Application of proteomic methods for identification of sperm immunogenic antigens

A. Domagała1, S. Pulido2, M. Kurpisz1,3 and J.C. Herr2

1Department of Reproductive Biology and Stem Cells, Institute of Human Genetics, Polish Academy of Sciences, 60-479 Poznan, Strzeszynska 32, Poland; 2CRCRH, Department of Cell Biology, University of Virginia Health System, Box 800732, Charlottesville, VA 22908, USA
3Correspondence address. Tel: +48 61 6579212; Fax: +48 61 8233235; E-mail: kurpimac@man.poznan.pl

Although the immunological properties of sperm have been explored for a few decades, none of the antigens studied so far appears to be an effective target, to inhibit the fertilization process or shown the full spectrum of sperm antigenic potential. Antisperm antibodies (ASA) collected from infertile individuals and prepubertal boys with cryptorchidism together with two-dimensional (2D) electrophoresis have been employed. Immunoreactive antigens were cored from silver stained 2D gels and analyzed by mass spectrometry (MS). The obtained sequences were searched in the published protein databases. Altogether, 35 different sperm entities were identified in accessible protein databases, out of which 10 appeared to be sperm-specific. Additionally, 6 amino acid sequences indicated novel (hypothetical) proteins. Seventeen sperm entities were detected in sera samples from immune infertile males and 18 entities in ASA-positive seminal plasma (SP). Interestingly, we identified a few sperm structures, none of them sperm specific in sera samples from infertile females. Although, infertile males from whom the ASA-positive SP samples were obtained, did not have ASA in their circulation, the range of sperm antigens detected by systematic and local antibodies overlapped to a great extent (six identical entities). Sera samples from prepubertal boys allowed to show antigens, previously thought to be only present on mature sperm. Three out of four detected were sperm-specific. Using serum and SP of ASA-positive infertile adults and sera samples of prepubertal boys with testicular failure, we have extended the range of known, immunogenic sperm proteins as well as identified some novel antigens (n = 6) of human sperm for further characterization.

Keywords: antigens; antisperm antibodies; infertility; proteome; sperm

Introduction

Serum or seminal plasma (SP) antisperm antibodies [(ASA)-positive samples] from infertile adults may be used as a tool for identification of auto- and isomune sperm antigens (Shetty et al., 1999). Antigenic targets derived from sperm were studied for their prospective diagnostic, therapeutic and/or immunococontraceptive potential.

From observation of in vivo sensitized individuals (infertile or vasectomized), there appears to be a lack of adverse effects (except from infertility) of continuous ASA presence in their circulation and/or genital tract. Thus, cognate antigens of ASA may be potentially used for immunococontraception based on sperm components.

For immunococontraceptive studies in humans, there must be selected well-defined sperm autoantigens, characterized by: (i) sperm-specificity; (ii) a strong potential to induce high titers of sperm-specific antibodies within genital tract secreteions, preferably in the female and (iii) a role in the fertilization process.

Sperm-specificity is the most essential requirement. Studies with the use of monoclonal and polyclonal ASA showed that they may recognize antigens present on unrelated cells (Kurpisz et al., 1989). Thus, taking into account the possible dramatic consequences of a cross reactivity of antisperm vaccines with antigens present on somatic cells, the cell-specificity of antibodies resulting from intended sensitization is an absolute must.

Many methods have been used in the past to identify sperm-specific targets (Domagała and Kurpisz, 2004b). Data resulting from these studies (mostly unidimensional Western immunoblotting) were, however, conflicting (Domagała et al., 2000; Domagała and Kurpisz, 2004a). The differences were explained either by a difference in methodology or by selected populations of infertile patients. Apart from that, characterization of relevant protein(s) was limited to their molecular weight. Therefore, experimental outcome was not at all satisfying.

Besides, it could not be possible to predict a role for antigens recognized by ASA-positive individuals in fertilization, because the majority of the published reports did not analyze the reactivity of sperm antigens with sera of fertile patients.

In this paper, we used the proteomic approach for characterization of sperm immunoactive components. Antigens isolated from sperm plasmalemma using Triton X-114 were subjected to 2D electrophoresis followed by Western blotting and probing with serum and SP samples, obtained from individuals with high concentrations of ASA.

Immunoreactive antigens were cored from silver stained 2D gels and analyzed by MS. The obtained sequences were searched for in the published protein databases (NCBI, Swiss-Prot) and analyzed by MS. The obtained sequences were searched in the published protein databases (NCBI, Swiss-Prot) and analyzed by MS. The obtained sequences were searched in the published protein databases (NCBI, Swiss-Prot) and analyzed by MS.
Material and Methods

Patient and control samples
Initially, we obtained serum and/or SP samples from 29 individuals with a positive result of indirect immunobead-binding test (IBT) as previously described (World Health Organization, 1992). This group consisted of 21 infertile men, four infertile women, two prepubertal boys with cryptorchidism and two vasectomized men.

All female patients were infertile and revealed ASA in their sera samples, and one of them concomitantly had ASA in her cervical mucus (CM). Other CM samples were ASA-negative. Out of the analyzed female samples, only two revealed reactivity sufficient to be identified by means of 2D electrophoresis.

In the group (n = 21) of immune infertile men (all of them positive by IBT according to WHO standards), six individuals were positive for ASA only in their serum, nine only in their semen specimens and in six patients ASA were present in serum as well as on the surface of sperm cells (as determined by IBT). Out of initially screened samples, four ASA-positive infertile men, two sera samples of vasectomized men and three ASA-positive SP samples revealed detectable 2D electrophoresis pattern of reactivity and were further subjected by MS.

The control adult group consisted of sera samples obtained from 10 healthy individuals (five males and five females), with proven in vivo fertility. Their sera did not contain detectable ASA by means of IBT.

Two ASA-positive sera samples were obtained from prepubertal boys (aged 4 and 9 years) with unilateral cryptorchidism. Five sera samples from healthy prepubertal boys were run concomitantly as controls for this age group.

Local Ethical Committee (Medical School of Poznan) permission was granted to perform the presented study and written consents were obtained from the individuals (or their parents) who agreed to provide their serum or SP samples to the experiments.

Indirect IBT
The procedure has already been described elsewhere (World Health Organization, 1992). Briefly, motile sperm cells were obtained from semen ASA-negative donors by ‘swim-up’ technique. Sperm cells were washed in HAM F-10 medium supplemented with 1% BSA and the concentration adjusted to 25–30 × 10^6 per ml. One hundred microliters of this suspension was mixed with 100 μl of a tested sample (serum or SP) and 200 μl of HAM F-10/1%BSA, and incubated for 1 h at 37°C. After incubation, the cells were washed in HAM F-10/1%BSA and resuspended to a concentration 25–30 × 10^6/μl.

Five microliters of immunobead suspension (5 mg/ml) was mixed with 10 μl of final sperm suspension, covered with a coverslip and incubated in a moist chamber for 8 min. The spermatozoa were observed in a phase-contrast microscope, at 400× magnification. Motile spermatozoa with at least one bead attached to their surface were considered positive. Binding of immunobeads to the particular regions of a sperm cell (head, midpiece, tail) was also documented.

Direct IBT
This test was used for estimation of the percentage of immunobead, sperm cells binding due to the ASA present on the spermatozoa surface. In this test, the autologous sperm is applied instead of the donor’s sperm; however, in general the procedure is similar to indirect IBT (Kamieniczana et al., 2003).

Sperm antigenic extraction
Swim-up sperm (30 × 10^6/ml) was washed twice in PBS and then resuspended in 1 ml of Triton/deoxycholate (TDOC) buffer (Snow and Ball, 1992). Extraction of antigenic fractions lasted for 1 h and was performed at 4°C, with constant agitation. After removal of non-solubilized particles by centrifugation, the antigenic preparations were dialyzed and concentrated in Centricon YM-10 (Millipore Corporation, Bedford, MA, USA) concentrators, against PBS (pH 7.6), with three buffer changes. The resulting sperm proteins were collected and the protein concentration was determined by means of the RCDC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer’s manual.

2D electrophoresis
Isolelectric focusing was performed in 11 cm long strips (Ready Strip™ IEP Strips, Bio-Rad Laboratories). Sample buffer consisted of 7 M urea, 2 M thiourea, 100 mM DTT, 2% NP-40, 0.2% Ampholines (Amersham Biosciences AB, Uppsala, Sweden) and protease inhibitors. Twenty-five micrograms of protein mixture were applied per one strip. The strips were allowed to passively rehydrate (2 h, RT) and then were placed in Protein EIE Cell (Bio-Rad Laboratories) apparatus for active rehydration (17 h, RT) and IEF (25 000 Vh).

For the second dimension, the strips were equilibrated in equilibration buffer I (6 M urea, 2% SDS, 0.05 M Tris/HCl, pH 8.8, 20% (vol/vol) glycerol and 2% DTT) for 10 min at RT and then in equilibration buffer II (6 M urea, 2% SDS, 0.05 M Tris/HCl, pH 8.8, 20% (vol/vol) glycerol and 2.5% iodoacetamide) and placed on top of the second dimension SDS–PAGE gels (Criterion™ Pre cast Gel, Bio-Rad Laboratories), using a linear gradient (8–16%) of acrylamide in Protean II Multi-Cell apparatus (Bio-Rad Laboratories).

After electrophoresis, the gels were either silver stained (Domagała et al., 1998) or transferred to nitrocellulose membranes (Naaby-Hansen et al., 1997).

Western blotting
Transfer was performed according to the standard sandwich technique, in the tank system. The procedure was performed overnight at 100 mA, followed by 300 mA for 2 h. Antigens were visualized on the membranes by staining them with MemCode Reversible Protein Stain Kit (Pierce) in order to ensure the efficacy of the transfer procedure.

Immunoblotting
Membranes with blotted antigens were incubated (1 h, RT) in the blocking solution (5% dry milk, 0.05% Tween-20 (vol/vol), PBS, pH 7.4). After washing, the blots were incubated (4°C, overnight) with sera samples, diluted 1:2000 or with SP samples, diluted 1:500 in blocking solution. The horse-radish peroxidase-conjugated secondary antibody was then applied and the enzyme products were visualized by enhanced chemiluminescence, using the manufacturer’s protocol (Amersham, Buckinghamshire, UK).

Mass spectrometry
The W.M. Keck Biomedical Mass Spectrometry Laboratory at The University of Virginia Biomedical Research Facility was responsible for the MS. Briefly, the procedure was as following. The gel piece was transferred to a siliconized tube and washed and destained in 200 μl of 50% methanol, overnight. The gel pieces were dehydrated in acetonitrile, rehydrated in 30 μl of 10 mM dithiothreitol in 0.1 M ammonium bicarbonate and reduced at room temperature for 0.5 h. The DTT solution was removed and the sample alkylated in 30 μl of 50 mM iodoacetamide in 0.1 M ammonium bicarbonate at room temperature for 0.5 h. The reagent was removed and the gel pieces dehydrated in 100 μl acetonitrile. Next, the acetonitrile was removed and the gel pieces rehydrated in 100 μl 0.1 M ammonium bicarbonate. The pieces were again dehydrated in 100 μl acetonitrile, the acetonitrile removed and the pieces completely dried by vacuum centrifugation. Afterwards, the gel pieces were rehydrated in 20 ng/μl trypsin in 50 mM ammonium bicarbonate on ice for 10 min. Any excess trypsin solution was removed and 20 μl of 50 mM ammonium bicarbonate and formic acid added. The sample was digested overnight at 37°C and the formed peptides extracted from the polyacrylamide in two 30 μl aliquots of 50% acetonitrile/5% formic acid. These extracts were combined and evaporated to 25 μl for MS analysis.
The LC-MS system consisted of a Finnigan LCQ ion trap mass spectrometer system with a Protana nanospray ion source interfaced to a self-packed 8 cm × 75 μm id Phenomenex Jupiter 10 μm C18 reversed-phase capillary column. About 0.5–5 μl volumes of the extract were injected and the peptides eluted from the column by an acetonitrile/0.1 M acetic acid gradient at a flow rate of 0.25 μl/min. The nanospray ion source was operated at 2.8 kV. The digest was analyzed using the double play capability of the instrument acquiring full scan mass spectra to determine peptide molecular weights and product ion spectra to determine amino acid sequences in sequential scans. This mode of analysis produces approximately 400 CAD spectra of ions ranging in abundance over several orders of magnitude. Not all CAD spectra are derived from peptides.

The data were analyzed by database searching using the Sequest search algorithm. Peptides that were not matched by this algorithm were interpreted manually and searched versus the EST databases using the Sequest algorithm.

Results

Sera samples of ASA-positive infertile individuals (including prepubertal boys with cryptorchidism) as well as ASA-negative fertile controls reacted with sperm membrane antigens that had been partitioned into the detergent phase. We assumed that the reactivity of ASA-negative serum samples could be neither specific nor relevant to fertilization. Therefore, for MS analysis, only spots recognized by ASA-positive samples and not recognized by ASA-negative samples were cored (Figs 1–5).

Altogether, we have identified 35 different sperm protein entities, accessible in available Search Data Bases (Tables S1–S5, Supplementary material), 10 of them were sperm-specific and additional six appeared to be novel, hypothetical proteins (Table 1).

Out of four sera samples of ASA-positive infertile females, only two allowed a clear identification of the immunoreactive spots in a silver-stained gel (Fig. 1). Similarly, as in a group of sera samples of infertile males, only a fraction initially IBT-positive specimens revealed sufficient affinity, in order to exhibit detectable reactivity in 2D electrophoresis. Three spots were cored from the gel and successfully analyzed by MS (Fig. 1). None of the antigens, reacting with sera samples of immune-infertile females, however, was sperm-specific (Table S3, Supplementary material). One serum sample reacted only with one sperm antigen, dihydrolipoamide dehydrogenase. This enzyme was recognized by both sera samples obtained from infertile women.

We blotted ASA-positive serum samples of infertile men with human sperm antigens that allowed detection of 17 different entities (Table S3, Supplementary material). The remaining sera samples from this group either did not specifically react with antigenic compounds (i.e. were detected by ASA-negative sera samples) in immunoblotting or the obtained sequences could not be identified by MS, mainly due to insufficient amount of protein in a cored spot. Nineteen spots were cored from the gel for MS (Fig. 2).

Sperm antigens reacting with ASA of infertile female patients differed from sperm antigens recognized by circulating ASA from infertile men (Figs 1 and 2; Tables S1 and S2, Supplementary material).

Sera samples of vasectomized patients also reacted with sperm antigens (Fig. 3). Five spots were cored from the gel for MS analysis. The antigens recognized by one serum sample of a vasectomized patient (KM) were sperm-specific (proacrosin binding protein sp32 and CRISP-2; Table S3, Supplementary material). The other identified antigens were not sperm-specific (Table S3, Supplementary material).

In general, sperm antigens recognized by antibodies contained in samples from vasectomized men differed from the antigens recognized by ASA-positive infertile males (Tables S2 and S3, Supplementary material).

Three ASA-positive SP samples of infertile men provided seven spots that were subsequently cored from the gel (Fig. 4). They recognized 15 different entities derived from sperm, some of them (n = 6) apparently testis-specific (Table S4, Supplementary material). Infertile men, from whom the ASA-positive SP samples were obtained, did not have ASA in their circulation. Therefore, we were unable to directly compare their systemic versus local immune responses against sperm. However, antibodies to alpha enolase, rab GDP-dissociation inhibitor, elongation factor 2, human 6-phosphogluconate dehydrogenase, decarboxylating and proacrosin binding protein sp32 were present in sera samples of ASA-positive infertile men (Tables S2 and S4, Supplementary material).

We have also blotted two ASA-positive sera samples obtained from prepubertal boys (both operated for unilateral cryptorchidism) reactive with human sperm antigens (Fig. 5). One of these boys (sample U16) was 9 years old at the time of blood sampling. His serum sample contained a high concentration of ASA of the IgAa (IBT result: 95%), directed to sperm head (99%) and midpiece (1%). ASA present in this serum sample reacted with heat shock 70 kDa protein (HSP-70) and testis-specific isoform of lactate dehydrogenase (Table S5, Supplementary material).

The second prepubertal patient (U25), aged 4 years, had 27% anti-sperm IgM in his serum, as determined by means of indirect IBT. ASA present in his serum were directed to antigens present on the sperm tail tip, as determined by IBT (data not shown). This serum sample reacted with malate dehydrogenase (mitochondrial precursor) and testis specific enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH-2) (Table S5, Supplementary material).

Discussion

Identification of sperm antigens recognized by ASA present in infertile individuals is a step toward understanding the development of autoimmune infertility and may also lead to immune contraception based on the components derived from male gametes when exploring novel detected entities.

Our studied populations consisted of adult, ASA-positive patients, suffering from infertility and prepubertal cryptorchid boys whose serum contained high levels of ASA, as determined by indirect IBT. The control populations consisted of age-matched healthy, male volunteers.

Table 1: Novel proteins detected by ASA using 2D electrophoresis and MS

<table>
<thead>
<tr>
<th>Source of ASA</th>
<th>Name of protein</th>
<th>Tissue expression</th>
<th>Possible function</th>
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<tbody>
<tr>
<td>Male serum</td>
<td>Hypothetical protein</td>
<td>Brain, testis, liver, spleen and tumors</td>
<td>Proteoglycan membrane glycoprotein</td>
</tr>
<tr>
<td>Male serum</td>
<td>Unnamed protein product</td>
<td>Tissues not well established</td>
<td>S 100 family Ca-binding like-protein</td>
</tr>
<tr>
<td>Male serum</td>
<td>Hypothetical protein</td>
<td>Several tissues</td>
<td>No data</td>
</tr>
<tr>
<td>Male serum</td>
<td>Unknown</td>
<td>No data in humans, homology with Danio rerio protein</td>
<td>No data</td>
</tr>
<tr>
<td>Vasectomized male serum</td>
<td>Unknown</td>
<td>Several tissues including testis and uterus</td>
<td>ATP synthase, H+ transporting</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td>Hypothetical protein</td>
<td>Placenta, ovary</td>
<td>Not known</td>
</tr>
</tbody>
</table>

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ASA-negative individuals and healthy fertile adult males and females.

The presence of ASA in sexually immature males is a disputable issue due to the fact that in these individuals differentiated sperm cells are not present. However, previous studies, published by our group (Kasprzak et al., 1996; Domagala et al., 2000) and by other authors (Mininberg et al., 1993; Sinisi et al., 1997), indicated the presence of ASA in prepubertal sera. There are at least several

Figure 1: Identification of the immunoreactive spots in a silver-stained gel. (A, B) Western immunoblotting of human sperm membrane antigens with serum samples of ASA-positive (arrows) and ASA-negative (circles) females. (C) Silver-stained gels containing sperm antigens, extracted with Triton X-114, and resolved by means of 2D electrophoresis. Arrows indicate immunoreactive sperm antigens subjected to MS. The approximate positions of the molecular weight and pI standards are indicated on the left and on the top, respectively.

ASA-negative individuals and healthy fertile adult males and females.

The presence of ASA in sexually immature males is a disputable issue due to the fact that in these individuals differentiated sperm

Figure 2: Immunoblotting of ASA-positive and ASA-negative serum samples of infertile men. (A–D) Representative examples of Western immunoblotting of human sperm membrane antigens with serum samples of ASA-positive (arrows) and ASA-negative (circles) men. (E) Silver-stained gels containing sperm antigens, extracted with Triton X-114, and resolved by means of 2D electrophoresis. Arrows indicate immunoreactive sperm antigens subjected to MS. The approximate positions of the molecular weight and pI standards are indicated on the left and on the top, respectively.
explanations for induction of ASA in prepubertal boys. First, we have to be aware of cross-reactive components on somatic cells and microbial agents (Kurpisz et al., 1989; Kurpisz and Alexander, 1995) that may subsequently react with testicular structures including those ones present prepuberty on spermatogenic stem cells (archispermatogonia).

Second, immunosuppressive ability of T cells toward sperm cell antigens seems to be significantly diminished (Kurpisz, 1999) due to the absence of spermatozoa. Third, anatomic barrier between testis and circulation does not exist, although elevated temperature (cryptorchid testis) may activate autoimmune response of the humoral nature.

Figure 3: Reaction of sera samples of vasectomized patients with sperm antigens. (A, B) Western immunoblotting of human sperm membrane antigens with serum samples of ASA-positive, vasectomized men. (C) Silver-stained gels containing sperm antigens, extracted with Triton X-114, and resolved by means of 2D electrophoresis. Arrows indicate immunoreactive sperm antigens subjected to MS. The approximate positions of the molecular weight and pI standards are indicated on the left and on the top, respectively.

Figure 4: ASA-positive SP samples of infertile men. (A, B) Western immunoblotting of human sperm membrane antigens with ASA-positive SP samples. (C) Silver-stained gels containing sperm antigens, extracted with Triton X-114, and resolved by means of 2D electrophoresis. Arrows indicate immunoreactive sperm antigens subjected to MS. The approximate positions of the molecular weight and pI standards are indicated on the left and on the top, respectively.
Figure 5: ASA sera samples obtained from prepubertal boys reactive with human sperm antigens. (A, B) Western immunoblotting of human sperm membrane antigens with serum samples of ASA-positive (arrows) and ASA-negative (circles) prepubertal boys. (C) Silver-stained gels containing sperm antigens, extracted with Triton X-114, and resolved by means of 2D electrophoresis. Arrows indicate immunoreactive sperm antigens subjected to MS. The approximate positions of the molecular weight and pI standards are indicated on the left and on the top, respectively.

(Mieusset et al., 1993) due to the uncontrolled cascade of events (mRNA synthesis, exposure of novel membrane antigens).

Using LC-MS and peptide matching, we identified a range of immunoreactive antigens (n = 35) in sperm. We excluded from the analysis antigenic spots that were recognized by antibodies, contained in sera samples of fertile, ASA-negative individuals, as non-related to infertility. In such analysis, 50% of recognized sperm antigens in our study were previously identified in two recent but separate proteomic approaches (Martinez-Heredia et al., 2006; Stein et al., 2006).

Peptide matching was not always achieved in 100%, it was frequently found that cored spots from immunoreactive spots from female sera did not contain any peptides. There was a substantial amount of false matches while using material originating from reactions with female sera. Protein identification could be achieved in almost 80% of spots obtained by reactions with male ASA.

Sperm antigens, recognized in individual patients, with only few exceptions, were distinct. It suggests a diversity in the ASA repertoire and different background of autoimmune reactions in individual patients, although generally speaking, some overlap among antigens identified by male ASA samples including sera and SP samples was found (Table 2). Eight out of nine proteins identified herein were also identified in recent proteomic searches for sperm antigens both in human and rodents (Martinez-Heredia et al., 2006; Stein et al., 2006). This may indicate immunodominance of these peptides (across the species) as well as fairly easy targeting by LC-MS technology. Another interesting aspect of these overlapping protein sequences is that they represent both intrinsic and surface antigens; therefore, they may act at different stages of development of autoimmune reactions. Indeed, some of these determinants have already been investigated for contraceptive approaches (l-lactate dehydrogenase C chain; GAPDH-2; Goldberg and Herr, 1999; Miki et al., 2004).

Having put emphasis on overlapping sperm entities it should be further noted that not all samples recognized them all. It is quite surprising that sera of prepubertal boys (Table S5, Supplementary material) recognized three germ cell specific entities previously identified in sperm (Hsp, LDHC, GAPDH-2) out of the overall four detected entities. The reactions found in sera samples from prepubertal boys were subsequently confirmed by reactivities of sera samples from ASA-positive infertile males and vasectomized males (Table 2). This fundamental finding negates the reservations of some authors (Mininberg et al., 1993; Sinisi et al., 1997; Domagala et al., 2000; Domagala and Kurpisz, 2004a) on sperm-specific antibodies initiated before puberty. On the contrary, it seems that such phenomenon may be an epidemiological reason for infertility in adults with a history of cryptorchidism (Urry et al., 1994).

Additionally, we have also observed reactivity of some sera samples from ASA-negative fertile adults and healthy prepubertal boys with sperm. However, we share the view that the presence of some reactions in fertile, (ASA-free) healthy individuals may reflect the physiological phenomenon of natural antibodies (Shetty et al., 1999; Domagala and Kurpisz, 2004a), which are not detrimental to fertility status of these individuals.

This paper thus produces several new interesting conclusions in addition to previous work of this group which pioneered potential 2D electrophoresis with MS to successfully search for sperm entities (Naaby-Hansen et al., 1997) as well as explored ASA-positive sera samples for the study of sperm immunogenic proteins (Shetty et al., 1999). As for the latter study, the present study underlines (i) interesting comparison between local (SP) and systemic immune responses, (ii) extends the immunogenic library of well-identified sperm entities linking them to autoimmune syndromes, present both in adults and prepuberty (this can be either used for diagnostic purposes of autoimmunity or for possible immune interventions against the antibodies developed to immunodominant proteins) and (iii) indicates several novel sperm-specific entities, possibly related to fertilization and therefore worth testing for immunocontraception.

A proteomic approach could lead to further interesting studies (similarly to transcriptome studies) that could differentiate expression...
Table 2: Overlapping sperm entities detected by examined ASA samples in 2D electrophoresis and MS

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Sperm entities</th>
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<tr>
<td>Prepubertal boys (sera)</td>
<td>Alpha enolase, GAPDH-2, GAPDH-2, L-lactate dehydrogenase C, ATP synthase beta chain mitochondrial precursor, Proacrosin binding protein sp32, CRISP-2</td>
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</table>


References

Supplementary Data

Supplementary data are available at http://molehr.oxfordjournals.org

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