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Thrombophilic & Immune Factors

Pregnancy Loss

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Recurrent Pregnancy Loss

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- Definition : 3 or more clinically recognized pregnancy losses before 20wks from LMP.

- Clinical investigation should be started after two consecutive spontaneous abortions, especially

when fetal heart activity had been identified prior to the pregnancy loss
when the women is older than 35 yrs of age
when the couple has had difficulty conceiving

Risk of Pregnancy Loss

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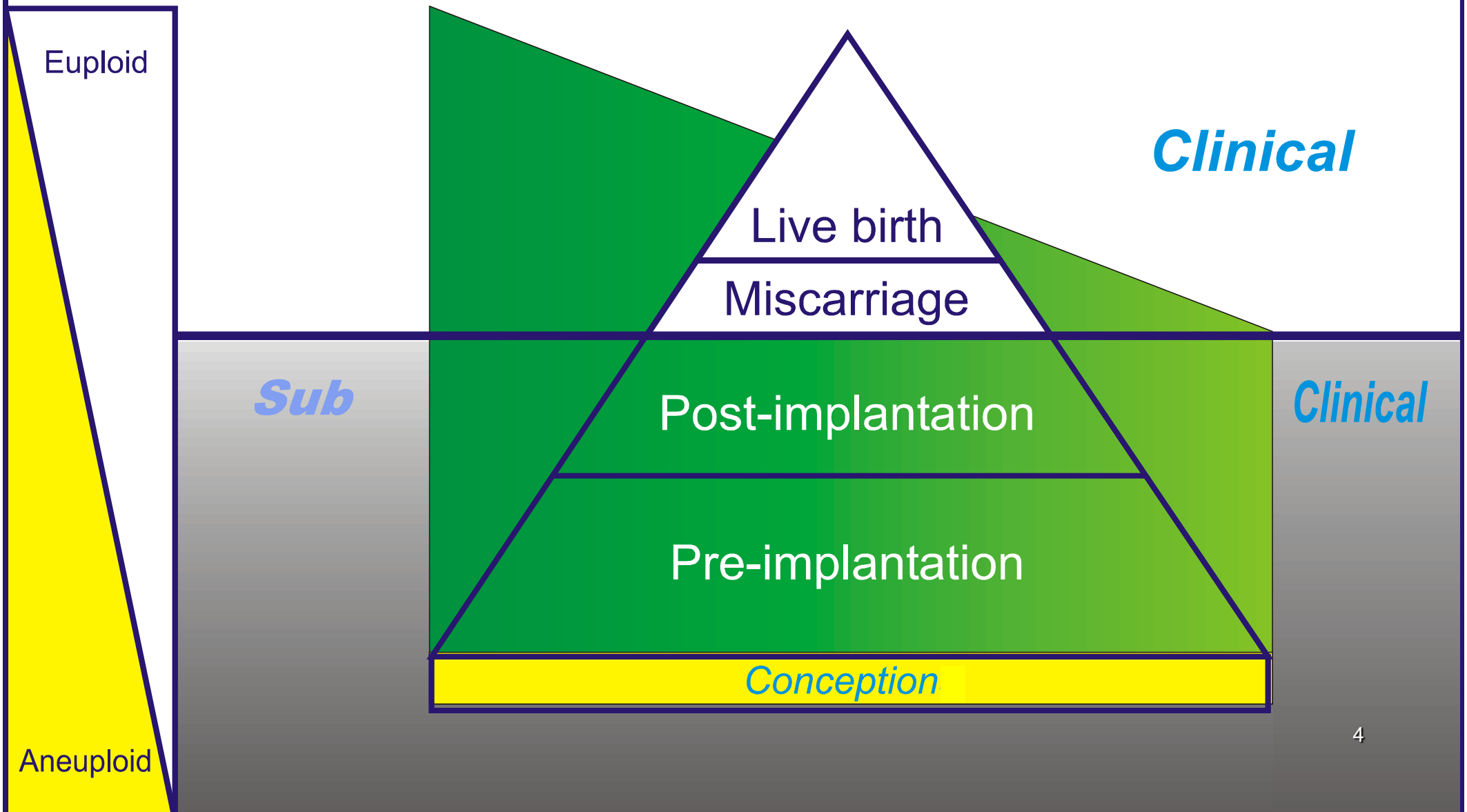
15-20% of all Pregnancy

11-13% in a First Pregnancy
13-17% after one abortion
38% after Two abortions

ACOG: Testing after two Miscarriage.

The Iceberg of Pregnancy Loss

(Macklon et al, Hum Reprod Update, 8:333-343)



Pregnancy loss

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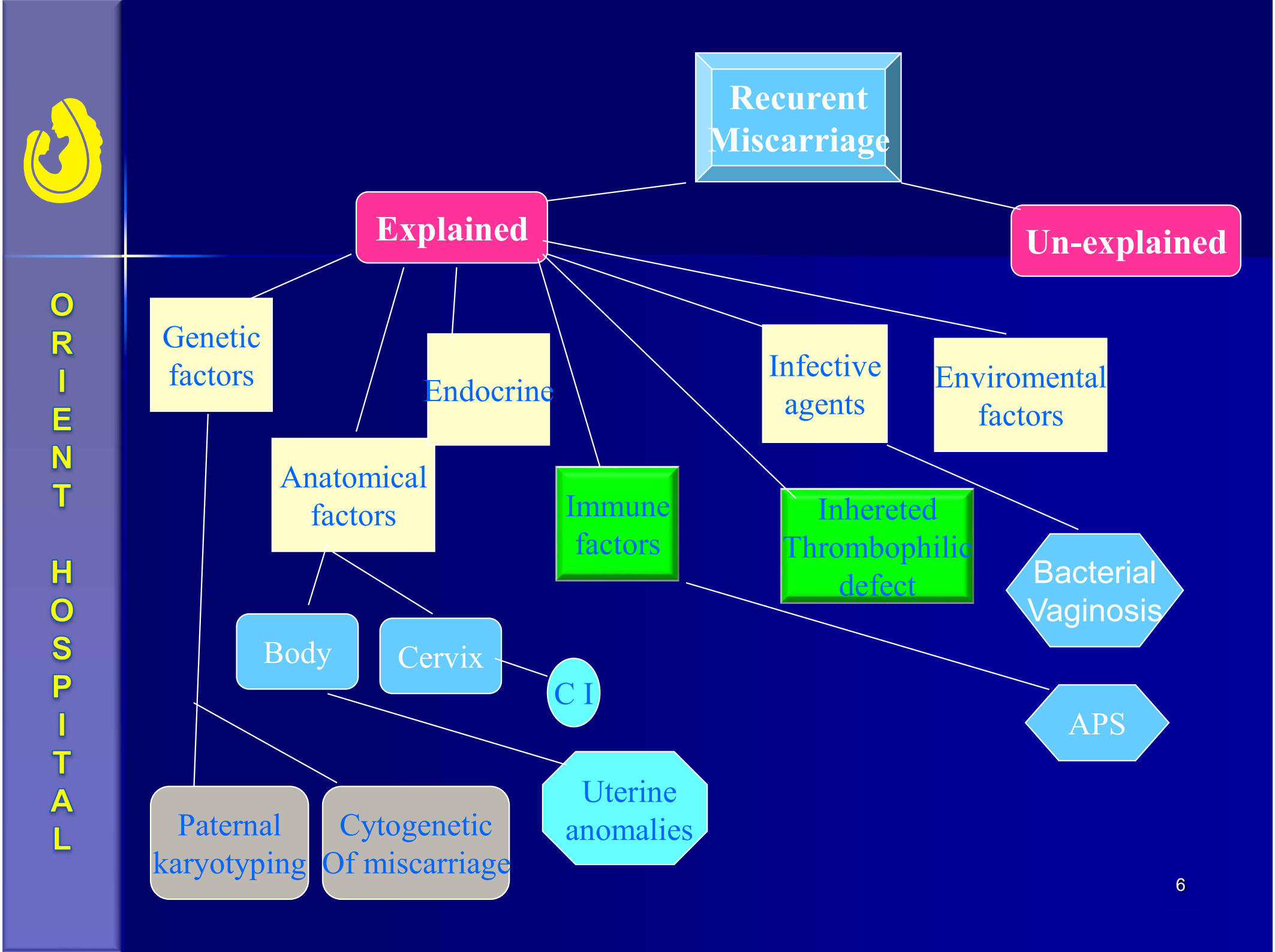
- Spontaneous pregnancy loss is, in fact, the most common complicadon of pregnancy.
- About 70% of human conceptions fail to achieve viability
- estimated 50% are lost before the first missed menstrual period.
- Most of preg. Losses are unrecognized.
- Actual rate of preg. Loss after implantation is 31%(by hCG assay)
- Clinically recognized, loss occures in 15 - 20% before 20wks of gestation.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Recurent Miscarriage
Explained
Un-explained
Genetic factors
Endocrine
Anatomical factors
Immune factors
Infective agents
Enviromental factors
Inhereted Thrombophilic defect
Bacterial Vaginosis
APS
Body
Cervix
C I
Uterine anomalies
Paternal karyotyping
Cytogenetic Of miscarriage

Causes of pregnancy loss

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Chromosomal

55% of occult and early losses

5% of recurrent losses.

environmental

hormonal

anatomical

Immunological

45% of early losses

95% of late losses

Immune Factors

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40% of unexplained Infertility.

80% of unexplained Pregnancy Losses.

Unfortunately for couples with immunological problems, their chances of recurrent loss increase with each successive pregnancy.

Immunological Factors

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1) Auto – Immune :

- Immunological response to pregnancy itself.
- The woman is rejecting her own proteins .
- Auto-antibodies attack own antigens.

2) Allo – Immune :

- Mother's response to the man's genetic contribution to the pregnancy
- Rejection of proteins from the man

Classical Definition of The Immune System

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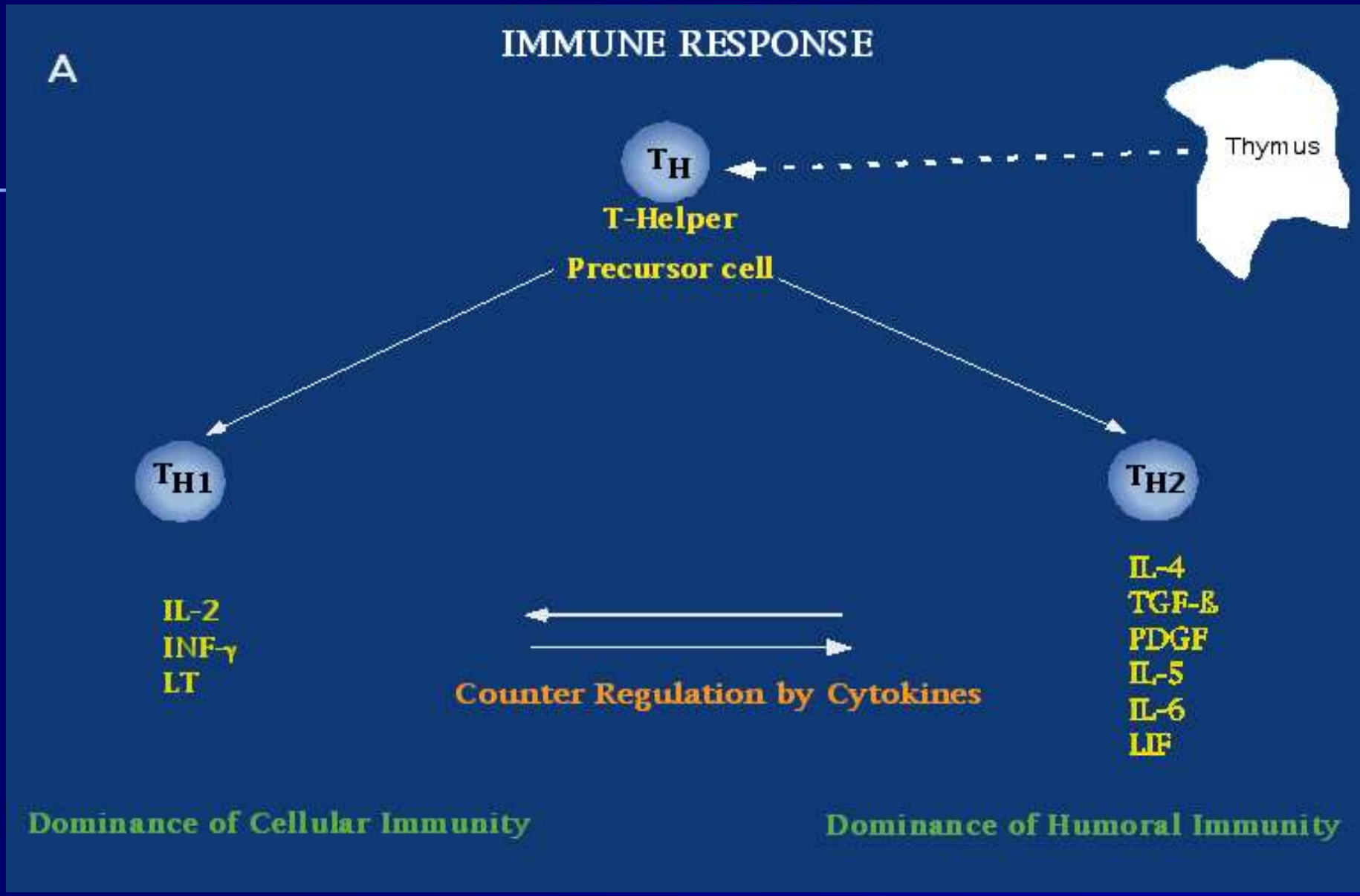
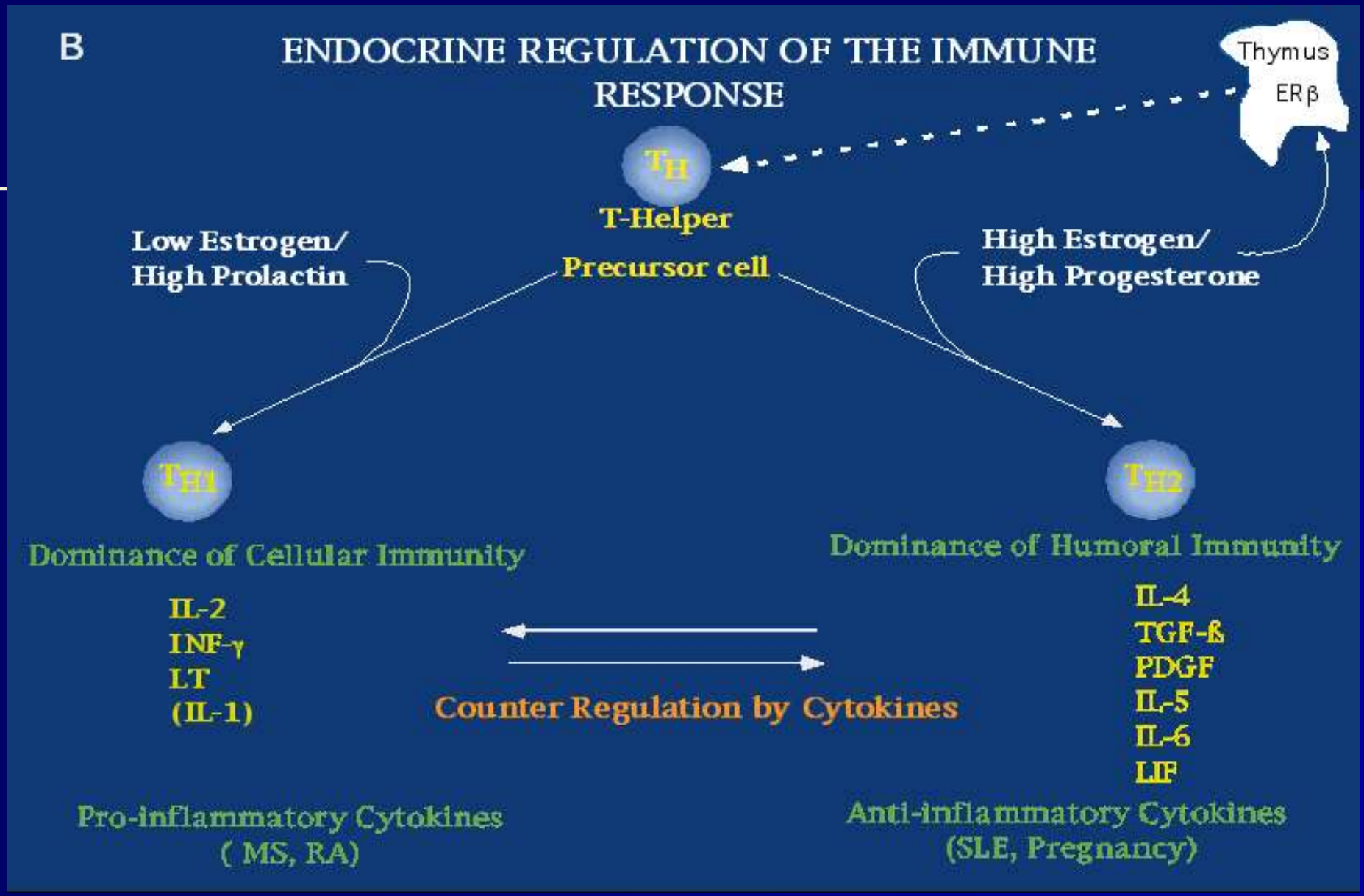
- Innate Immune response .
  - Macrophage
  - Granulocyte
  - They patrol and phagocytize foreign material .
- Adaptive immune response .
  - Cellular: cytolytic response by Natural Killer and cytotoxic T cells .
  - Humoral: Antibody Production by cells


A
IMMUNE RESPONSE
Thymus
TH
T-Helper
Precursor cell
TH1
TH2
IL-2
INF-γ
LT
IL-4
TGF-ß
PDGF
IL-5
IL-6
LIF
Counter Regulation by Cytokines
Dominance of Cellular Immunity
Dominance of Humoral Immunity


B
ENDOCRINE REGULATION OF THE IMMUNE RESPONSE
Thymus
ERβ
TH
T-Helper
Precursor cell
Low Estrogen/
High Prolactin
High Estrogen/
High Progesterone
TH1
TH2
Dominance of Cellular Immunity
Dominance of Humoral Immunity
IL-2
INF-γ
LT
(IL-1)
Counter Regulation by Cytokines
IL-4
TGF-ß
PDGF
IL-5
IL-6
LIF
Pro-inflammatory Cytokines
(MS, RA)
Anti-inflammatory Cytokines
(SLE, Pregnancy)

Adaptive immune response

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- T helper cells :

  - TH1: Interferon gamma – to increases cell-mediated immune response and inhibits the humoral immune response.

  - TH2: Interleukin 4- to increase humoral immune response and inhibits the cell mediated immune response.

- Is highly specific and MHC dependent .

Immunology of Pregnancy

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- Mechanical Barrier .
- Suppression of the maternal Immune System .
- Absence of MHC class I molecules .
- Th-2 type immune response
- Local Immune suppression :
  - Fas/Fas Ligand system .
  - Macrophages and cytokines

Auto – Immune Factors

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- Anti phospholipid antibodies (APL).
- Anti Thyroid antibodies (ATA)
- Anti Nuclear antibodies (ANA).

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Antiphospholipid syndrome

Antiphospholipid Syndrome

In the antiphospholipid antibody syndrome the body recognizes phospholipids (part of a cell's membrane) as foreign and produces antibodies against them.

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Antiphospholipid Syndrome

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APA syndrome is an acquired autoimmune Factor in which vascular thrombosis and/or recurrent pregnancy losses occur in patients having laboratory evidence for antibodies against phospholipids or phospholipid-binding protein cofactors in their blood.

Antiphospholipid Syndrome

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Antiphospholipid antibodies are a family of approximately 21 antibodies directed against negatively charged phospholipid binding proteins.

only the Lupus Anticoagulant and Anticardiolipin antibodies (IgG and IgM subclass, but not IgA) have been shown to be of clinical significance.

Antiphospholipid Syndrome

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The mechanism of aPL-associated pregnancy loss is related to the adverse effect of these antibodies on:

- embryonic implantation.
- trophoblast function.
- trophoblast differentiation.
- placental vasculopathy.

Placental Vasculopathy

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Placental pathologists use the term placental vasculopathy to describe pathological placental changes were found to be associated with some clinical conditions such as preeclampsia, IUGR, placental abruption and some cases of fetal loss and preterm labor .

Placental Vasculopathy

- villous infarcts.
- multiple infarcts.
- fibrinoid necrosis of decidual vessels.
- fetal stem vessel thrombosis.
- placental hypoplasia.
- spiral artery thrombosis .

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PRINCIPAL PATHOGENIC MECHANISMS MEDIATED BY APL

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Interference with:	
a) soluble coagulation factors:	protein C/S pathway inhibition; fibrinolysis inhibition
b) coagulation cells:	induction of a pro-adhesive, pro-inflammatory and pro-coagulant endothelial phenotype; induction of a procoagulant phenotype in monocytes
c) trophoblast cells:	reduction of proliferation and differentiation; gonadotrophin secretion impairment

Antiphospholipid Syndrome

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- 15-17 % from RPL (in general).
- primary (53%)
- secondary (47%) .
- (37%) Secondary APS associated with SLE or SLE-like syndrome.
- Females are more frequently affected than males. It mainly affects the second and third decades of life.

"Euro-Phospholipid Project Group". in a cohort of 1000 patients. Arthritis Rheum 2002;

Laboratory criteria

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The laboratory criteria are medium or high titer, not low titer, IgG or IgM anticardiolipin antibody, and/or a lupus anticoagulant on two or more occasions at least six weeks apart.

Sapporo criteria (RCOG)

Antiphospholipid syndrome

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- Adverse pregnancy outcomes include

(a) three or more consecutive miscarriages before ten weeks of gestation,

(b) one or more morphologically normal fetal deaths after the tenth week of gestation and

(c) one or more preterm births before the 34th week of gestation due to severe pre-eclampsia, eclampsia or placental insufficiency.

Anti Thyroid Antibodies (1)

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- 30% From RPL.
- Double risk for Pregnancy Loss or implantation failure (IVF)

- Tow Kinds of Antibodies :
  1- Thyroglobuline (Anti TG).
  2- Thyroid microsomal (Anti TPO).

- Seleniume , Prednisolone
- Thyroid Hormone Supplementation.

Anti Thyroid Antibodies (2)

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- Aetiology :
  - Link with other autoimmune problems.
  - Direct involvement of the antibodies .
  - Effect of age.
  - Sub-clinical hypothyroidism .
  - Natural killer cells hyperactivity .
  - Marker for T-lymphocyte function.

Anti Nuclear Antibodies

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- Histones : Smallest building blocks of DNA.
- ANA-Positivity : auto immune process that affects the development of the placenta .
- ANA-Positivity :
  - * SLE on lupus .
  - * Progressive Systemic Sclerosis .
  - * Sclerodermo Polymyositis .
  - * Drugs : Isoniazide, Hydralazin …
- Idiopathic Mechanism .
- Treatment : Prednisolone .

Allo-Immune Causes of RPL

- Problems with embryo signaling .
  - Soluble HLA-G.
- Problem with maternal immune Response :
  - NK cells.
  - T cells (Th_1 , Th_2).
  - B cells function.

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HLA - Genotyping

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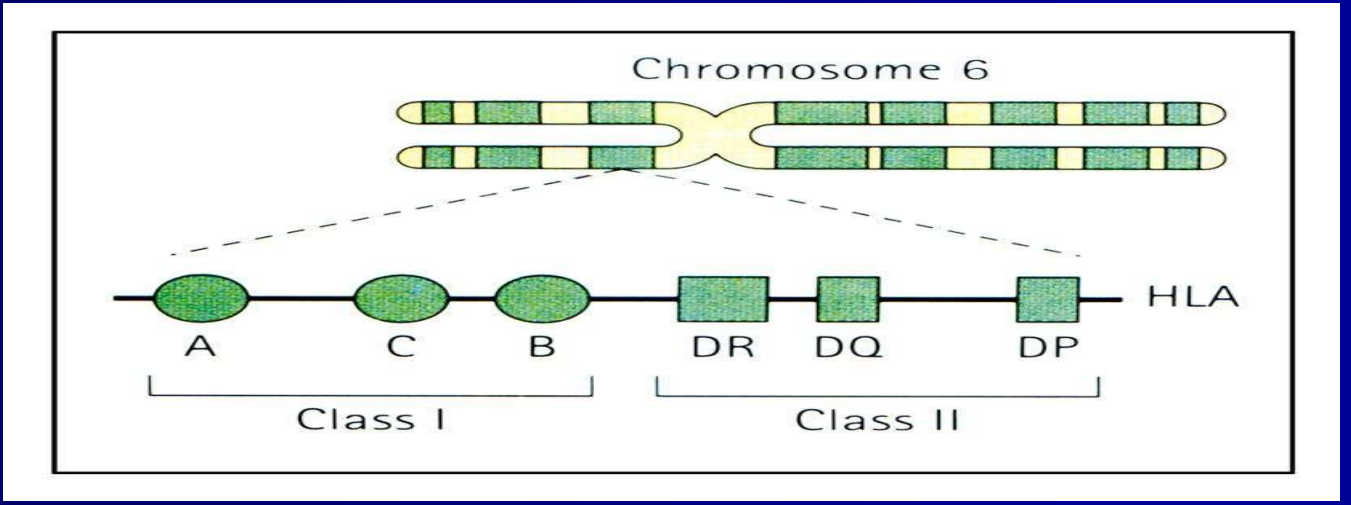
- HLA (Human Leukocyte antigens) , Class II .
- DQ Alpha Genotyping .
- Identify couples who look too much "alike".
- Blocking antibodies deficiency.



Natural Killer Cells

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- Immune cells witch kill anything perceived as foreign
- TNF (tumor necrosis factor), other cytokines
  * Like chemotherapy
  * embryo toxic
- NK Cells (> 12%) :
  High risk for abortion and Implantation Failure .
- The Test :
  RIP = Reproductive Immuno Phenotype (CD 56 +).
  NK assay.

• Enbrel (Etanercept) Supress TNF Alpha
  Supress TH-1 Embryo – Toxic Cytokins

Embryo Toxicity

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- Cytokines .

- Embryo Toxic Cytokines :
  - 60 % from RSA .
  - Endometriosis .
- The Test :

  ETA = Embryo Toxicity Assay

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Thrombophilia

What is “thrombophilia”?

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“Any disorder (inherited or acquired) associated with increased tendency to venous thrombosis “

Egeberg 1965

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The contact between placenta and maternal circulation is crucial for the success of pregnancy.Pro-thrombotic changes and thrombosis may interfere with these processes leading to adverse pregnancy outcomes at any gestational age

Thrombophilia

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Inherited

- Actived Protein C resistance Factor V Leiden.
- Prothrombin Mutation.
- (MTHFR) Hyperhomocystenemia.
- Protein C deficiency
- Protein S deficiency
- Anti Thrombin deficiency

Acquired

- Antiphospholipid synd
- Advancing age
- Malignancy
- Immobilization
- Trauma, Postoperative
- Pregnancy
- Estrogen use
- Hematologic diseases
- Nephrotic syndrome

Combined:

- Hyperhomocystenemia

Inherited Thrombophilia

Factor V Leiden - APC Resistence

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- APCR results from a point mutation in the FV gene, which causes resistance to degradation by activated PC (AD)
- The partial resistance of the mutant factor Va to inactivation by PC causes half life of FVa to prolonged, and the hemostatic balance to shift toward thrombosis
- Most common inherited thrombophilia,
  5-8% of healthy general population,
  20-30% of patients with thrombosis
- Thrombotic risk x7 in heterozygotes
  x80 in homozygotes
- Common with other types of thrombophilia

Activated Protein C Resistance

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Factor V Leiden is the cause of APCR in95% of cases

Other Causes: (5%)

Pregnancy

Oral contraceptives

Increased levels of factor VIII

Anti phospholipid antibodies

cancer

Other mutations in factor V

Factor V Leiden (A506G) mutation

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adenine 506 guanine (A506G) mutation in factor V (factor V Leiden) (a substitution of glutamine for arginine at amino acid 506 of factor V) **Factor V Leiden (FVL) is a mutation in the factor V molecule, rendering it resistant to cleavage by activated protein C. Factor V remains a procoagulant and thus predisposes the carrier to clot formation.**

It has been linked with an increased risk for venous thromboembolism due to Resistance to activated protein C and is responsible of 20–30% of venous thromboembolism events

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Prevalence of factor V Leiden mutation and its relation with recurrent spontaneous pregnancy loss in a group of Syrian women

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ABSTRACT

Objective: The aim of our study was to investigate the prevalence of factor V Leiden and its relation with RPL in a group of Syrian women.

Materials and Methods: The study group included 35 women with a history of recurrent pregnancy loss (two or more abortions before 20th week of gestation) were referred to Orient hospital for obstetrics, gynecology and assisted reproduction, Damascus, Syria, for investigation between December 2005 and July 2006. All women with known causes of pregnancy loss after convenient investigations were excluded. The control group included 45 healthy women from the same ethnic background, who had at least one successful pregnancy, and none of them had a history of fetal loss or complicated pregnancy. FVL mutation was screened by Real-time PCR method.

Results: The results show that 10 women out of 35 with RPL and 4 women out of 45 controls had FVL mutation (28.6 versus 8.9 %,P=0.022, Odds ratio 4.1, 95% CI: 1.16-14.4). From the 25 women who were primary RPL, eight patients had the factor V Leiden (32 versus 8.9%, P=0.014, OR: 4.8, 95%CI: 1.2, 18.17). From the 10 women who were secondary RPL, two patients had the factor V Leiden (20 versus 8.9%, P=0.30, OR: 2.5, 95% CI: 0.4-16.4). All patients and controls carrying the factor V Leiden were heterozygote.

Conclusion: Our results revealed that the prevalence of FVL was significantly higher in women with RPL in comparison with controls, particularly in the subgroup with primary RPL, and there is an association between factor V Leiden mutation and recurrent pregnancy loss.

Key Words: Factor V Leiden mutation, recurrent pregnancy loss, Prevalence, Syrian women

Prothrombin (G20210A) mutation

A change of G to A at position 20210 in prothrombin (prothrombin 20210A) elevates baseline prothrombin levels and thrombin formation.

Hyperhomocystinemia

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- Homocysteine-

  intermediary amino acid

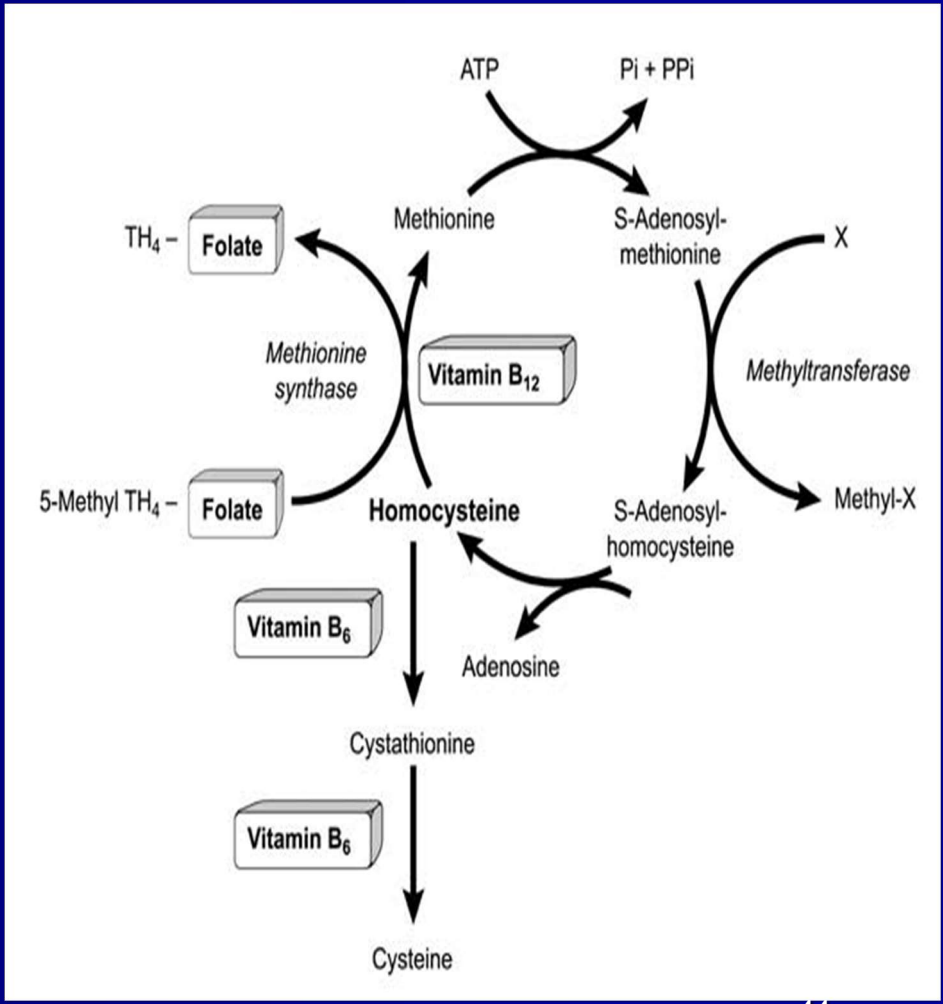
  formed by the conversion of

  methionine to cysteine

- Cofactors: folate, vitamin

  B12,

  vitamin B6



Hyperhomocysteinaemia

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- Reduced activity of MTHFR
- homozygous for mutation C677T
- Deficiencies of folate, vitamin B6, vitamin B12
- Increased risk of thromboembolic disease: meta-analysis OR 2.95 (CI 2.08-4.17)

Methylene tetrahydrofolate reductase (MTHFR)

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- Most common form of genetic hyperhomocysteinemia
- Point mutation- alanine-valine aa677
  reduced enzymatic activity
- Homozygotes- increased homocysteine levels (10% of normal population), confers a x2-3 increased
  risk for thrombosis
- Risk factor for atherosclerotic disease and
  recurrent VTE
- Heterozygotes- normal homocysteine levels, no increased risk for thrombosis

MTHFR (C677T) mutation

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A homozygous methylenetetrahydrofolate reductase (MTHFR) mutation, present in 1-4% of the general population, is associated with a three fold increased risk for DVT or PE, as well as preeclampsia and placental abruption.

Protein S deficiency

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Protein S deficiency (PSD), present in up to:

2 % of the general population, is found in approximately

15% of individuals with a DVT or PE.

6% of women with obstetrical complications.

including a relatively high risk for stillbirth.

Protein C deficiency

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- Protein C deficiency (PCD), present in about 1.5% of the general population, is associated with a lower risk for obstetrical complications than PSD and is found in 3-5% of individuals with a DVT or PE.
- Furthermore, PCD combined with a FVL mutation is a relatively common cause of DVTs and show a higher risk for thrombosis compared to FVL alone.

Antithrombin III deficiency

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- Antithrombin III deficiency (ATIII), present in less than 0.5 % of the general population, as with PSD and PCD, may rarely result from mutational events
- Because of its relative rarity, actual risks for thrombotic events are difficult to estimate, but without question this entity contributes to thrombotic risks during pregnancy.

Changes in Normal Pregnancy

- Protein S: free levels fall to 40%-60% of normal in the first trimester
- Protein S deficiency requires confirmation 3 months post partum
- Protein C: constant in all 3 trimesters
- Antithrombin: unchanged by pregnancy but can fall in severe pre-eclampsia
- Homocysteine: falls by 30%-50%
- Prothrombin levels increase

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Prevention

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In the past the obstetrical art focused mainly on how to deal with complications.

but now by the remarkable advance in modern obstetrics ,immunology, and hematology, **the goal is how to prevent them.**

Maternal risk assessment

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Maternal risk assessment can be firstly identified from

history

Maternal risk assessment

Recurrent pregnancy loss is not just a Bad Luck and must be investigated .

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Maternal risk assessment

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But on other hand some conditions need no recurrence to be alarming, and to be investigated.

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one unexplained fetal deaths after ten weeks of pregnancy

one preeclampsia or placental insufficiencies occurring before 34 weeks

One previous preterm birth

one or more confirmed episodes of venous or arterial thrombosis.

any of these must invite a big question mark

?

Pregnancy loss after 10wk

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one pregnancy loss more than 10wk. Gestation or pregnancy associated with late adverse outcome

need no recurrence to be investigated.

Pregnancy loss after 10wk

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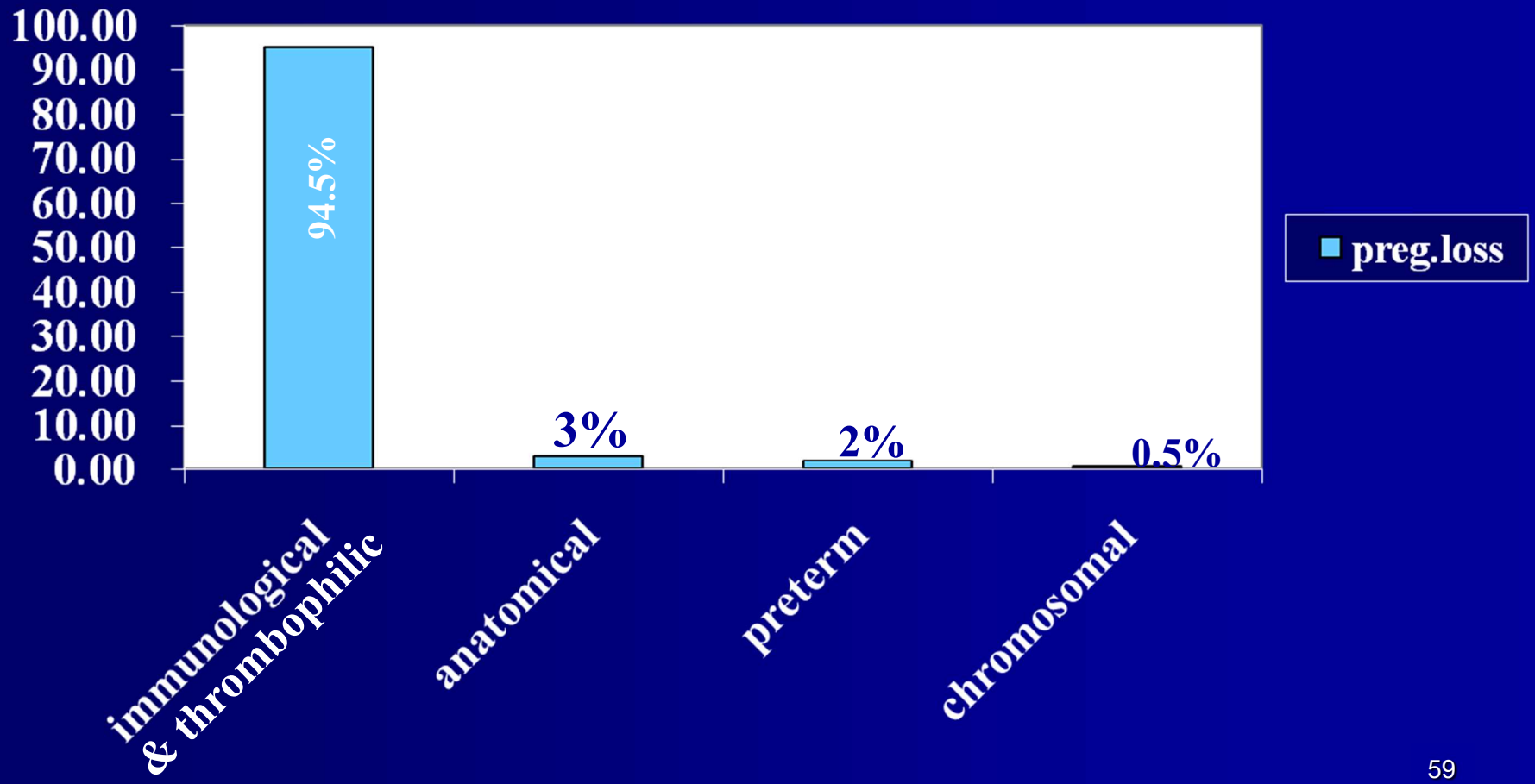

Pregnancy Loss after 10wk

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How much is thrombophilia common among general population

Inherited thrombophilia

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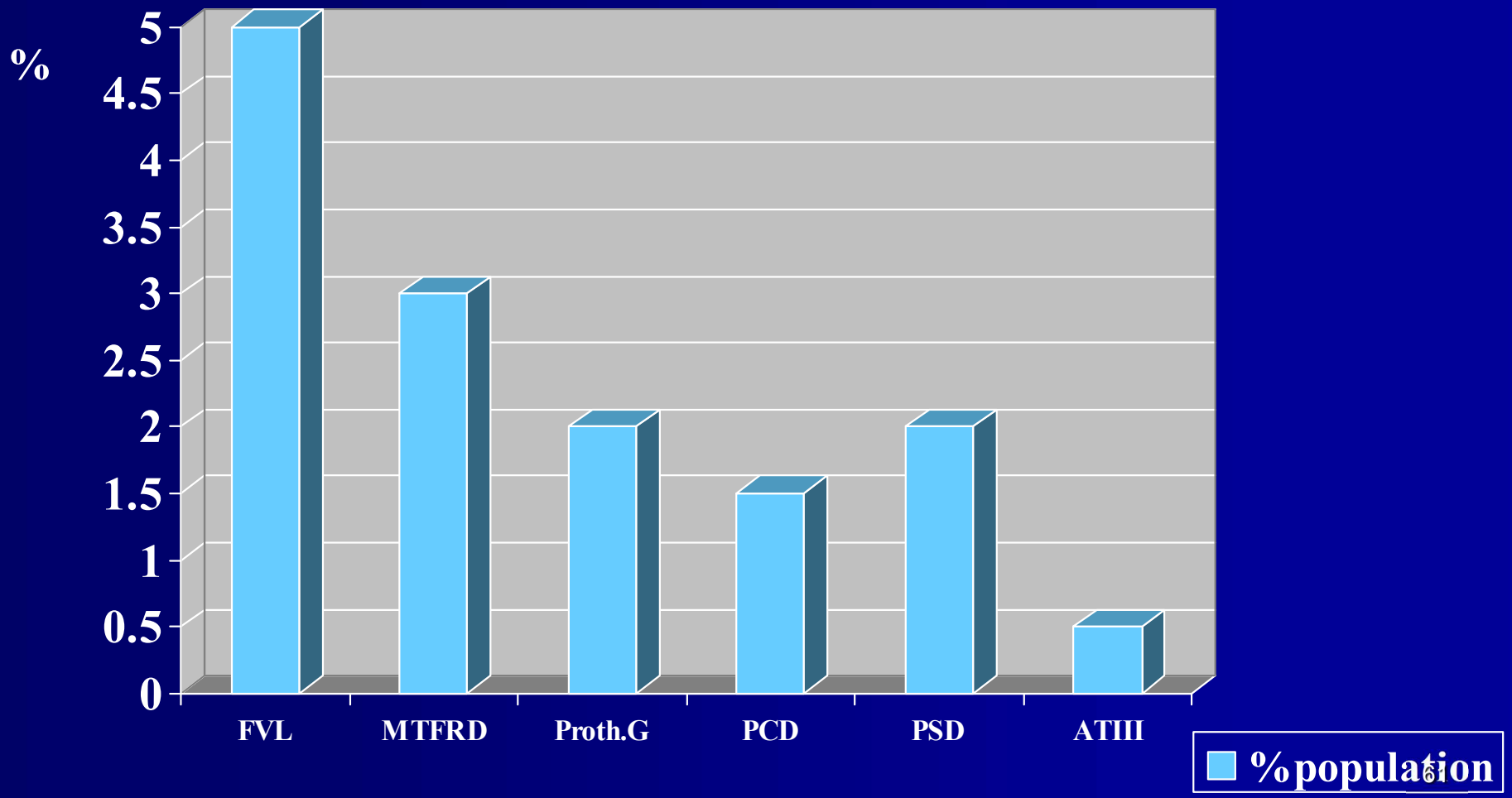

Thrombophilia and fetal loss

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Recent case-control studies and meta analyses attempted to quantify the risks associated with different thrombophilic defects and adverse clinical events in pregnancy,

Thrombophilia and fetal loss

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A meta analysis published in LANCET 15 march 2003 included 31 studies published between 1975 and 2002 (by Medline search).

Relative risk is quantified by odd ratio

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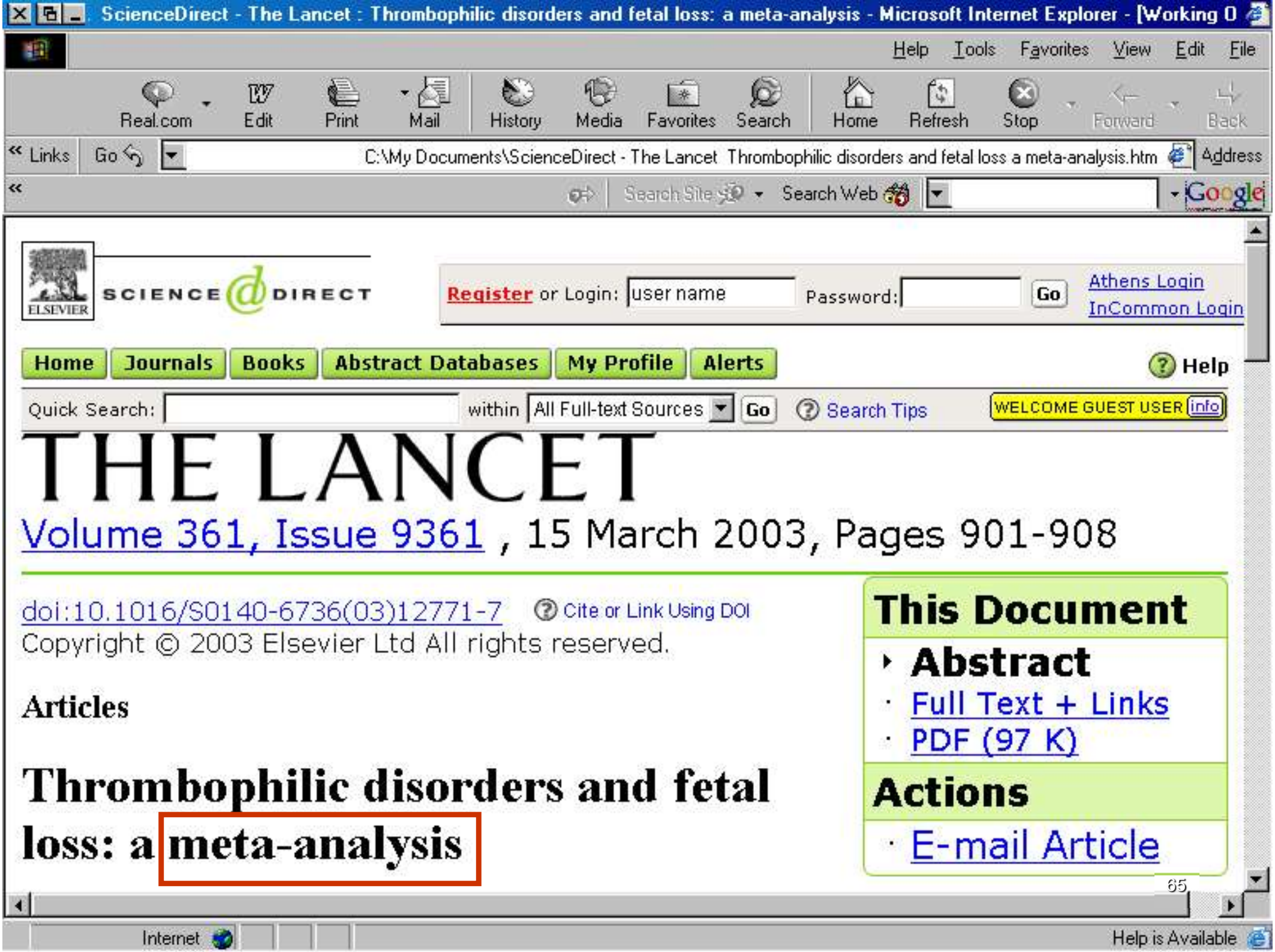
- If OR=1, the exposure is not related to the disease (no association)
- If OR>1, the exposure is positively related to the disease (possible causal)

If OR<1, the exposure is negatively related to the disease (possible protective)


ScienceDirect - The Lancet : Thrombophilic disorders and fetal loss: a meta-analysis - Microsoft Internet Explorer - [Working O
Help Tools Favorites View Edit File
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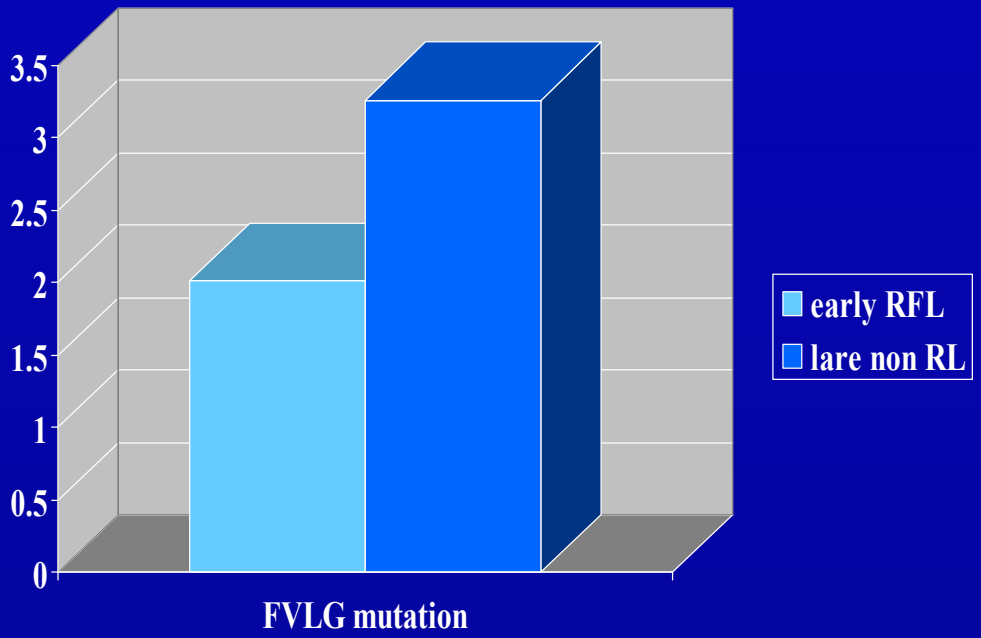
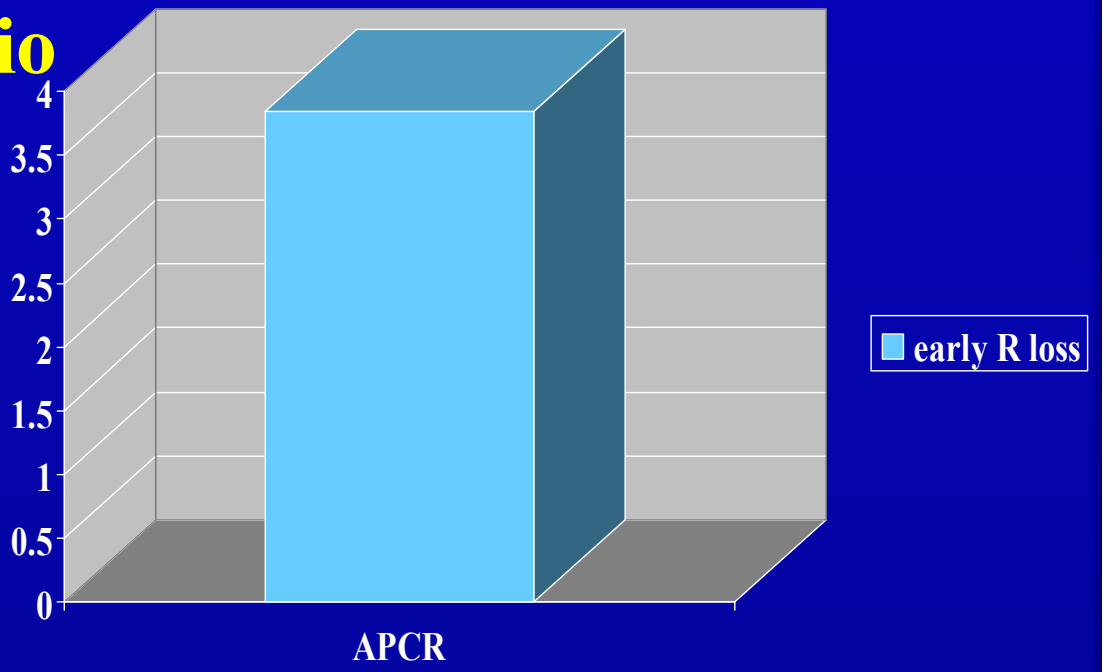
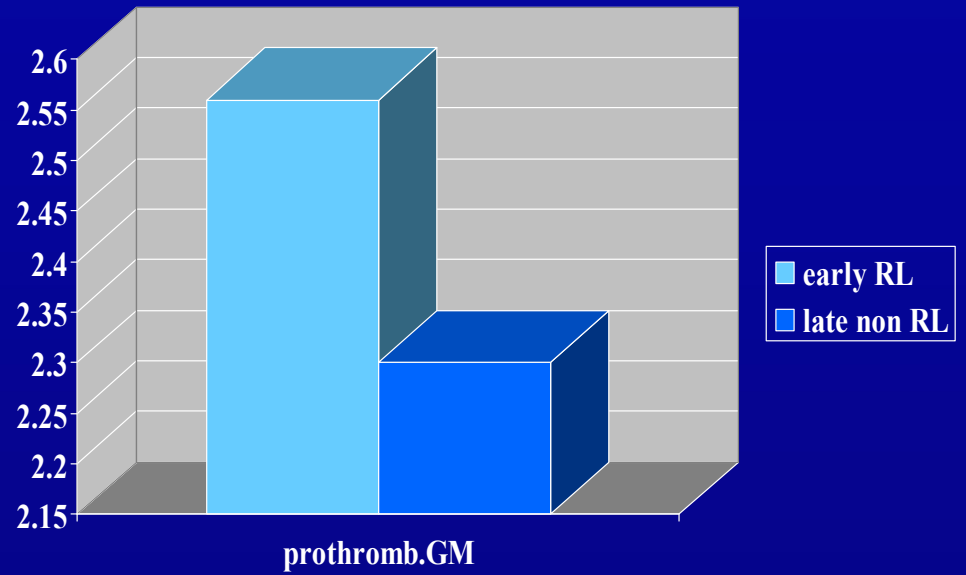
Thrombophilia and fetal loss

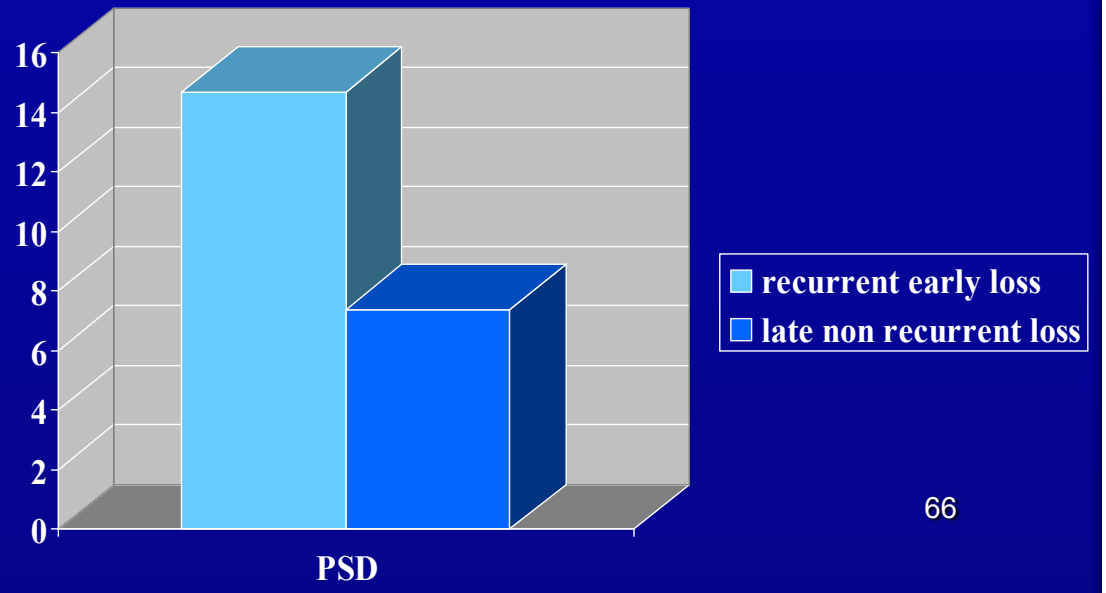
Odd ratio



Odd ratio







Thrombophilia and fetal loss

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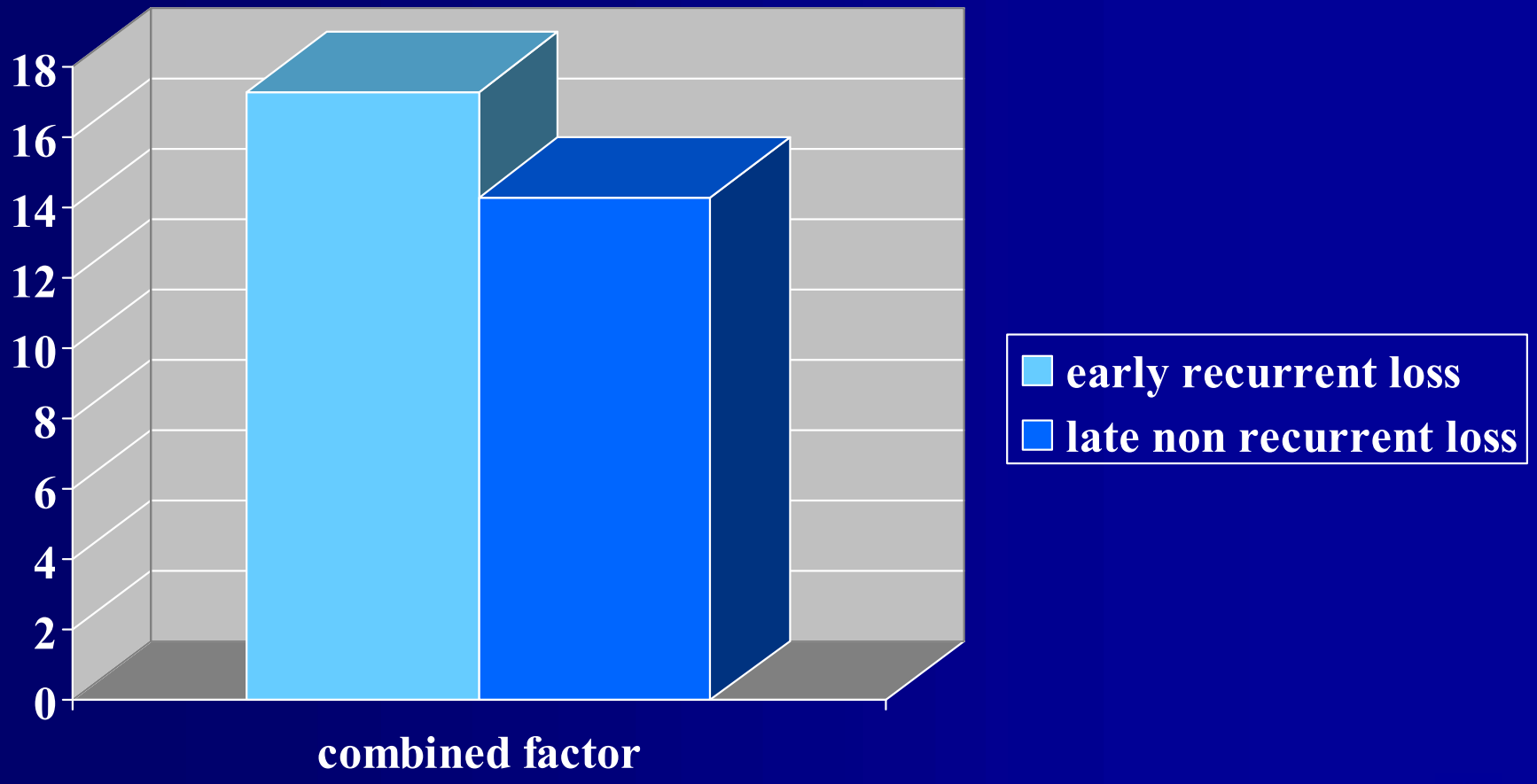
Odd ratio



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Assessment of maternal risk and prediction of risk factors is the gate for prevention of adverse pregnancy outcomes.

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Management

Antiphospholipid Antibodies

- In women with a history of recurrent miscarriage and aPL, future live birth rate is significantly improved when a combination therapy of **aspirin** plus **heparin** is prescribed.

(RCOG-A)

Antiphospholipid Antibodies

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Currently there is no reliable evidence to show that steroids improve the live birth rate of women with recurrent miscarriage associated with aPL.

their use may provoke significant maternal and fetal morbidity.

(RCOG-C)

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Antiphospholipid Antibodies

Pregnancies associated with aPL treated with aspirin and heparin remain at high risk of complications during all three trimesters.

(RCOG-B)

Thrombophilias

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The combination of **aspirin and heparin** is effective in recurrent fetal loss in **APS** and could be considered for women with **inherited thrombophilias** and history of severe preeclampsia, IUGR, abruptio placentae or fetal loss, although no controlled studies on the subject are currently available

***Cochrane Review* 2003**

Anti-thyroid Antibodies

- **Routine screening for thyroid antibodies in women with recurrent miscarriage is not recommended.**

(RCOG-B)

Alloimmune factors

- Immunotherapy, including paternal cell immunisation, third-party donor, trophoblast membranes and intravenous immunoglobulin (IVIG), in women with previous unexplained recurrent miscarriage does not improve the live birth rate

(RCOG-B)

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- Anti Cardiolipin (IgM)
- Anti Cardiolipin (IgG)
- Lupus Anti Coagulant (LAC)
- Anti Thyroid Microsomal (Anti TPO)
- Anti Thyroglobulin (Anti TG)
- Anti Toxoplasmosis (Total)
- Anti Phospohlipid (IgM)
- Anti Phospholipid (IgG)
- β2 (Glycoprotein)
- Anti Cardiolipin (Total)
- Protein S
- Protein C
- Anti Thrombin III
- Homocysteine (F)
- Protein C Resistance
- F. V Leiden Mutations
- MTHFR Mutation
- Prothrombin Mutation
- PT
- PTT

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N. ABO HASSAN	Androlgist
D. GHRAWI	Executive Secretary
N. OLABI	Presentation Design
F. HAMAD	Administration Manager
A. ALKHATEB	M.D Micro Biologist
R. ALKHATEB	MD. Gyn. Obs. Ph. D.

Thank You