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Original paper

INSULIN-LIKE GROWTH FACTOR-I AND -II AND THEIR BINDING PROTEINS IN HUMAN EJACULATES

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Summary: The insulin-like growth factor (IGF)/IGF-binding protein (IGFBP) system was investigated in ejaculates from thirty men aged 23 46 years. Mean ejaculate volume was 3.3 mL (range 1 6 mL). Viscosity was increased moderately in six samples and greatly in four more. The number of motile sperm (0 165 million/mL; mean 49; median 39) was highly correlated with total number ($P < 0.001$). Concentrations of IGF-I and -II determined by displacement radioimmunoassay were positively correlated ($P = 0.001$). IGF-I ranged from 2.7 to 29.7 nmol/L (mean 9.6; median 6.5) and increased with viscosity. IGF-II ranged from 5.7 to 39.0 nmol/L (mean 16.0; median 13.5). IGFBP-2 and putative IGFBP-4 were detected by autoradiography of ligand blots after polyacrylamide gel electrophoresis. These binding proteins were stable in ejaculates stored at 18 °C with protease inhibitors and remained associated with higher molecular weight complexes during molecular size chromatography with 1 mol/L acetic acid. They eluted at least partially in the expected fractions with 1 mol/L sodium chloride but further attempts at purification were unsuccessful due to progressive degradation. It is suggested that ionic bonds between IGFBP-2 and other components, such as proteoglycans, provide protection from proteolysis in a similar way as shown for IGFBP-1 and α_2-macroglobulin.

Key words: semen, insulin-like growth factors, binding proteins

Introduction

Insulin-like growth factors (IGF-I and IGF-II) are produced by many tissues and may have mitogenic and metabolic actions on surrounding or distant cells. Seminal plasma IGF-I probably derives mainly from Sertoli cells in the testis and its concentration has been positively related to sperm content and the percentage of normal spermatozoa (1, 2). Moreover, infertility associated with a low sperm count has been alleviated by growth hormone treatment, which simultaneously increased sperm motility and IGF-I concentration (3). IGF-II has also been detected in human seminal plasma apparently in much higher amounts than IGF-I (4).

The actions of IGFs are modulated by a family of different binding proteins (IGFBP), also synthesised in a specific manner by different types of cell. The predominantly inhibitory IGFBP-2 and putative IGFBP-4 were detected in seminal plasma (5), the former in relatively high concentrations (6), while IGFBP-3 was found only as fragments unable to bind IGFs (7). Human ejaculates contain large amounts of the serine protease, prostate specific antigen (PSA), which readily degrades IGFBP-3 (8).

The aim of this work was to investigate the IGF/IGFBP system in human ejaculates of different quality, with particular emphasis on quantitating IGF-II. An attempt was made to separate and estimate IGFBP-2.

Material and Methods

Ejaculates were obtained from thirty apparently healthy men aged 23 46 years. Each subject consented that the specimen could be used in this study. Volume, viscosity, total and motile sperm counts were estimated by standard methods after liquefaction at 36 °C for 30 min. The protease inhibitors phenylmethylsulphonyl fluoride (PMSF) and disodium ethylenediaminetetraacetic acid (EDTA) were added at final concentrations of 1 mmol/L and 6.7 mmol/L, respectively. Samples were then stored in closed containers at 18 °C until required.

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Protein concentration was estimated spectrophotometrically from optical density (OD) at 280 and 260 nm or by the biuret reaction, albumin with bromcresol green and PSA by radioimmunoassay. Concentrations of IGF-I and -II in the ejaculates were determined by displacement radioimmunoassay (RIA) after extraction with acid-ethanol, neutralisation and cryoprecipitation (9, 10). Thus, IGF-I was determined using a polyclonal rabbit anti-IGF-I antiserum (Biogenesis, Poole, UK), which showed 0.054% cross-reactivity with IGF-II at 50% inhibition of ^{125}I-IGF-I tracer binding. Sufficient IGF-II (ICN Biomedicals Inc., Aurora, USA) was added to the reaction mixture to release IGF-I from any IGFBP carried through the extraction procedure and allow reaction with the reagent antiserum. Conversely, IGF-I (ICN Biomedicals Inc., Aurora, USA) could be included in the assay for IGF-II where a mouse monoclonal anti-IGF-II antibody (Biogenesis, Poole, UK) was used as the reagent, because cross-reactivity with IGF-I at 50% inhibition of ^{125}I-IGF-II tracer binding was 0.4%. Samples of the respective reference IGF preparations (WHO 87/518 and WHO 96/538) were included in each test series. Recovery of added ligand was 88.2 ± 12.5% (n = 9) in the IGF-I assay and 86.3 ± 5.2% (n = 7) in the IGF-II assay. Interassay and intrassay coefficients of variation were below 12% and 10% respectively. When human IGFBP-2 was tested in the IGF-I RIA instead of IGF-I standards, a parallel inhibition curve 46-fold less sensitive was obtained.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of seminal plasma was performed as previously described for blood serum (11, 12). Proteins were electrotransferred to nitrocellulose membranes (0.45 μm, Schleicher and Schuell, Einbeck, Germany), followed by autoradiography after incubation with ^{125}I-IGF-I of specific activity approximately 36 MBq/nmol. Protein bands were putatively identified according to the mobility of reference standards: transferrin (80 kD), bovine serum albumin (BSA, 66 kD), ovalbumin (OA, 45 kD), glyceraldehyde-3-phosphate dehydrogenase (36 kD), carbonic anhydrase (29 kD), chymotrypsin (24 kD). Human IGFBP-2 (34 kD, GroPep Ltd., Adelaide, Australia) was also used as a reference standard.

Ejaculate samples (0.5 mL) were subjected to molecular size chromatography on a column (31.5 × 0.86 cm) of Sephadex G-75 (bed volume 27 mL) with 1 mol/L acetic acid or 1 mol/L NaCl in 0.05 mol/L phosphate buffer (PB; pH 7.5) as the eluant. Protein fractions were also separated on a Sephacryl S-300 column (90 × 1.5 cm; bed volume 160 mL) calibrated with blue dextran (2000 kD), OA and insulin (6 kD). Protein concentration in eluate fractions was estimated spectrophotometrically from the optical density at 280 nm. The position of IGF and/or IGFBP was detected by elution in the presence of ^{125}I-IGF or by direct IGF-I RIA without previous acid-ethanol extraction. Fractions containing IGFBP were bulked, reduced in volume by centrifugation through a Millipore filter (10,000 kD cut-off) or by Amicon (UM-10) ultrafitration. They were then separated from glycoproteins by chromatography on Concanavalin-A Sepharose (10 × 0.6 cm; bed volume 5 mL) using 0.14 mol/L TRIS buffer (pH 7.2) with appropriate mineral supplements as eluant.

Data were statistically analysed using the MSTATC (U.S.A.) programme. Values obtained were checked for normal distribution within groups and subjected to square root or logarithmic transformation, as appropriate, before analysis by parametric methods. Student's t-test for the significance of differences between two groups was preceded by an F-test for equality of variance. Probability values less than 0.05 were considered statistically significant.

Results

Mean ejaculate volume was 3.3 mL (range 1 6 mL). The number of motile spermatozoa (0 165 million/mL; mean 49; median 39) was highly correlated ($r = 0.95$; $P < 0.001$) with total number (0 300 million/mL; mean 88; median 83). Concentrations of IGF-I ranged from 2.7 to 29.7 nmol/L (mean 9.6; median 13.5 nmol/L). IGF-II levels were slightly higher, ranging from 5.7 to 39.0 nmol/L (mean 16.0; median 13.5 nmol/L), and positively correlated with IGF-I levels ($r = 0.584$; $P < 0.001$).

Viscosity was increased moderately (grade 2) in six samples and greatly (grade 3) in four more. When the results for these ten ejaculates were grouped and compared with those for the twenty samples with normal viscosity (*Table I*), it was apparent that the mean

Table I Characteristics and IGF levels [mean (SD)] in human ejaculates with normal or increased viscosity

Variable	Normal viscosity	Increased viscosity	Significance of t-test
Number of subjects	20	10	
Subject age (yrs)	32.0 (6.5)	34.7 (6.8)	NS
Volume (mL)	3.35 (1.46)	3.35 (1.29)	NS
Sperm conc. ($\times 10^6$ /mL)	95.3 (78.2)*	73.7 (65.5)	NS
Motile ($\times 10^6$/mL)	55.3 (52.0)*	36.0 (37.9)	NS
IGF-I (nmol/L)	7.5 (5.1)**	13.8 (9.1)	P = 0.027**
IGF-II (nmol/L)	14.5 (8.3)**	18.9 (8.6)	NS
IGF molar sum	22.0 (12.3)**	32.7 (14.8)	P = 0.041**

* Distribution normalised by square root transformation.
** Distribution normalised by logarithmic transformation. Differences between means remained statistically significant.

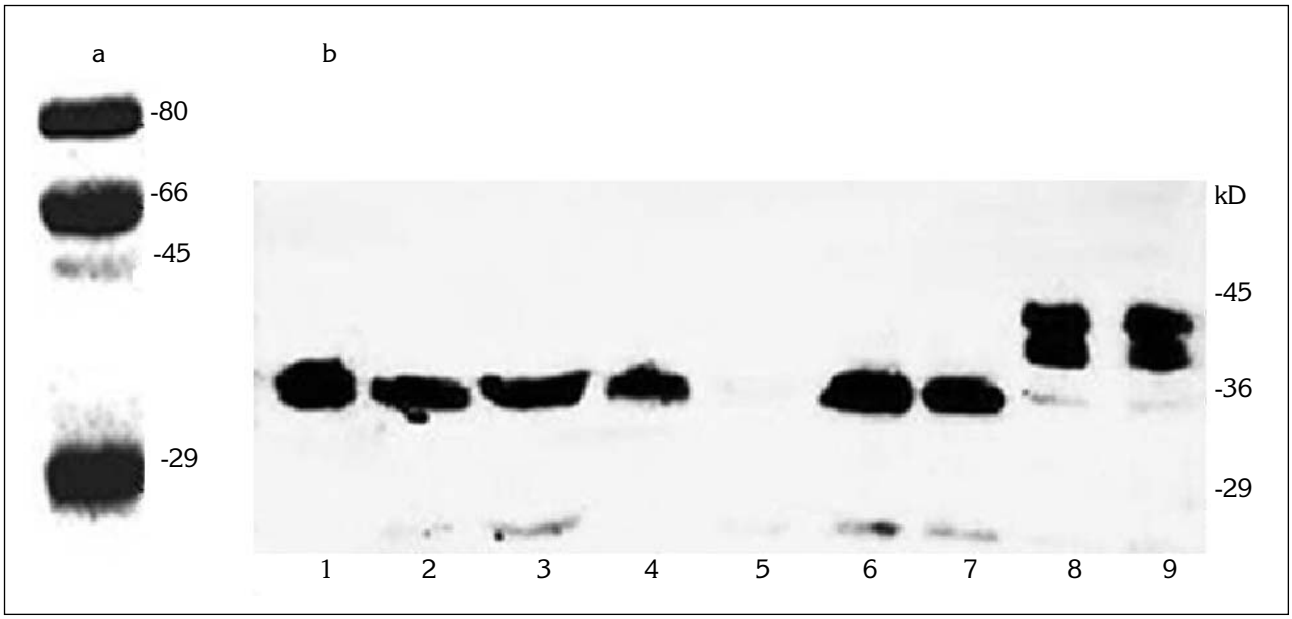

Figure 1. SDS-PAGE of seminal plasma (SP) showing
a) the main protein fractions and b) fractions binding ^{125}I-IGF-I: lane 1 – IGFBP-2; lanes 2, 4, 6 and 7 – SP samples; lanes 3 and 5 – IGFBP remaining complexed after chromatography of adjacent SP on Sephadex G-75 with 1 mol/L acetic acid; lanes 8 and 9 – blood serum.

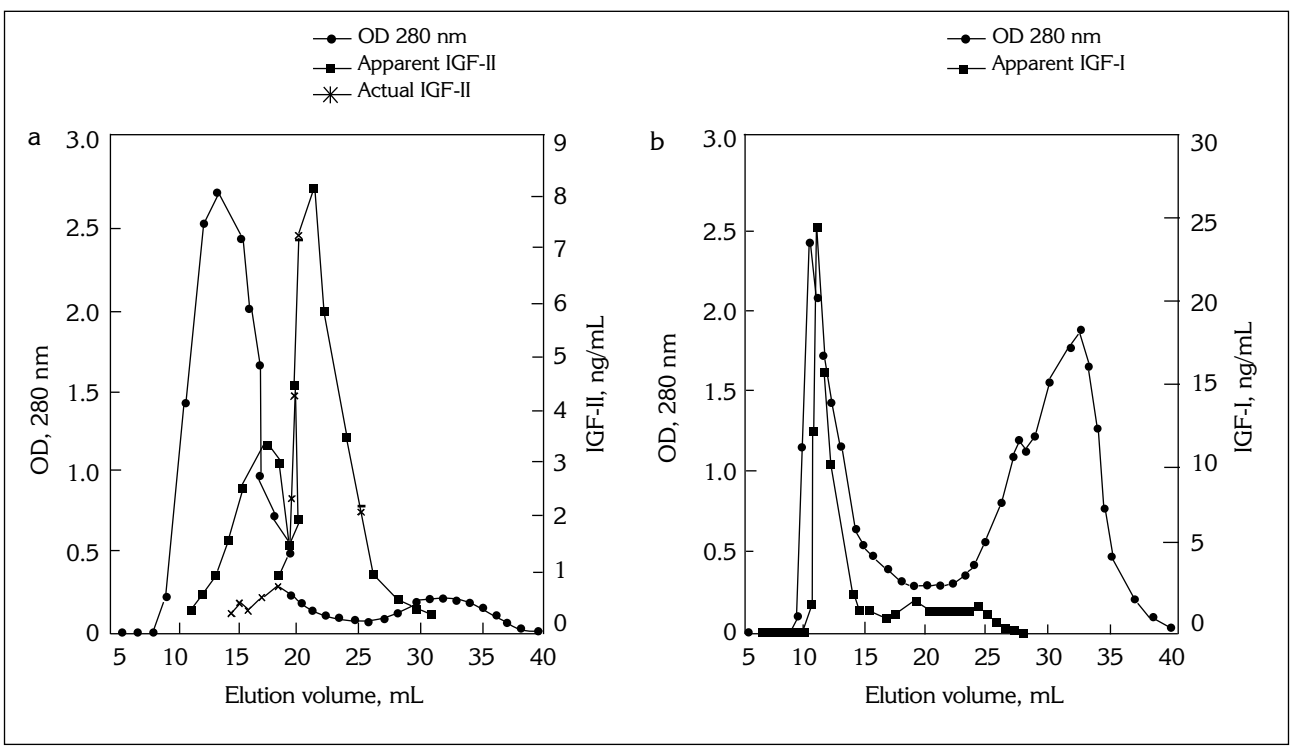

Figure 2. Elution of a) blood serum and b) seminal plasma with 1 mol/L acetic acid through Sephadex G-75 (31.5 × 0.86 cm).
Apparent IGF was measured by direct RIA without extraction.
Actual IGF was determined in the presence of the contraligand (displacement RIA).

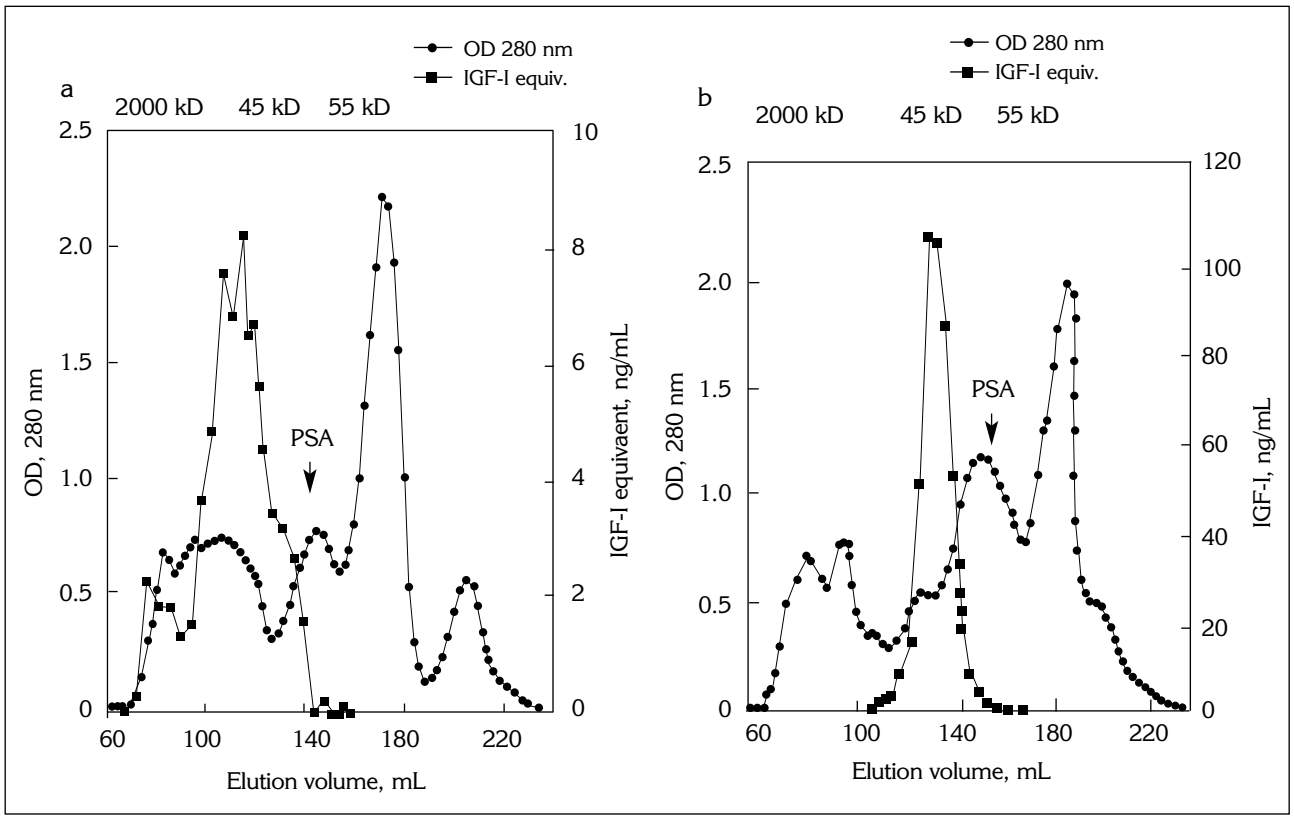

Figure 3. Elution of pooled seminal plasma (4 mL) through Sephacryl S-300 (90 × 1.5 cm) with a) 50 mmol/L phosphate buffer (pH 7.4) + NaCl (50 mmol/L) and b) 10 mmol/L TRIS/EDTA (pH 7.1) buffer + NaCl (1 mol/L). IGF equivalents were determined by direct RIA without extraction.

concentration of IGF-I (13.8 nmol/L) was significantly higher in the former than in the latter group (7.5 nmol/L). Mean IGF-II concentration was slightly but not significantly higher. Values for t were statistically significant whether the data were mathematically transformed to eliminate skewedness and kurtosis or not.

The electrophoretic profile of seminal plasma showed dominant proteins at 80, 66 and 30 kD (*Figure 1a*) consistent with the presence of albumin (21.2 mg/mL) and PSA (1.8 mg/mL) as well as lactoferrin (13). The IGFBP profile for several samples is shown in *Figure 1b* in comparison with human serum. Bands with the same mobility as IGFBP-2 (34 kD) were dominant, putative IGFBP-4 (24 kD) bands were present but faint, while the IGFBP-3 doublet (40 45 kD) characteristic for serum was absent.

While acid chromatography of blood serum on Sephadex G-75 clearly separated free IGF (7 kD) from IGFBP (25 40 kD) nearly all IGF/IGFBP in seminal plasma remained associated with higher molecular mass proteins (*Figure 2*). Elution through Sephacryl S-300 with neutral PB containing 0.05 mol/L NaCl gave four main OD peaks, the second containing PSA. Practically all IGF/IGFBP eluted in a broad irregular peak before PSA (*Figure 3a*). However, increasing the salt concentration to 1 mol/L concentrated most IGF/IGFBP into a sharp peak eluting at the position expected for IGF/IGFBP binary complexes (*Figure 3b*) without much modification to the OD elution profile. Chromatography of the IGF/IGFBP containing fractions on ConA removed glycoproteins, including IGFBP-4, and the presence of IGFBP-2 in the non-bound fraction was confirmed by SDS-PAGE and autoradiography of the ligand blot. However, purification from enzymes, such as PSA, was not complete and separation of IGFBP from the higher molecular mass complexes appeared to facilitate degradation. While subsequent elution of the Con-A non-bound fraction on Sephadex G-75 showed IGF-equivalents in higher molecular mass complexes, as well as at the positions expected for IGFBP-2 and IGF-I, the yield was very low and OD values indicated extensive degradation to small molecular mass compounds. This was not prevented by including SDS, high zinc concentrations (specific PSA inhibitor) or EDTA in the media used.

Discussion

The IGF-I levels found in human ejaculates in this study are of the same order as those recorded

by other authors. Thus, mean concentrations varying from 2.7 nmol/L to 14.8 nmol/L have been reported for normal human seminal plasma (1, 2, 14). Similar mean values were found in bovine (18.8 nmol/L), porcine (2.3 nmol/L) and equine (2.6 nmol/L) seminal plasma (15, 16).

Concentrations of IGF-II were of the same order and positively correlated with those for IGF-I. While boar and bull seminal plasma also contain similar amounts of each IGF (15, 17), an early study reported much higher IGF-II concentrations in human seminal plasma (4). IGF-II is synthesised in the prostate gland largely in the high molecular mass (15 kD) form (18, 19). The total molar sum of IGF ligand ranged from 9 to 61 nmol/L in our subjects. This is much less than the reported concentration of IGFBP-2 (355 nmol/L) (6). It is probable that the high IGF-II level previously reported for human seminal plasma was actually IGFBP-2 (4). We have found that IGFBP will give standard inhibition curves in both IGF RIA. In our study, including the contraligand in all tests (displacement RIA) prevented interference by IGFBP carried through the extraction procedure. This was not done in the early study (4).

The semen IGFBP profile appears to be species specific as IGFBP-2 and -5 were found in equine, IGFBP-3, -4 and -5 in bovine and IGFBP-2 and -4, plus IGFBP-3 fragments in human seminal plasma (1, 5, 7, 15, 20) but quantitative data are rare. Both IGFBP-2 and -4 are produced by the prostate gland, which also secretes acid and neutral proteases that can rapidly degrade these IGFBPs (8, 18, 21 23). Degradation appears to be limited by various broad-spectrum protease inhibitors, such as α_2-macroglobulin (α_2M), which is both present in seminal plasma and has been shown to bind IGFBP-1 without affecting IGF-binding (1, 24). Since IGFBP-1 and -2 share many structural similarities, it may be supposed that some seminal plasma IGFBP-2 may be bound to α_2M. However, IGFBP-2 has been shown to bind to the glycosoaminoglycans (GAG), chondroitin-4 and -6-sulphate (CS), keratan sulphate and heparin, as well as the proteoglycan aggrecan (25). This binding, which reduced the affinity for IGF-I, could be inhibited by high salt concentrations. Since, normal human seminal plasma contains proteoglycan and several GAGs including CS-C, hyaluronic acid and heparitin sulphate (26, 27) and we have shown that IGFBP/IGF activity is moved from high molecular mass complexes by 1 mol/L NaCl, it appears that most seminal plasma IGFBP-2 is bound in this way without IGF ligand. If it is assumed that all apparent 'IGF-I' eluting in high molecular mass complexes during column chromatography was actually IGFBP-2 then, using the factor 46 found for pure IGFBP-2 in the IGF-I RIA, it may be estimated that our human ejaculates contained from 15 to 50 mg/L. This overestimate is not much higher than the value of 11.1 ± 5.1 mg/L recorded by Blum et al. (6) and confirms the molar excess of IGFBP-2 in human seminal plasma.

While IGFBP-2 is primarily regarded as a protein which inhibits IGF action, protecting the ligands from degradation but preventing them from acting on the specific receptor, independent actions affecting cell adhesion, migration and proliferation through reaction of the RGD-motif with $\alpha_2\beta_1$-integrin have been reported (28). Serum IGFBP-2 levels may be elevated in a variety of human malignancies including active prostate carcinoma but not in benign prostate hyperplasia (29, 30) and over-expression of IGFBP-2 has been associated with an increased proliferation rate in some cancer cell lines (31). The functions of IGF-II and IGFBP-2 in human seminal plasma are not at present known. On the other hand, many authors have concluded that IGF-I has a role in fertility, particularly in connection with normal maturation and motility of the spermatozoa (1 3, 14, 32).

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INSULINU SLIČNI FAKTORI RASTA I NJIHOVI VEZUJUĆI PROTEINI U HUMANOJ SEMINALNOJ PLAZMI

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Kratak sadržaj: Izučavan je sistem insulinu sličnih faktora rasta (IGF) i IGF-vezujućih proteina (IGFBP) u seminalnoj plazmi trideset ljudi starosti 23 – 46 godina. Prosečna zapremina uzorka bila je 3,3 mL (opseg 1 – 6 mL). Viskoznost je bila povećana blago u šest uzoraka i dosta u još četiri. Broj pokretljivih spermatozoida (0 – 165 miliona/mL; srednja vrednost 49; medijana 39) bio je u visokoj korelaciji sa ukupnim brojem ($P < 0,001$). Koncentracije IGF-I i -II određivane radioimunoesejom bile su u pozitivnoj korelaciji ($P = 0,001$). IGF-I se kretao od 2,7 do 29,7 nmol/L (srednja vrednost 9,6; medijana 6,5) i povećavao se sa viskoznošću. IGF-II se kretao od 5,7 do 39,0 nmol/L. IGFBP-2 i verovatno IGFBP-4 detektovani su na ligand-blotovima nakon gel-elektroforeze. Ovi vezujući proteini su bili stabilni u seminalnoj plazmi sačuvanoj na – 18 °C u prisustvu inhibitora proteaza. Ostali su vezani u kompleksima velike molekulske mase pri hromatografiji sa 1 mol/L sirćetnom kiselinom. Kompleksi su bili razgrađeni hromatografijom sa 1 mol/L natrijum hloridom ali dalje prečišćavanje je bilo bezuspešno zbog brze proteolize. Pretpostavka je da jonske veze izmedju IGFBP-2 i drugih sastojaka seminalne plazme, kao što su proteoglikani, sprečavaju proteolizu na sličan način kao što je pokazano za kompleks IGFBP-1 i α_2-makroglobulina.

Ključne reči: seminalna plazma, insulinu slični faktori rasta, vezujući proteini

References

1. Glander H-J, Kratzsch J, Weisbrich C, Birkenmeier G. Insulin-like growth factor-I and alpha2-macroglobulin in seminal plasma correlate with semen quality. Hum Reprod 1996; 11: 2454 – 60.
2. Colombo JB, Naz RK. Modulation of insulin-like growth factor-I in the seminal plasma of infertile men. J Androl 1999; 20: 118 – 25.
3. Ovesen PG, Jorgensen JO, Ingerslev J, Orskov H, Christiansen JS. Growth hormone treatment of men with reduced sperm quality. Ugeskr Laeger 1998; 160: 176 – 80.
4. Ramasharma K, Cabrera CM, Li CH. Identification of insulin-like growth factor-II in human seminal and follicular fluids. Biochem Biophys Res Commun 1986; 140: 536 – 42.
5. Rosenfeld RG, Pham H, Oh Y, Lamson G, Giudice LC. Identification of insulin-like growth factor binding protein-2 (IGFBP-2) and a low molecular weight IGFBP in human seminal plasma. J Clin Endocrinol Metab 1990; 70: 551 – 3.
6. Blum WF, Horn N, Kratzsch J, Jørgensen JOL, Juul A, Teale D, Mohnike K, Ranke MB. Clinical studies of IGFBP-2 by radioimmunoassay. Growth Regulation 1993; 3: 100 – 4.
7. Lee KO, Oh Y, Giudice LC, Cohen P, Peehl DM, Rosenfeld RG. Identification of insulin-like growth factor-binding proteins-3 (IGFBP-3) fragments and IGFBP-5 proteolytic activity in human seminal plasma: a comparison of normal and vasectomized patients. J Clin Endocrinol Metab 1994; 79: 1367 – 72.
8. Réhault S, Monget P, Mazerbourg S, Tremblay R, Gutman N, Gauthier F, Moreau T. Insulin-like growth factor binding proteins (IGFBPs) as potential physiological substrates for human kallikreins hK2 and hK3. Eur J Biochem 2001; 268: 2960 – 8.
9. Nikolić JA, Ratković M, Nedić O. Determination of insulin-like growth factor-I by radioimmunoassay. J Serb Chem Soc 1996; 61: 1147 – 55.
10. Nikolić JA, Nedić O, Masnikosa R. Determination of IGF-II in human serum. J Serb Chem Soc 1998; 63: 805 – 15.
11. Hossenlopp P, Seurin D, Segovia-Quinson B, Hardouin S, Binoux M. Analysis of serum insulin-like growth factor binding proteins using Western blotting. Use of the method for titration of the binding proteins and competitive binding studies. Anal Biochem 1986; 154: 138 – 43.
12. Nikolić JA, Nedić O, Gavrović M, Stojanović D, Živković B. Different serum insulin-like growth factor-I (IGF-I) binding characteristics accompany reduced growth associated with low thyroid hormone and/or low total IGF-I status in young pigs. Acta Vet Beograd 1998; 48: 3 – 18.
13. Sensabaugh GF, Crim D. Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification. J Forens Sci 1977; 4: 106 – 115.
14. Baxter RC, Martin JL, Handelsman DJ. Identification of human semen insulin-like growth factor-I/somatomedin-C immunoreactivity and binding protein. Acta Endocrinol (Copenh) 1984; 106: 420 – 7.
15. Hoeflich A, Reichenbach H-D, Schwartz J, Grupp T, Weber MM, F ll J, Wolf E. Insulin-like growth factors and

IGF-binding proteins in bovine seminal plasma. Dom Anim Endocrinol 1999; 17: 39 51.

16. Lackey BR, Gray SL, Henricks DM. Measurement of leptin and insulin-like growth factor-I in seminal plasma from different species. Physiol Res 2002; 51: 309 11.
17. Hirai M, Boersma A, Hoeflich A, Wolf E, Foll J, Aumriller TR, Braun J. Objectively measured sperm motility and sperm head morphometry in boars (Sus scrofa): relation to fertility and seminal plasma growth factors. J Androl 2000; 22: 104 10.
18. Cohen P, Peehl DM, Rosenfeld RG. The IGF axis in the prostate. Horm Metab Res 1994; 26: 81 4.
19. Monti S, Di Silverio F, Iraci R, Martini C, Lanzara S, Falasca P, Poggi M, Stigliano A, Sciarra F, Toscano V. Regional variations of insulin-like growth factor I (IGF-I), IGF-II and receptor type 1 in benign prostatic hyperplasia tissue and their correlation with intraprostatic androgens. J Clin Endocrinol Metab 2001; 86: 1700 6.
20. Macpherson ML, Simmen RC, Simmen FA, Hernandez J, Sheerin BR, Varner DD, Loomis P, Cadario ME, Miller CD, Brinsko SP, Rigby S, Blanchard TL. Insulin-like growth factor-I and insulin-like growth factor binding protein-2 and -5 in equine seminal plasma: association with sperm characteristics and fertility. Biol Reprod 2002; 67: 648 54.
21. Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW. Human kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related but distinct kallikreins in the prostate. Crit Rev Clin Lab Sci 1998; 35: 275 368.
22. Frenette G, Deperthes D, Tremblay RR, Lazure C, Dube JY. Purification of enzymatically active kallikrein hK2 from human seminal plasma. Biochim Biophys Acta 1997; 1334: 109 15.
23. Nunn SE, Peehl DM, Cohen P. Acid-activated insulin-like growth factor binding protein protease activity of cathepsin D in normal and malignant prostatic epithelial cells and seminal plasma. J Cell Physiol 1997; 171: 196 204.
24. Westwood M, Aplin JD, Collinge IA, Gill A, White A, Gibson JM. Alpha2-macroglobulin: A new component in the insulin-like growth factor/insulin-like growth factor binding protein-1 axis. J Biol Chem 2001; 276: 41668 74.
25. Russo VC, Bach LA, Fosang AJ, Baker NL, Werther GA. Insulin-like growth factor binding protein-2 binds to cell surface proteoglycans in the rat brain olfactory bulb. Endocrinol 1997; 138: 4858 67.
26. Bonnet F, Perin JP, Maillet P, Jolles P, Alliel PM. Characterisation of a human seminal plasma glycosaminoglycan-bearing polypeptide. Biochem J 1992; 288: 565 9.
27. Binette JP, Ohishi H, Burgi W, Kimura A, Suyemitsu T, Seno N, Schmid K. The content and distribution of glycosaminoglycans in the ejaculates of normal and vasectomised men. Andrologia 1996; 28: 145 9.
28. Schuett BS, Langkamp M, Elmlinger MW, Ranke MB. Integrin mediated IGF-independent action of IGFBP-2 and IGFBP-2 fragments. First Joint Symposium GH-IGF 2002, Boston, USA, 5-9 Oct 2002. Growth Horm IGF Res 2002; 12: 275 (Abstract 154).
29. Ho PJ, Baxter RC. Insulin-like growth factor binding protein-2 in patients with prostate carcinoma and benign prostatic hyperplasia. Clin Endocrinol 1997; 46: 333 42.
30. Shariat F, Lamb DJ, Kattan MW, Nguyen C, Kim J, Beck J, Wheeler TM, Slawin KM. Association of preoperative levels of insulin-like growth factor I and insulin-like growth factor binding proteins-2 and -3 with prostate cancer invasion, progression and metastasis. J Clin Oncol 2002; 20: 833 41.
31. Wex H, Hohmann B, Mittler U, Vorwerk P. Higher cell proliferation of IGFBP-2 over-expressing cancer cell lines. First Joint Symposium GH-IGF 2002, Boston, USA, 5 9 Oct 2002. Growth Horm IGF Res 2002; 12: 250 (Abstract 078).
32. Miao ZR, Lin TK, Bongso TA, Zhou X, Cohen P, Lee KO. Effect of insulin-like growth factors (IGFs) and IGF-binding proteins on in vitro sperm motility. Clin Endocrinol 1998; 49: 235 9.

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