نام عداید دانستند
کار از خاک آدم نمیدارند
ما هم جهان حبیب به آن دوشیت
که بر سرعت فرسش اورنگ کشیدن.
Immunological Infertility & Subfertility in the Male Patient

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Session on the LABORATORY & INFERTILITY
Ordibehesht 1391, 10th iqicl, Tehran
Physiologically Proven but Clinically Unproven!

Reproduction Immunology:

• Immu. disorders in Male
• in Female
• In Pregnancy
• In Fetus & Newborn
• Immunological Contraception
INFERTILITY

- Inability to conceive after a defined period of MARRIAGE
- 90% of healthy couples within 1 yr.
- 95% within 2 yrs.
Normal Fertility

depends on

- spermatogenesis
- maturation in epididymis
- coitus
- transport through female genital tract
- fertilisation
- implantation
Prize for 40 weeks of pregnancy!
Thanks God, Praise belongs to Allah
FERTILITY vs STERILITY

<table>
<thead>
<tr>
<th>MALE PARTNER</th>
<th>FEMALE PARTNER</th>
</tr>
</thead>
<tbody>
<tr>
<td>STERILE</td>
<td>SUBFERTILE</td>
</tr>
<tr>
<td>not pregnant</td>
<td>probably pregnant</td>
</tr>
<tr>
<td>not pregnant</td>
<td>possibly pregnant</td>
</tr>
<tr>
<td>not pregnant</td>
<td>not pregnant</td>
</tr>
</tbody>
</table>
اتیولوژی:

عوامل ژنتیک

10 - 5

10 - 5

10 - 15

15 - 20

علل عفونی

عوامل محيطي

3

عیوب آناتومیک

اختلالات اندوکرین

عوامل محيطي
A.S.A.
When R They formed?

• Autoimmunity & Alloimmunity
• Evidences (in Human & in Animal Models)
<table>
<thead>
<tr>
<th>Defence</th>
<th>Surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunodeficiency (&amp; Infection)</td>
<td>Allergy</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Autoimmunity</td>
</tr>
</tbody>
</table>

Hypersensitivity Immunopathology
Deletion

Negative Selection
Central Tolerance
Unresponsiveness

Anergy

Inhibitory Cytokines

T

B

Tr

T

B
• Sequestration
• X-reaction, Molecular Mimicry
### Immunologically privileged sites

<table>
<thead>
<tr>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Eye</td>
</tr>
<tr>
<td>Testis</td>
</tr>
<tr>
<td>Uterus (fetus)</td>
</tr>
<tr>
<td>Hamster cheek pouch</td>
</tr>
</tbody>
</table>

These Sites Sequester Self Antigens, But Few Autoimmune Diseases Are Due to Release Of Hidden Self Antigens.
<table>
<thead>
<tr>
<th>Disease</th>
<th>HLA allele</th>
<th>Relative risk</th>
<th>Sex ratio (♀:♂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankylosing spondylitis</td>
<td>B27</td>
<td>87.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Acute anterior uveitis</td>
<td>B27</td>
<td>10</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Goodpasture's syndrome</td>
<td>DR2</td>
<td>15.9</td>
<td>~1</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>DR2</td>
<td>4.8</td>
<td>10</td>
</tr>
<tr>
<td>Graves' disease</td>
<td>DR3</td>
<td>3.7</td>
<td>4-5</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>DR3</td>
<td>2.5</td>
<td>~1</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>DR3</td>
<td>5.8</td>
<td>10-20</td>
</tr>
<tr>
<td>Type I insulin-dependent diabetes mellitus</td>
<td>DR3/DR4 heterozygote</td>
<td>~25</td>
<td>~1</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>DR4</td>
<td>4.2</td>
<td>3</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td>DR4</td>
<td>14.4</td>
<td>~1</td>
</tr>
<tr>
<td>Hashimoto's thyroiditis</td>
<td>DR5</td>
<td>3.2</td>
<td>4-5</td>
</tr>
</tbody>
</table>
Blood-testis barrier

- Sertoli cells
- Tight junction
- Adluminal compartment
- Basal compartment
Patient characteristics and prevalence of ASA

- Spermatocele
- OAT
- Cryptorchidism
- Primary testicular failure
- Testicular trauma
- Epididymitis
- Varicocele
- Idiopathic infertility
- Vasectomy
- VV
Indications for antisperm antibody testing in infertile man

**Abnormal semen analysis**
- Clumping/agglutination
- Low motility
- Shaking in place motility
- Poor sperm viability

**Abnormal postcoital test**
- Low number of sperm in mucus
- Poor motility
- Shaking in place motility

**Identifiable risk factors**
- Abnormal in vitro cervical mucus penetration tests
- Failed or low fertilization during IVF
- Abnormal sperm penetration assay
- Unexplained infertility after couple’s evaluation
پاراژوانی چند مفهوم:

<table>
<thead>
<tr>
<th></th>
<th>Antigen</th>
<th>Hapten</th>
<th>Immunogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>--</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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</tr>
</tbody>
</table>
Placental expression of complement regulatory proteins

- Membrane co-factor protein (CD46)
- Decay accelerating factor (CD55)
- Protectin (CD59)
Complement regulation

CDs 55 & 59 inhibit MAC formation
Fas  Fas-L interaction

Apoptosis
Fas Fas-L interaction

- Fas structure and function
- Fas-L structure and function
- Fas-L tissue distribution
Cells expressing FAS-L (CD178):

- Activated T cell
- Activated NK cell
- Tumor cells
- Retinal cells
- Corneal cells
- Endothelialial cells
- Placental cells
- Sertoli cells
Non Responsiveness in the Feto- Maternal Interface:

1. Activation of Innate Immunity
2. Lack of Classical MHC molecules
3. Functional Sequestration (Fas Dependent)
4. Presence of Specific Immune Factor (NK, TH2, Treg, Steroidal Suppressors, α-FP, hcc)
Immunological infertility

The role of antisperm antibodies in male infertility
Risk Factors for A. S. A. production:

- Hereditary, in men
- Acquired (including iatrogenic)
  
  Trauma, Varicocele, Tumors, Infection, Homos
• Auto or homo-sensitization in animals (and in male volunteers) can be obtained with testicular homogenate or epididymal spermatozoa and complete Freund's adjuvant.

• Immune orchitis in spontaneous human pathology has also been reported.

• Vasectomy for the voluntary control of male fertility may be considered a particular form of experimental autoimmunization; and many vasectomized individuals develop antisperm antibodies.
Antisperm antibodies can:

(i) be a mere epiphenomenon;
(ii) be a factor aggravating a pathologic situation already able to cause infertility;
(iii) play a pathogenetic role
If the antisperm autoimmune reaction represents the causal factor of infertility, immunosuppressive therapy seems to give the most satisfactory results, administered either in high doses for a very short time period or in low doses over a prolonged period, or even after transient pharmacologically induced azoospermia.
A.S.A.
Where R They Found?

- Male:
  Serum, Seminal Fluid, Sperm Surface
- Female:
  Serum, Mucous, Secretions, Follicular Fluids
اساس تستهای ASA

- وجود آگلوتیناسیونان (Preformed Agglu)
  (Head to Head, Tail to Tail, Mixed)

- اختلال در عملکرد اسپرم

- تشخیص حضور خود ab
Variations in

**A. S. A. Detection Systems:**

1. Immuno Bead Tests
2. MAR Test
4. Sperm Immobilization Test
5. P.C.T.
6. ELISA & ELISA_LIKE
7. IFM
8. FCM
The secretory immune system of the Female Genital Tract (from Stites)
The diagram illustrates the relationship between the implanting blastocyst, uterus, and decidua, and the response types:

- **T<sub>H1</sub>-type response**
  - Cell infiltrates
  - Cytotoxicity to embryo
  - Pregnancy loss

- **T<sub>H2</sub>-type response**
  - Antibody production
  - Developing embryo
  - Successful pregnancy
Positive mixed antiglobulin reaction (MAR) test

Raw semen sample with latex beads coated with IgG seen bound to sperm surfaces, mainly tails (phase contrast, ×40)
Immunobeads are polyacrylamide spheres with covalently bound rabbit antihuman immunoglobulins. Test is considered positive if at least 20% of motile spermatozoa have immunobead binding and is considered clinically significant when at least 50% of the motile spermatozoa are coated with immunobeads.
<table>
<thead>
<tr>
<th><strong>Table 2.4.1</strong> Suggested risk factors for ASA formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic obstruction of the MRT</td>
</tr>
<tr>
<td><em>Congenital</em></td>
</tr>
<tr>
<td>Congenital bilateral absence of the vas deferens (CBAVD)</td>
</tr>
<tr>
<td>Müllerian prostatic cysts</td>
</tr>
<tr>
<td><em>Acquired</em></td>
</tr>
<tr>
<td>Vasectomy</td>
</tr>
<tr>
<td>Iatrogenic obstruction of the epididymis and/or vas deferens</td>
</tr>
<tr>
<td>Inflammation and/or infection of the male reproductive tract</td>
</tr>
<tr>
<td>Varicocele</td>
</tr>
<tr>
<td>Cryptorchidism</td>
</tr>
<tr>
<td>Testicular trauma</td>
</tr>
<tr>
<td>Testicular torsion</td>
</tr>
<tr>
<td>Testicular surgery</td>
</tr>
<tr>
<td><em>Testicular sperm extraction (TESE)</em></td>
</tr>
<tr>
<td><em>Testicular biopsy</em></td>
</tr>
<tr>
<td><em>Organ-sparing surgery for testicular tumors</em></td>
</tr>
<tr>
<td>Testicular tumors</td>
</tr>
<tr>
<td>Homosexuality</td>
</tr>
<tr>
<td>Risk factor</td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Chronic obstruction of the MRT</td>
</tr>
<tr>
<td>Inflammation/infection of the MRT</td>
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<tr>
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</tr>
<tr>
<td>Testicular tumors</td>
</tr>
<tr>
<td>Homosexuality</td>
</tr>
</tbody>
</table>
Fig. 2.7.1 Immunofluorescent stainings of formalin-fixed human sperm with monoclonal antibodies. Mab H6–3C4 reacts exclusively with sperm (a) but not with lymphocytes (b), while campath-1 recognizes the lymphocytes (d) as well as sperm (c).
Agglutination

- Reported when **motile** sperm stick to each other in a definite pattern.
  - Head-head
  - Tail-tail
  - Head-tail
- Immunological cause of infertility
- Done on several HPF
Tests for A. S. A.

1. ELISA & ELISA-Like Titer & Isotype- Topology?
2. Microscopic Immobilization
3. Agglutination Based (with or without indicators) usually qualitative rather than quantitative
Spermatozoa “loaded” with spermagglutininins stick to the glycoprotein filaments as soon as they come in contact with cervical mucus.

Cervical mucus containing spermagglutininins provides the penetrating spermatozoa with the spermagglutininins and afterwards the spermatozoa stick to the glycoprotein filaments.
Diagnosing Immunological Infertility

- Antibodies in semen: IgG and IgA-class
- Antibodies in serum: agglutinating, cytotoxic (requiring complement)
- Current techniques in routine analysis of semen and serum
Detecting Antisperm Antibodies attached to Spermatozoa

(direct tests)
Mixed Antiglobulin Reaction (MAR)
Schematic representation of the direct MAR test

(A) 1 drop of coated Latex particles
(B) 1 drop of fresh, untreated semen
(C) 1 drop of antiserum to IgG
Immunobead test

bead with rabbit anti-human IgG or IgA
Detecting Antisperm Antibodies in Serum

(indirect tests)
Indirect SpermMAR test

BUFFER
DONOR SEMEN ➔ SWIM-UP ➔ 25 µl DONOR SPERMATOZOA (20 million/ml)

PATIENT ➔ DECOMPLEMENT ➔ DILUTE 1/16 ➔ 25 µl DILUTED SERUM
SERUM (56°C/30 min) INCUBATE 37°C/60 min

(SpermMAR) 10 µl (A) ➔ + 1 DROP PARTICLES (B)

B ➔ A ➔ C ➔ + 1 DROP ANTI-IgG (C)

READ & MOTILE SPERMATOZOA WITH PARTICLES
Reyleigh - Debye d ≈ WL

Mie phenomenon d ≈ WL

d << WL

d ~ WL

d >> WL
## Antisperm Antibodies

<table>
<thead>
<tr>
<th></th>
<th>Immunobead</th>
<th>SpermMAR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Motility</strong></td>
<td>rapidly</td>
<td>good</td>
</tr>
<tr>
<td><strong>Preparation</strong></td>
<td>time</td>
<td>non</td>
</tr>
<tr>
<td><strong>S. Volume</strong></td>
<td>0.5-2.0 ml</td>
<td>10 µl</td>
</tr>
<tr>
<td><strong>Shelf life</strong></td>
<td>1 month</td>
<td>1 year</td>
</tr>
<tr>
<td><strong>Sensitivity-specificity</strong></td>
<td></td>
<td>Better</td>
</tr>
<tr>
<td><strong>Price</strong></td>
<td>~ X 2</td>
<td></td>
</tr>
</tbody>
</table>
Disturbances due to A. S. A.

- Sperm Cytotoxicity
- Sperm Maturation
- Sperm Motility
- Sperm- Oocyte interactions
- Sperm fusion to Ovum
- Zygote Development
- Implantation
- Embryonic Growth (Recurrent Spontaneous Abortion)
Presence of ASA should be evaluated if:

1. Report of Sperm Clump/ Agglutination in the Semen Analysis
2. High Count of Round Cells are proven to be WBCs
3. Low Motility, especially with a Hx of Trauma
4. Idiopathic/ Unexplained Infertility
5. Recurrent Spontaneous Abortion
ایمنی نسبت به اسپرمرم:

• "Not All Or None"
• نسبی است
• تیتر آنتی بادی؟
• ایزوتایپ آن؟
• میل اتصال فردی؟
• محل اتصال؟

Concentration
• انتخاب میزان

Ig Class
• انتخاب نوع Ig

Affinity
• اندازه گیری افزایش دمای مخصوص

Epitope Location
• اندازه گیری موقعیت اپتیپ
درمان اختلالات و نازایی‌های ناشی از ASA:

- کاهش تولید (با ایمونوساپر سورها) (Elution)
- پاک کردن متصل ASA (ART)
از توجه شما متشکرم
Questions?