patients with azoospermia are necessary to examine whether analysis of PGDS in seminal plasma will aid in the differential diagnosis of obstructive versus nonobstructive azoospermia. Patients with very low PGDS levels in seminal plasma appear to suffer from post-testicular obstruction or a severe abnormality in spermatogenesis; in patients with normal PGDS in seminal plasma, obstruction can be excluded. These suggestions need confirmation with a larger series of patients.

The source of PGDS in seminal plasma has been examined by immunohistochemistry. It appears that the Sertoli cells are the major source of PGDS production and secretion into the semen. The finding that Sertoli cells synthesize PGDS is not surprising, since these cells produce a number of other secreted proteins, including androgen-binding protein,[38] transferrin,[39] inhibin,[40,41] aromatase,[42] and plasminogen activators.[43] The actual role of PGDS in semen is currently speculative. Previous reports suggest that PGDS is a transporter of lipophilic molecules, being a member of the lipocalin family.[16] In this respect, this protein may serve as a small lipophilic ligand transporter that supports the germ cells. Since PGDS binds and transports retinoids,[44] and retinoids are essential for germ cells, we speculate that PGDS is a molecule that facilitates retinoid transport to germ cells. This proposal needs further investigation.

In conclusion, it appears that PGDS concentration in seminal plasma is a novel marker that may aid in the differential diagnosis of obstructive and nonobstructive azoospermia. Since PGDS was shown here to be produced by the Sertoli cells, it will be interesting to investigate the biologic role of PGDS in spermatogenesis.

REFERENCES

1. Greenberg SH, Lipshultz LI, and Wein AJ: Experience with 425 subfertile male patients. J Urol **119:** 507–510, 1978.
2. Killian GJ: Fertility factors in seminal plasma. Proceeding of the 14th Technical Conference on Artificial Insemination and Reproduction. National Association of Animal Breeders, Columbia, Missouri, 1994, pp 33–38.
3. Wang MC, Valenzuela LA, Murphy GP, *et al:* Purification of a human prostate specific antigen. Invest Urol **17:** 159–163, 1979.
4. Watt KWK, Lee PJL, M'Timkulu T, *et al:* Human prostate-specific antigen: structural and functional similarity with serine proteases. Proc Natl Acad Sci USA **83:** 3166–3170, 1986.
5. McCormack RT, Rittenhouse HG, Finlay JA, *et al:* Molecular forms of prostate-specific antigen and human kallikrein gene family: a new era. Urology **45:** 729–744, 1995.
6. Lilja H: A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. J Clin Invest **76:** 1899–1903, 1985.
7. Lilja H, Abrahamsson PA, and Lundwall A: Semenogelin, the predominant protein in human semen: primary structure and identification of closely related proteins in the male accessory glands and the spermatozoa. J Biol Chem **264:** 1894–1899, 1989.
8. Lilja H, Oldbring J, Rannevik G, *et al:* Seminal vesicle-secreted proteins and their reactions during gelatin and liquefaction of human semen. J Clin Invest **39:** 499–510, 1987.
9. Lee C, Keefer M, Zhao ZW, *et al:* Demonstration of the role of prostate-specific antigen in semen liquefaction by two-dimensional electrophoresis. J Androl **10:** 432–438, 1989.
10. Malm J, and Lilja H: Biochemistry of prostate specific antigen, PSA. Scand J Clin Lab Invest Suppl **221**: 15–22, 1995.
11. Ferguson RA, Yu H, Kalyvas M, *et al:* Ultrasensitive detection of prostate-specific antigen by a time-resolved immunofluorometric assay and the Immulite immunochemiluminescent third generation assay: potential applications in prostate and breast cancers. Clin Chem **42:** 675–684, 1996.
12. Henttu P, Liao S, and Vihko P: Androgens up-regulate the human prostate-specific antigen messenger ribonucleic acid but down-regulate the prostatic acid phosphatase mRNA in the LNCaP cell line. Endocrinology **130:** 766–772, 1992.
13. Nagata A, Suzuki Y, Igarashi M, *et al:* Human brain prostaglandin D synthase has been evolutionarily differentiated from lipophilic-ligand carrier proteins. Proc Natl Acad Sci USA **81:** 4020–4024, 1991.
14. Clausen J: Proteins in normal cerebrospinal fluid not found in serum. Proc Soc Exp Biol Med **107:** 170–172, 1961.
15. Killian GJ, Chapman DA, and Rogowski LA: Fertility associated proteins in Holstein bull seminal plasma. Biol Reprod **49:** 1202–1207, 1993.
16. Peitsch M, and Boguski M: The first lipocalin with enzymatic activity. Trends Biochem Sci **16:** 363, 1991.

17. Urade Y, Fujimoto N, and Hayaishi O: Purification and characterization of rat brain prostaglandin D synthase. J Biol Chem **260:** 12410–12415, 1985.

18. Melegos DN, Diamandis EP, Oda H, *et al:* Immunofluorometric assay of prostaglandin D synthase in human tissue extracts and fluids. Clin Chem **42:** 1984–1991, 1996.

19. Lundquist F, and Seedorff HH: Pepsinogen in human seminal fluid. Nature **170:** 1115–1116, 1952.

20. Samloff IM, and Liebman WM: Purification and immunochemical characterization of group II pepsinogens in human seminal fluid. Clin Exp Immunol **11:** 404–414, 1972.

21. Sanchez LM, Freije JP, Merino AM, *et al:* Isolation and characterization of a pepsin C zymogen produced by human breast tissues. J Biol Chem **267:** 24725–24731, 1992.

22. Diez-Itza I, Merino AM, Toliva J, *et al:* Expression of pepsinogen C in human breast tumors and correlation with clinicopathologic parameters. Br J Cancer **68:** 637–640, 1993.

23. Liotta LA, Steeg PS, and Stetler-Stevenson WG: Cancer metastasis and angiogenesis. An imbalance of positive and negative regulation. Cell **64:** 327–336, 1991.

24. Chiang L, Contreras L, Chiang J, *et al:* Human prostatic gastricsinogen: the precursor of seminal fluid acid proteinase. Arch Biochem Biophys **210:** 14–20, 1981.

25. Reese JH, McNeal JE, Redwine EA, *et al:* Differential distribution of pepsinogen II between the zones of the human prostate and the seminal vesicle. J Urol **136:** 1148–1152, 1986.

26. Tang J, and Wong RN: Evolution in the structure and function of aspartic proteases. J Cell Biochem **33:** 53–63, 1987.

27. Diamandis EP, Nadkarni S, Bhaumik B, *et al:* Immunofluorometric assay of pepsinogen C and preliminary clinical applications. Clin Chem **43:** 1365–1371, 1997.

28. Baxter RC, and Martil JL: Structure of the Mr 140,000 growth-hormone-dependent insulin-like growth factor binding protein complex: determined by reconstitution and affinity labeling. Proc Natl Acad Sci USA **86:** 6898–6902, 1989.

29. Blum WF, and Ranke MB: Insulin-like growth factor binding proteins with special reference to IGF-BP3. Acta Pediatr Scand **367:** 55–62, 1990.

30. Barreca A, Artini PG, Cesarone A, *et al:* Interrelationships between follicle stimulating hormone and the growth hormone-insulin-like growth factor-IGF-binding protein axes in human granulosa cells in culture. J Endocrinol Invest **19:** 35–42, 1996.

31. Marx J: Possible function found for breast cancer genes. Science **276:** 531–532, 1997.

32. Jensen RA, Thompson ME, Jetton TL, *et al:* BRCA1 is secreted and exhibits properties of a granin. Nature Genet **12:** 303–308, 1996.

33. Lianidou ES, Melegos DN, and Diamandis EP: BRCA1 tumor suppressor gene product shares immunoreactive epitopes with a protein present in seminal plasma. Clin Biochem **30:** 425–432, 1997.

34. Mann T, and Rottenberg DA: The carbohydrate of human semen. J Endocrinol **34:** 247–259, 1966.

35. World Health Organization: *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction,* 3rd ed. Cambridge, Cambridge University Press, 1992.

36. Hoffmann A, Conradt HS, Gross G, *et al:* Purification and chemical characterization of beta-trace protein from human cerebrospinal fluid: its identification as prostaglandin D synthase. J Neurochem **61:** 451–456, 1993.

37. Diamandis EP, Yu H, and Sutherland DJA: Detection of prostate specific antigen immunoreactivity in breast tumors. Breast Cancer Res Treat **32:** 301–310, 1994.

38. Hansson V, Reusch E, Trygstad O, *et al:* FSH stimulation of testicular androgen binding protein. Nature **246:** 56–58, 1973.

39. Skinner MK, and Griswold MD: Secretion of testicular transferrin by cultured Sertoli cells is regulated by hormones and retinoids. Biol Reprod **27:** 211–221, 1982.

40. Le Gac F, and de Krester DM: Inhibin production by Sertoli cells. Mol Cell Endocrinol **28:** 487–498, 1982.

41. Bicsak T, Vale W, Vaughan J, *et al:* Hormonal regulation of inhibin production by cultured Sertoli cells. Mol Cell Endocrinol **49:** 211–217, 1987.

42. Dorrington JH, and Armstrong DT: Follicle stimulating hormone stimulates estradiol-17β synthesis in cultured Sertoli cells. Proc Natl Acad Sci USA **72:** 2677–2681, 1975.

43. Lacroix M, Smith FE, and Fritz IB: Secretion of plasminogen activator by Sertoli cell enriched culture. Mol Cell Endocrinol **9:** 227–236, 1977.

44. Tanaka T, Urade Y, Kimura H, *et al:* Lipocalin-type prostaglandin D synthase (beta-trace) is a newly recognized type of retinoid transporter. J Biol Chem **272:** 15789–15795, 1997.