LYBIA 2010

RECURRENT ABORTION: ETIOLOGY AND PREVENTION

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Outline

- Statistics

- Epidemiology

- Etiology
  - -Genetic factors
  - -Anatomic causes
  - -Endocrin factors
  - -Infectious causes
  - -Immunologic problems
  - -Thrombophilias

- Counseling on RPL

Recurrent Pregnancy Loss (RPL)

Abortus: Sporadic – Recurrent

RPL: <20 week and three consecutive losses

RECURRENT MISCARRIAGE

• 3 OR MORE MISCARRIAGES	• 2 MISCARRIEGES IN WOMEN >35 yr OR WITH SUBFERTILITY

INCIDENCE =0.3-0.5% OF PREGNANCY

WILCUXA, N Engl J Med 1998

Most published studies
on management contain
important confounding variables
which either weaken or totally
invalidate their conclusions.

Among the defects are:
inconsistent definitions (common);
different investigative protocols (universal);
lack of normal controls (difficult to achieve);
and time over which the study population was gathered (inevitable).
There is also considerable psychological pressure to find a “cause” so that “something can be done about it”.

Premises

Spectrum of pregnancy loss

Preclinical loss

- Developmental failure – fertilized ovum does not divide
- Preimplantation – blastocyst does not implant
- Preclinical – blastocyst lost with next menstruation

Clinical loss

- Embryonic – loss before the 9 gestational wks
- Fetal – loss at or after 9 gestational wks
- Miscarriage (abortion) – loss before 20 gestational wks
- Stillbirth – loss after 20 gestational wks

Frequency of Pregnancy Losses Throughout Pregnancy

		Loss Rate
Preimplantation Embryo		~ 50%
Implanted Embryo:		30%
Preclinical	~ 20%	
Clinical	10 - 12%	
Clinical Pregnancy:		10 - 12%
Before 8 weeks	7 - 8%	
8 - 16 weeks	2%	
> 16 weeks	1%	

Likelihood Repeated Pregnancy Loss*

	Prior Abortions	Risk
Prior Liveborns	0	5 - 10%
	1	20 - 25%
	2	25%
	3	30%
	4	30 - 35%
No Prior Liveborns	3	40%

** Maternal-age dependent*

Simpson, 2002

1) Statistics

- 50% of all pregnancies end in SAB since 2-4 wk pregnancies will often go unnoticed.
- 30% lost between implantation and the 6th week.
- 70% of first trimester losses due to chromosomal abnormalities.

1) Statistics

- Rate of loss increases with age and is as great as 75% over age 40.

- Once FHT are seen on USG rate of loss in normal women 3-5%.

- With RPL-once FHT seen loss rate is 4-5 times greater (22.7%).

2) Epidemiology

- Sporadic %15
- 2 consecutive ab. %5
- **3 consecutive ab. %1**

of all pregnancies…

- Sporadic %15
- 2 consecutive %2.3
- 3 consecutive %0.34

by chance…

ACOG 2002

- Previous pregnancy affects the current pregnancy…
- 2 abortus %24
- 3 abortus %50
- 4 abortus %60
- 5 abortus %73

* * *Risk of abortus after 3 previous ab. similar to seen after 2 ab.'s. Therefore, 2 ab.'s warrants a RPL workup.

2) Epidemiology

- Maternal Age
  - 20-24 %9 sporadic
  - 45Y → %75
- Smoking
  - OR:1.8
- Alcohol
  - OR:4.84
- Caffeine ?
  - OR:2.21
- Environmental factors

  -Anesthetic gases

  -ETOH

  -Hg

  -Tetrachloroethylene (used in dry cleaning)

3) Etiology

Even an extensive workup will fail to find a recognizable cause in up to half of cases

Etiologic mechanisms historically presumed for recurrent pregnancy loss

Genetic Factors
1. Chromosomal
2. Single gene disorders
3. Multifactorial

Anatomic Factors
1. Congenital
2. Acquired

Endocrine Factors
1. Luteal phase insufficiency
2. Androgen disorders, including luteinizing hormone disorders
3. Prolactin disorders
4. Diabetes mellitus

Infectious Factors
1. Bacteria
2. Viruses
3. Parasites
4. Zoonotics
5. Fungal

Immunologic Factors
1. Autoimmune
   a. Antiphospholipid antibodies
   b. Other antibodies
2. Alloimmune
   a. Th 1 immunity (immunodystrophism)
   b. Blocking antibody deficiency

Miscellaneous Factors
1. Environmental
2. Stress
3. Placental abnormalities
4. Medical illness
5. Male factors
6. Dyssynchronous fertilization
7. Coitus
8. Exercise

CAUSES OF RECURRENT MISCARRIAGE

GENETIC	2-5%
ENDOCRINE	10-17%
ANATOMIC	10%
INFECTIOUS	5%
IMMUNOLOGICAL	UP TO 30%
OTHERS (TROMBOPHILIA)	13%
UNEXPLAINED	30%

It has been assumed that a large proportion of idiopathic RSA is genetic in origin. To date, however, the standard genetic evaluation consists solely of parental and abortus karyotyping. This identifies parental defects, such as balanced translocations, than can cause RSA, and it ascertains fetal aneuploidy, a common cause of spontaneous abortion that intrinsically has little recurrence risk.

MECHANISMS OF EMBRYONIC REJECTION

Four potential sites at which a lethal problem can develop

Site 1: fetus itself (paternal MHC antigens)

Site 2: fetal placental – circulations (maternal antipaternal endothelial cell antibodies)

Site 3: fetal trophoblast (NK cells, like activated Th1 cells,
could release cytokines dystrophic to trophoblast)

Site 4: maternal vascular target (hemorrhagic necrosis
at the trophoblast-maternal uterine tissue interface
in the CBA x DBA/2 system)

3) Etiology

Genetic factors

Genetic Factors

- 70% 1st trimester losses, 30% of 2nd, and 3% of 3rd trimester losses due to chromosomal abnormalities.

- If abnormal karyotype with SAB then 50% chance next fetus is normal.

- Most abnormalities are due to translocation and nondisjunction.

- Most common chromosomal abnormality is autosomal trisomy (most commonly Tr 16).

- 2nd most common 45X followed by polyploidies.

Translocation carriers

		Abortus and cytogenetic analysis		
	Abort. Numb.	Diploid & balanced structural disorders	Aneuploid & Poliploidy	Unbalanced structural disorders
RPL (1)	420	% 54	% 45	% 2
General Population (2)	7182	% 52	% 46	% 2
≥2 First trim. Abort. & Translocation carriers (3)	33	% 28	% 3	**% 39**
≥2 First trim. Abort. & resiprocal translocation carriers (4)	25	% 44	% 20	**% 36**

(1) Stephenson 2002;
(2) Jacobs & Hassold 1987
(3) Siera 2003
(4) Sugiura-Ogasawara 2004

TRISOMY IN SPONTANEOUS ABORTIONS

Trisomy	N		Trisomy	N	
1	0		7	46	4.5%
2	53	5.2%	8	37	3.6
3	8	<1.0	9	32	3.2
4	24	2.4	10	20	2.0
5	1	<1.0	11	2	<1.0
6	3	<1.0	12	9	<1.0

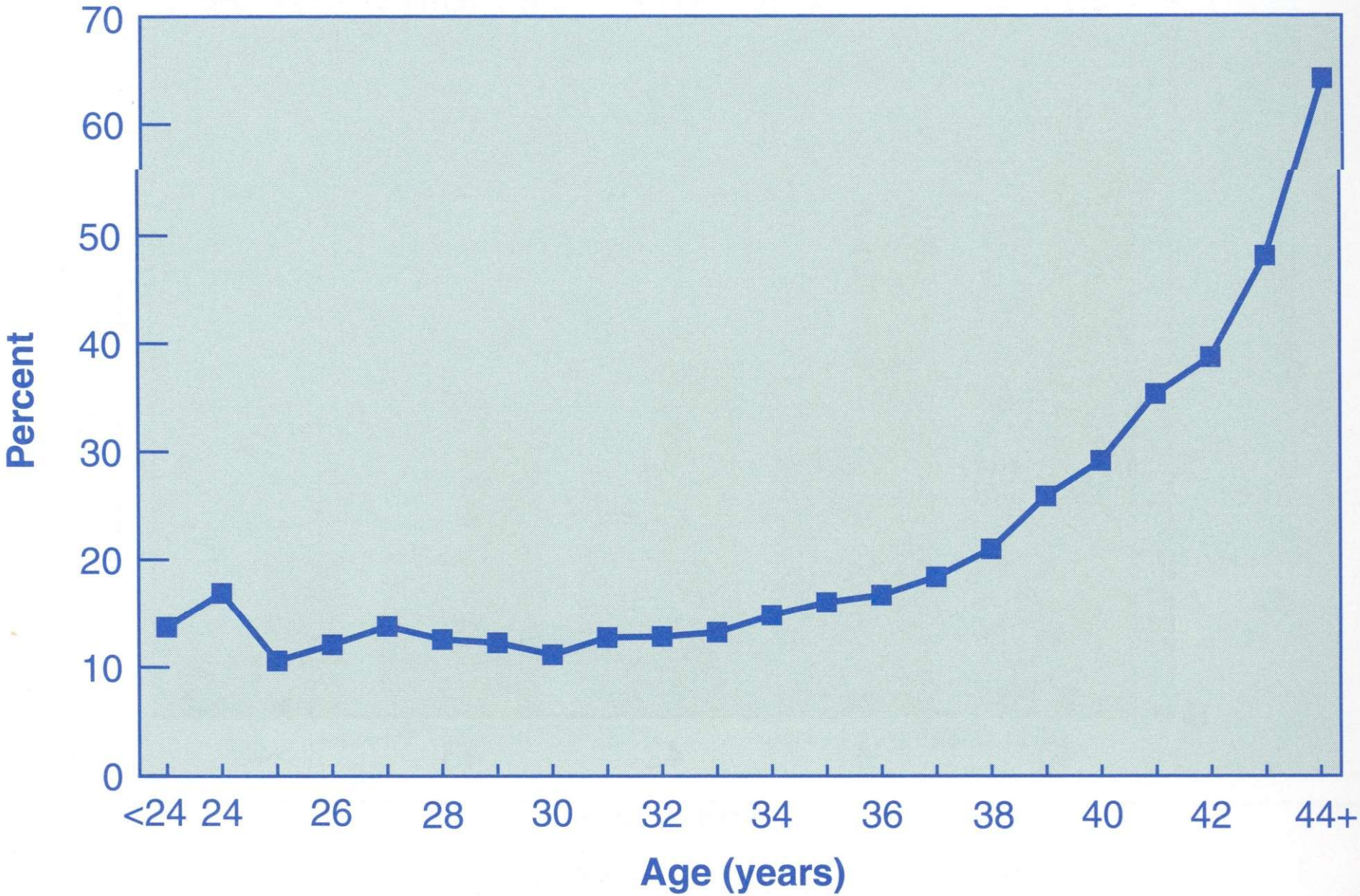
TRISOMY IN SPONTANEOUS ABORTIONS

Trisomy	N		Trisomy	N	
13	59	5.8%	18	51	5.0
14	51	5.0	19	1	<1.0
15	73	7.2	20	27	2.7
16	320	31.6	21	85	8.4
17	7	<1.0	22	103	10.2

AXIOMS

- Fetal loss is usually genetic in origin.
- The earlier in the gestation the loss, the higher the likelihood of genetic etiology.
- The older maternal age in the first trimester, the higher risk of having chromosomal abnormality.

MISCARRIAGE IN ASSISTED REPRODUCTION



Genetic Factors

- All pts with RPL should be offered karyotype testing of parents. %3-6 balanced translocation (Reciprocal, Robertsonian etc.)

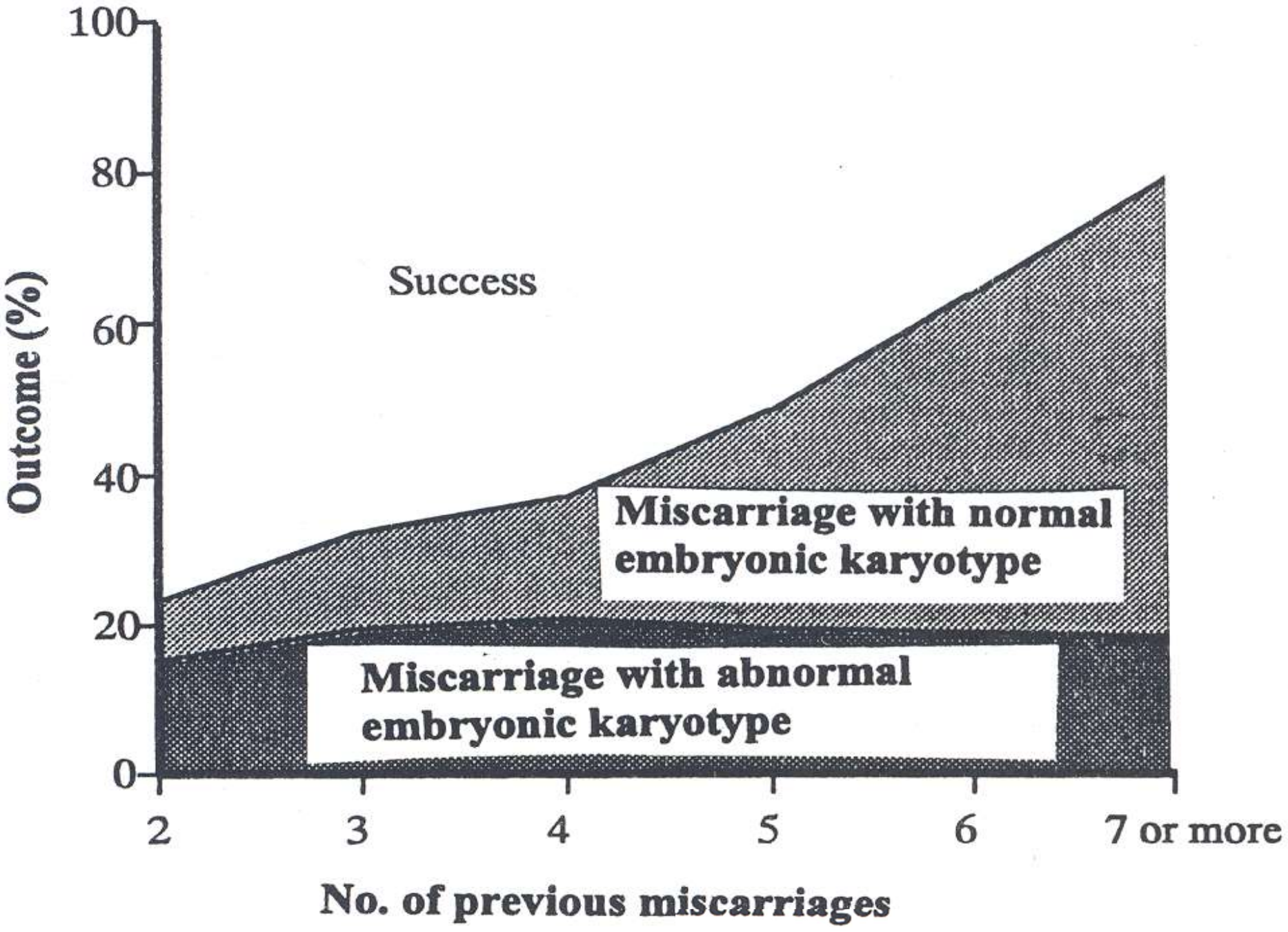
- Karyotyping of POC's is controversial and definite recommendations for routine karyotyping has not been proven. Benefits are, if aneuploid a maternal cause of pregnancy loss is excluded and results may provide comfort to the pt.

OUTCOME BY KARYOTYPE OF ABORTUS

Karyotype of Index Abortus	Live Birth
Normal	82.3%
Abnormal	88.0%

Karyotyping of spontaneous losses in the first trimester beginning **with the patient's second loss** provides clinically important etiologic information and decreases the number of evaluations necessary for recurrent pregnancy loss.

Hogge et al., 2003



Ogasawara et al., 2000

Retrospective analysis.

A total of **1,309** women with a history of 2-20 consecutive first-trimester abortions.

Patients with a previous normal embryonic karyotype aborted more frequently than those with an abnormal karyotype.
The frequency of normal embryonic karyotypes significantly increases with the number of previous abortions, and a normal karyotype in a previous pregnancy is a predictor of subsequent miscarriage.

AXIOMS

- **Even if karyotype normal, genetic etiology likely still paramount [mutant gene(s)].**

- **Evaluation can never exclude genetic etiology because not all mendelian and polygenetic causes can be tested.**

COROLLARIES

- Prognosis generally good (60-70% liveborn) because genetic causes rarely obligatorily recurrent.

- Because causation usually genetic, treatment usually futile and perhaps unwise.

Clinical Options for Treatment of Recurrent Spontaneous Abortion

Diagnosis	Evidence of value	Value being tested
•Genetic		
1.Genetic counseling		X
2.Donor gametes		X
•Anatomic/uterine		
1.Septum transection		X
2.Cervical cerclage		X
3.Myomectomy		X
4.Polypectomy		X
5.Lysis of adhesions		X
•Endocrinology		
1.Progesterone	X	X
2.Clomiphene citrate		X
3.HMG		X
4.Thyroid replacement	X	X

Clinical Options for Treatment of Recurrent Spontaneous Abortion

Diagnosis	Evidence of value	Value being tested
•Microbiology		
1.Antibiotics		X
•Immunologic		
1.Aspirin		X
2.Aspirin + heparin	X	
3.Aspirin + prednisone	X	
4.IVIg		X
5.Mononuclear cell immunization (paternal)	X[a]	
•Metabolic		
1.As indicated		X
•Iatrogenic		
1.Eliminated consumption or exposure		X

[a] This procedure has been shown to enhance live birth rates by 8-10%.

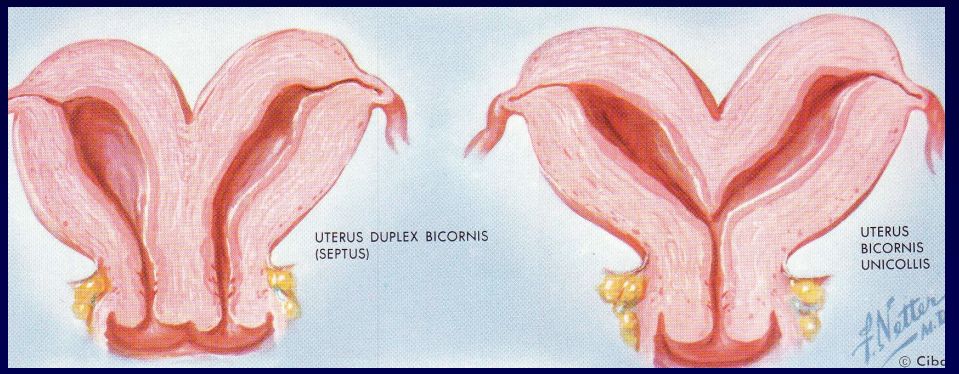
3) Etiology

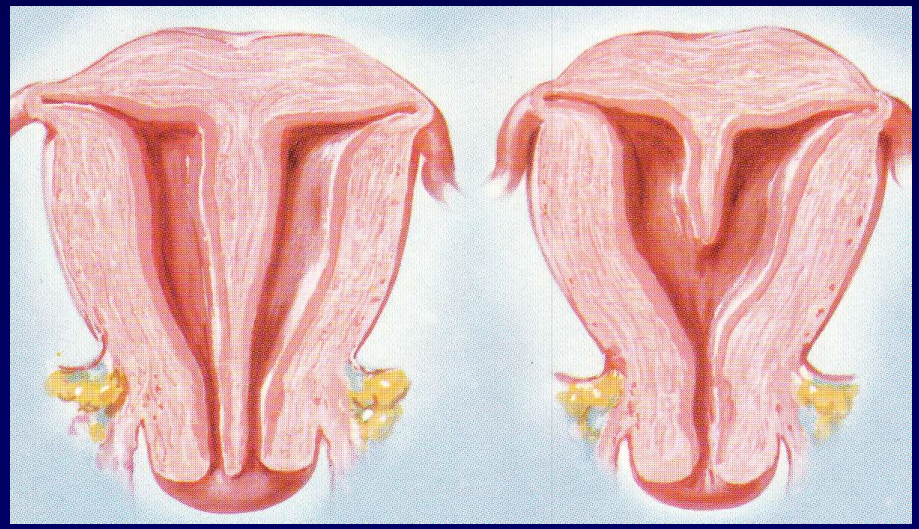
Anatomic causes

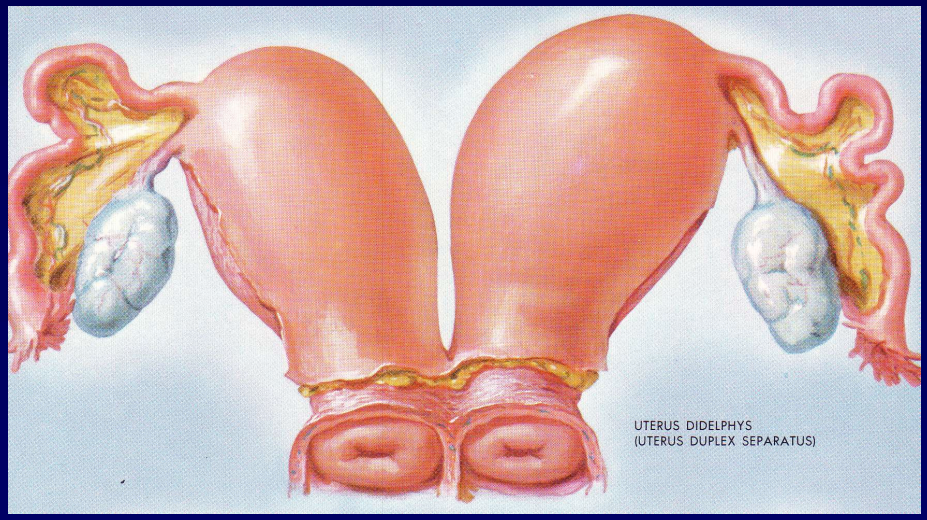
Anatomic causes

%10-20 of RPL cases

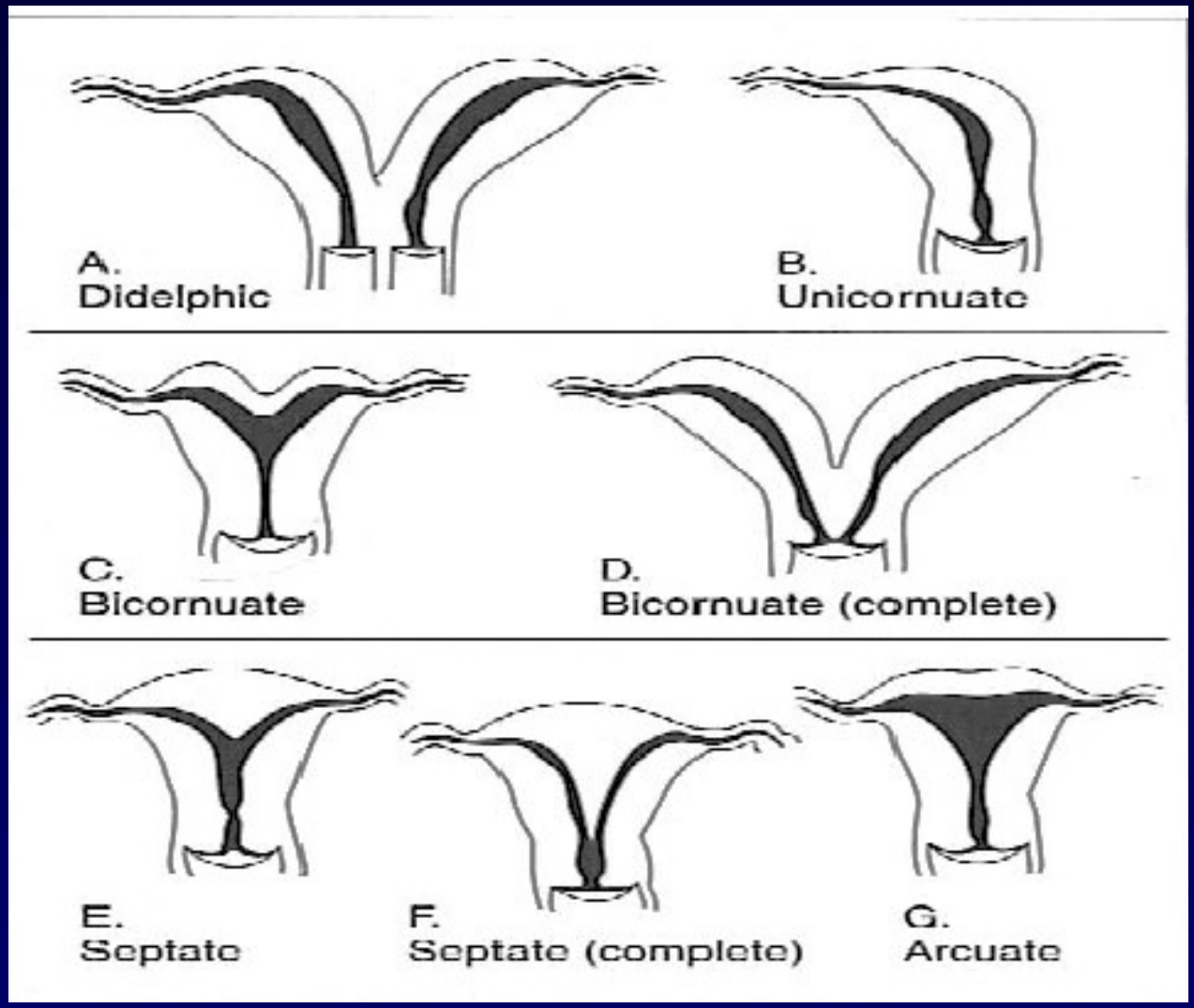
Probst 2000







Anatomic causes



Unicornuate, Bicornuate, Didelphic uterus do **NOT** usually cause RPL.

Anatomic causes
Uterine anomalies

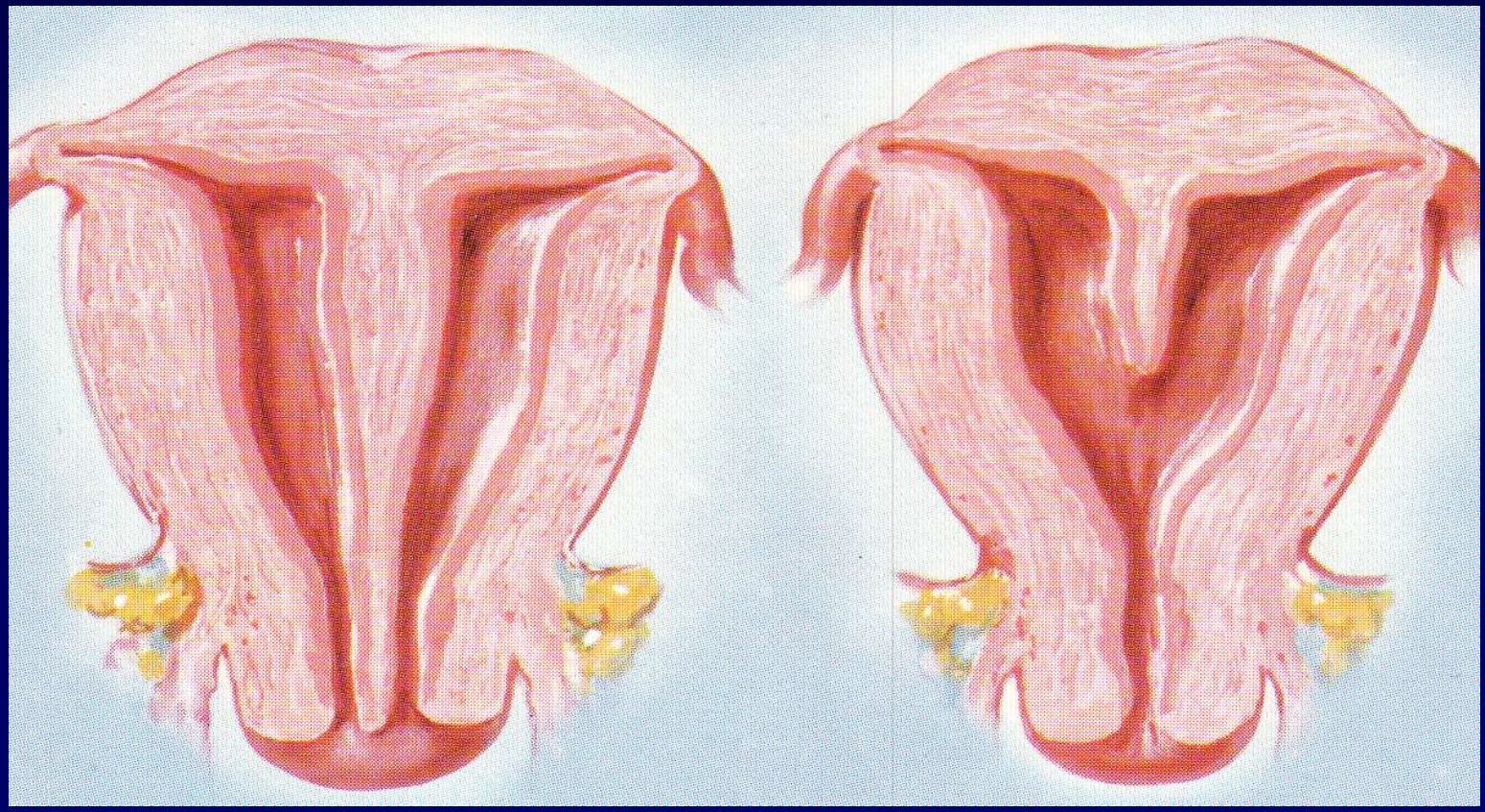
Uterine anomalies	Patient (n)	Abortus %	Preterm %	Term Labor %	Live Birth %
Unicornuate	151	**36.6**	16.2	44.6	54.2
Didelfis	114	32.2	28.3	36.2	55.9
Bicornuate	261	36	23	40.6	55.2
Septate	198	**44.3**	22.4	33.1	50.1
Arcuate	102	25.7	7.5	62.7	66

Grimbizis GF, Hum Reprod Update 2001

Anatomic causes

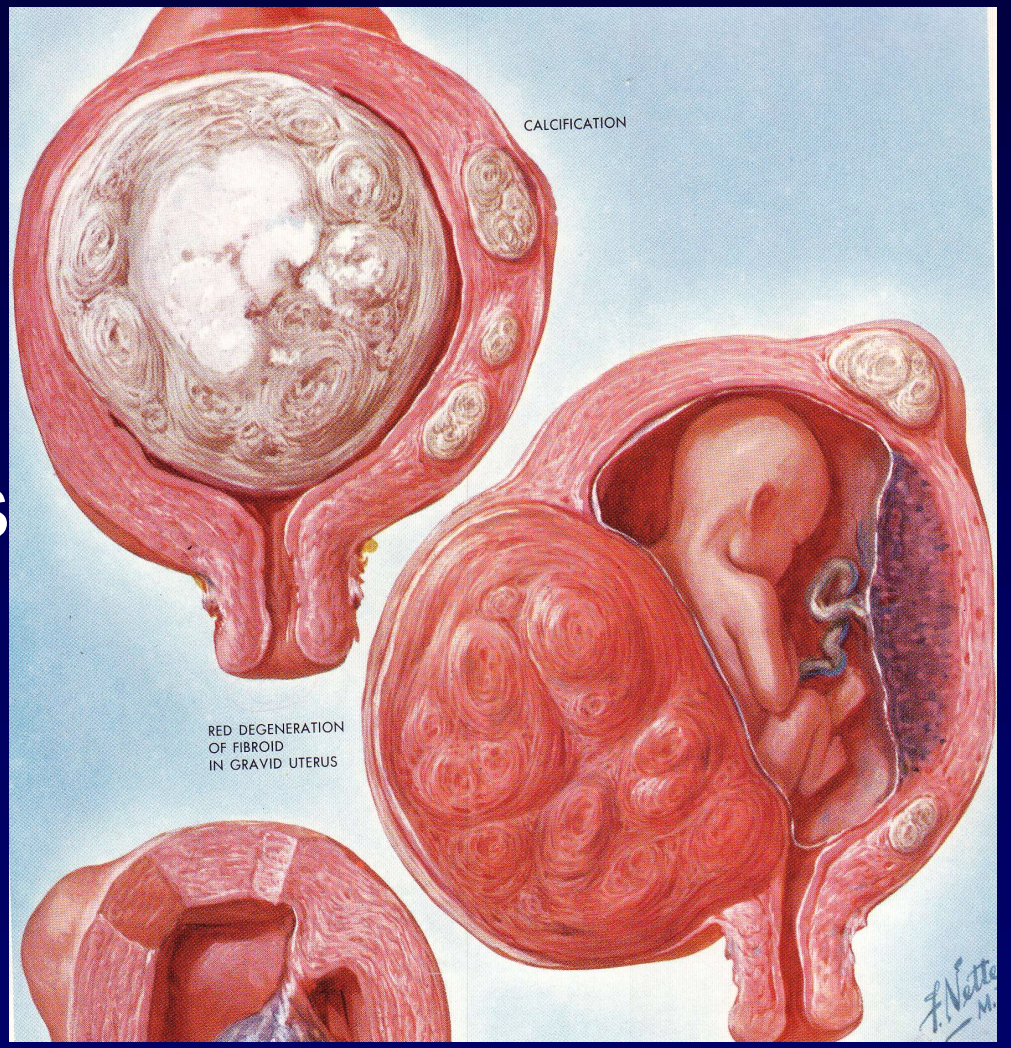
Septum, %60 PPL

Buttram, 1983



Anatomic causes

- Cervical insufficiency (most commonly seen 2nd trimester)
- Intrauterine adhesions
- Leiomyoma



Anatomic causes

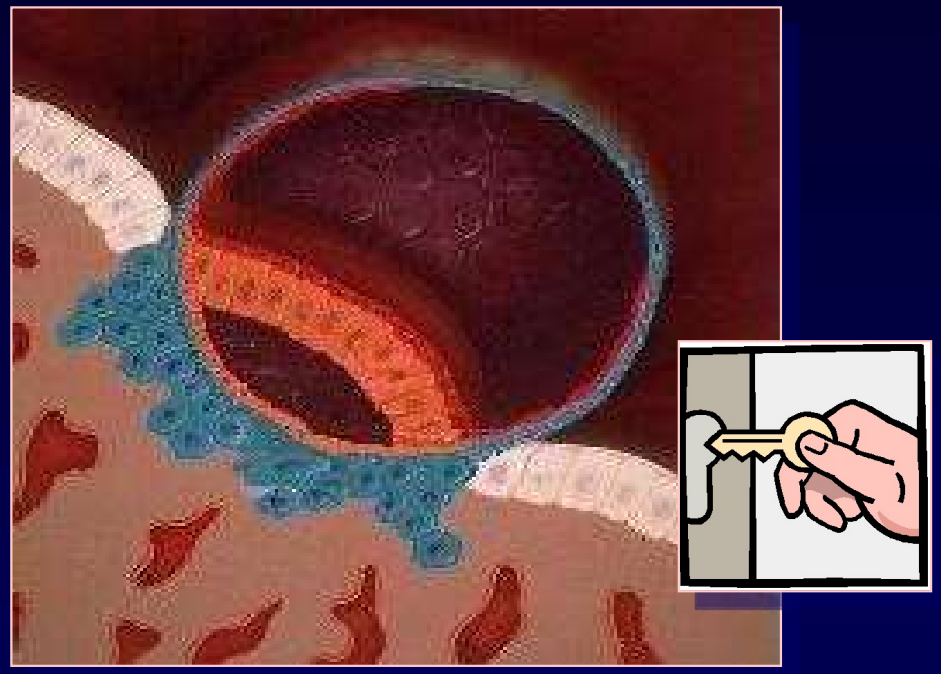
- **Probably not associated with early first trimester losses.**
- **More plausible explanation for late *(10-12 weeks)* first trimester or second trimester losses**

Surgical correction not usually recommended following early first trimester losses…

3) Etiology

Endocrine factors

IMPLANTATION



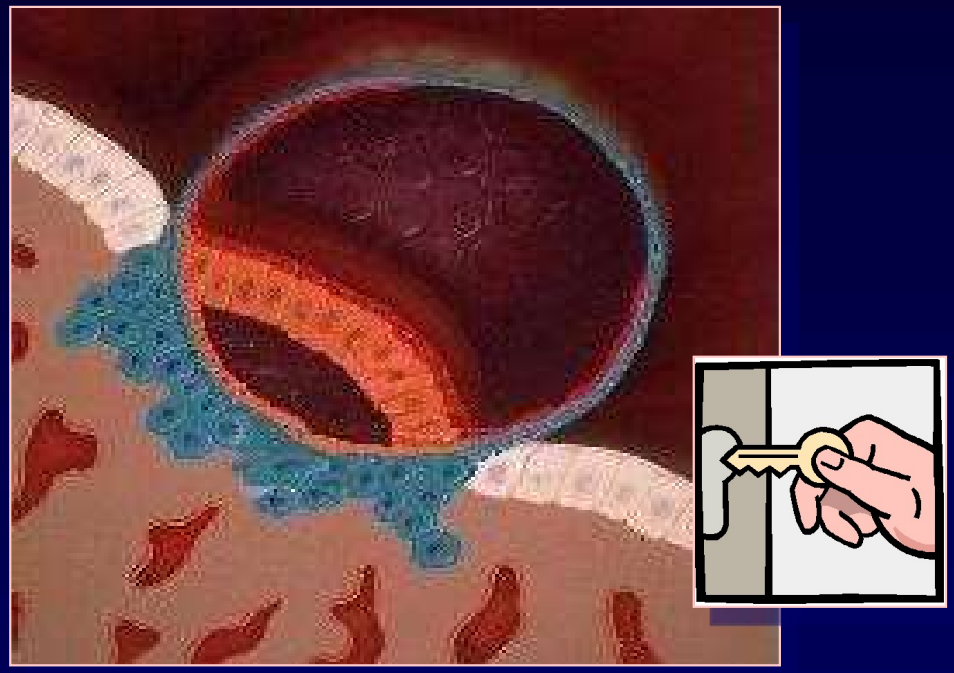
75% of ABORTION S

is due to a failure of implantation

23-60% ARS I trimester

Luteal Phase Defect

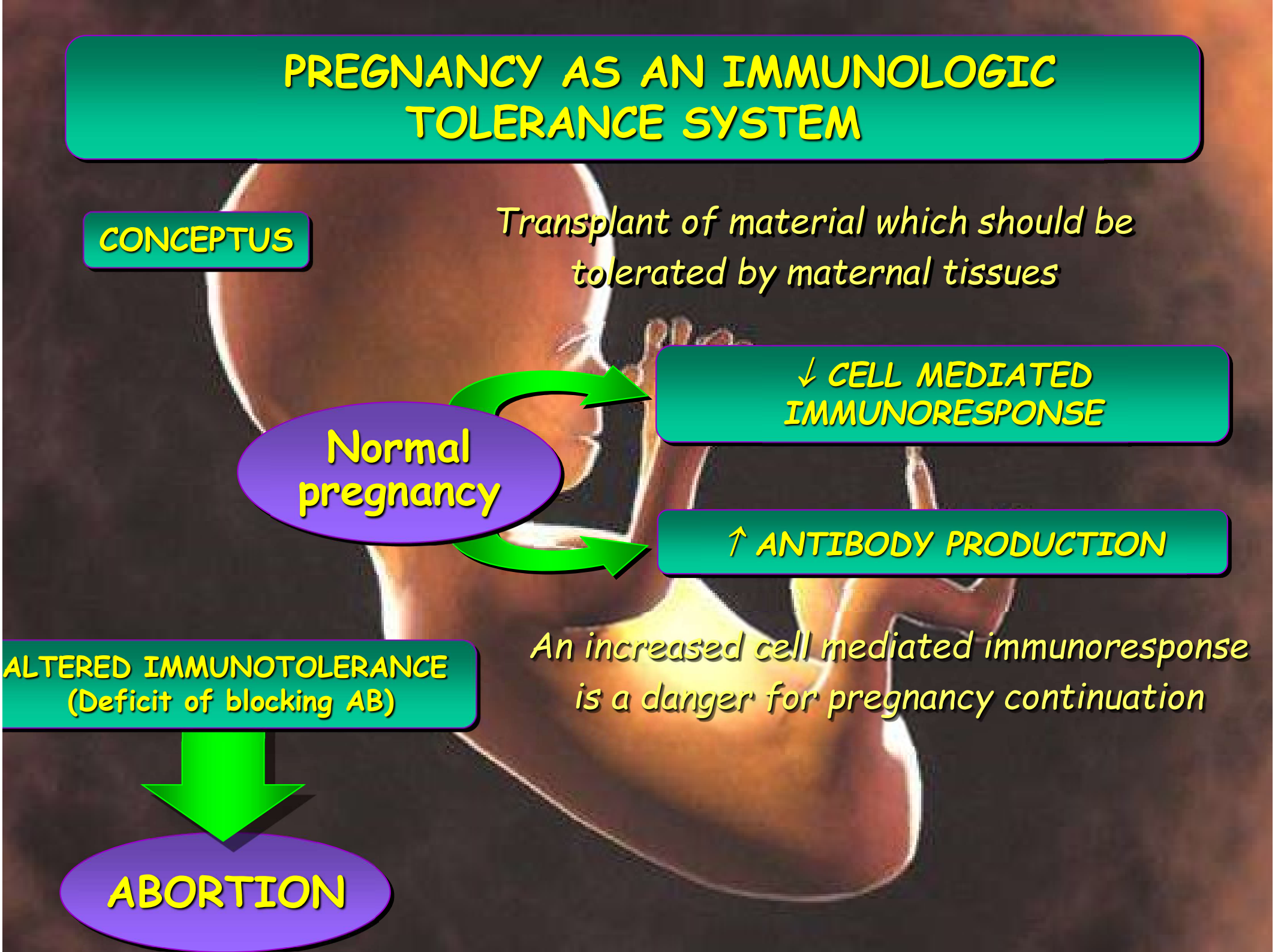
IMMUNE FACTORS FOR ARS



Immune response: key role in pregnancy, and in particular during implantation

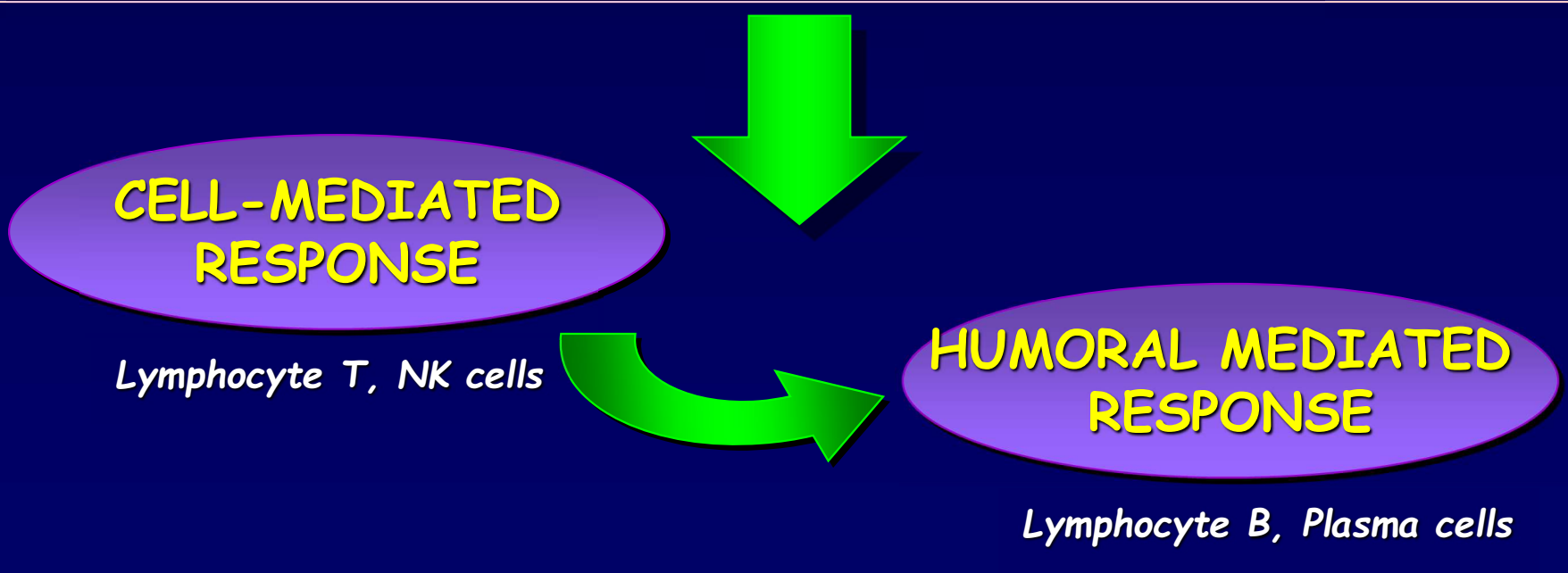
The alteration of the complex local immune network is responsible for implantation failure and AS

Autoimmune disorders: 15-20% ASR

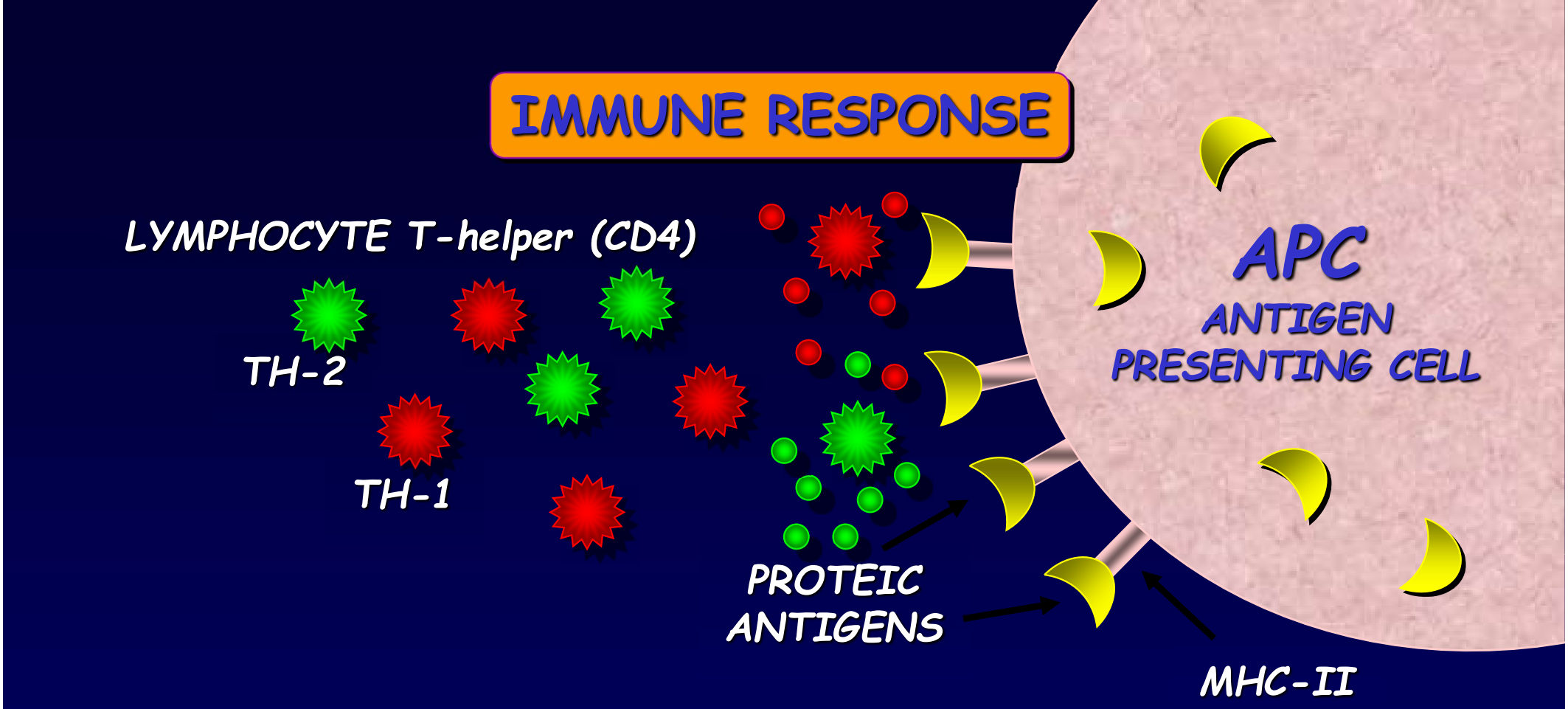

PREGNANCY AS AN IMMUNOLOGIC TOLERANCE SYSTEM
CONCEPTUS
Transplant of material which should be tolerated by maternal tissues
↓ CELL MEDIATED IMMUNORESPONSE
Normal pregnancy
↑ ANTIBODY PRODUCTION
An increased cell mediated immunoresponse is a danger for pregnancy continuation
ALTERED IMMUNOTOLERANCE (Deficit of blocking AB)
ABORTION

PROGESTERONE: IMMUNOLOGIC PROPERTIES

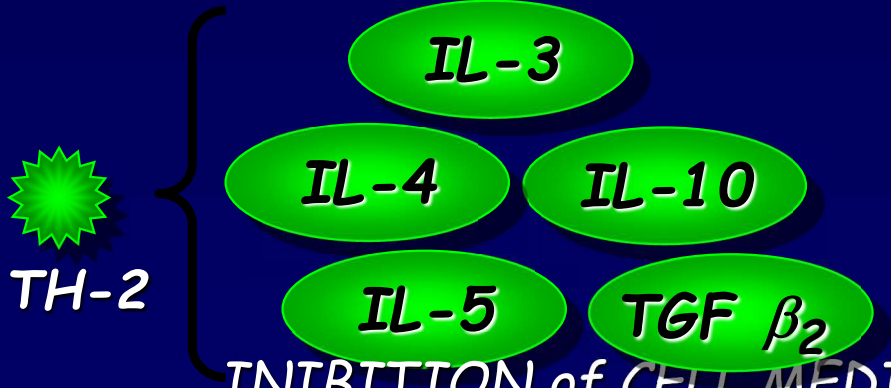
- ⇑ **CITOCHINE TH2** *(IL-3, IL-4, IL-10…)*
- ⇑ **PIBF** *production by TH2 Lymphocytes*
- *Direct inhibition of NK cells*
- ⇑ **LIF** production by Lymphocyte
- ⇑ **PP14** endometrial production
- ⇑ *T-Suppressor (CD4)/T-Cytotoxic (CD8) RATIO*



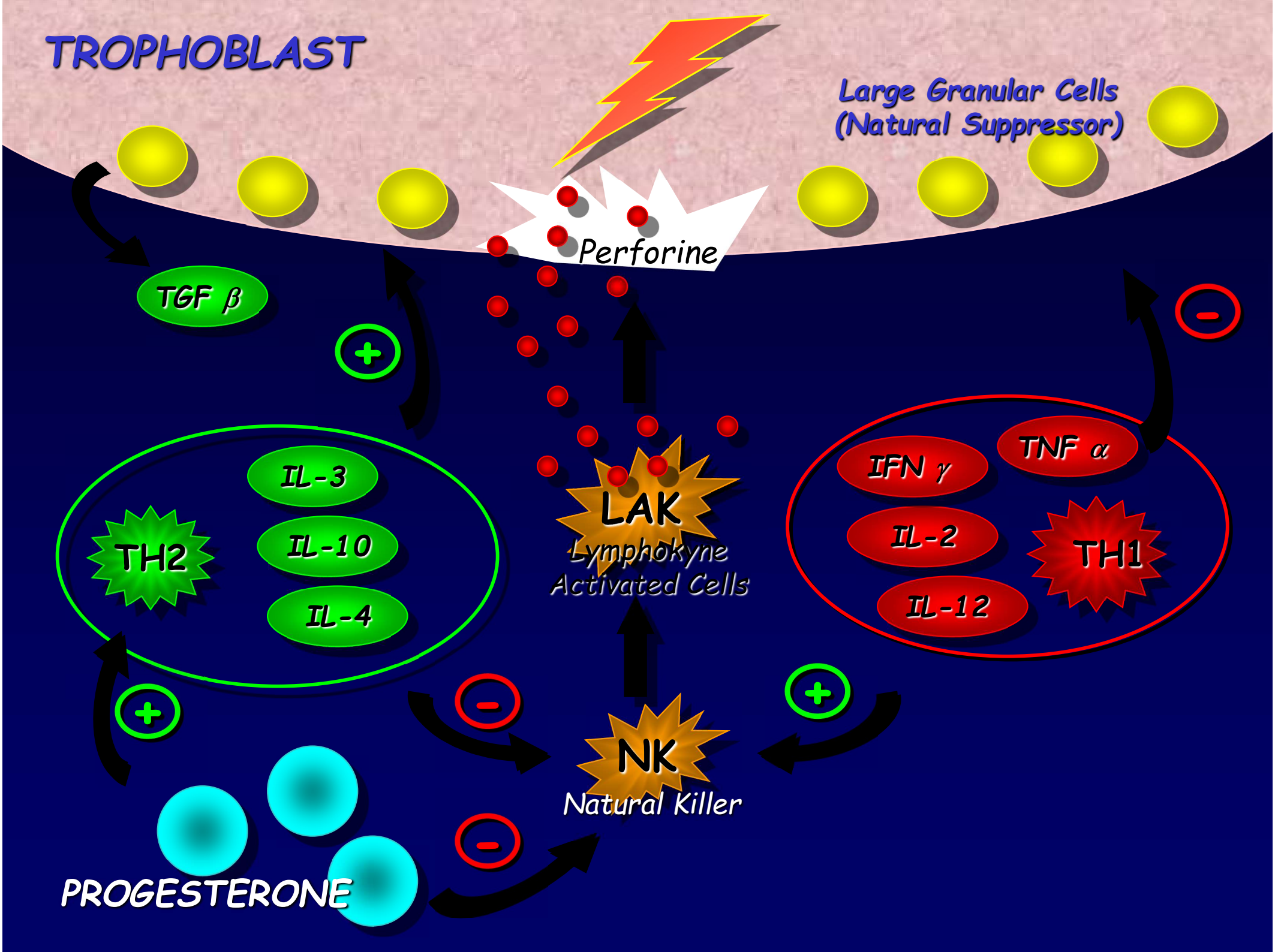
IMMUNE RESPONSE

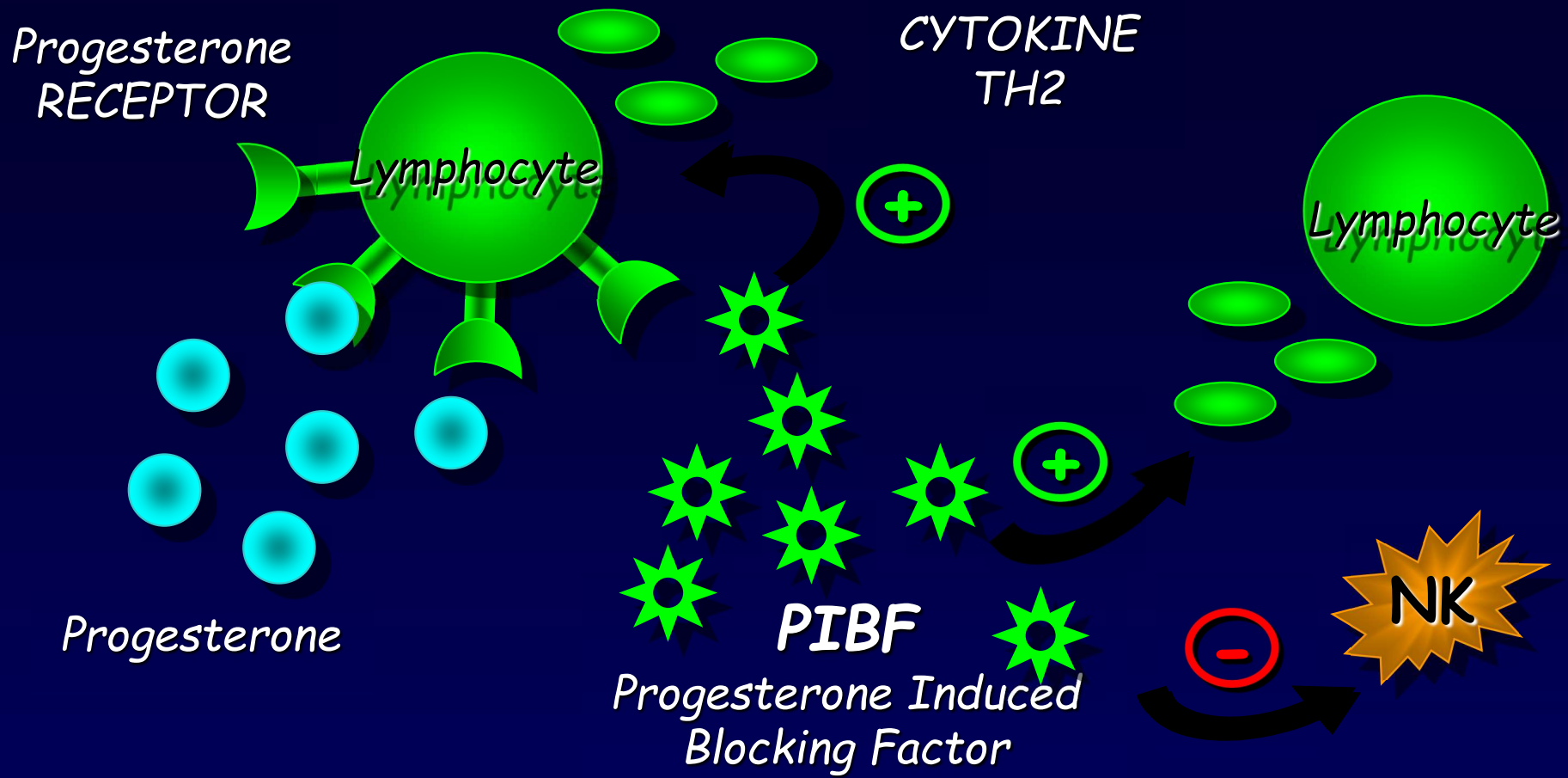


STIMULATION of
CELL MEDIATED RESPONSE



INIBITION of CELL MEDIATED
RESPONSE and
STIMULATION of HUMORAL
MEDIATED RESPONSE

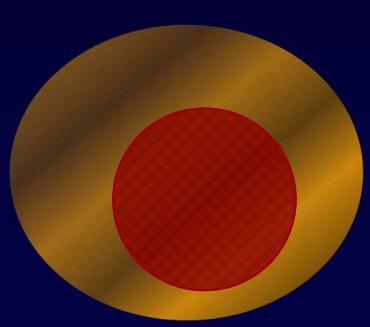

TROPHOBLAST
Large Granular Cells
(Natural Suppressor)
Perforine
TGF β
+
IL-3
TH2
IL-10
IL-4
LAK
Lymphokyne
Activated Cells
IFN γ
TNF α
IL-2
TH1
IL-12
−
+
−
+
NK
Natural Killer
−
PROGESTERONE



PIBF

- 34K Da protein
- Immunomodulator and antiabortive
- Downregulation of NK cells
- Arachidonic acid release inhibition (PGF2α)
- **ABSENT OR SIGNIFICANTLY LOW IN THREATENED ABORTION, RECURRENT ABORTION OR PRETERM DELIVERY**

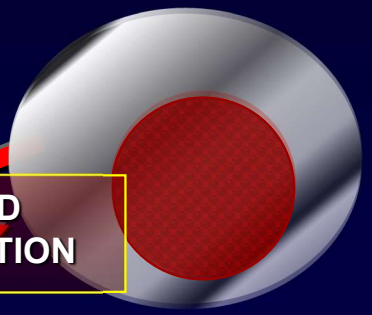
Balance between Th1 & Th 2 responses



TH1

IL - 10

INHIBITED PROLIFERATION

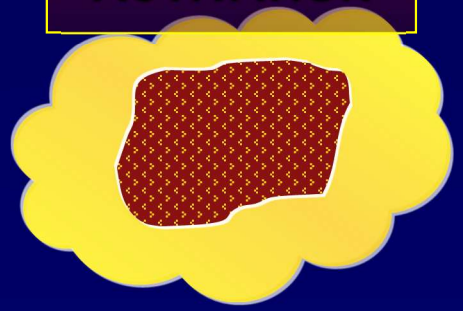


TH2

INHIBITED PRODUCTION

IFN Y

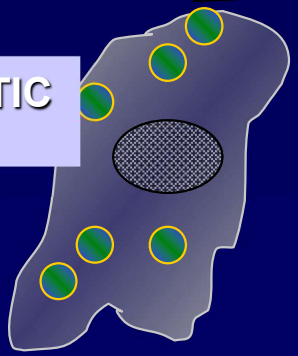
ACTIVATION



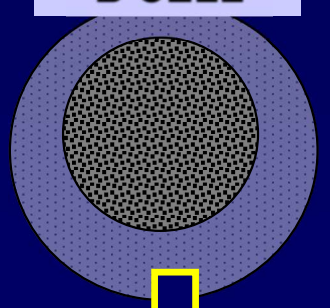
MACROPHAGE

IL – 4
IL - 5

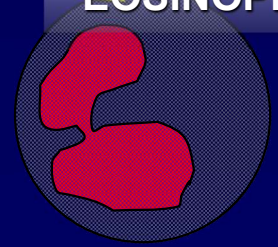
PLASMATIC CELL



B CELL



EOSINOPHYLE

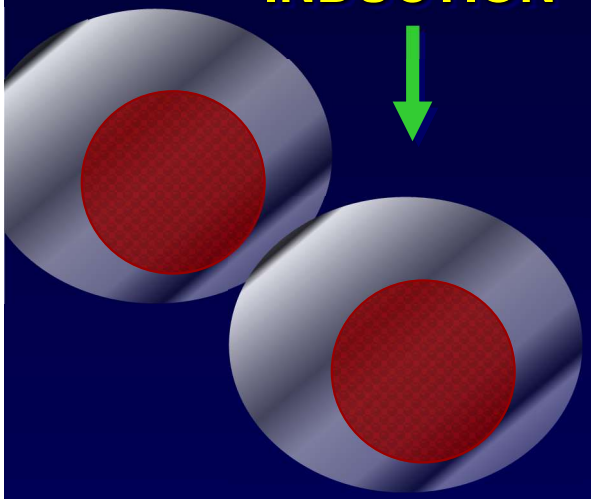


HUMORAL RESPONSE
IMMUNOGLOBULINS

Progesterone

Immunomodulation

FETAL ALLOANTIGENS
INDUCTION



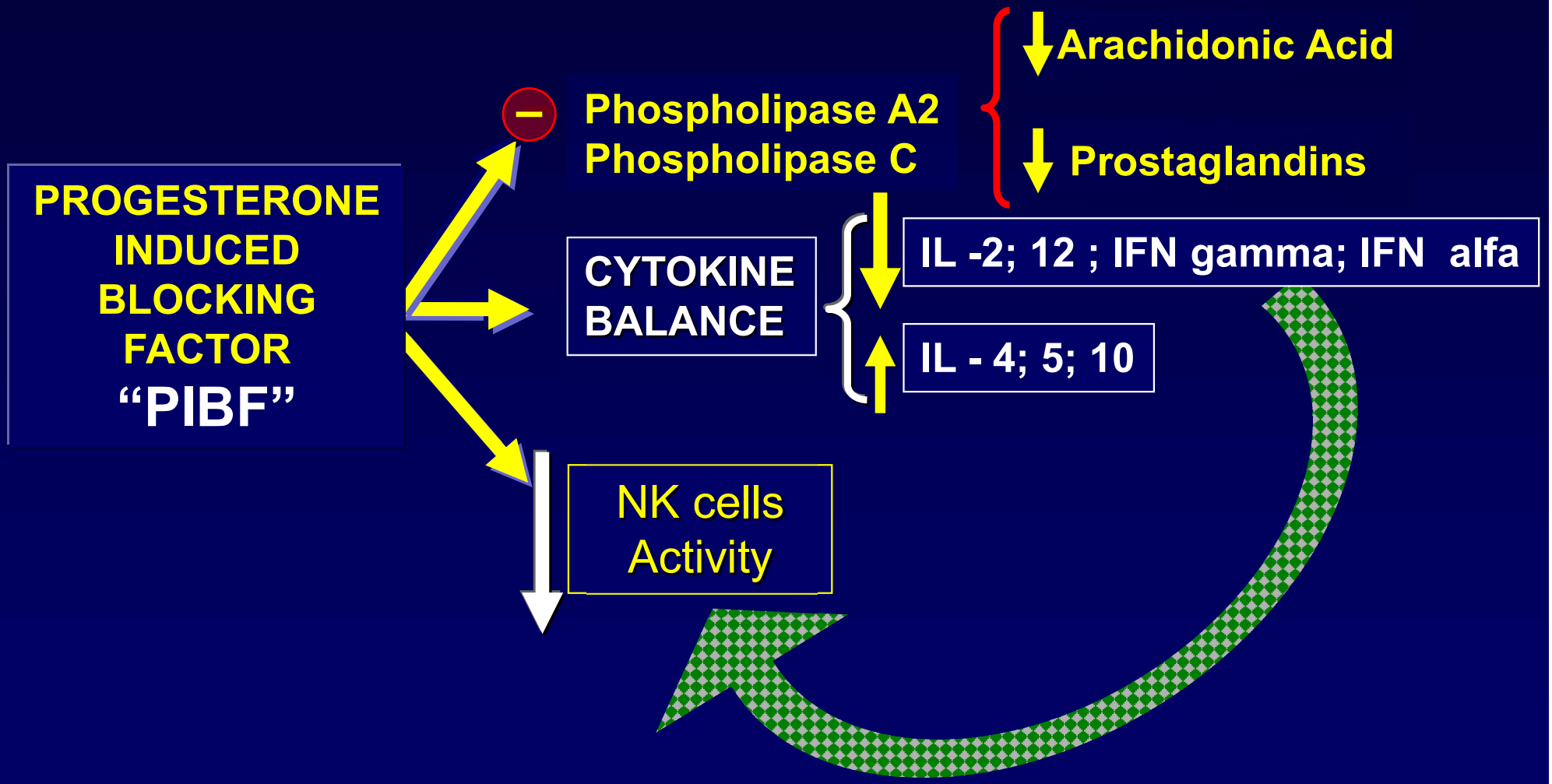
PROGESTERONE INDUCED BLOCKING FACTOR "PIBF"

LYMPHOCYTE ACTIVATION

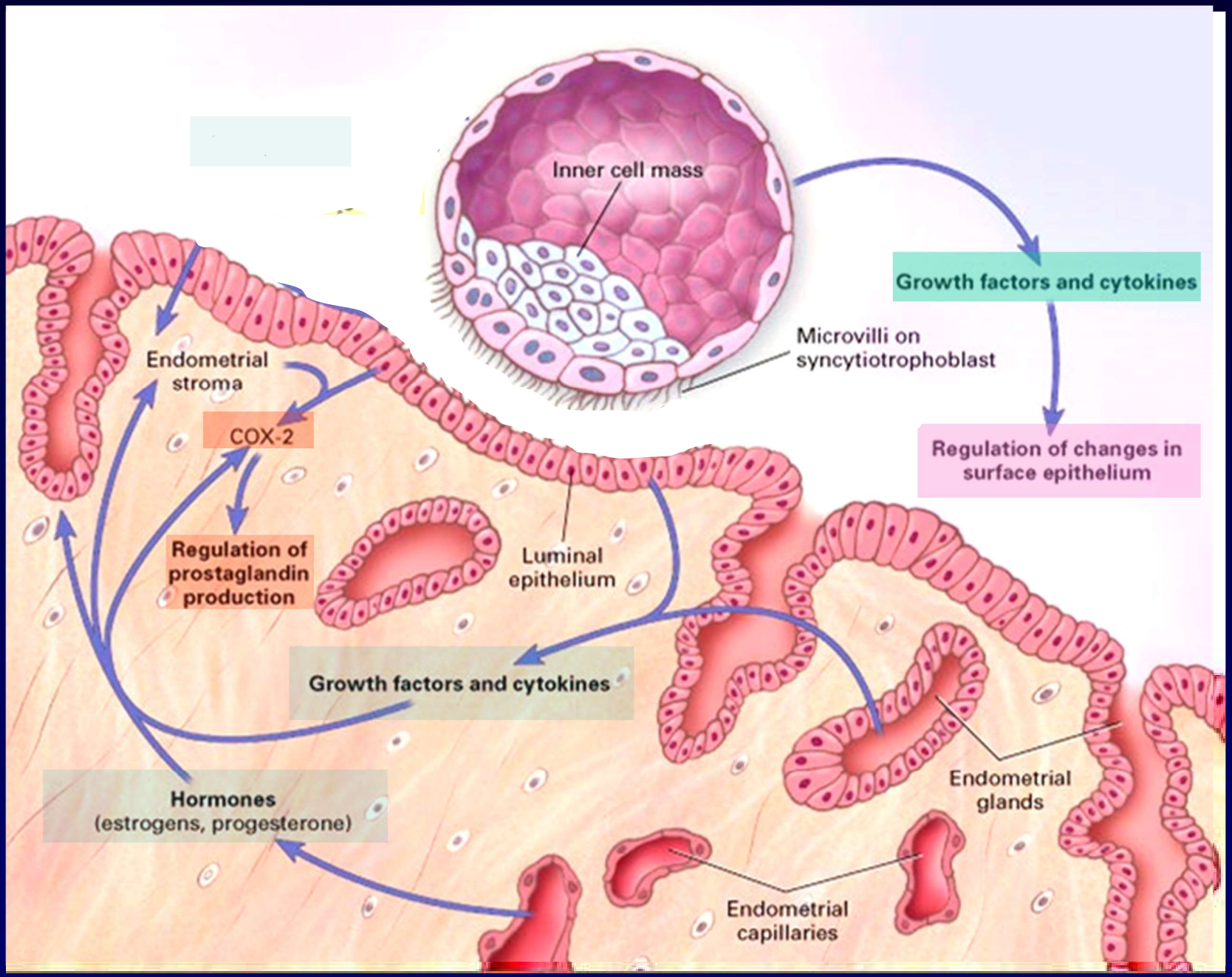
PROGESTERONE-PR COMPLEX

Progesterone

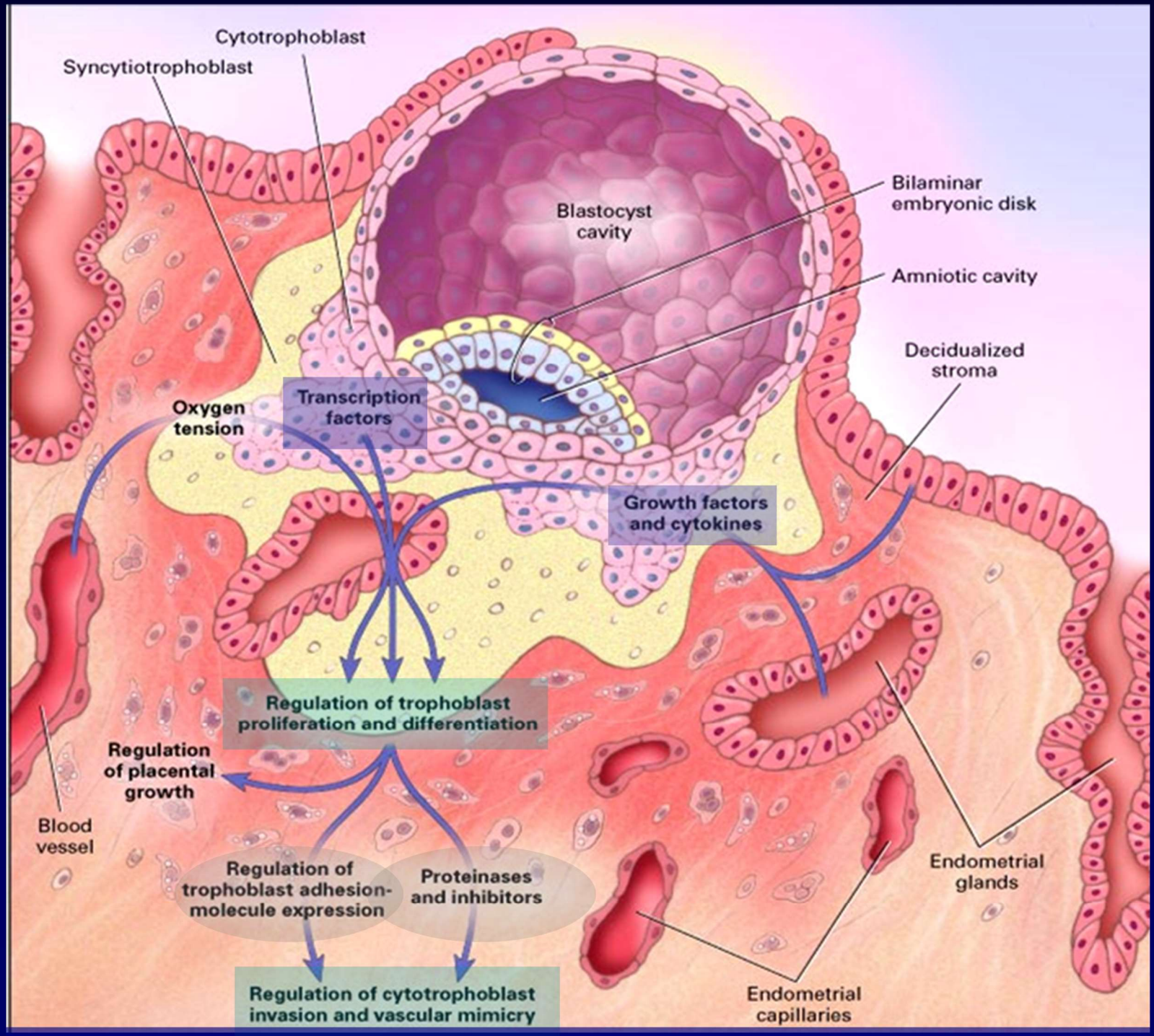
Immunomodulation



IMPLANTATION



Maternal Tolerance of the Fetal Semi - Allograft



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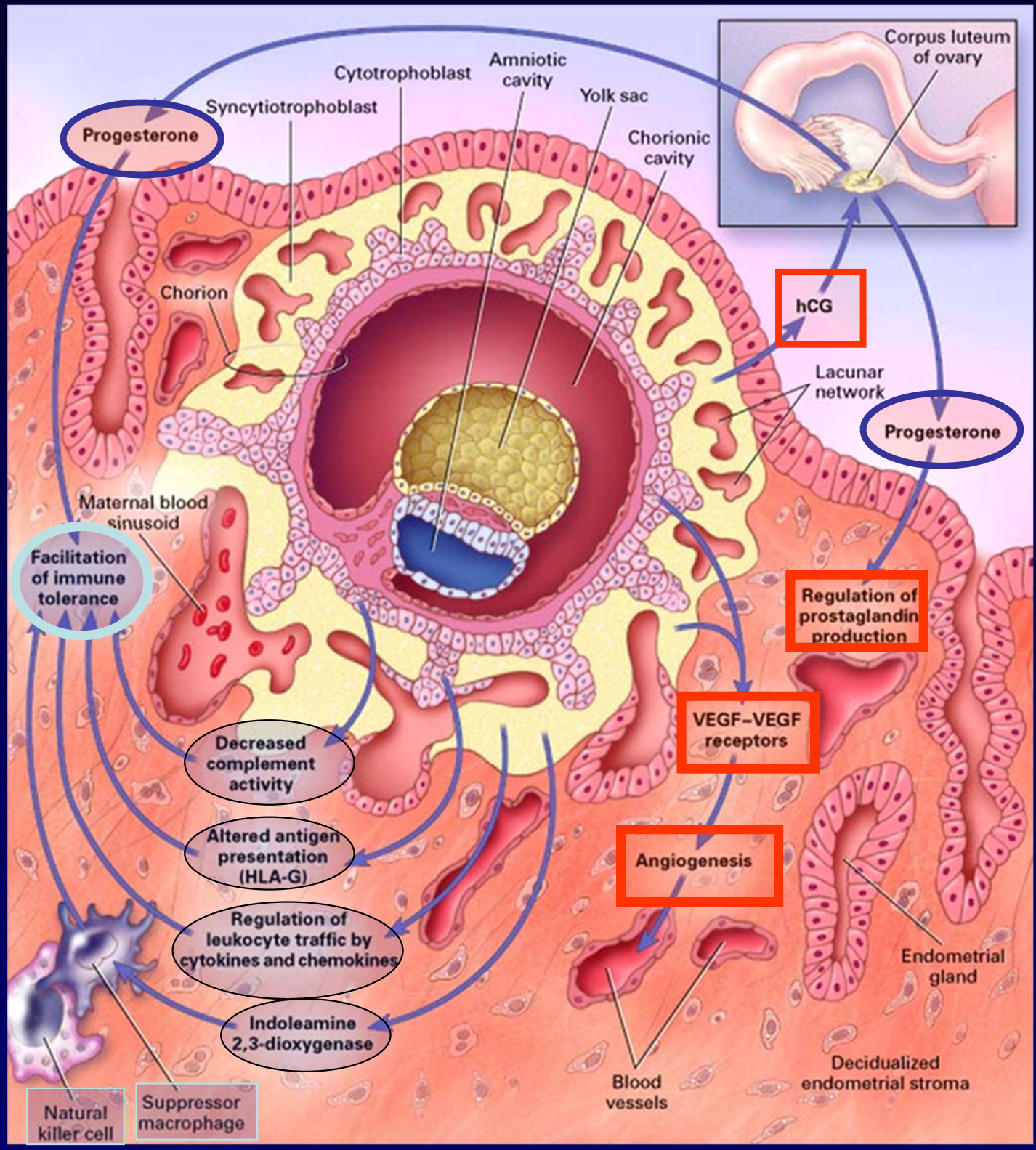

Corpus luteum of ovary
Progesterone
Syncytiotrophoblast
Cytotrophoblast
Amniotic cavity
Yolk sac
Chorionic cavity
Chorion
hCG
Lacunar network
Progesterone
Maternal blood sinusoid
Facilitation of immune tolerance
Regulation of prostaglandin production
VEGF–VEGF receptors
Decreased complement activity
Altered antigen presentation (HLA-G)
Angiogenesis
Regulation of leukocyte traffic by cytokines and chemokines
Endometrial gland
Indoleamine 2,3-dioxygenase
Blood vessels
Decidualized endometrial stroma
Natural killer cell
Suppressor macrophage

PUTATIVE ROLE OF PROGESTERONE IN IMPLANTATION

PROGESTERONE

CRF

trophoblast

endometrium

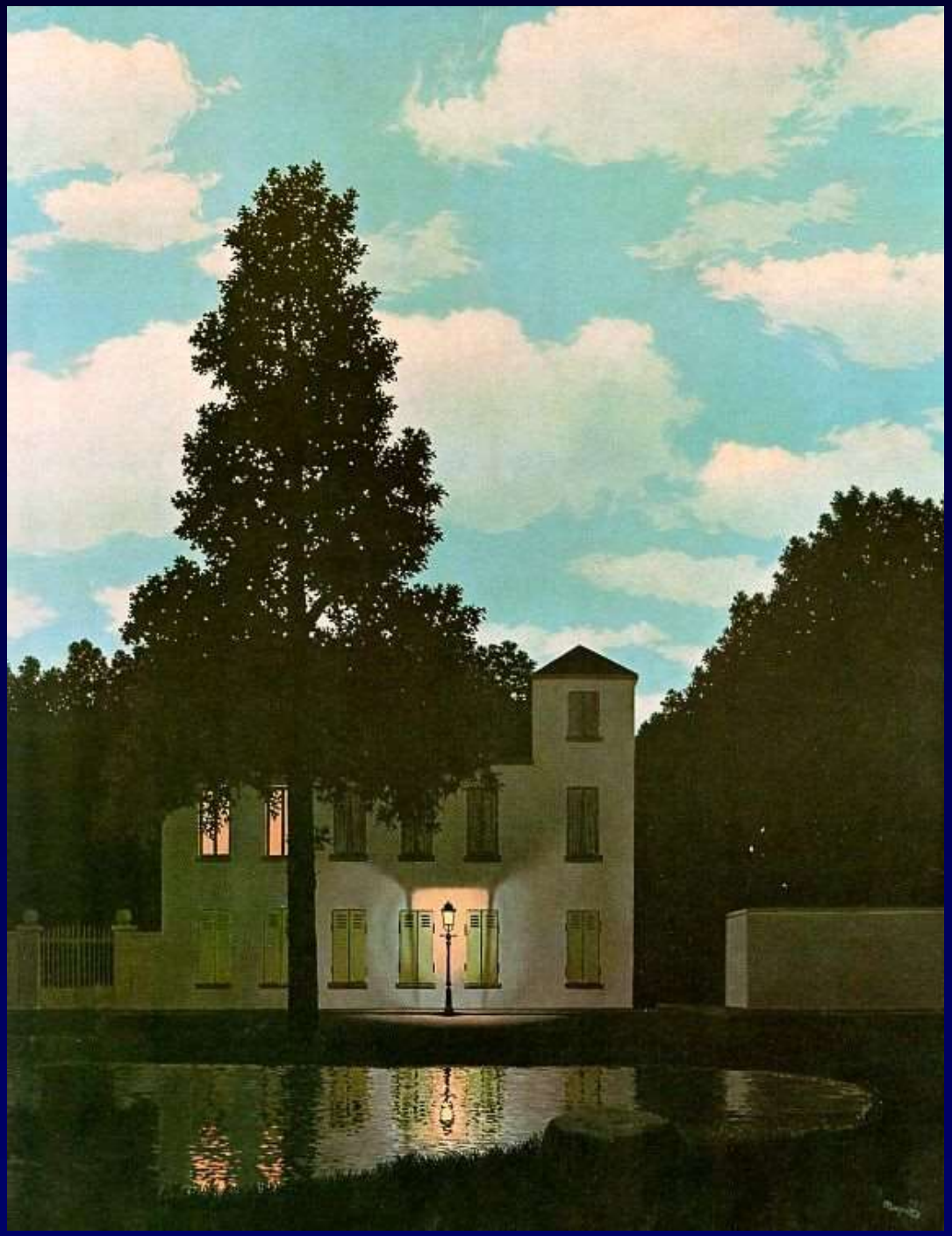
improves blastocyst implantation

modulates early maternal tolerance

modulates cytotrophoblast migration into maternal tissue

stimulates T lymphocytes apoptosis

PROGESTERONE
&
RECURRENT
MISCARRIAGE



The progesterone controversy

Prognosis for viable birth*

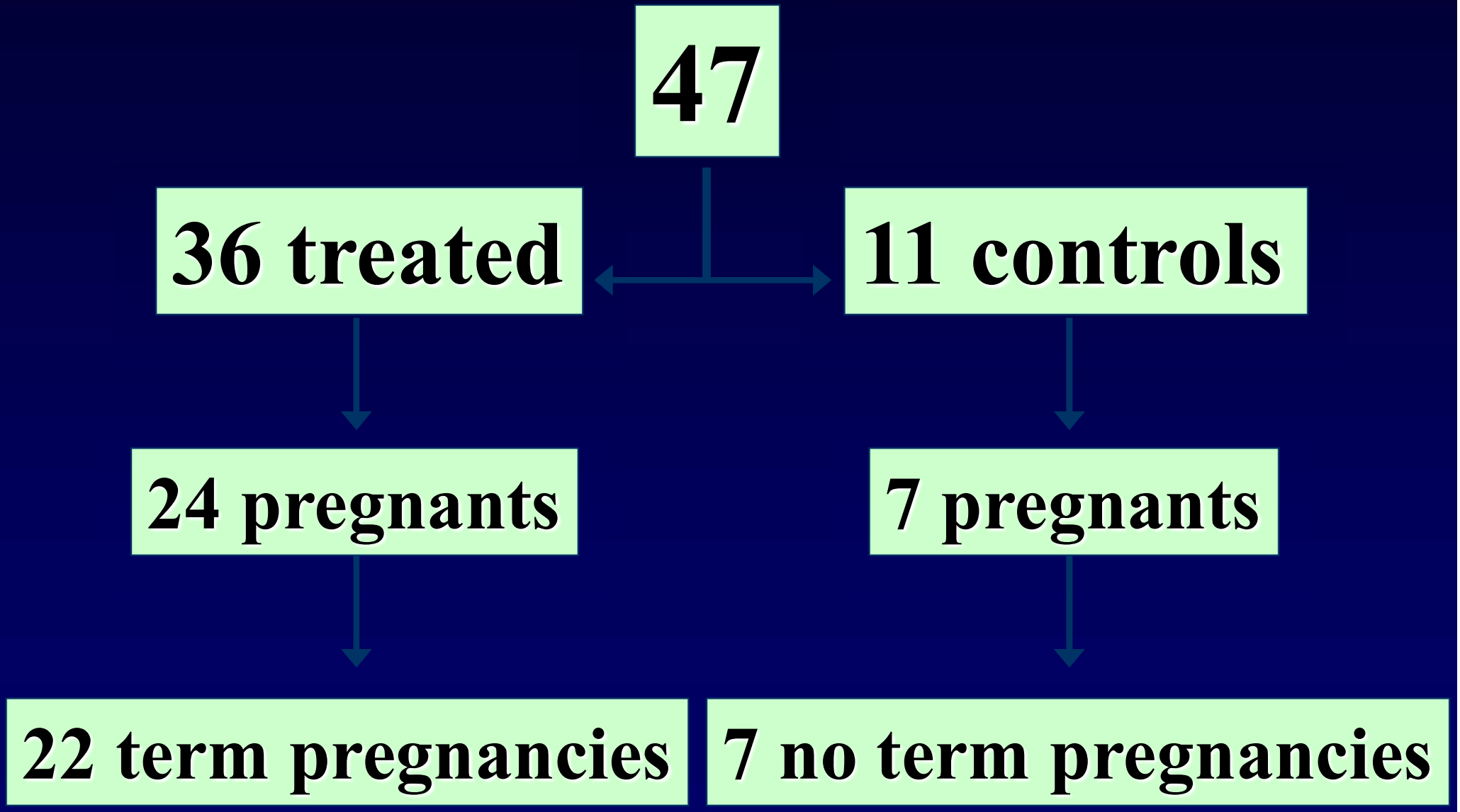
STATUS	INTERVENTION	PER CENT
Genetic factors	Timed intercourse and supportive care	20 - 90
Anatomic factors	Surgery and supportive care	60 - 90
Endocrine factors		
Luteal phase deficiency	**Progesterone and/or ovulation induction with or without pituitary desensitization and supportive care**	**80 - 90**
Hypotyroidism	Thyroid replacement and supportive care	80 - 90
Infections	Appropriate antibiotics and supportive care	70 - 90
Antiphospholipid syndrome	Aspirin, heparin, and supportive care	70 - 90
Th1 cellular immunity	Progesterone immunosoppression and supportive care	70 - 90
Unknown factors	Timed intercourse and supportive care	60 - 90

* Derived from more than 1000 cases .

47 cases	
age	28-45
mean	37
previous abortion	>2
APA	32%
ATA	33%
ANA	28%
AOA	2%
↑ NK cells	40%
↑ CD4/CD8	15%

Di Renzo, 2003

Successful treatment of immunologic abortion with progesterone (high doses)

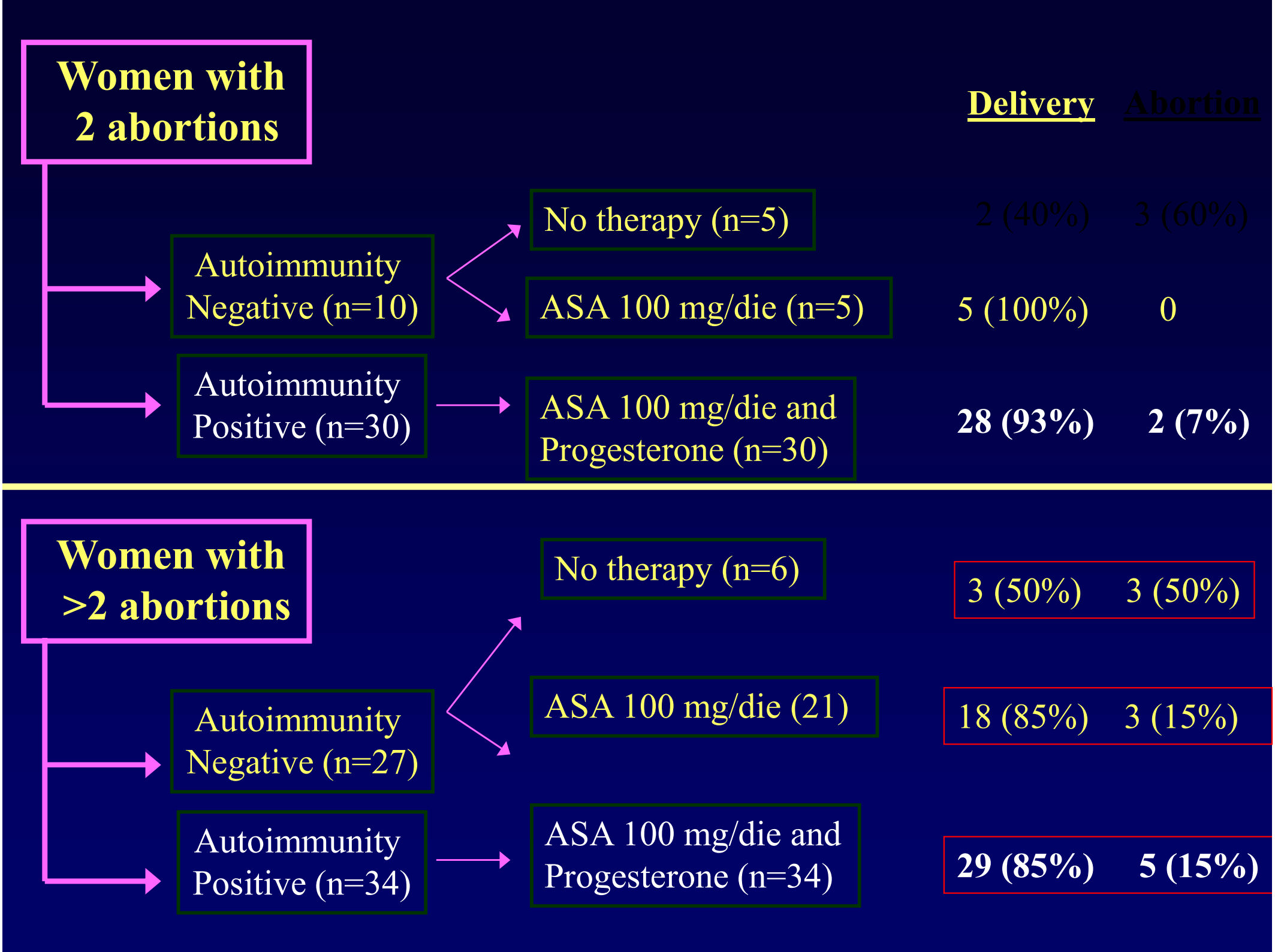


Di Renzo et al., 2003

% of patients with etiologic diagnosis of miscarriage

Etiology	Women with 2 abortions	Women with 3 abortions or mor
Karyotype anomalies of the couple	6 (2%)	9 (4%)
Anomalies of the uterine cavity	11 (4%)	19 (8%)
Genital infective pathology	78 (27%)	9 (4%)
Hyperprolactinemia	23 (8%)	19 (8%)
Immunological pathology	147 (51%)	138 (68%)
Not identified pathology	23 (8%)	19 (8%)
Total	288	213

Di Renzo et al., 2002


Women with 2 abortions
Delivery
Abortion
Autoimmunity Negative (n=10)
No therapy (n=5)
2 (40%)
3 (60%)
ASA 100 mg/die (n=5)
5 (100%)
0
Autoimmunity Positive (n=30)
ASA 100 mg/die and Progesterone (n=30)
28 (93%)
2 (7%)
Women with >2 abortions
No therapy (n=6)
3 (50%)
3 (50%)
Autoimmunity Negative (n=27)
ASA 100 mg/die (21)
18 (85%)
3 (15%)
Autoimmunity Positive (n=34)
ASA 100 mg/die and Progesterone (n=34)
29 (85%)
5 (15%)

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

A case-control study has shown that women with recurrent miscarriages are no more likely than fertile controls to have circulating thyroid antibodies. A prospective study has shown that the presence of thyroid antibodies in euthyroid women with a history of recurrent miscarriage does not effect future pregnancy outcome.

RCOG, 17, 2006

Endocrine factors

The truth about **COMMON MISCONCEPTIONS**

- Mild or subclinical endocrine disease is **NOT** associated with RPL, but significant thyroid dysfunction or uncontrolled DM may lead to increased risk of SAB, therefore, routine screening for TSH, PRL, and DM is questionable.

- Endometriosis is **NOT** associated with increased RPL.

- Elevated LH from PCOS does **NOT** increase SAB risk it is associated with anovulation and infertility.

There is insufficient evidence to evaluate the effect of progesterone supplementation in pregnancy to prevent a miscarriage

However, the only meta-analysis to assess progesterone support for pregnancy in recurrent miscarriage found progesterone to have a beneficial effect.

RCOG, 17, 2006

Endocrine factors

THE TRUE FACTS

- Inadequate luteal phase **IS** associated with RPL, but treatment with progesterone or it's metabolites has not been proved to significantly prevent RPL.

- Although luteal phase progesterone is always checked with RPL, studies have **NOT** proven it's efficacy.

"THERE IS NO EVIDENCE"

THERE IS NOT AFTER RESEARCH

EVIDENCE WAS NOT STUDIED

Levels of evidence

THIS IS THE CASE FOR PROGESTERONE

3) Etiology

Infectious causes

Infectious causes

There has been **NO** true association found between Bacterial or viral organisms and RPL,
although Chlamydia, Mycoplasma, Listeria, Toxoplasma, rubella, HSV, CMV, measels, coxackievirus have been associated with SAB's.

Endometrial Ureaplasma has been associated with RPL.

Antichlamydial Ab has been found in women with RPL, but chlamydia is **NOT** associated with RPL.

Rarely explanation for first trimester losses

3) Etiology

Immunologic problems

Immunologic problems

- **Autoimmunity:** An immunological response is directed against a specific component of the host (Antiphospholipid syndrome etc.).
- **Alloimmunity:** immunologic differences between individuals (foreign antigens).

Immunologic problems

Factors suggesting immunologic causes

- Many SAB's
- No recent full term pregnancies
- Less than 35yo
- SAB with normal karyotype
- One loss after 1st trimester

Immunologic problems

Alloimmunity

- **HLA sharing: Little if any association overall.**
- **Even if statistical association with selected HLA antigens exists, practical significance unclear.**

Treatment: No immunotherapy even if HLA sharing or given antigen found.

Antiphospholipid Syndrome (APS)

Responsible for %15 of RPL.

Clinical findings: Vascular thrombosis
Pregnancy complications
(1≥missed Ab. >10W,
3≥missed Ab. İn the first trimester,
1 ≥ PIH before 34W).

Lab. findings: LA (+),
ACA Ig M and/or G (+)

Antiphospholipid syndrome: Presence of Lupus anticoagulant and/or anticardiolipin Ab (IgG, IgM), on 2 occasions 6 wks apart.

Antiphospholipid Syndrome

- Acquired thrombophilia
- Syndrome of thrombotic phenomena, thrombocytopenia, pregnancy loss associated with presence of phospholipid antibodies
  - Primary
  - Secondary (associated with another autoimmune disease eg SLE)

Mechanisms proposed for the antiphospholipid syndrome

- Altered eicosanoid synthesis
- Injury to endothelium
- Induction of receptors for cell adhesion molecules on endothelium
- Induction of tissue factor expression on endothelium and monocytes
- Induction of apoptosis
- Interference with protein C / protein S pathway
    - aPL recognition of proteins C and S
    - Inhibition of activation of protein C
    - Inhibition of heparin – antithrombin III complexes
- Crossreactivity to oxidized LDL
- Disruption of annexin V shield

Reasons to suspect aPL

- Recurrent misc.
  - Esp after 1 normal delivery
- Unexplained IUFD
- Severe PET< 34 weeks
- Severe IUGR
- Arterial thrombosis
- Venous thrombosis
  - Especially of in unusual site
- SLE
- False positive syphilis titre
- Prolonged APTT
- ITP

Laboratory criterion:- The Problem

- There are many phospholipid antibodies directed against phospholipid antigens which are associated with pregnancy and other pathology
  - Only able to test for ACA, LA (APTT), syphilis, or beta$_2$ glycoprotein 1 (ß$_2$ -GP$_1$)
  - Antiphosphatidylserine, -ethanolamine, -inositol, -glycerol, antiphosphatidic acid

Not always present with aCL

The Problem

- May need to use other indirect laboratory markers of aPL disease to make a possible diagnosis

Indirect aPL tests

- ↓C3, ↓C4
- ESR > 100

The Problem

- If no laboratory confirmation, may need to use high index of clinical suspicion to make a tentative diagnosis and justify a therapeutic trial

Pathology

- Antiphospholipid antibody in the presence of $ß_2$-$Glycoprotein_1$ binds to vascular endothelium
- Causes damage through cascade of molecules
- Thromboxane release
- Clot formation
  - (At placenta due to Annexin V deficit)

aPL – Maternal Risk in Pregnancy

- Pre-eclampsia occurs in 18 – 48%
- Risk of thrombosis in pregnancy is raised in all women

  - Increase in risk over that of normal pregnancy:
    - ACA - OR 3.2 (CI 1.1 – 9.28)
    - LA - OR 11.1 (CI 3.8 – 32.3)

aPL – Fetal/Neonatal Data

- Recurrent Miscarriage:- 5 – 20% aPL positive (higher if first pregnancy went to term)
- Fetal Death:- 15-18% aPL positive

- IUGR occurs in 15-30%
- Abnormal FHR occurs in 46-52%
- Preterm labour occurs in 12-35%

APA thrombogenic effects include:

- Inhibition of endothelial anticoagulant activity including interference with thrombomodulin, PC, PS and/or ATIII activitya
- Platelet hyperaggregability
- Impairment of fibrinolysis
- Increased endothelial synthesis of procoagulant tissue factor, and von Willebrand factor multimers

Although APA may arise from conditions likely to be associated with poor pregnancy outcomes (e.g., SLE), these antibodies seem to directly promote placental thrombosis and vasculopathy, by interfering with a variety of phospholipid-associated anticoagulant proteins.

Pathology

- Antiphospholipid antibody in the presence of $ß_2$-$Glycoprotein_1$ binds to vascular endothelium
- Causes damage through cascade of molecules
- Thromboxane release
- Clot formation
  - (At placenta due to Annexin V deficit)

Placental Pathology

Out and associates evaluated 47 placentas

from 45 patients having stillbirths

and noted that among the 17 patients with APA all but three had evidence of placental thrombosis and infarction. These latter findings were present significantly more often in patients with APA. Patients with APA also display evidence of spiral artery vasculopathy (i.e. “acute atherosis”).

Pathogenic mechanisms for Placental Thrombosis

Evidence that APA have direct pathogenic effects on implantation, placental function and fetal growth include:

• mice riceiving high concentrations of APA positive-IgG more frequently abort their fetuses, have lower embryonic and placental weights and display evidence of decidual necrosis and intravascular decidual fibrin deposition

• immunization of mice with an IgM monoclonal ACA results in low pregnancy rates, low numbers of fetuses and a high rate of resorptions

• exposure of cultured human trophoblast cells to APA inhibits gonadotropin releasing hormone-induced hCG release

• APA-associated placental pathological findings include placental thrombosis and infarction as well as evidence of spiral artery vasculopathy

Obstetrical conditions associated with APA

Median prevalence of APA in RPL patients:

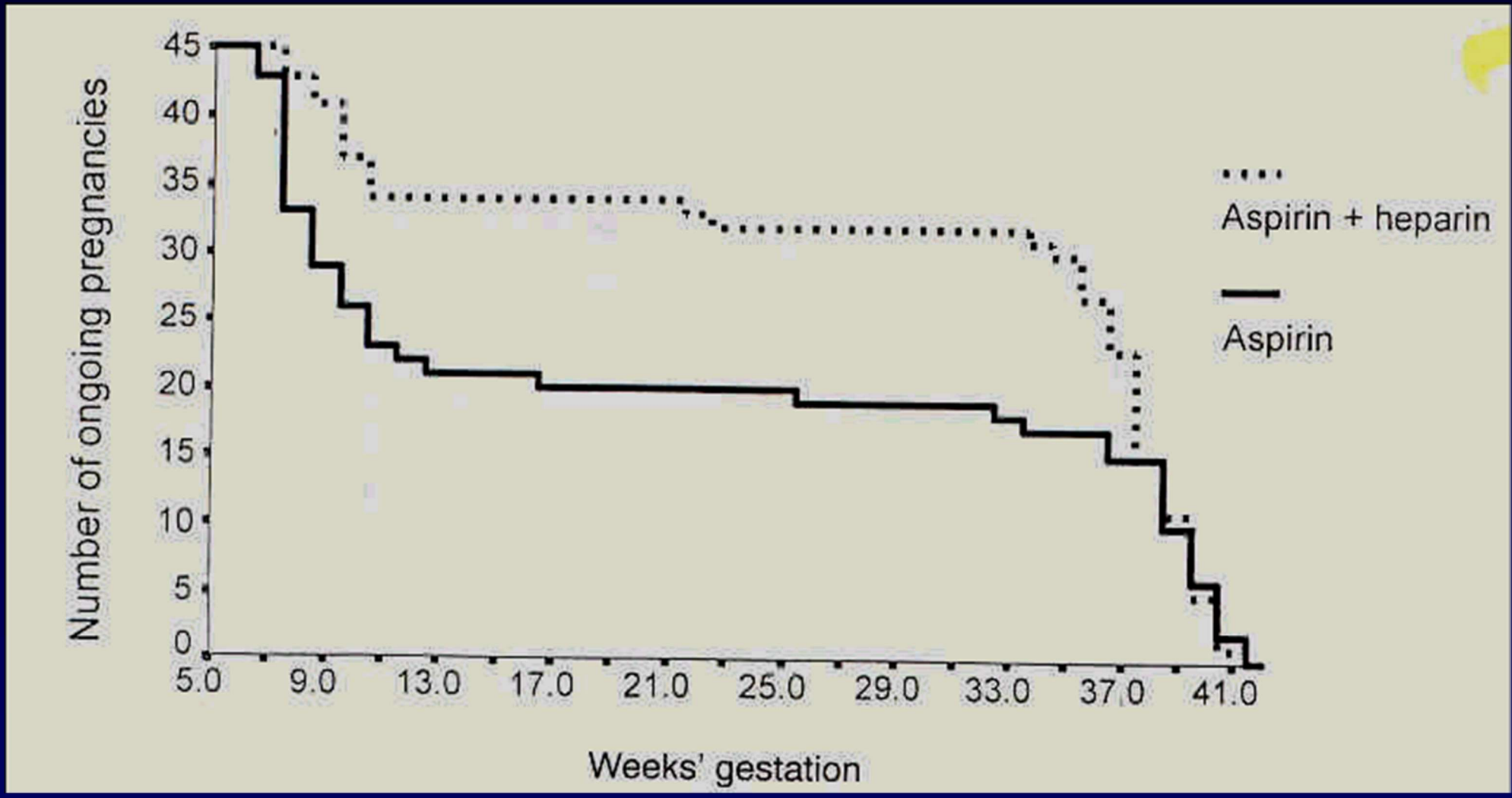
- **ACA: 8%**
- **LAC: 7%**

APS: Treatment
Placebo vs Aspirin

	Placebo	*Aspirin*	*Statistical significance*
Antenatal complications			
Bleeding (any in pregnancy, No.)	7 (35%)	9 (45%)	NS
Hypertension or preeclampsia* (No.)	3 (18%)	3 (19%)	NS
Preterm birth* (24-37 wk, No.)	0 (0%)	2 (13%)	NS
Cesarean delivery* (No.)	5 (29%)	5 (31%)	NS
Outcome			
Live birth (No.)	17 (85%)	16 (80%)	NS
Neonatal outcome			
Birth weight (g, mean ± SD)	3367 ± 582	3038 ± 790	NS
Small for gestational age*† (No.)	4 (24%)	1 (6%)	NS
Neonatal admission* (No.)	2 (12%)	2 (13%)	NS
Congenital anomalies* (No.)	1 (6%)	1 (6%)	NS

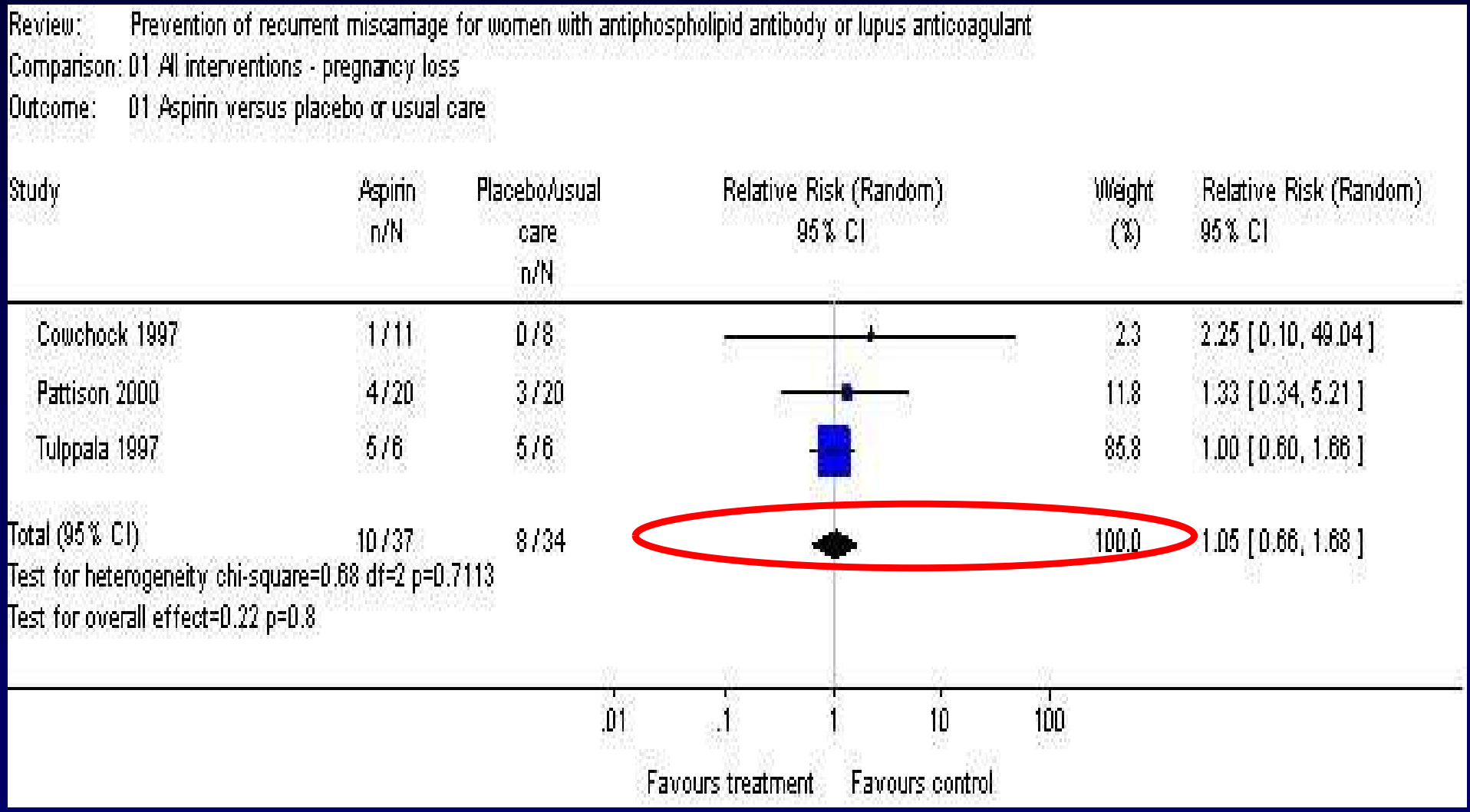
NS, Not significant.
*Percentage calculated for live births only.
†As defined by birth weight <5th percentile.

Pattison Am J Obstet Gynecol 2000



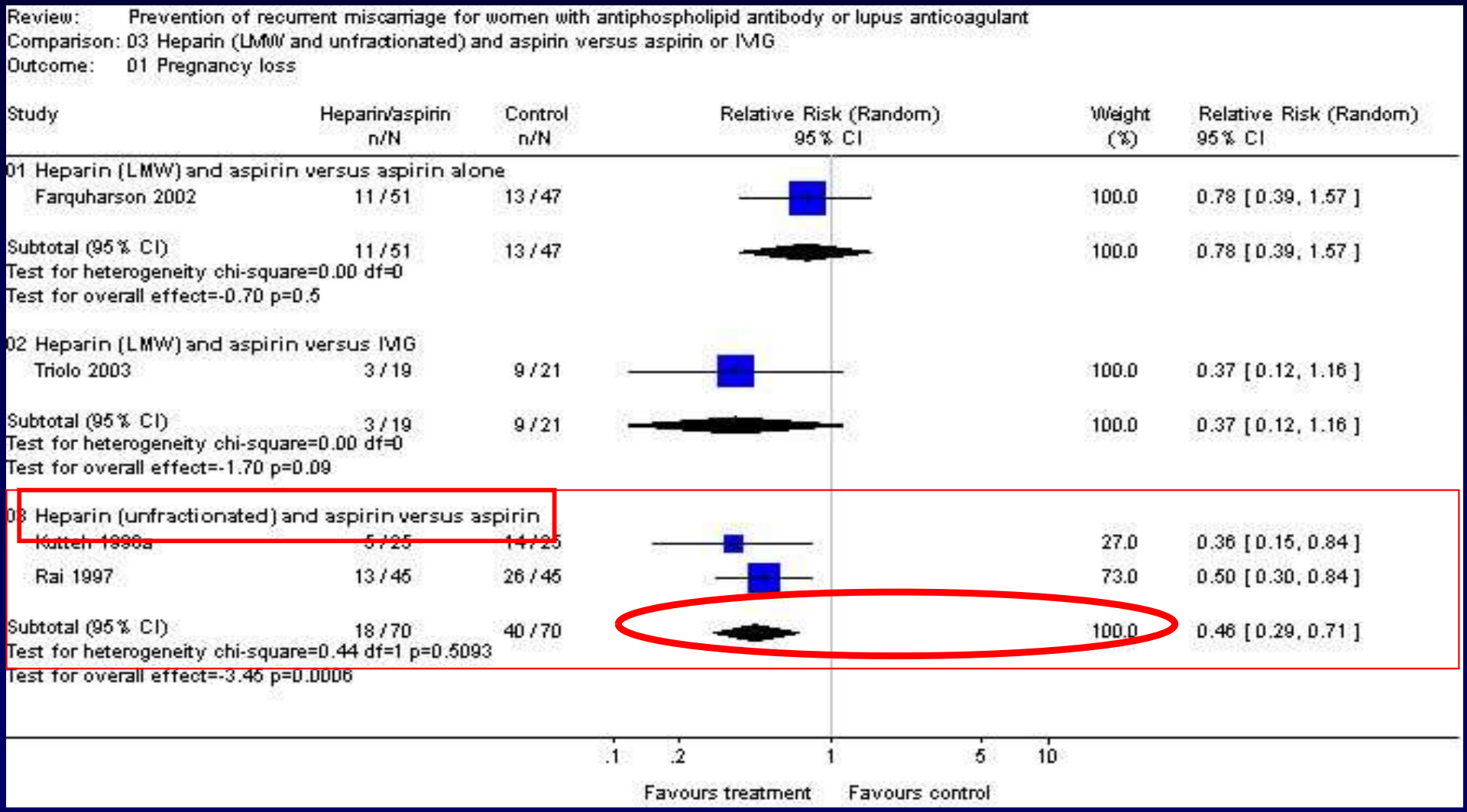
Pregnancy outcome in recurrent miscarriers with antiphospholipid antibodies (aPL) treated with aspirin and heparin

APS: Treatment
Placebo vs Aspirin



Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. (**Cochrane Review, 2005**)

APS: Treatment
Heparin + Aspirin vs Aspirin



Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. **(Cochrane Review, 2005)**

Guidelines for Heparin + Aspirin

Treatment of patients with Antiphospholipid Antibodies and Recurrent Pregnancy Loss

1. Baseline nonpregnant studies of antiphospholipid antibodies, CBC with platelets, partial thromboplastin time (PTT) and lupus anticoagulant should be obtained. The antiphospholipid antibody assay may be repeated in early pregnancy at 20 wks and again at 30 wks.

2. Aspirin (60-100 mg) daily, beginning at the time of ovulation in the planned conception cycle. Aspirin should be discontinued one week before delivery.

Guidelines for Heparin + Aspirin

Treatment of patients with Antiphospholipid Antibodies and Recurrent Pregnancy Loss

3. Subcutaneous heparin (5000-6000 U b.i.d.) should be initiated sfter the missed period and a confirmed pregnancy test. Midinterval platelets and PTT should be obtained for 2 consecutive wks following the initiation of heparin therapy and 1 week following and adjustment in heparin dosage. During the remainder of pregnancy, PTT and platelets can be checked monthly. Calcium carbonate (1.5 g/day) should be initiated when heparin is started.

4. The pregnancy should be documented by ultrasonography at ~7 wks for the detection of fetal heart motion. Further sonography may be performed at 16-20 wks.

Guidelines for Heparin + Aspirin

Treatment of patients with Antiphospholipid Antibodies and Recurrent Pregnancy Loss

5. Antenatal testing should begin at 28-30 wks, based on the increased risk of IUGR and stillbirth. This may include kick counts, nonstress test or serial biophysical profiles. Serial scans for growth rate may be indicated.

6. Heparin should be continued until full term and should be discontinued when the patient initiates spontaneous labor. One heparin dose may be skipped the night before amniocentesis or the night before any scheduled induction or operative delivery. Heparin should be reinitiated 2-3 days postpartum at 5000 U b.i.d. in those patients with previous thromboembolic events, anticoagulation should continue for 6 weeks.

Guidelines for Heparin + Aspirin

4

Treatment of patients with Antiphospholipid Antibodies and Recurrent Pregnancy Loss

7. If the patient is fully anticoagulated and delivery is emergent, 1% protamine sulfate can be administered by slow i.v. over 10 min (2.5 mg protamine / 1000 U heparin, maximum 50 mg protamine) if coagulation indicators are elevated

8. Patients should not use estrogen-containing oral contraceptives for contraception. Aspirin (60-100 mg/day) can be advised until further recommendations are available.

Prognosis

- 70% to 80% live birth rate for patients treated heparin and aspirin
- However, even among patients with live births there is an increased risk of complications including pre-eclampsia, prematurity, fetal distress and IUGR

Refractory cases:

1) Prednisone (40 mg p.o., q.d.)but associated with an increased incidence of: - PPROM (40%) and PTD (64%)

   - osteopenia; 8%reduction in bone density with doses as low as 10 mg / day x 20 weeks in nonpregnant patients – need extra calcium supplementation

   - gestational diabetes: 30%-50%

2) High dose IVIG (e.g., 0.5 g/kg x 2 days q.4 weeks or 1 gm/kg x 1 course q.4 weeks)

Results

Parameter	Group A n=52	Group B n=50
Term pregnancy (>36 weeks) and a healthy newborn	46 (88.5)	45 (90.0)
Spontaneus abortions	3 (6.1)	2 (4.2)
Preterm delivery (23-36 weeks)	3 (6.1)	3 (6.3)
Gestational hypertension	4 (8.2)	5 (10.4)
Median of the birth weights (and range)	3069g (730-4300)	3120 (800-4500)
Small for gestational age babies (<3°centile)	5 (10.2)	4 (8.3)
Others proslems (thrombocytopenia, 3°trimester vaginal bleeding, etc)	2 (4.1)	2 (4.2)

(Di Renzo, 2008)

aPL - Treatment

- Trials of aspirin and heparin show improved outcome over aspirin alone.
- Use Aspirin and heparin as for congenital thrombophilia
- Add oral steroids only for evidence of other autoimmune dysfunction
- No place for IgG ?
- Full anticoagulation required for
  - Severe thrombocytopenia
  - History of recent thrombosis

Treatment

- When no laboratory anomaly known/found using treatment with Aspirin +/- Heparin in next pregnancy
  - Recurrent miscarriage
    - Take home baby 92% vs 60% $p<0.005$
  - Severe recurrent pregnancy problems
    - Recurrence 2.3% vs 15.6%

Recurrent miscarriage associated with antiphospholipid antibodies :prophylactic treatment with low-dose aspirin and fish oil derivates

Among patients treated with low-dose aspirin, 12 out of the 15 (80%) pregnancies ended in live births. In the fish oil derivate group 11 out of the 15 (73.3%) ended in live births ($p>0.05$). There were no significant differences between the low-dose aspirin and the fish oil derivates groups with respect to gestational age at delivery (39.9 +/-0.4 vs 39 +/-1.5 weeks), fetal birth weight (3290 +/-200g vs 3560 +/-100g), number of cesarian sections (25% vs 18%), or complications.

(Carta et all. 2005)

Treatment

- Where no tests are available
  - Folate 5mg/day
  - Aspirin 60-150mg/day
  - Heparin
    - Enoxaparin
    - Non-fractionated 5000 – 7500 units 3x daily
      - Needs to be stopped for delivery

To diagnose APS it is mandatory that the patient should have two positive tests at least six weeks apart for either lupus anticoagulant or anticardiolipin (aCL) antibodies of IgG and/or IgM class present in medium or high titre

RCOG, 17, 2006

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

A randomised controlled trial showed that the live birth rate of women with recurrent miscarriage associated with aPL treated with low-dose aspirin only is 40% and this is significantly improved to 70% when they are treated with low-dose aspirin in combination with low-dose heparin

RCOG, 17, 2006

Cochrane systematic review of 18 randomised controlled trials has shown that the use of various forms of immunotherapy, including paternal cell immunisation, third-party donor leucocytes, trophoblast membranes and IVIG, in women with unexplained recurrent miscarriage provides no significant beneficial effect over placebo in preventing further miscarriage.

Another meta-analysis indicated that IVIG treatment does not improve the live birth rate in women with unexplained recurrent miscarriage. Moreover, immunotherapy is expensive and has potentially serious adverse effects including transfusion reaction, anaphylactic shock and hepatitis. The use of immunotherapy should no longer be offered to women with unexplained recurrent miscarriage and routine tests for HLA type and anti-paternal cytotoxic antibody should be abandoned

RCOG, 17, 2006

3) Etiology

Thrombophilias

Congenital Thrombophilias

- Protein S deficiency
- Protein C deficiency
- Protein Z deficiency
- Antithrombin III deficiency
- Factor V Leiden mutation
- *Activated Protein C resistance (APCR)*
- MTHFR (Methylene tetrahydrofolate reductase) mutation
- *Hyperhomocysteinaemia*
- Prothrombin gene mutation
- Platelet membrane integrin alpha2beta1 polymorphism
- Plasmin activator inhibitor anomaly
- Sticky platelet syndrome

- **ALL IMPLICATED IN EXCESS RATES THROMBOSIS IN NONPREGNANT STATE**

Acquired Thrombophilia

- Antiphospholipid syndrome
  - +/- SLE

THROMBOPHILIA

Deficiencies - Hypercoagulable

- Protein C
- Protein S
- Antithrombin

THROMBOPHILIA

Mutations - Hypercoagulable

- Factor V Leiden (G1691A)
- Factor II - Prothrombin G20210A
- Hyperhomocysteinemia

  (MTHFR C677T, A1298C)

Thrombophilias

Screening Tests

- Activated protein C resistance or F V Leiden DNA analysis
- Prothrombin gene mutation DNA analysis
- Antithrombin III activity test
- Protein C activity test
- Protein S activity test
- Homocysteine level
  - MTHFR DNA analysis
- Antiphospholipid syndromes
  - aPTT
  - Lupus Anticoagulant
  - Anticardiolipin anticor IgG - IgM

Thrombophilia: Early Pregnancy Losses (1st - 2nd trimester)

Disorders	OR	(95% CI)
Factor VL Homozygot	2.71	1.32–5.58
Factor VL Heterozygot	1.68	1.09-2.58
Prothrombine Heterozygot	2.49	1.24-5.0
ACA	3.40	1.33-8.68
Lupus anticoagulant	2.97	1.03-8.56
MTHFR Homozygot	1.40	0.77-2.55
AT III	0.88	0.17-4.48
Protein C	2.29	0.20-26.43
Protein S	3.55	0.35-35.72

Thrombophilia in pregnancy : a systematic review. BJH 2005

Thrombosis: Risk and economic assessment of thrombophilia screening (TREATS) study

Factor V Leiden and RPL

FVL	RPL	LIVE BIRTHS (%)
heterozyg	<12 weeks	6/16 (37.5%)
normal	<12 weeks	106/153(69.3%)

Observational ,non-treatment outcome study:

Unexplained RPL with FVL heterozygous had lower chance of live birth.

Rai et al. Human Reprod 17:442-445, 2002

Thrombophilia: Reccurrent Early Pregnancy Losses (1st trimester)

Disorder	OR	(95% CI)
Factor VL*	1.91	1.01–3.61
Prothrombin Heterozygot	2.70	1.4-5.0
ACA	5.05	1.82-14.05
MTHFR Homozygot	0.86	0.44-1.69

*FVL heterozigot ve homozigot Combined

Thrombophilia in pregnancy : a systematic review. BJH 2005

Thrombosis: Risk and economic assessment of thrombophilia screening (TREATS) study

Thrombophilia: Non-recurrent 2nd Trimester Pregnancy Losses

Disorder	OR	(95% CI)
Factor VL*	4.12	1.93–8.81
Prothrombin Heterozygot	8.60	2.18-33.95
Lupus anticoagulant	5.05	1.82-14.05
MTHFR Homozygot	0.86	0.44-1.69

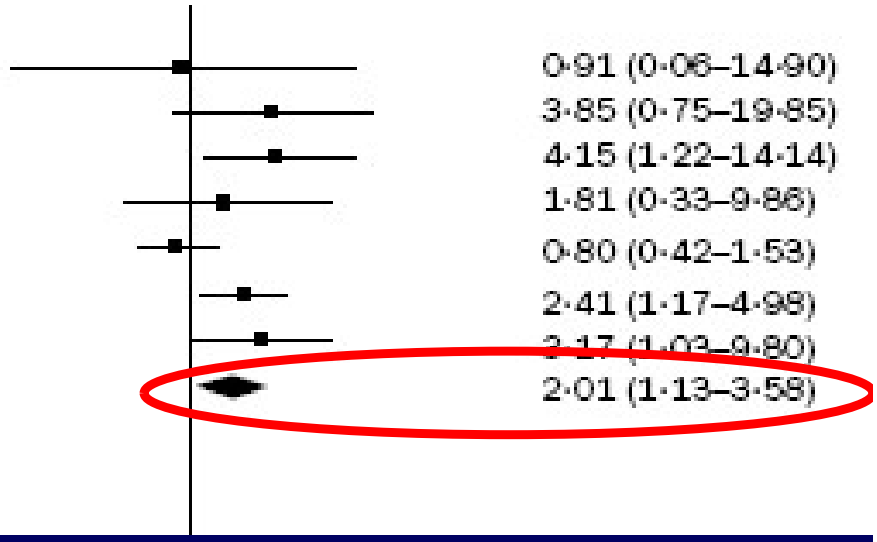
*FVL heterozigot ve homozigot combined

Thrombophilia in pregnancy : a systematic review. BJH 2005

Thrombosis: Risk and economic assessment of thrombophilia screening (TREATS) study

Factor V Leiden, RPL<13W

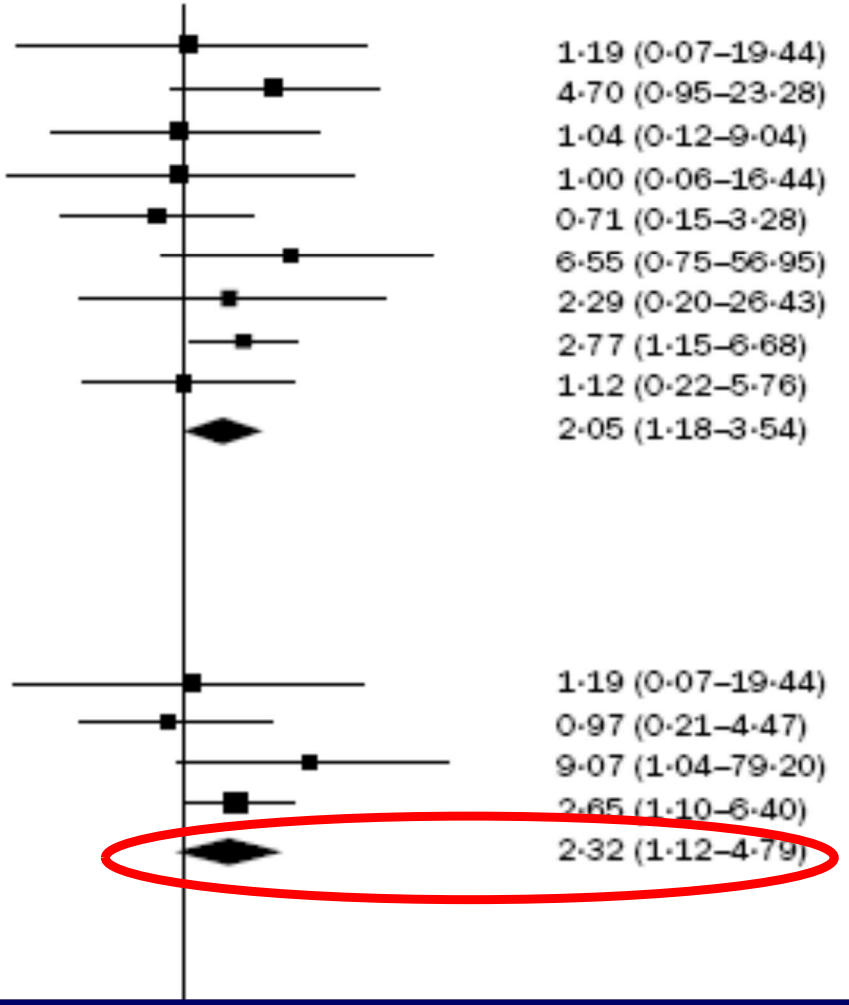
Study	FVL positive n/N	FVL negative n/N	Odds ratio (95% CI)	Odds ratio (95% CI)
FVL and recurrent fetal loss before 13 weeks				
Balasch[8]	1/2	54/103		0·91 (0·06–14·90)
Fatin[9]	6/8	53/121		3·85 (0·75–19·85)
Foka[10]	9/13	52/148		4·15 (1·22–14·14)
Grandone[11]	2/7	25/138		1·81 (0·33–9·86)
Ral[20]	59/71	845/983		0·80 (0·42–1·53)
Reznikoff[22]	27/38	233/462		2·41 (1·17–4·98)
Younis[25]	6/14	31/162		3·17 (1·03–9·80)
Subtotal (95% CI)	110/153	1293/2117		2·01 (1·13–3·58)
Test for heterogeneity p=0·11				
Test for overall effect p=0·02				



Rey 2003

Prothrombine, RPL

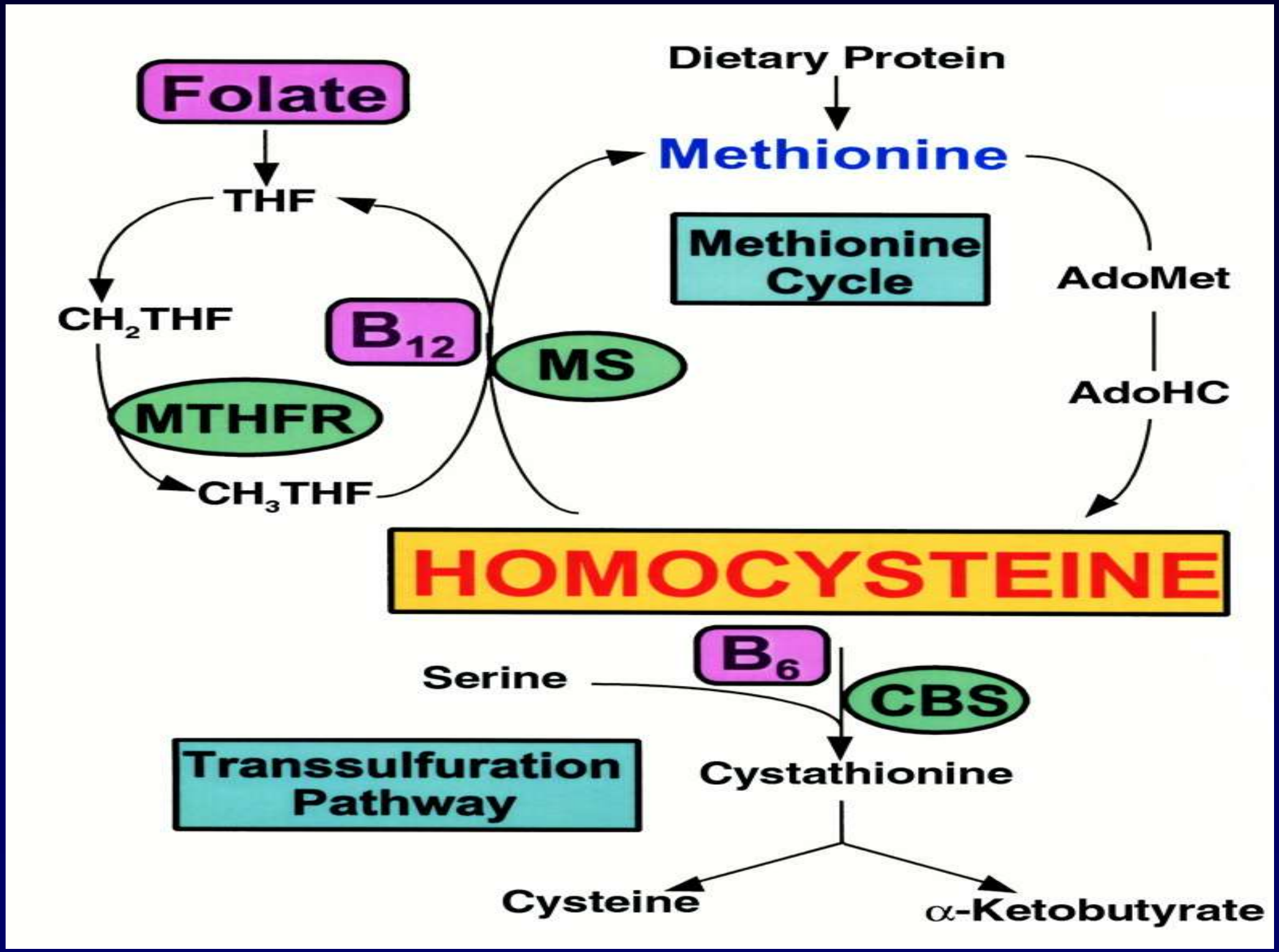
Study	PTm positive n/N	PTm negative n/N	Odds ratio (95% CI)	Odds ratio (95% CI)
PTm and recurrent fetal loss				
Fatini[9]	1/2	58/127		1·19 (0·07–19·44)
Foka[10]	7/9	73/171		4·70 (0·95–23·28)
Gris[27]	1/5	88/547		1·04 (0·12–9·04)
Kutteh[15]	1/2	49/98		1·00 (0·06–16·44)
Pickering[18]	4/7	118/181		0·71 (0·15–3·28)
Plhusch[19]	5/6	97/224		6·55 (0·75–56·95)
Raziel[21]	2/3	34/73		2·29 (0·20–26·43)
Reznikoff[22]	20/27	240/473		2·77 (1·15–6·68)
Wramsby[24]	3/6	59/125		1·12 (0·22–5·76)
Subtotal (95% CI)	44/68	816/2019		2·05 (1·18–3·54)
Test for heterogeneity p=0·67				
Test for overall effect p=0·01				
PTm and recurrent fetal loss before 13 weeks				
Fatini[9]	1/2	58/127		1·19 (0·07–19·44)
Pickering[18]	4/7	87/150		0·97 (0·21–4·47)
Plhusch[19]	5/6	70/197		9·07 (1·04–79·20)
Reznikoff[22]	20/27	240/463		2·65 (1·10–6·40)
Subtotal (95% CI)	30/42	455/937		2·32 (1·12–4·79)
Test for heterogeneity p=0·38				
Test for overall effect p=0·02				



Rey 2003

Hyperhomocysteinemia

- Normal level: less than 10.4 umol/L
- Frequency: 1/10
- Inheritance MTHFR autosomal recessive
- Severity: causes 10 to 20 % of all VTE
- MTHFR gene mutation C677t and A1298C
- Dietary deficiency of folate, B6, and B12
- Causes venous and arterial thrombosis


Folate
Dietary Protein
Methionine
THF
Methionine Cycle
AdoMet
CH_2THF
B_{12}
MS
AdoHC
MTHFR
CH_3THF
HOMOCYSTEINE
B_6
Serine
CBS
Transsulfuration Pathway
Cystathionine
Cysteine
α-Ketobutyrate

Hyperhomocysteinemia & RPL

- Meta-analysis 1/92 to 11/99
- RPL <16 weeks and $\geq$ 2 losses
- English language case-control studies
- fasting tHcy, afterload tHcy, and MTHFR
- Pooled risk estimates of 2.7 (fasting) to 4.2 (afterload)

Nelen et al. Fert Steril 2000

Homocysteinemia and RPL

Diagnostic test	+/total	OR (95% CI)
Fasting homocysteine	53/403	2.7 (1.4-5.2)
Afterload homocysteine	54/351	4.2 (2.0-8.8)
MTHFR C677T,A1298C	85/599	1.4 (1.0-2.0)

Nelen et al. Fert Steril 2000

Thrombophilias and RPL

- Systematic review of 79 studies
- Publication within last 23 years
- Well-defined study criteria, quality scores
- All women had $\geq$ one thrombophilia
- Evaluated associations with early RPL
- Not associated with AT, PC, PS, MTHFR

Robertson et al. Br J Haemat 2005

COROLLARIES

- Factor V Leiden mutation has been related to the RPL.
- Activated Protein C Resistance has been corraleted to the RPL.
- Prothrombin G20210A mutation has been associated with the RPL.
- MTHFR mutation, deficiency of Protein C, S and Antithrombin III have NOT been related to RPL.

Rey 2003

Thrombophilias

- Effects of thrombophilia
  - Age dependant
  - Smoking dependant
  - Rise exponentially in the presence of a second thrombophilia especially anticardiolipin antibodies

Congenital Thrombophilias

- Literature
  - Pregnancy outcomes have not taken into account co-morbidities and maternal age
  - Have looked at single isolated anomalies eg Protein S
  - Assumed that decreased APCR = Factor V Leiden
  - Hyperhomocysteinaemia = MTHFR mutation

Congenital Thrombophilias

- Result is that the literature is
  - CONFUSING

Congenital Thrombophilias

- Thrombophilic disorders and fetal loss: a meta-analysis
  - Rey et al, Lancet, 2003, 361, 901.
    - Looked at methodology, heterogeneity of studies, confounding factors etc.

Congenital Thrombophilias

- Those involved in pregnancy complications (Summary)
  - Factor V Leiden mutation
  - Decreased Activated Protein C (Not Leiden)
  - Prothrombin gene
  - Decreased Protein S
  - Hyperhomocysteinaemia.

Prevalence of inherited thrombophilia (377 patients with thrombophilia work-up)

	Thrombophilia cases/total (%)	p-value
Recurrent loss	28/118 (24)	n.s
Isolated loss	33/147 (22)	
Aneuploid loss	8/45 (18)	n.s.
Euploid loss	11/48 (23)	
Previous stillbirth	18/51 (35)	0.05
No previous stillbirth	74/326 (23)	
2nd trimester loss	22/66 (33)	0.01
No 2nd trimester loss	58/312 (19)	

Lockwood & Di Renzo, 2001

THROMBOPHILIA & DVT

Deficit	Prevalence	RR	Risk
ATIII	0.02%	50-100	70-90%
PC	0.2-0.4%	5-50	50%
PS	0.02%	5-50	50%
FV Leiden	4-7 %	7-80	30%
PT 20210A	1-2%	5	8%
Hcy	1-11%	2-3	10%

U Seligsohn, NEJM 2001;344:1223; Colman, Hirsh, Marder, Clowes, George: Hemostasis and Thrombosis, Lippincott 2001.

Trombophilia	Miscarriage	IUFD	PE	HELLP
AT III defic.	++	++	+	
Protein C defic.	+	++	+	
Protein S defic.	+	++	+	+
Dysfibrinogenemia	+	+		
APC-Resistance	+	++	++	
f V Leiden	++	++	++	+
Hyper HCY	+	+	+	+
F II mutation		+		
A-PL Syndrome	++	++	++	+
Combined	++	++	+	+

Blumenfeld and Brenner Fertil Steril 1999; 72: 765-74

Placental vascular complications associated with thrombophilia

	Miscarriage	IUFD	Pre-eclampsia	Placenta abruption
Antithrombin III deficiency	++	++	+	
Protein C deficiency	++	++	+	+
Protein S deficiency	++	++	+	+
Dysfibrinogenem	++	+		
APC resistance	++	++	++	+

Placental vascular complications associated with thrombophilia

	Miscarriage	IUFD	Pre-eclampsia	Placenta abruption
Factor V Leiden	++	++	++	++
MTHFR 677TT	+	+	+	+
Hyperhomocysteinaemia	+	+	++	++
Factor II G20210A	+	+	+	++
Antiphospholipid syndrome	++	++	++	++
Combined defects	++	++	++	++

THROMBOPHILIA, EITHER CONGENITAL (FV Leiden, PTG 20210A) OR ACQUIRED (hyper-HCY, a-PL antibodies) WAS FOUND ASSOCIATED WITH RECURRENT ABORTION

ONLY IN LATE GESTATION

- ✓ **Rai et al 1995**
- ✓ **Green 1999**

BOTH IN EARLY AND LATE GESTATION

- ✓ **Younis et al 2000**
- ✓ **Pihusch, 2001**
- ✓ **Rai et al 2001**
- ✓ **Reznikoff - Etievant et al 2001**

EARLY RECURRENT ABORTION

- **INCLUSION CRITERIA**

- **At least 3 miscarriages or 2 in women older than 35**
- **Pregnancy documented by US**
- **Spontaneous abortion occurring between 6th and 13th week of pregnancy**

- **EXCLUSION CRITERIA**

- **Ectopic pregnancy**
- **Pregnancy documented by b-hCG only**
- **Blighted ovum**

ROUTINE SCREENING IN COUPLES WITH RECURRENT ABORTION

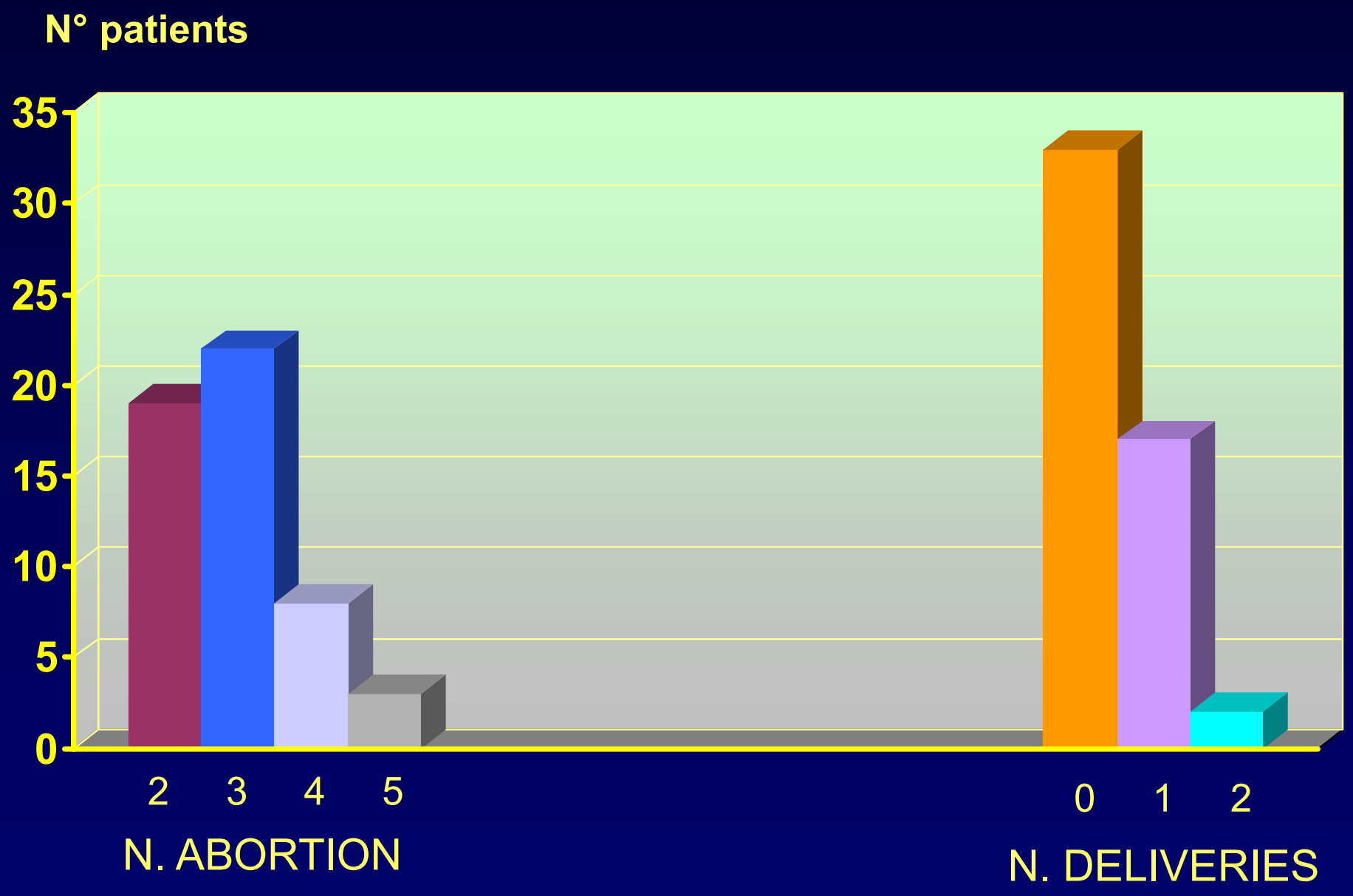
1° LEVEL:

- **Menstrual/reproductive hystory**
- **ATIII, PC, PS, APCr**
- **OCGT**
- **Vaginal and Cervical Swabs**
- **Autoimmunity (ACA, ANA, ENA, LAC, a-PL)**
- **Thyroid (TSH, f-T3, f-T4, Ab-TPO, Ab-TG)**
- **ULTRASOUND SCAN OF THE PELVIS**

2° LEVEL:

- **HYSTEROSCOPY**
- **KARIOTYPE (in both partners)**

CASES SERIES



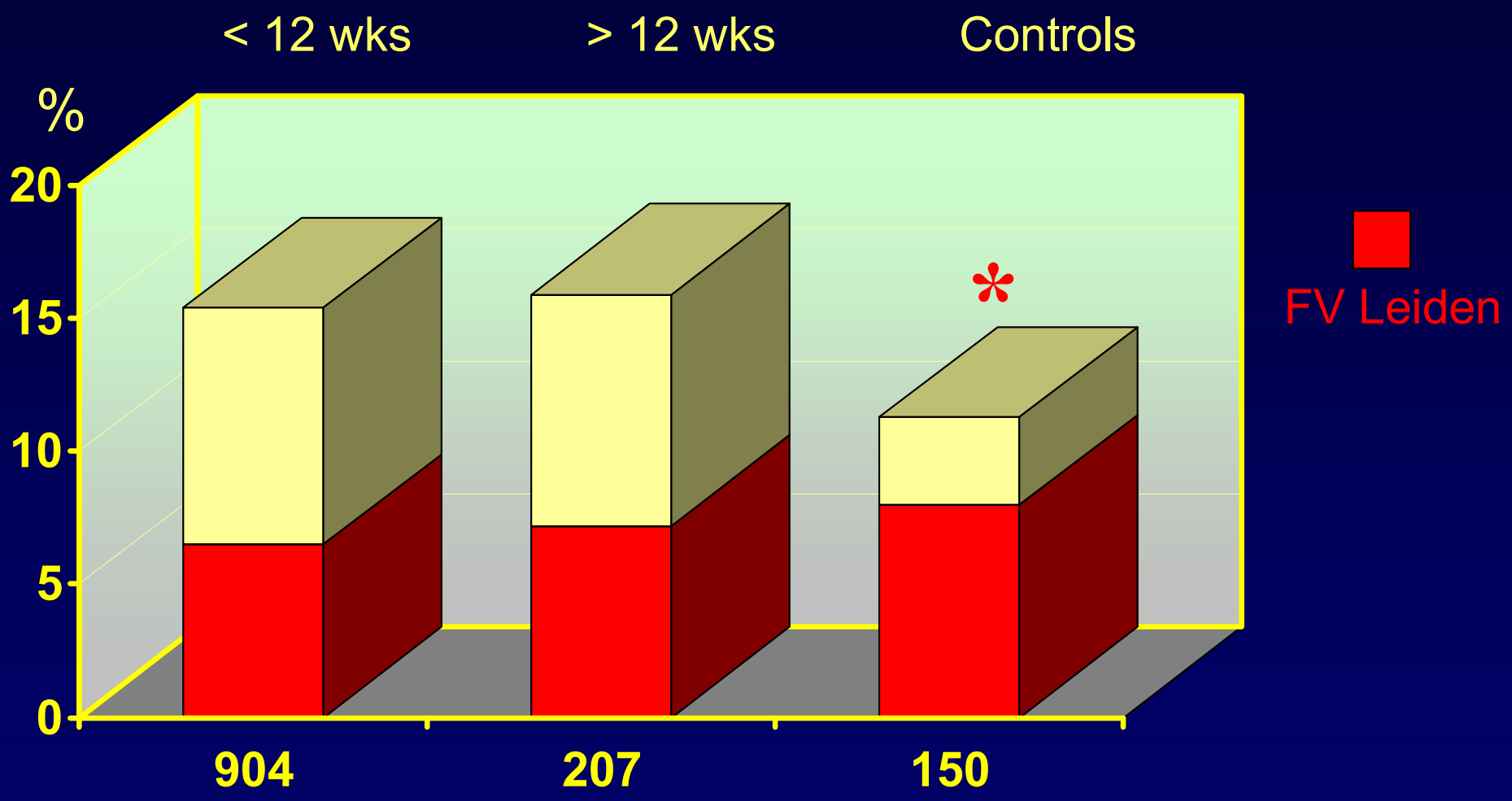
Normal Ranges	Patients with RSA
TSH (0.35-4.50 µUI/ml)	2.03 ± 1.07
FT3 (1.7-4.2 pg/ml)	3.27 ± 0.45
FT4 (6.1-16.7 pg/ml)	10.8 ± 0.7
PS (70-140%)	81.48 ± 18.40 *
PC (70-140%)	103.54 ± 12.92
ATIII (80-120%)	95.9 ± 21.8
FVIII (50-150%)	128.02 ± 30.94
FIX (60-150%)	109.56 ± 18.47
FXI (60-150%)	106.41 ± 16.2

*** One case has a deficit (< 41%)**

ACQUIRED THROMBOPHILIA

TEST	POSITIVE PATIENTS	%
ACA	2	3.8
ANA	5	9.6
ENA	0	0
LAC	0	0
APC-r	3 * (1 fV Leiden)	5.7
HYC (>15)	4	7.6

APC-r and EARLY RECURRENT ABORTION (3+)



Hum Reprod 2001; 16: 961-5

ACTIVATED PROTEIN C RESISTANCE could be

CONGENITAL when associated with the single point mutation (G→A) at nucleotide position 1691 of Factor V

ACQUIRED when associated with:

- ✓Antiphospholipid antibodies/LAC
- ✓Combined oral contraceptive
- ✓Pregnancy
- ✓Elevated levels of FACTOR VIII

Factor V Leiden (Odds Ratio)

- Early pregnancy loss (12-20 weeks) 2.01 (1.13-3.58)
- Late pregnant (recurrent) 7.83 (2.83-21-67
- Late pregnant (nonrecurrent) 3.26 (1.82-5.83)
- Severe preeclampsia 4.90 (1.30-18.30)
- Still birth 3.60 (1.40-9.40)
- Severe complications 3.70 (1.50-9.60)
  - (PET/Abruption/IUGR/SB)

Activated Protein C

- Leiden negative (Odds Ratio)

- Early pregnancy loss (12-20 weeks) 3.48 (1.58-7.70)
- Late pregnant (recurrent)
- Late pregnant (nonrecurrent)
- Severe preeclampsia
- Still birth
- Severe complications (PET/Abruption/IUGR/SB)

Prothrombin Gene (Odds Ratio)

- Early pregnancy loss (12-20 weeks) 2.58 (1.04-29.0)
- Late pregnant (recurrent)
- Late pregnant (nonrecurrent) 2.30 (1.09-4.87)
- Severe preeclampsia
- Still birth
- Severe complications 3.90 (1.10-14.6)
  (PET/Abruption/IUGR/SB)

Protein S

- Early pregnancy loss (12-20 weeks)
- Late pregnant (recurrent) 14.7 (11.6-28.0)
- Late pregnant (nonrecurrant)
- Severe preeclampsia
- Still birth
- Severe complications (PET/Abruption/IUGR/SB)

Hyperhomocysteinaemia
(incl MTHFR)

- Early pregnancy loss (12-20 weeks)
- Late pregnant (recurrent) 1.8 (1.1-3.5)
- Late pregnant (nonrecurrant)
- Severe preeclampsia
- Still birth
- Severe complications 3.1 (1.4-7.1)
  (PET/Abruption/IUGR/SB)

Recurrent Miscarriage

- Hyperhomocysteinaemia
- PAI anomaly
- Protein S
- Factor V Leiden
- Sticky platelet syndrome
  - Account for 40% of treatable disorders

- Antiphospholipid syndrome (Acquired)
  - 60% of treatable disorders
    - » Bick et al, Clin Appl Thromb Hemost, 2005, 11, 1.

Congenital Thrombophilia

- Protein S/ Protein C/ APCR/ ATIII/ Hyperhomocysteinaemia/Prothrombin gene/
  - OR for Post partum DVT 7.01 (3.58-15-74)

Management – Congenital Thrombophilia

- Protein S, APCR/Factor V Leiden, Prothrombin gene, Antithrombin III (in the presence of clinical pathology)

  - Trials of aspirin and heparin show improved outcome over aspirin alone.
  - Aspirin 60-150mg/day:
    - Start from evidence of pregnancy
    - Some stop aspirin at 34 weeks, but watch placental function. Recent trials show no harm in continuing to term
    - Treats the fetus and the mother!

Heparin

- In absence of recent thrombosis, prophylactic doses of heparin alone are sufficient
  - Heparin:- Start after evidence of fetal heart on ultrasound
  - Low molecular weight heparin Enoxaparin (Clexane) 1mg/Kg/day
  - Continue to 1 week postpartum
    - No need to cease for delivery
  - Where thrombosis treatment is required dose of Heparin is doubled.

Management

- Homocysteinaemia
  - Folate, Aspirin, Pyridoxine
  - Add heparin with history of maternal DVT

In the absence of a randomised trial, the poor pregnancy outcome associated with FVL mutation, coupled with the maternal risks during pregnancy, may justify routine screening for FVL and offering thromboprophylaxis for those with FVL mutation and evidence of placental thrombosis.

RCOG, 17, 2006

Conclusions

- Future research in this field will most likely deal with a number of aspects. Verification of the potentil associations of the various genetic thrombophilias with gestational pathologies is rapidly emerging. Currently 30-50% of vascular gastational patologies cannot be accounted for by thrombophilia. Whether yet unknown novel genetic or acquired thrombophilia will be found to play a role remains to be determined. While the mechanism has not been established it is intriguing to speculate whether antithrombotic strategies will be of value in this setting

Conclusions

- In view of the potential association of thrombophilia and RFL, and the high prevalence of thrombophilia in Caucasian populations, issue of screening are raised. As complete thrombophilia work-up is currently and costly, screening test are highly warranted. One such potential assay is the protein C global test, which, in preliminar study, was abnormal in 70% of 61 women with RFL compared to only 11% of 60% controls. The test successfully identified all women with thrombophilia. Morever , protein C globally identified 15/29 (52%) of women with RFL who did not have any thrombophilic defect, suggesting that this assay may have an additive value in diagnosis of women with pregnancy loss

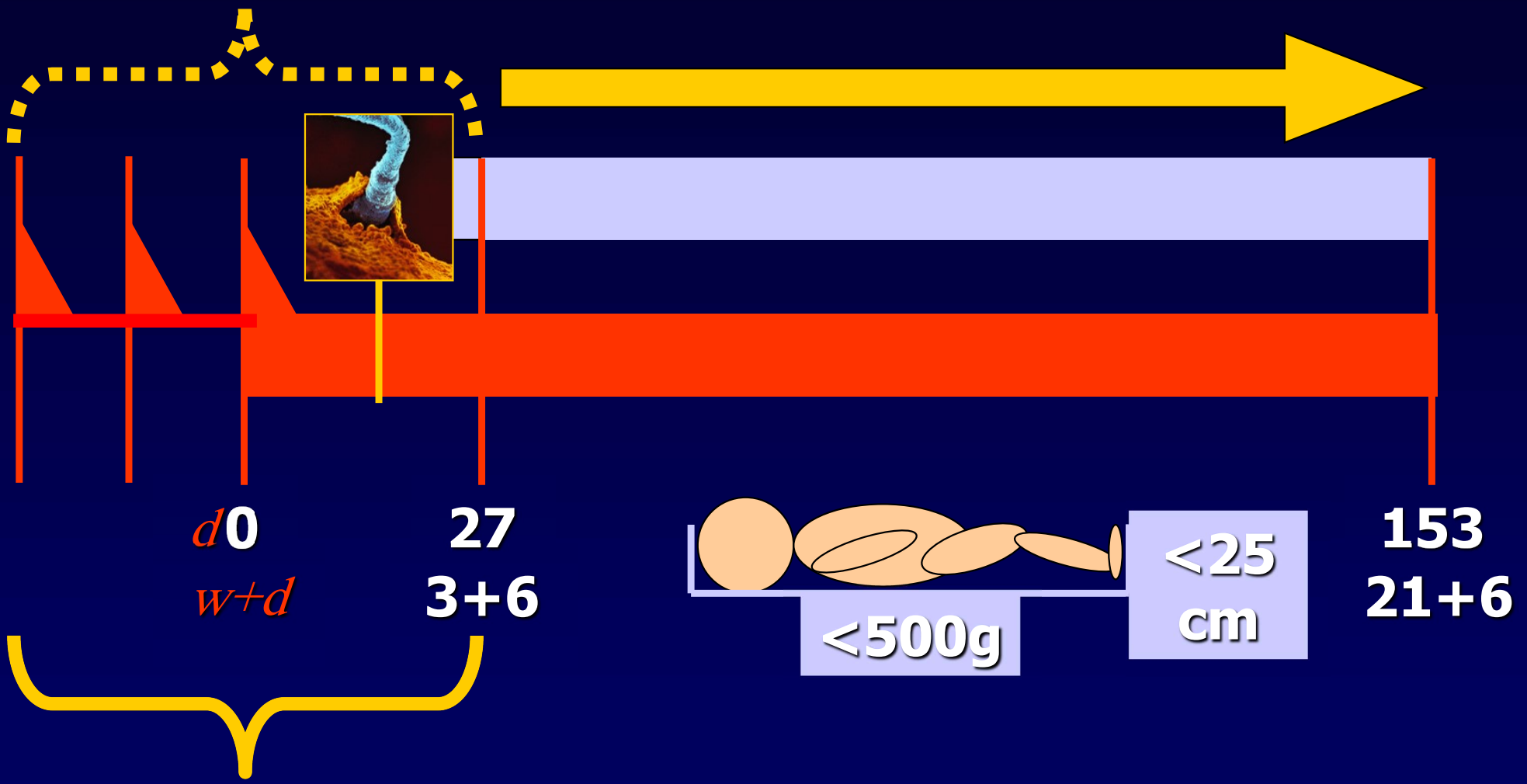
Conclusions

- The patogenetic mechanism responsible for placental vascular pathologies in women with thrombophilia have not been fully elucidated. Moreover, it is yet unknown why only some women with thrombophilia express vascular gestational pathologies while others do not. It is possible that this may relate to local factors affecting coagulation, fibrinolysis and vascular tone at tha level of placental vessels

Conclusions

- The role of antithrombotic therapeutic modalities deserves prospective clinical trials, several of which are currently ongoing, in order to improve outcome for a large population of women who currently experience poor gestational outcome. Because novel inherited and acquired prothrombotic abnormalities are currently under investigation, the question of prophilaxis in women with RFL who do not have a specific thrombophilic defect is currently often addressed by clinicians. This question should be answered by prospective randomized trials

PERICONCEPTIONAL MEDICINE



1° PREGNANCY: COUNSELING

RPL: COUNSELING→ PREGNANCY PLANNING →

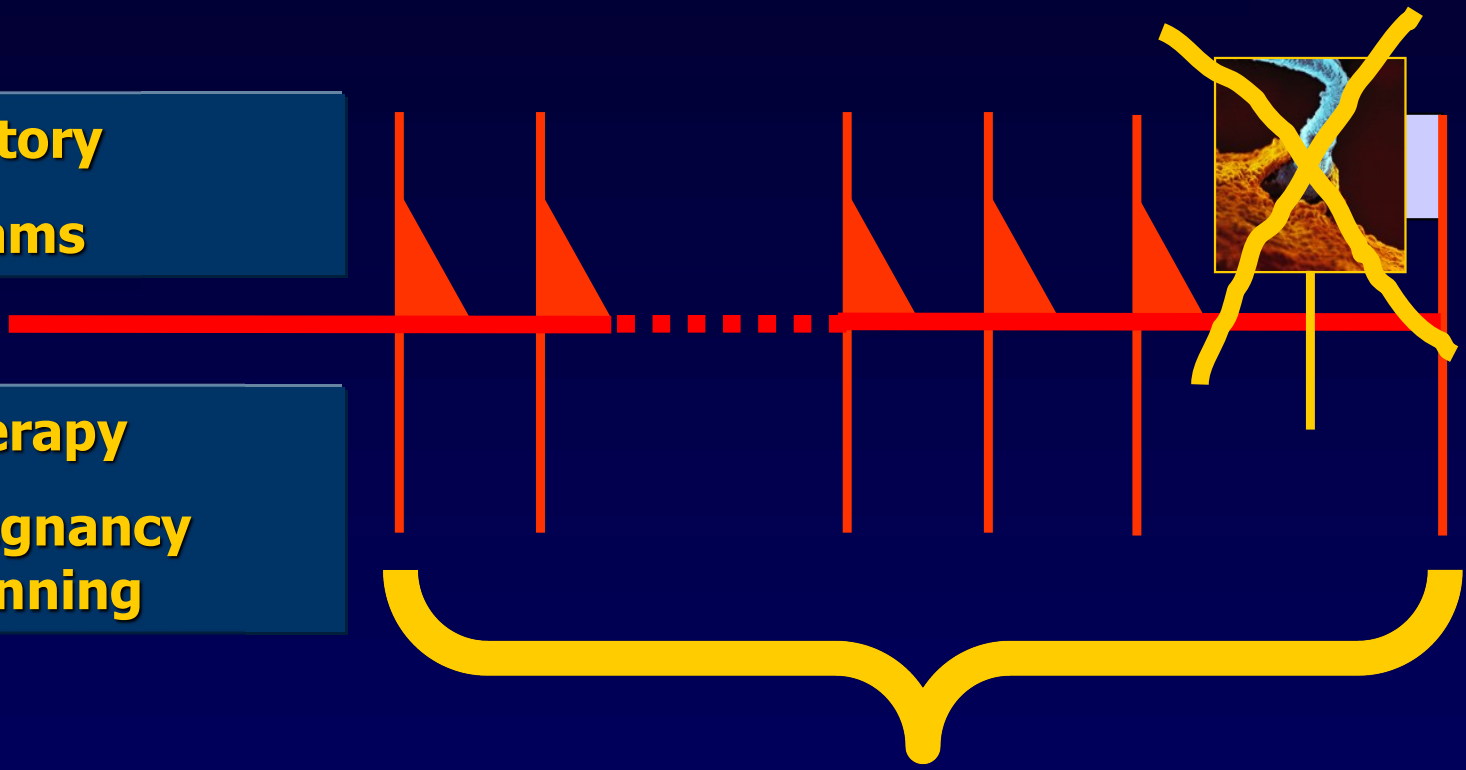
PERICONCEPTIONAL MEDICINE

History

Exams

Therapy

Pregnancy planning



1–2 years

REPRODUCTIVE MEDICINE

R Reproductive awareness

E Environmental toxicants and teratogens

F Folic acid suppementation

R Review genetic history

A Alcohol, tobacco and other substance use

M Medical conditions

E Evaluate immunizations and infectious diseases

D Domestic violence and psychosocial issues

Key Elements of Periconceptional Counseling

Medical history and medication review 1

- **Diabetes**: identify prior to pregnancy and optimize control
- **Hypertension**: avoid ACE inhibitors, angiotensin II receptor antagonist, thiazide diuretics
- **Epilepsy**: optimize control, folic acid, 1 mg/day
- **DVT**: switch from warfarin (Coumadin) to heparin
- **Depression / anxiety**: avoid benzodiazepines

Key Elements of Periconceptional Counseling

Genetic history

2

- Review family history of genetic issues
- Carrier screening (ethnic background): sickle cell anemia, thalassemia, Tay-Sachs disease
- Carrier screening (family history): cystic fibrosis, nonsyndromic hearing loss (connexin-26)

Key Elements of Periconceptional Counseling

Reproductive and Gyn history

- Review prior pregnancy outcomes and counsel about recurrence
- Review prior gyn history
- Review history of PID or other tubal issues

Key Elements of Periconceptional Counseling

Living environment, diet, weight, food and medications 4

- Folic acid supplement (400 mcg routine, 1 mg diabetes/epilepsy, 4 mg prev neural tube defect)
- Assess weight, calculate BMI, advise optimal BMI
- Recommend regular exercise in moderation
- Avoid hyperthermia (hot tubs, overheating)
- Assess risk of nutritional deficiencies (vegan, pica, milk intol, calcium or iron deficiency)
- Avoid overuse of: vitamine A (limit to 3,000 IU per day) and vitamine D (limit to 400 IUday)
- Limit caffeine to two cups of coffee or six glasses of soda per day
- Screen for domestic violence
- Household chemicals: avoid paint thinners and strippers, other solvents, pesticides
- Smoking cessation and avoidance of secondary smooking
- Screen for alcoholism and use of illegal drugs

Key Elements of Periconceptional Counseling

Fertility review and optimization

5

- Assess for regular ovulation, signs of PCOS
- Discuss fertility after birth control
- Review frequency and timing of sexual intercourse
- Refrain from use lubrificants

Key Elements of Periconceptional Counseling

Medical examination and testing: Immunization

- Test for infectious disease (e.g. HIV, syphilis)
- Hepatitis B immunization for at-risk patients
- Preconception immunizations (rubella, varicella)
- Counsel about avoidance of infections (e.g. toxoplasmosis, cytomegalovirus, parvovirus B19)
- Suggest a dental examination

Key Elements of Periconceptional Counseling

Male periconception counseling and assessment 1

- Medical history and review
- Review of potentially harmful exposure
- Avoid exposure to heat
- Avoid alcohol and smoking and other harmful substances
- Suggest sperm analysis

Ethnic origin	Screening recommended	Test	Frequency (%)
Black	Sickle cell trait β-thalassemia	Sickle cell smear MCV < 70	10% 5%
European jewish	Tay-Sachs disease carrier; Canavan, Cystic fibrosis, Familial Dysautonomia	Hexosaminidase A	4%
French canadian	Tay-Sachs disease carrier	Hexosaminidase A	> 5%
Mediterranean	α-, β -thalassemia	MCV < 70	10 to 20%
Southest asian (Laotian, Thai, Cambodian, Hmong)	α-, β -thalassemia	MCV < 70	20 to 40%
Indian, Middle Eastern	Sickle cell trait α-, β -thalassemia	Sickle cell smear MCV < 70	Unknown Unknown

MCV = mean corpuscolar volume.

Screening for infectious diseases

- Human immune deficiency virus (both in husband and wife)
- Syphilis (both in husband and wife)
- Hepatitis B antigen and antibody (both in husband and wife)
- Gonorrhea
- Chlamydia
- Testing for prior exposure to Parvovirus
- Testing for prior exposure to cytomegalovirus
- Testing for prior exposure to toxoplasma
- Testing for prior exposure to herpes (both in husband and wife)
- Testing for varicella immunity
- Testing for rubella immunity

The prognosis worsens with increasing maternal age and the number of previous miscarriages. The value of psychological support in improving pregnancy outcome has not been tested in the form of a randomised controlled trial. However, data from several nonrandomised studies have suggested that attendance at a dedicated early pregnancy clinic has a beneficial effect, although the mechanism is unclear

RCOG, 17, 2006

Work-up

- **Detailed history and physical examination**
- **TSH/thyroid hormones/antimicrosomal antibodies**
- **Hysterosalpingogram (HSG) or saline-instillation Sonohysterogram (SIS)**
- **LAC/ACA**
- **Cervico-vaginal-urethral microbiological evaluation**
- **Parental karyotypes**
- **If missed abortion diagnosed, obtain karyotype on villi, and NK cell count, If no specimen is available send paraffin blocks of prior D&E to rule out evidence of aneuploidy or immunopathy**

If the patient has a history of thromboembolism, severe IUGR, with or without fetal deaths after 16 weeks obtain the following:

* Antithrombin III
* Protein C
* Protein S
* Activated Protein C resistance/Factor V Leyden
- Heparin Co-factor
- PAI 1

Tests potentially useful in the evaluation of recurrent pregnancy loss

Parental and abortal karyotype

Intrauterine cavity assessment

Luteal phase endometrial biopsy

Thyroid hormones

Lupus anticoagulant

Anticardiolipin antibody IgG, IgM

Antiphosphatidylserine antibody IgG,IgM

Embriotoxic factor-lymphocyte assay

Platelet count

Proposed therapies

Gamete donation for Robertsonian translocation of homologous chromosomes

Synchronizing fertilization with ovulation

Supportive care

Oocyte donation for advanced maternal age

Surgical correction of an intrauterine filling defect

Correction of luteal phase insufficiency

- Progesterone
- Ovulation induction with or without pituitary desensitization

Correction of hypothyroidism-thyroid replacement

Correction of hyperprolactinemia-bromocriptine

Eradication of infection, if detected

Aspirin and heparin for antiphospholipid syndrome

Other immunotherapies given hystorically

- Immunization-leukocytes, intravenous immune globulin
- Immunosuppression-corticosteroids, progesterone

Management options for recurrent miscarriage before pregnancy

Condition	Management options
•Parental chromosome rearrangement	•Gamete donation
•Uterine septum or adhesions	•Hysteroscopic resection/division and temporary intrauterine device
•Thick uterine septum/double uterine horns, single cervix	•Abdominal metroplasty
•Presumed cervical incompetence with failed cerclage in previous pregnancy	•Cervical cerclage before conception
•Fibroids	•GnRH analogue and myomectomy
•LH hypersecretion	•GnRH analogue with gonadotrophins or laparoscopic ovarian diathermy /laser
•Thrombophilia	•Low-dose aspirin and/or low-dose heparin

SUMMARY

- Genetic causes predominate early in pregnancy and likely persist throughout . Both recurrent and sporadic first trimester losses are usually chromosomal in etiology (50-60%).

- "Non-genetic" causes rarely explain first trimester losses, and "treatment" is not usually indicated.

SUMMARY

- **In asymptomatic women** "non-genetic" causes rarely explain first trimester losses.
- **In asymptomatic women** even a positive screening test (hysterosalpingography, thrombophilia panel, HLA sharing) cannot be assumed to provide explanation.
- **In clinically normal individuals**, treatment rarely indicated.

SUMMARY

- In the cases of RPL: To diagnose APS → LA and ACA positivity, 6-8 wks intervals. If positive, give LWMH + Aspirin next pregnancy.
- Neither Leucocyte immunization nor IVIG do apply for a treatment in cases with RPL.
- We need further studies to use LWMH and /or aspirin in prevention of RPL (hereditary thrombophilia), apart from APS.

SUMMARY

- These patients require an understanding, sympathetic and supportive doctor.
- Multiple visits during the first trimester.
- 50 % of pts with 1st trimester RPL will have a successful pregnancy.
- Pts with 2nd trimester losses have a poorer prognosis.

“Why did your mother reject you?”

one asks, while peering down a microscope tube at the remnants of a spontaneously aborted embryo.

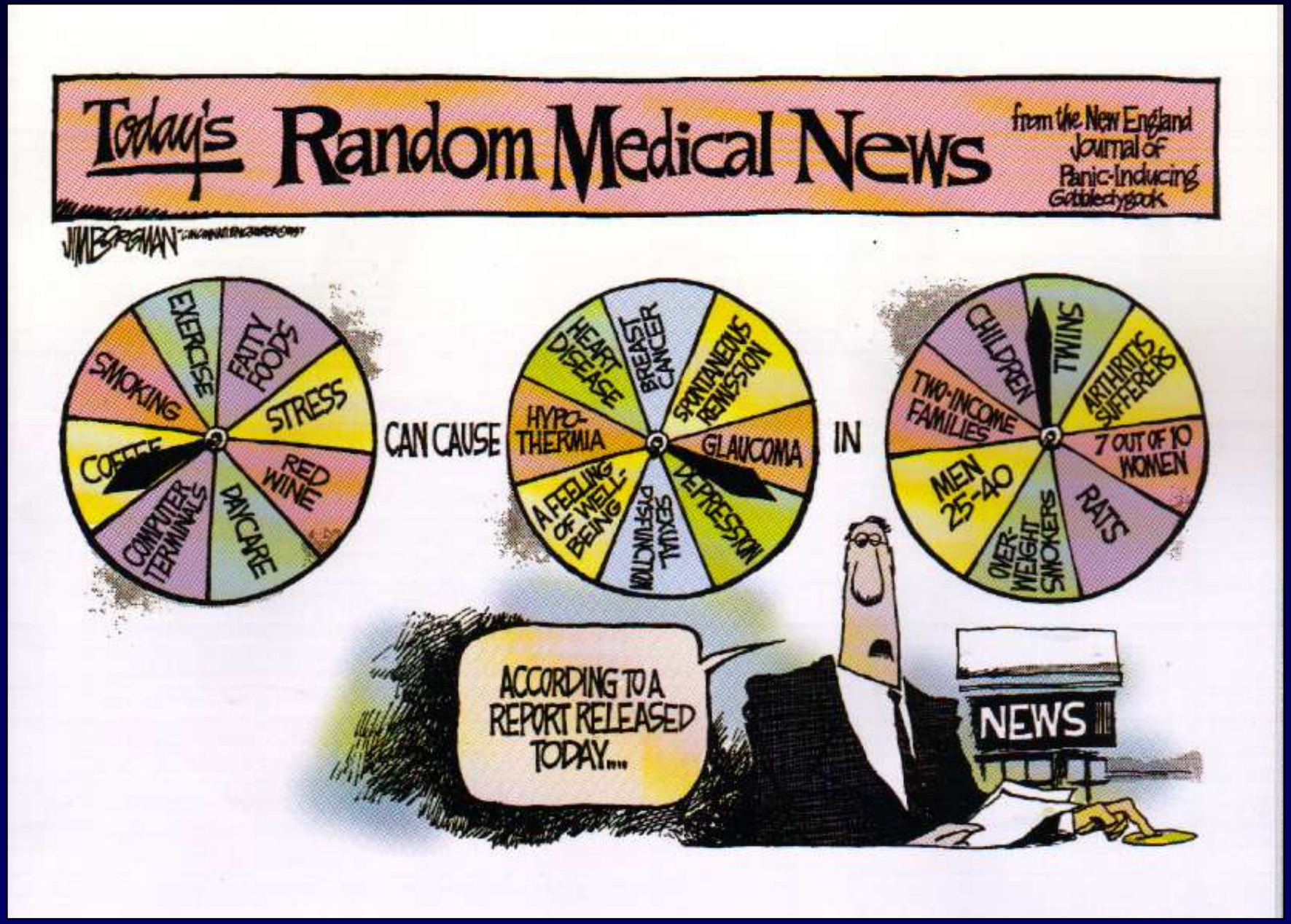
Recurrent pregnancy loss is emotionally frustrating for couples experiencing loss and often is a frustrating challenge for their physicians.

This frustration often results in the recommendation of unsubstantiated tests and therapies of dubious efficacy.

We risk adding still more unsubstantiated tests and therapies to an already cumbersome therapeutic repertoire, resulting in further expansion of health care expenditures and the potential exploitation of couples seeking care.

Recommendations regarding
the routine clinical use of the therapy
for recurrent pregnancy loss
should rely on level-one evidence from
the outcome of well-designed clinical trials
based on testable hypotheses using
diagnostic tests assessed by their performance.

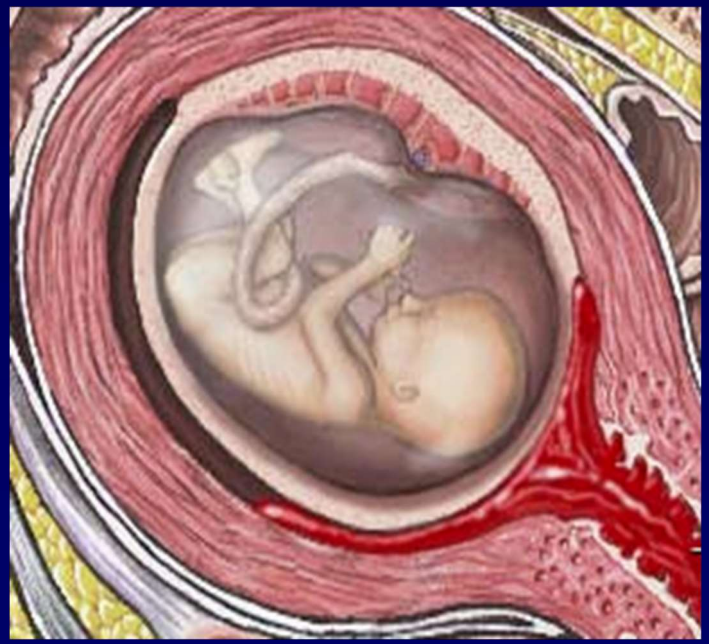
Understanding the potential mechanisms involved in recurrent pregnancy loss together with a caring, empathetic attitude toward the couple will enable amelioration of the emotional distress these couples encounter and facilitate a rational, cost-effective evaluation leading to appropriate consultation and effective therapy.



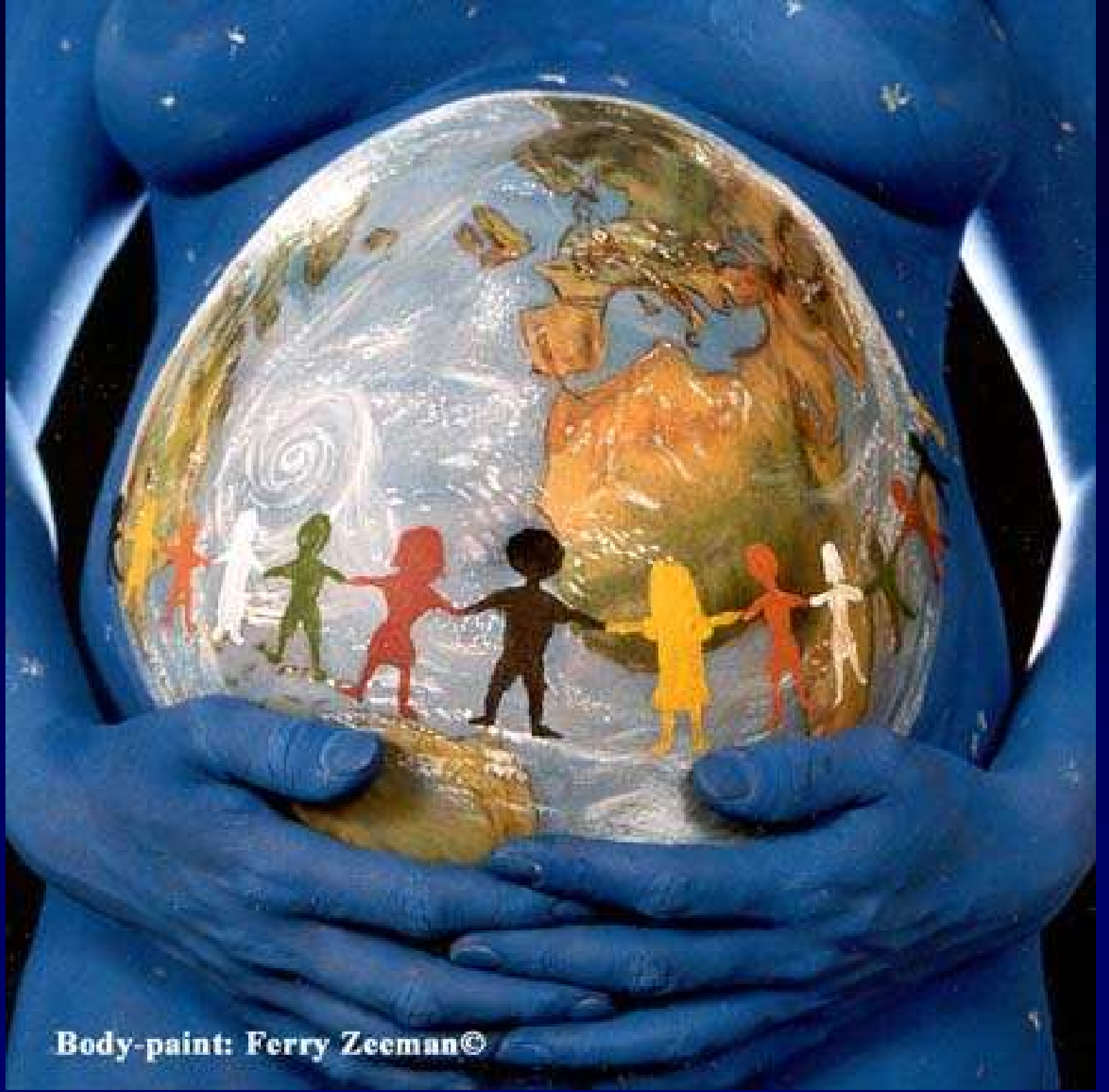
American Journal of Public Health, March 2004

CONCLUSIONS

NO CONSENSUS ON THERAPEUTIC REGIMENS
IN APA ASPIRIN AND HEPARIN IS THE TREATMENT OF CHOICE
IN THROMBOPHILIC PATIENTS STILL OPTIONS
ROLE OF PROGESTERONE INCREASINGLY ACCEPTED
ROLE OF STEROIDS CONTROVERSIAL
UNDER INVESTIGATION ANTIOXIDANTS AND PUFA



ARE HIGH RISK PREGNANCY A CONSEQUENCE OF MEDICAL PROGRESS


Body-paint: Ferry Zeeman©

Thank You
for your
attention

Comparision of aspirin versus aspirin and heparin therapy in IgG positive pregnancies

	Aspirin (n= 39)	Aspirin & Heparin (n= 33)	P
No. of live births	24 (61,5%)	28 (84.8%)	0.04
Mean gestational age (weeks) at delivery	37.04±0.8	36.98±0.81	0.8
No. of deliveries before 37 weeks gestation	5	4	>0.05
Mean birth wt (kg)	2.77±0.14	3.21±0.3	<0.001
Major complications at delivery	None	none	
Cogenital anomalies at birth	Hemangioma on chest in one baby	none	

(Goel et all. 2006)

Results from the Live-Enox study

The Live-Enox study was performed to compare the efficacy and safety of enoxaparin 40 mg/day and 80 mg/day on pregnancy outcomes in women with thrombophilia and history of RPL.

(Brenner et all. 2005)

Results from the Live-Enox study

Both doses of enoxaparin were shown to be equally effective and safe, with live birth rates of 84% and 78% in the enoxaparin 40mg/day and 80mg/day groups respectively

(Brenner et all. 2005)

Birth details of mothers and infants after enoxaparin 40 mg/d or 80 mg/d

	Enoxaparin 40 mg/d	Enoxaparin 80 mg/d	P
No. of women in group, n	89	91	
Details of mothers			
Live born neonates, n	70	65	.310
Gestational period, n (%)			
< 30 wk	3 (4.3)	2 (3.1)	.521
30-33 wk	0 (0)	1 (1.5)	.971
34-36 wk	4 (5.7)	9 (13.8)	.112
> 36 wk	63 (90.0)	53 (81.5)	.076
Preeclampsia, n (%)	3 (3.4)	4 (4.4)	.722
Placental abruptio, n (%)	4 (4.5)	3 (3.3)	.677

(Brenner et all. 2005)

Birth details of mothers and infants after enoxaparin 40 mg/d or 80 mg/d

	Enoxaparin 40 mg/d	Enoxaparin 80 mg/d	P
No. of women in group, n	89	91	
Details of newborns			
Birth weight			
Mean, g	3051	2998	.653
< 1500 g, n (%)	2 (3.1)	2 (3.2)	.953
1500-2000 g, n (%)	9 (13.8)	8 (12.7)	.813
> 2500g, n (%)	54 (83.1)	53 (84.1)	.513

(Brenner et all. 2005)

Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant

- Unfractionated heparin combined with aspirin (two trials; n=140) significantly reduced pregnancy loss compared to aspirine alone (relative risk (RR) 0.46, 95% confidence interval (CI) 0.29 to 0.71)
- Low molecular weight heparin (LMWH) combined with aspirin compared to aspirin (one trial; n=98) did not significantly reduce pregnancy loss (RR 0.78,95% CI 0.39 to 1.57)
- There was no advantage in high-dose, over low-dose, unfractional heparin (one trial; n=50)
- Three trials of aspirin alone (n=135) showed no significant reduction in pregnancy loss (RR 1.05, 95% CI 0.66 to 1.68)
- Prednisone and aspirine (three trials; n=286) resulted in a significant increase in prematurity when compared to placebo, aspirin, and heparin combined with aspirin

(Empson et all. 2005)

Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant

Combined unfractionated heparine and aspirin may reduce pregnancy loss by 54%.

(Empson et all. 2005)

Outcome data from women who had live births

Variable	LMWH + LDA	UFH + LDA	P
Live births	21/25 (84)	20/25 (80)	1.00
EGA at birth (wk)	37.3 ± 1.09	38.1 ± 1.65	.08
Birth weight (g)	3047 ± 339	2973 ± 319	.47
Vaginal delivery	14/21 (66.7)	15/20 (75)	1.00

(Noble et all. 2005)

Outcome data from women who had miscarriages

Variable	LMWH + LDA	UFH + LDA	P
Miscarriages	4/25 (16)	5/25 (20)	1.00
EGA at miscarriages (wk)	8.7 ± 1.2	7.2 ± 1.3	< .05
Blighted ovum	1/4 (25)	2/5 (40)	1.00
No fetal heart motion	3/4 (75)	3/5 (60)	1.00
Abnormal karyotypes	2/4 (50)	2/5 (40)	1.00

(Noble et all. 2005)

Treatment outcome in women suffering from recurrent miscarriage and antiphospholipid syndrome

148 observed women suffering from recurrent abortion with presence of lupus anticoagulant antibodies (LA) and/or high moderate concentration of anticardiolipin antibodies (ACA) have been divided randomly into followed three groups :

1) 56 patients treated by low-dose of acetylsalicylic acid (LDA, 75 mg daily)
2) 39 patients treated by low-molecular weight heparin (applied in dose of 20 g daily)
3) 53 patients treated by LDA and low molecular weight heparin simultaneously

(Malinowsky et all. 2003)

Treatment outcome in women suffering from recurrent miscarriage and antiphospholipid syndrome

In the group where only low-dose of acetylsalicylic acid was applied the success of pregnancy equaled 89.3%, in the group where only low molecular weight heparin was applied the successful pregnancy equaled 81.1% and in the group with acetylsalicylic acid and low molecular weight heparin being applied together the successful pregnancy equaled 92.5%.

(Malinowsky et all. 2003)

Factor Leiden in cohorts of women with pregnancy loss (PL)

Author (ref)	PL	Controls	OR	95% CL	P
Ridker et al	9/113 (8%)	16/437 (3.7%)	2.3	1.0-5.2	0.05
Grandone et al	7/43 (16%)	5/118 (4%)	4.4	1.3-1.47	0.01
Brenner et al	24/76 (32%)	11/106 (10%)	4.0	1.8-8.8	0.001
Wramsby et al	13/84 (15.5%)	2/69 (2.9%)	7.2	1.5-34.0	0.008

(Brenner&Kupferminc, 2003)

Practice points

- Women with recurrent miscarriages or IUFD should be evaluted for thrombophilia
- In patients with placental abruption or placental infarction it is advisable to analyse for thrombophilia
- Women with severe IUGR should be considered for evalutation of thrombophilia
- Patients who, following gestational vascular complications, are found to harbour thrombophilia should be offered participation in prospective randomized trials or should be treated on an individual basis

(Brenner&Kupferminc, 2003)

Clinical efficacy in abortion

- Recurrent miscarriage is a vexing clinical problem facing about 1% of couples
- In 50%of cases the exact underlying physio-pathology mechanisms remain unknown
- In up to 28% of the cases it is due to a luteal phase defect
- Progesterone might also play a significant role in establishing an adequate immune environment.
- Progesterone stimulates the lymphocytes of a pregnant woman to synthesize a 34-kDa protein known as progesterone-induced blocking factor (PIBF), which mediates both the immunomodulatory and anti-abortive properties of progesterone

Mechanisms for the maintenance of pregnancy

- Signs of apoptosis, already apparent by d26 of the menstrual cycle can be reduced with either hCG or progesterone treatment.
- The clinical utility of these findings includes a rational use of luteal-phase support for treatment of women with infertility and/or recurrent pregnancy loss

Lovely et al. *J Clin Endocrinol Metab 2006.*

Risk Factors for Spontaneous Miscarriage

Parameter	*N*	*Coding*	*Odds ratio*	*95% confidence interval*	*p-value*
Age (years)	862	Ref. 0-≤ 33/>33	1.76	1.00 - 3.09	0.051
BMI (kg/m^2)	837	Ref. >20/0-≤ 20	2.33	1.30 – 4.19	0.005
Progesterone (ng/ml)	862	Ref. >12/0 -≤12	2.24	1.26 – 4.00	0.006
PSQ demands (score)	844	Ref. 0-≤43/>43	1.12	0.61 - 2.05	0.117

Arck et al. *Reprod Biomed online 2008*

Identification of Patients at Risk for Subsequent Miscarriage

Birthday:	1964-1-1
Age of patient's partner:	44
Smoker:	No
Number of children:	0
Height in cm:	172
Weight in kg:	70
Gestational age:	7
Expect more than one child:	No
Progesteron in ng/ml:	12
You feel that too many demands are being made on you?	often
You have too many things to do?	sometimes
You feel that you are in a hurry?	sometimes
You have enough time for yourself?	sometimes
You feel under pressure from deadlines?	sometimes

Calculated miscarriage risk:

Please print this page and keep it with your patient file.

Print page | Finish

Definition

- Recurrent Spontaneous Abortion (RSA) is defined as **three or more consecutive** pregnancy losses prior to the 20th week of gestation.

Threatened abortion

- Vaginal bleeding while cervix is closed
- Evolution:
  - Spontaneous abortion
  - Pregnancy may proceed normally

Etiology

- One in five of all pregnancies concerned
- Often unclear and may be multifactorial

Prevalence and etiology

- **50 to 60% of chromosomal abnormalities, maternal infections, genital tract or endocrine abnormalities, anti-phospholipids antibodies, cigarette smoking or environmental factors**

Role of Immune Mediators, Hormones and neurotransmitters

- The feto-placental unit as a semi-graft
- Up-regulation of P4 receptors and release of P4-induced blocking factor (PIBF)
- Effector mechanisms in the maternal immunoresponse
- The cellular T cell system
- Natural killer (NK) cells

Role of Immune Mediators, Hormones and neurotransmitters

- Immunology of pregnancy
  - There is a maternal immune reaction to the allogeneic pregnancy
  - The trophoblast is able to eliminate abortogenetic maternal B cell and T cell responses
  - The presence of progesterone and its interaction with progesterone receptors at the decidual level appears to play a major role in this defense strategy

Role of Immune Mediators, Hormones and neurotransmitters

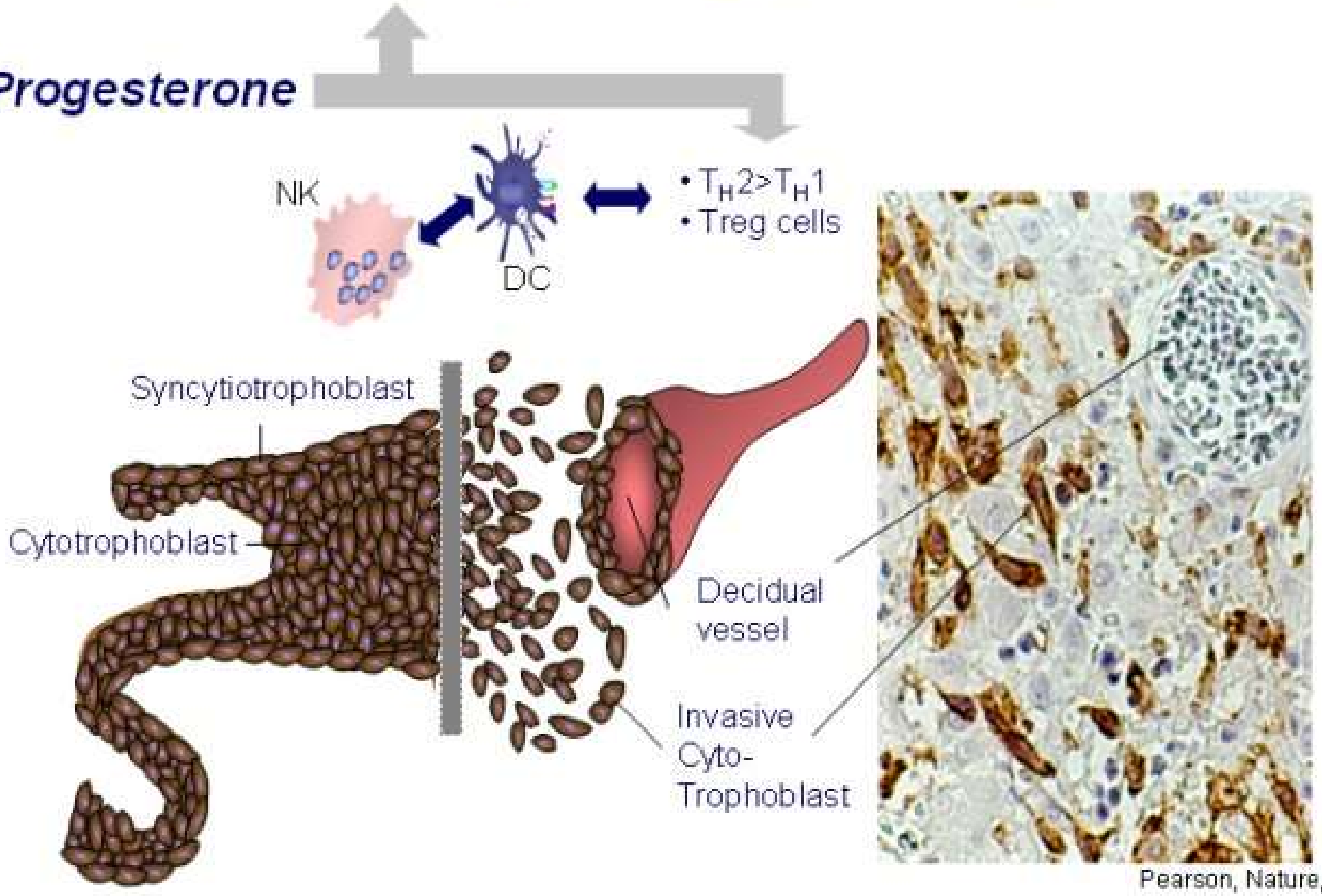
- Immunology of pregnancy
  - Activation of immune system is necessary in normal pregnancy
  - This is possible through an up regulation of progesterone receptors on natural killer (NK) cells in the decidua or on lymphocytes amongst placental cells
  - Progesterone-induced blocking factor (PIBF) exerts a substantial anti-abortive effect in-vivo.

Role of Immune Mediators, Hormones and neurotransmitters

- Immunology of pregnancy
- Several cytokines are involved in the immunomodulatory effects in early pregnancy
- Th-1 cytokines induce several cell-mediated cytotoxic and inflammatory reactions.
- Th-2 cytokines are necessary for the trophoblast to secrete hPL and hCG
- In successful pregancy, the normal profile is a Th2 type immunity.

'Immunity's Pregnant Pause'

Progesterone



Pearson, Nature, (2002)
Arck *et al.* J Reprod Fert Med (2006)

Role of Immune Mediators, Hormones and neurotransmitters

- Immunological recognition of pregnancy is important for the maintenance of gestation
- Inadequate recognition of fetal antigens might result in failed pregnancy
- Normal human pregnancy is characterized by low peripheral NK activity
- ↑ NK activity seems to play a role in spontaneous abortion of unknown etiology

Role of Immune Mediators, Hormones and neurotransmitters

- **Role of P4 in immunomodulation of pregnancy**
- Blood lymphocytes and decidual CD56+ and T cells develop specific progesterone receptors
- P4 exert a significant and dose-dependant inhibitory effect on lymphocyte cytotoxicity
- In presence of progesterone, activated lymphocytes and decidual CD56+ cells synthetise progesterone-induced blocking factor (PIBF), which exerts a substantial anti-abortive effect in-vivo (stimulating Th-2 cytokines production)

Role of Immune Mediators, Hormones and neurotransmitters

- P4 and immunology of pregnancy
  - Suppression of T-cell reactions
  - Inhibition of NK cells
  - Synergistic action with Pg E2

Conclusions

- Role of P4 and immunology of pregnancy:

- **Unexplained spontaneous abortion might be attributable to deleterious immune response of the mother toward the fetus**
- **Progesterone (P4) might play a significant role in establishing an adequate immune environment during the early stages of pregnancy**

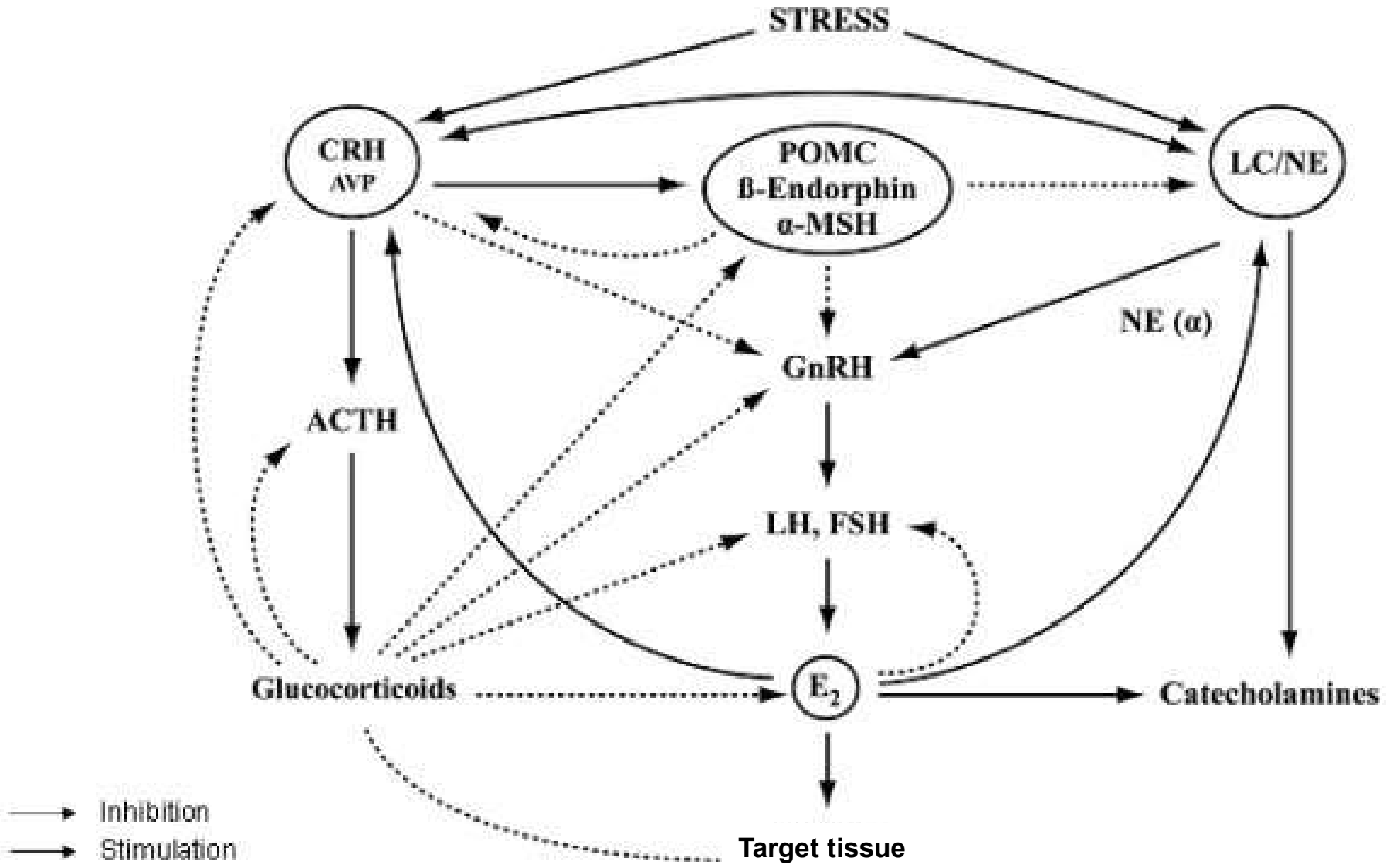
Environmental factors

•Maternel stress is linked to Early Pregnancy Loss (mostly within the 3 weeks of conception)

- 22 pregnancies
- 13 miscarriages (mean time of 16 days from ovulation to fetal loss)
- RR = 2,7-times more likely to experience a miscarriage if maternal urinary cortisol were above their usual baseline value.

Nepomnaschy et al. *Proc Natl Acad Sci USA 2006*
Arck et al. *Am J Reprod Immunology 2001*

Stress Inhibits HPG Axis via Activation of the HPA Axis and the Sympathetic System



Nepomnaschy *et al.* *Ann N Y Acad Sci 2007*

5α Pregnanolone (*) has an anxiolytic effect

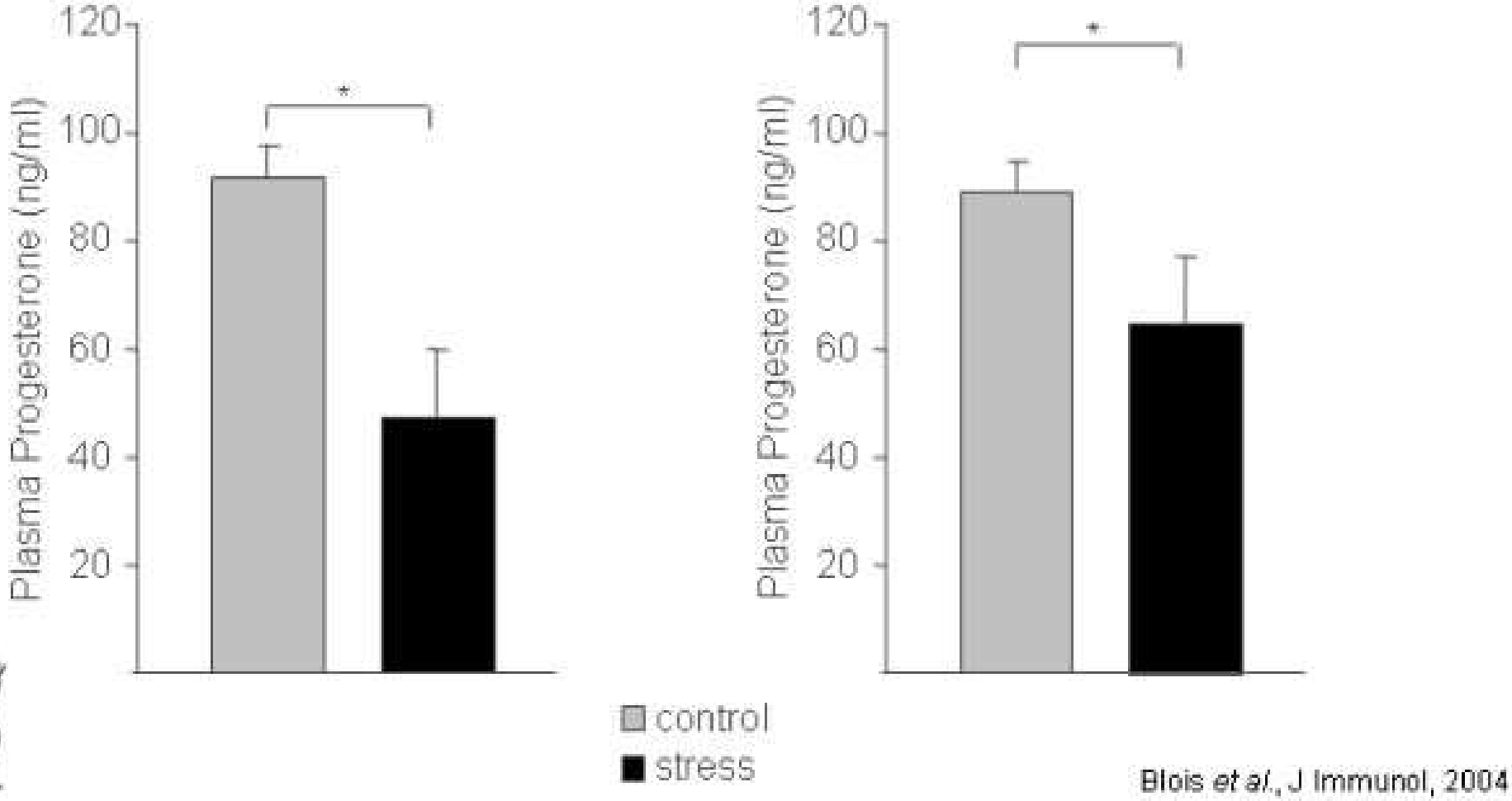
- Rapid effect (non genomic)
- Mediated by the $GABA_A$ Rec

Stress ↑ Th-1 cytokines (abortive) and ↓ P4 conc. and PIBF

(*) oral progesterone metabolite

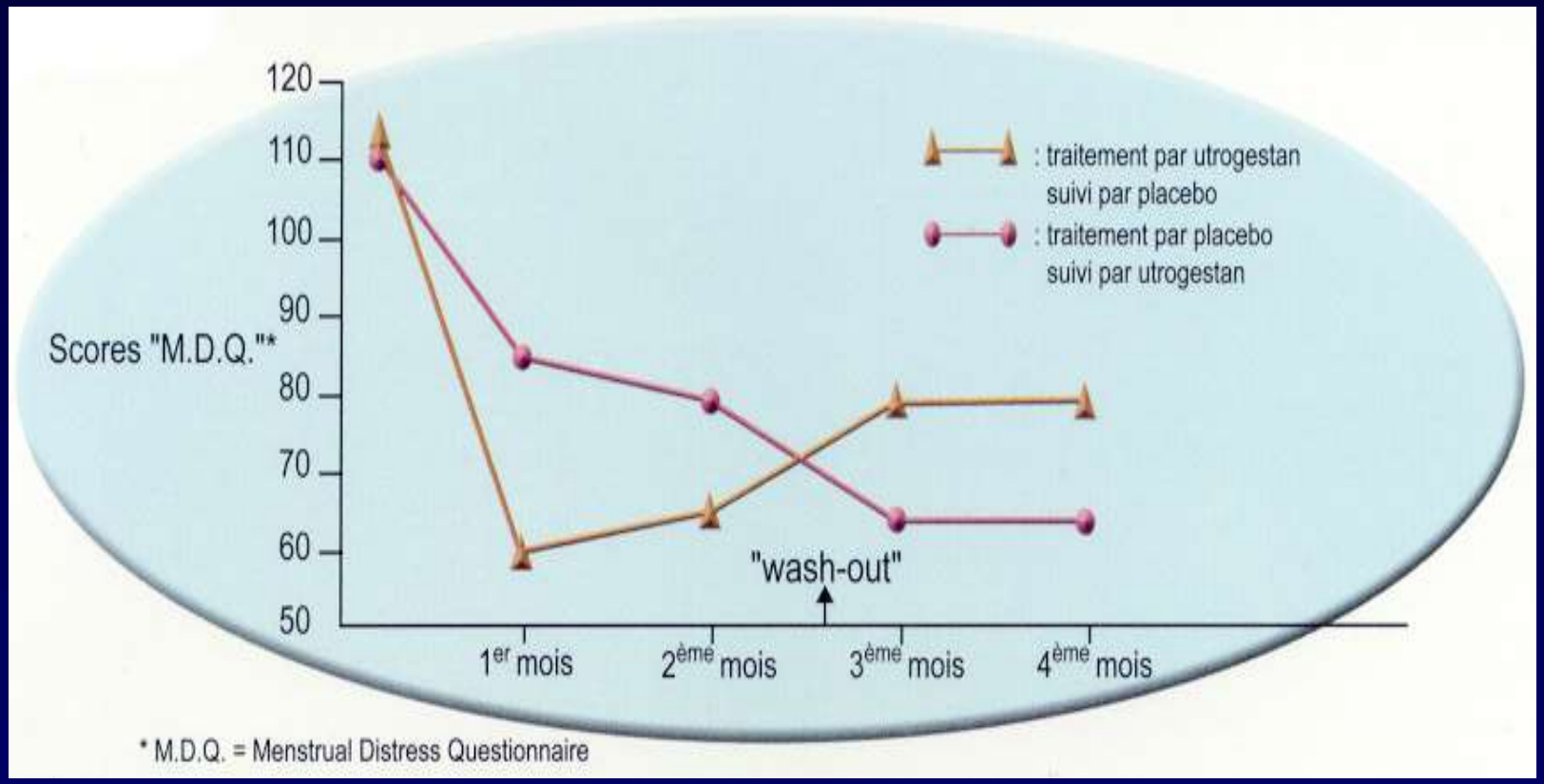
Bitran et al 1995. Rapkin et al 1997
Blois et al. *J Immunol 2004*

Prenatal Stress Alters Endocrine Hemostasis



Blois *et al.*, J Immunol, 2004
Unpublished data

Tranquilizing effect of PROGESTERONE against stress and depression



Dennerstein L et al. *BMJ 1985; **290**: 1617-1621*

Luteal Phase Defect and P4 blood levels

~ MID-LUTEAL PHASE
measurements of progesterone below 10 ng/ml (Blood measurements of progesterone are not always the best way to diagnose a LPD).

- Women with normal cycles compared to women with LPD
- Optimal P4 serum discriminatory level between luteal phase defect and normal cycles:

  ≤ 21 nmol/L

- 70% of sensitivity and 71% of specificity
- **In women with recurrent abortion**
  - **Incidence of LPD:** **40%**
  - **Successful pregnancies** **81%** (after P4 treatment)

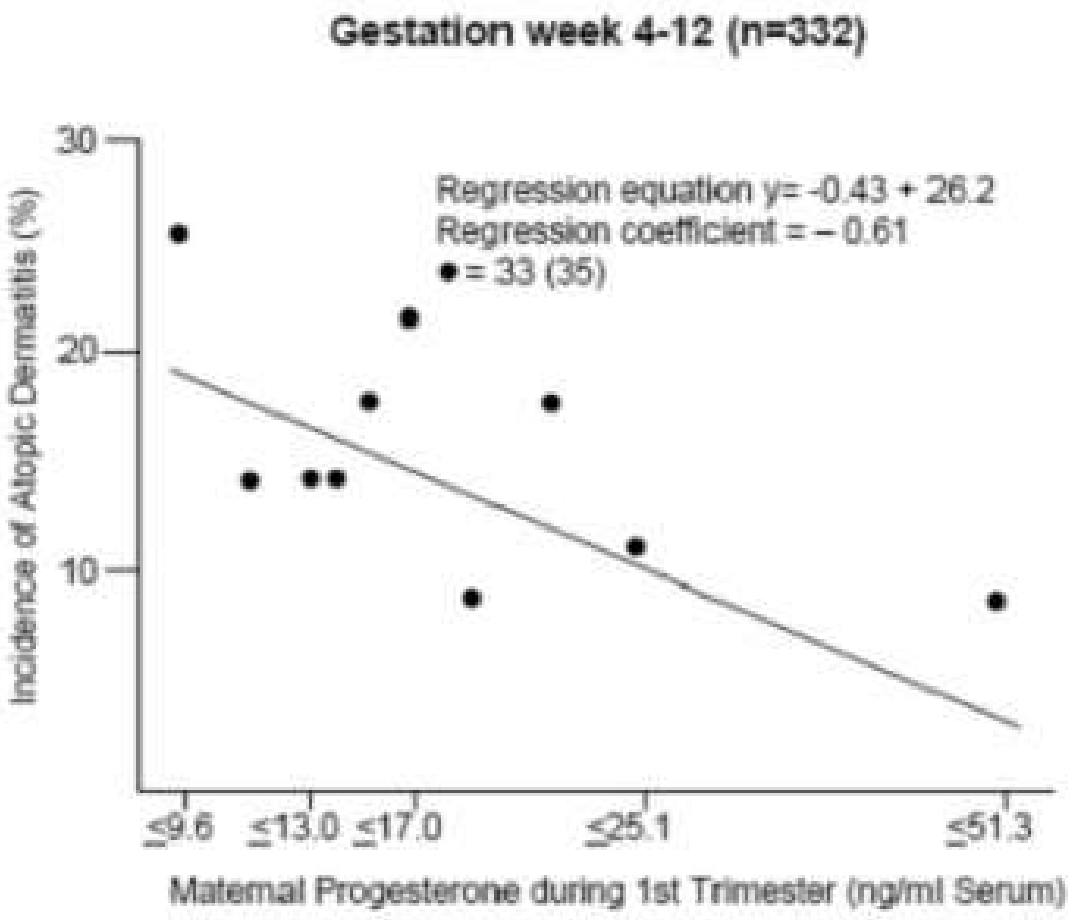
Clinical efficacy

Pregnancy rates after a single treatment course of clomiphene citrate followed by oral or vaginal micronized progesterone supplementation (300 mg/day, at bedtime). Values are expressed as numbers (%)

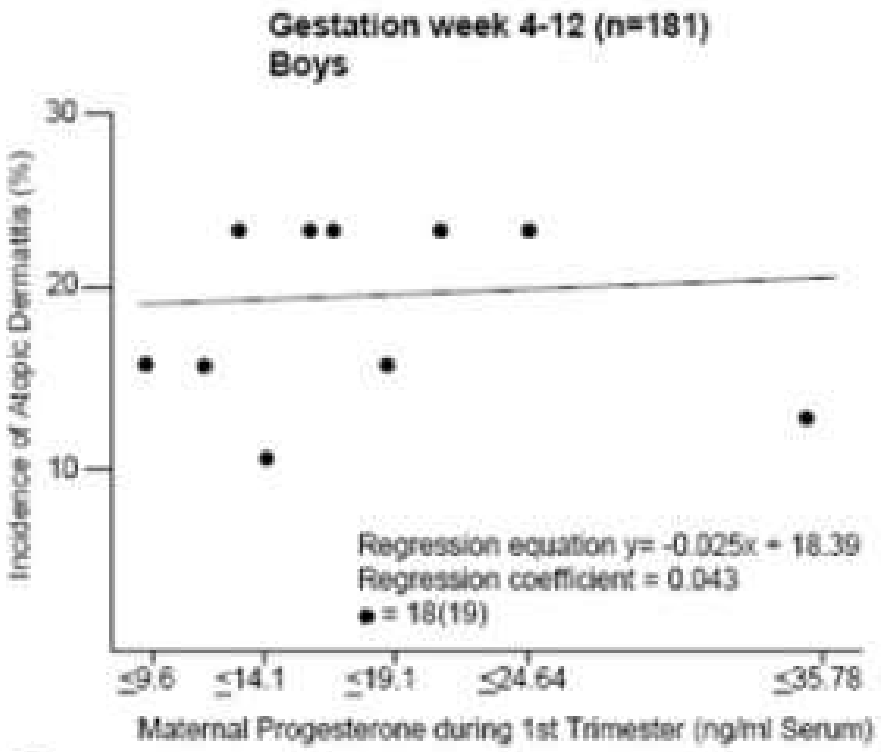
	Route of progesterone supplementation	
	Oral	*Vaginal*
Total cases	153 (100)	146 (100)
Pregnancy	17 (11.1)*	8 (5.5)
Twin pregnancy	1 (0.7)	0
Ectopic pregnancy	1 (0.7)	1 (0.7)
Spontaneous abortion	1 (0.7)	1 (0.7)
Intrauterine fetal death	1 (0.7)	0
Continuing pregnancy	14 (9.2)†	6 (4.1)

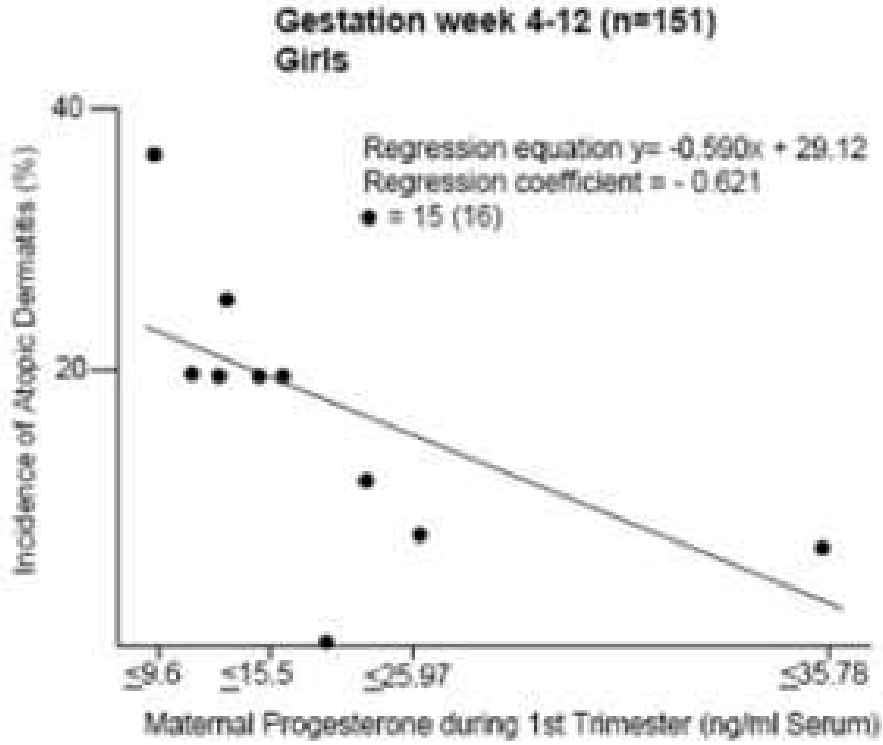
*$z = 2.11$, $p < 0.04$; †$z = 2.07$, $p < 0.04$; both by the z-test for instances (the zI-test)

Increased Incidence of Atopic Dermatitis Is Associated with Low Levels of Progesterone during First Trimester



Girls Reveal a Higher Vulnerability to Develop Atopic Dermatitis upon Low Progesterone During 1st Trimester





Obstetrical conditions associated with APA

Median prevalence of APA in RPL patients:

- ACA: 8%
- LAC: 7%

Immunologic tests of no clinical benefit

Antinuclear antibodies
IgA isotype of anticardiolipin and antiphosphatidylserine
Antiphosphatidic acid
Antiphosphatidylcholine
Antiphosphatidylethanolamine
Antiphosphatidylglycerol
Antiphosphatidylinositol
Antihistones
Anti-DNA (SS DNA, DS DNA)
Rheumatoid factor
Complement
Smooth muscle antibodies
Antiovarian and antiendometrial antibodies
Antibodies to gonadotropins and gonadal steroids
Antipaternal cytotoxic antibodies (leukocyte antibody detection assay)
Mixed lymphocyte culture reactivities
Human leukocyte antigen (HLA) profiles, including HLA-DR, -DP, -DQ
Peripheral blood immunophenotypes, including $CD56^{+}$ cells
Embriocytotoxicity of peripheral serum
Serum cytokines and adhesion molecules

THREATENED ABORTION

RATIONALE

Suboptimal progesterone production in pathologic pregnancies.

- **Serum progesterone (P)** levels were determined at the time of routine prenatal registration (227 patients) or upon presentation for evaluation of vaginal bleeding and/or abdominopelvic cramping/pain (135 patients).
- **P** associated with a **normal intrauterine gestation** was **24.63** +/- 4.19 (SD) **ng/mL** as **compared** with **6.29** +/- 2.43 ng/mL and **6.02** +/- 2.39 ng/mL for **spontaneous abortions** and **ectopic gestations,** respectively.
- Further, **P** differed between **asymptomatic** (**11.92** +/- 9.61 ng/mL) and **symptomatic** patients (**4.81** +/- 3.92 ng/mL) who were subsequently shown to have an abnormal gestation.
- By establishing a P cutoff point of **< or = 14.2 ng/mL** and **< or = 10.5 ng/mL** in **asymptomatic** and **symptomatic** patients, respectively, **100%** screening **sensitivity** was reached.
- P is therefore an excellent adjunctive marker for prediction of early pregnancy outcome, and in some cases qualitative abnormalities in chorionic gonadotropin may dictate its production.

Spontaneous and Habitual Abortion

CARL T. JAVERT M.D.

Professor of Clinical Obstetrics and Gynecology, College of Physicians and Surgeons of Columbia University; Director of Obstetrics and Gynecology, Woman's Hospital Division of St. Luke's Hospital; Attending Obstetrician and Gynecologist, New York Hospital. Formerly Associate Professor of Clinical Obstetrics and Gynecology, Cornell University Medical College; Obstetrical and Gynecological Pathologist, Woman's Clinic (Lying-In Hospital), New York Hospital

THE BLAKISTON DIVISION
McGraw-Hill Book Company, Inc.
NEW YORK TORONTO LONDON
1957

1957

This book is dedicated to the millions of men, women, and children who escaped the fate of an abortus in their first struggle for existence (in utero) and are now engaged in their second struggle for life (ex utero) which, even with the additional hazards of war, marriage, pregnancy, accident, and disease, is much less dangerous

OBSTETRIC CAUSES OF VAGINAL BLEEDING IN SPONTANEOUS ABORTIONS (1947 TO 1954)

Cause	*Number*	*Per cent*
Decidual hemorrhage	1,085	54.25
Abortion other than spontaneous	815	40.75
Placenta previa	35	1.75
Premature separation	32	1.6
Hydatidiform mole	21	1.05
Bicornuate uterus	11	0.55
Cervical pregnancy	1	0.05
Total	2,000	100.00

DECIDUA: NORMAL AND ABNORMAL HISTOLOGIC STATUS IN SPONTANEOUS ABORTIONS

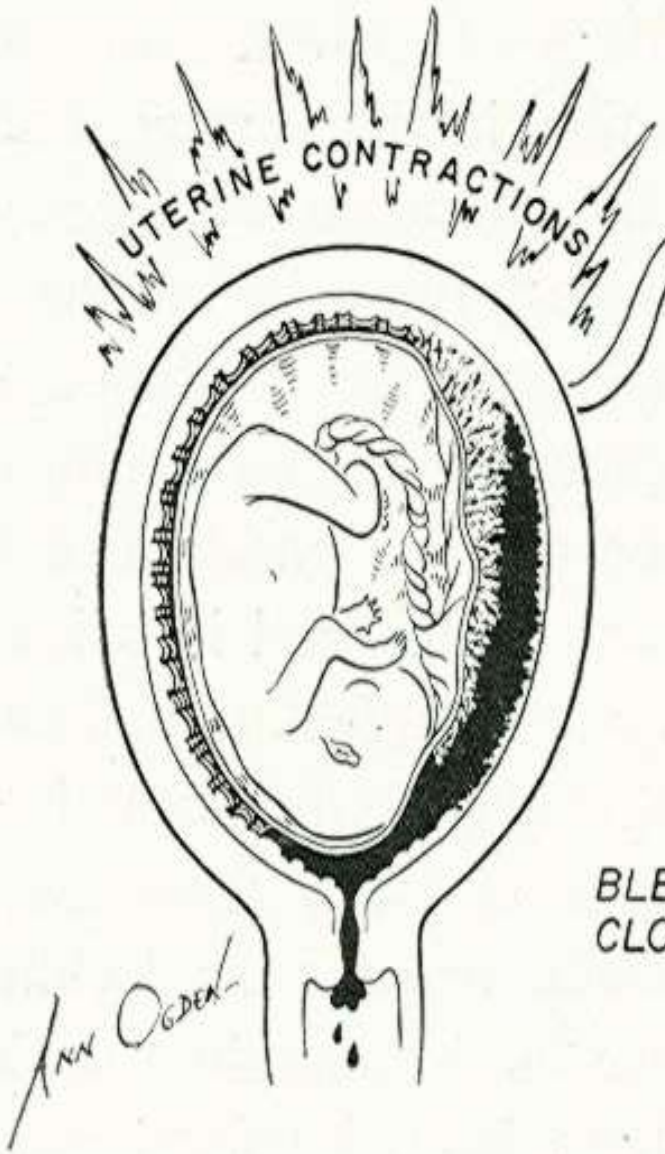
Status	*Spontaneous abortion*		*Control material* *	
	Number	*Per cent*	*Number*	*Per cent*
Normal	130	7.4	285	71.9
Abnormal:				
Hemorrhage	1,085	61.4	40	10.1
Degeneration	892	50.5	89	22.5
Infection	724	41.0	18	4.5
Total †	2,831		432	

* Therapeutic and unintentional abortion data from Tables 49 and 53.

† More than one lesion per specimen. Percentages calculated on basis of 1,769 and 396 cases, respectively, from Tables 5 and 49.

INCIDENCE OF DECIDUAL HEMORRHAGE, BY DURATION OF PREGNANCY

Pregnancy, weeks	*Cases*	*Decidual hemorrhage*	
		Number	*Per cent*
0–4	24	6	25.0
5–8	276	183	66.3
9–12	523	361	69.0
13–16	190	111	58.4
17–20	88	29	32.9
21–22	35	13	37.1
Total.....	1,136	703	61.9



UTERO-PLACENTAL MECHANISM

Concentric growth of placenta
Uterine contractions
Faulty implant _ Placent Praevia

VASCULAR MECHANISM

Vasodilation, rupture,
Hyperemia, stretching,
Trophoblastic invasion

BLEEDING
CLOTTING } *MECHANISM IMBALANCE*

Scurvy (Vitamin C, P deficiency)
Hypoprothrombinemia (K)
Afibrinoginemia
Calcium deficiency
Hormone deficiency (C.L.)
Thromboplastin
Platelets

Etiology of decidual hemorrhage and premature separation of the placenta includes a number of mechanisms.

Throfoblastic invasion

Rpture of decidual veins

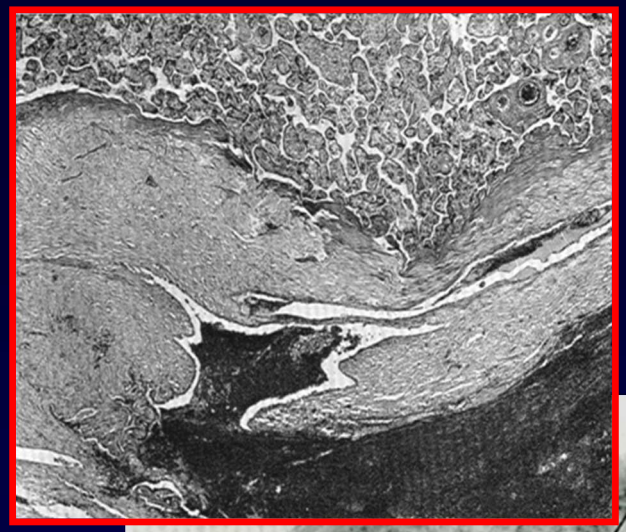
Decidual hemorrhage

PG production

Uterine contractions

P

Chorio-decidual detachment



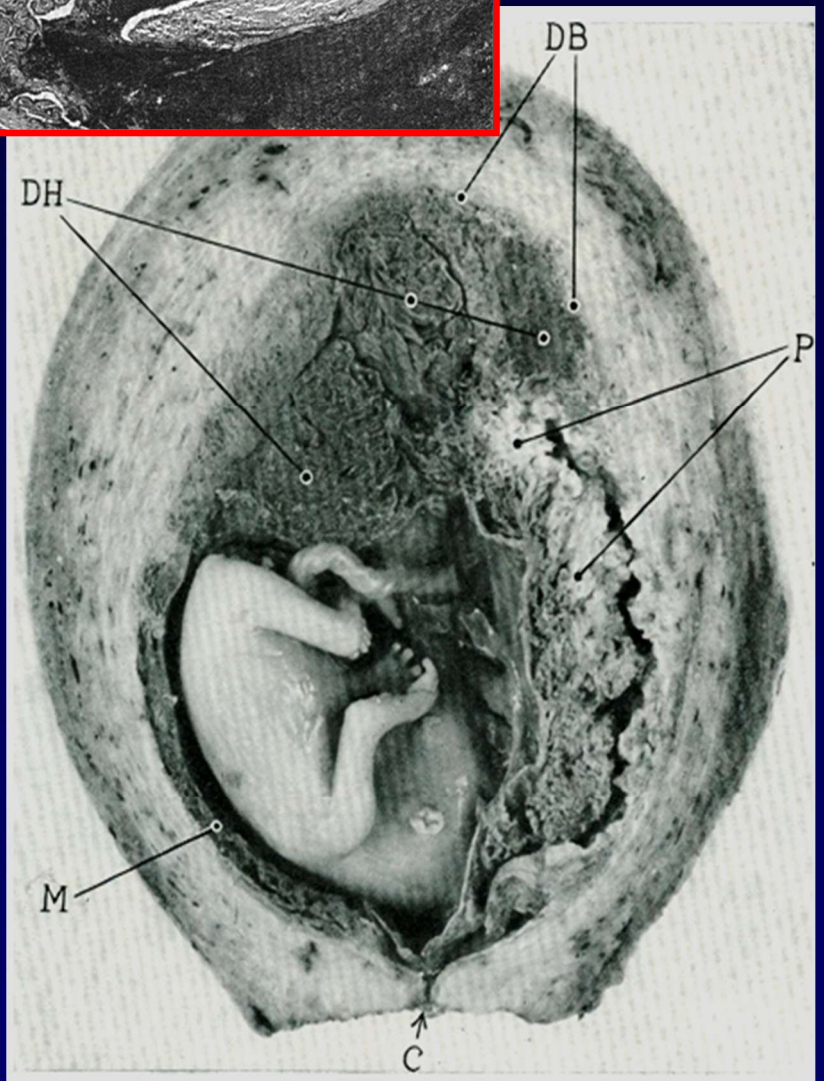
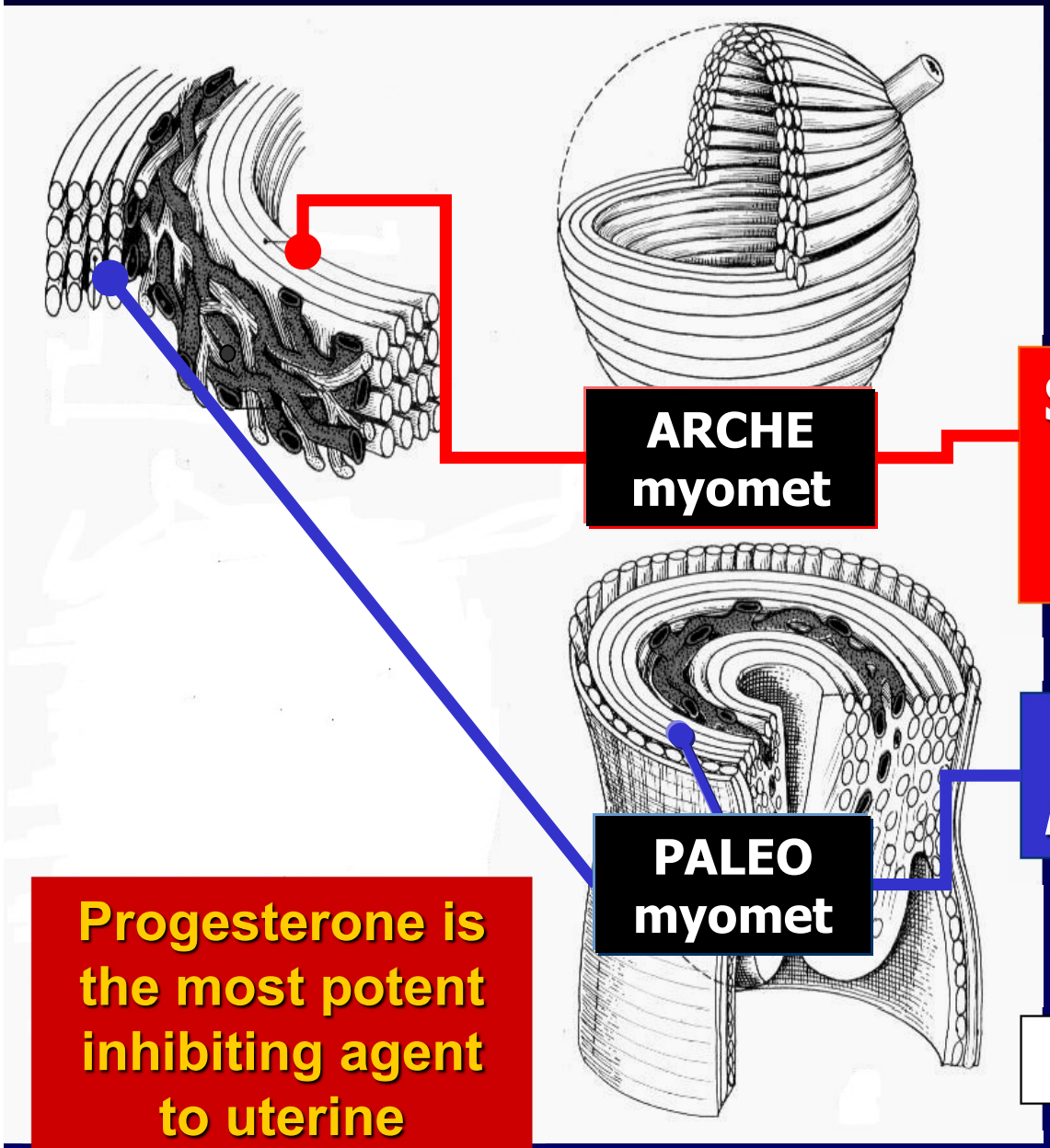


FIG. 58. Conceptus uteri with partial premature separation of the placenta in the fundus. Hysterectomy for therapeutic abortion and sterilization. *C*, cervix; *M*, membranes; *P*, placenta; *DB*, decidua basalis; *DH*, decidual hemorrhage.



ARCHE myomet

PALEO myomet

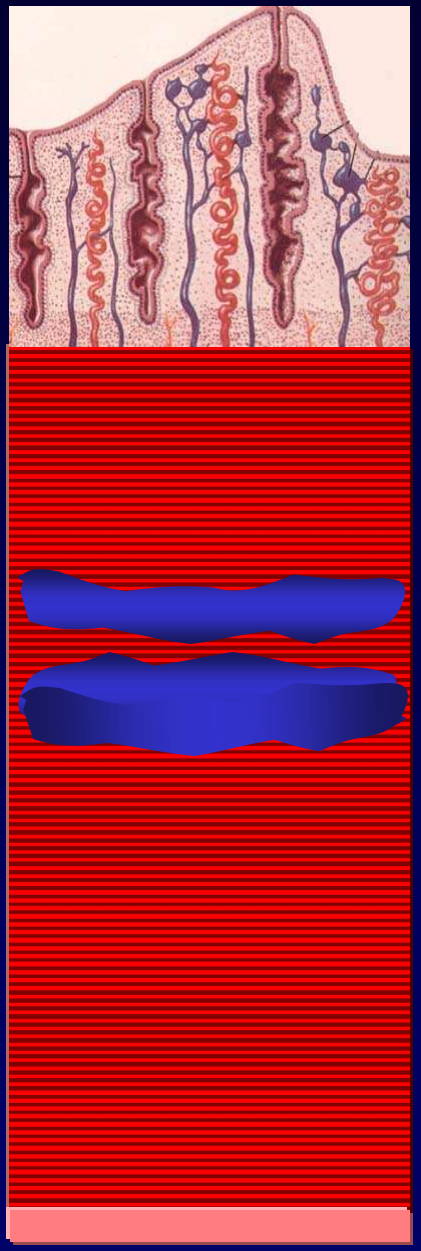
Layers

Subvascular & cyclic E_2R-PR

Vascular E_2R-PR (1/4 int)

SupraVascular

NEO myomet



Progesterone is the most potent inhibiting agent to uterine contractions up to half pregnancy

PRACTICE

Donati et al. (2000) – Italy → 1/3 of threat. abortions treated by Progest.

Malm et al. (2003) – Finland → 1/5 of threat abortions treated by Progest.

Olesen et al. (1999) – Denmark → 3.4-5% of pregnants receives Progest.

Beyens et al. (2003) – France → Progest. most used drug in pregnancy

American Journal of Obstetrics and Gynecology (2004) 191, 398–407

Prescription drug use in pregnancy

Susan E. Andrade, ScD,[a,k,*] Jerry H. Gurwitz, MD,[a,k] Robert L. Davis, MD, MPH,[b,k] K. Arnold Chan, MD, ScD,[c,d,k] Jonathan A. Finkelstein, MD, MPH,[e,k] Kris Fortman, PhD,[f,k] Heather McPhillips, MD, MPH,[b,k] Marsha A. Raebel, PharmD,[g,k] Douglas Roblin, PhD,[h,k] David H. Smith, PhD, MHA,[i,k] Marianne Ulcickas Yood, DSc, MPH,[j,k] Abraham N. Morse, MD,[a] Richard Platt, MD, MS[c,e,k]

Table I The most common drugs that were dispensed in the 270 days before delivery (n = 152,531)*

Generic drug	Dispensings (n)	Deliveries (n)
Progesterone	7,024	2,324 (1.5%)

EVIDENCE

1. Haemorrhage in early pregnancy - Miscarriage and ectopic pregnancy

1e. Hormone administration (Progestogens or HCG) should be used **only within controlled clinical trials** until the ratio of benefits to hazards has been more clearly established

→ Prendiville W. Human chorionic gonadotropin (HCG) for recurrent miscarriage. *Cochrane Database of Systematic Reviews. Cochrane Library,* 1997 Issue 4 (Type I evidence – systematic review)

→ Chalmers I, Enkin M, Kierse MJNC. Effective care in pregnancy and childbirth. Oxford: Oxford University Press, 1989 pp. 615-619 (in Enkin…1996)
(Type I evidence - systematic review)

bmj.com

Threatened miscarriage: evaluation and management

Alexandros Sotiriadis, Stefania Papatheodorou and George Makrydimas

BMJ 2004;329;152-155
doi:10.1136/bmj.329.7458.152

We searched literature in English with

→ Medline (January 1965 to April 2005),

→ Embase (January 1980 to April 2005),

→ the Cochrane database using the keywords

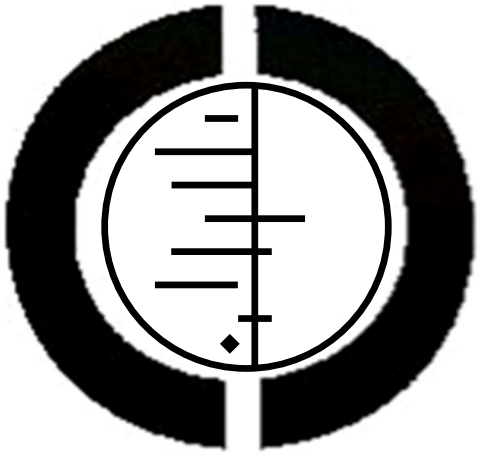
"**threatened**" *and* "**abortion**" *or* "**miscarriage**" *and* "**pregnancy**" *and* "**first trimester**" *or* "**early**" *and* "**bleeding**".

Table 2 Pregnancy outcome in studies with various therapeutic regimens

First author	Design	N	Sonography at presentation	Intervention	Successful continuation of pregnancy		
					Intervention group	Controls	P value
Moller[vi11]	Randomised controlled trial	260	No	Medroxyprogesterone (three regimens)	60/123	71/137	0.62
Tognoni[vi12]	Randomised controlled trial	145	No	Hydroxyprogesterone caproate	49/74	50/71	0.58
Berle[vi13]	Randomised controlled trial	300	No	Hydroxyprogesterone caproate	96/154	100/146	0.26
Gerhard[vi14]	Randomised controlled trial	52	Yes	Progesterone	23/26	21/26	0.44
Soltan[vi16]	Randomised controlled trial	35	Yes	Buphenine	19/23	4/12	<0.01
Harrison[21]	Randomised controlled trial	61	Yes	hCG	14/20	11/21	0.24
Harrison[21]	Randomised controlled trial	61	Yes	Bed rest	5/20	11/21	0.07
Giobbe[23]	Retrospective	226	Yes	Bed rest	123/146	64/80	0.41
Ben-Haroush[24]	Prospective observational	230	Yes	Bed rest	180/200	23/30	0.03

Progestogen for treating threatened miscarriage (Review)

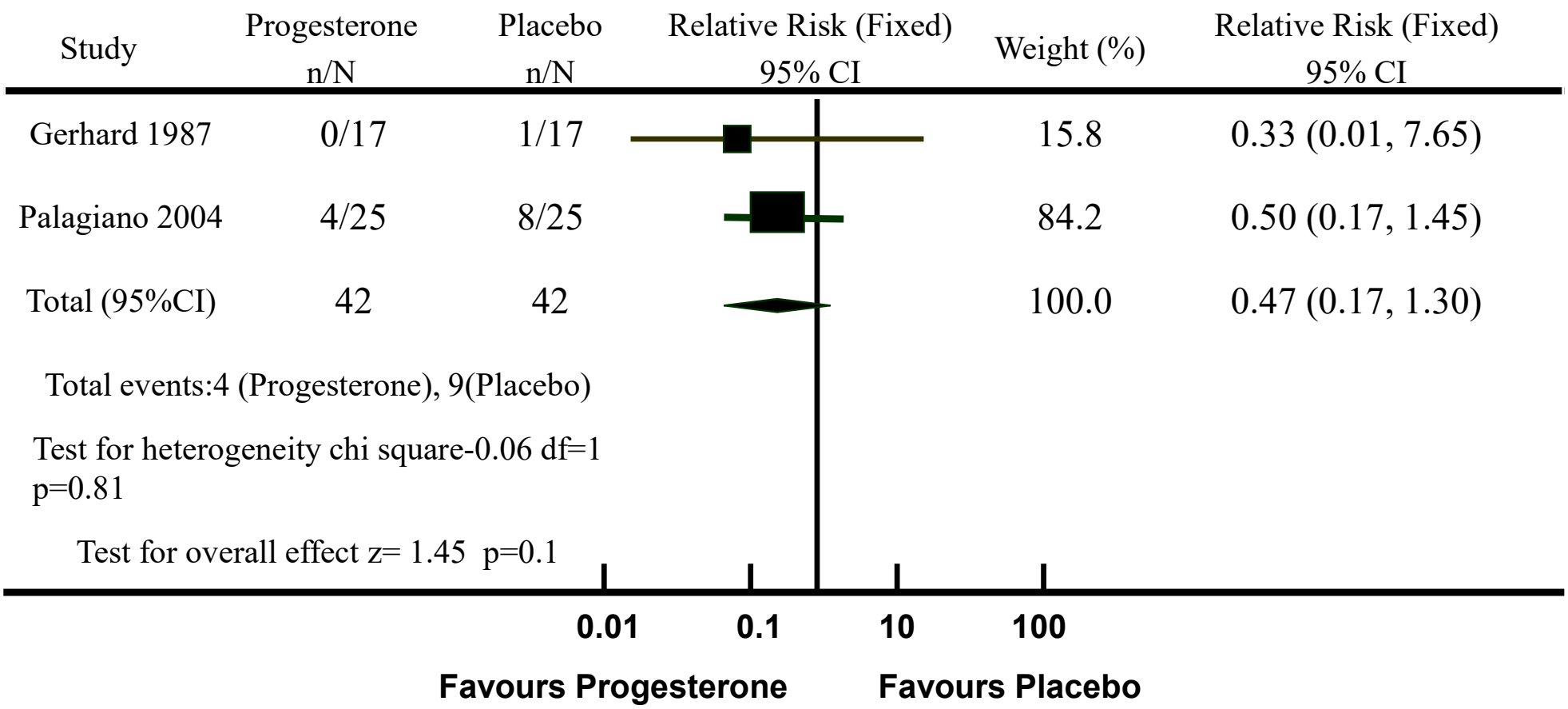
Wahabi HA, Abed Althagafi, Elawad M



THE COCHRANE
COLLABORATION®

Progestogen for treating threatened miscarriage (Review)

Analysis 01.01 Comparison o1 Progesterone versus placebo, Outcome 01 Miscarriage



The Cochrane Database of Systematic Reviews 2006 Issue 1

Progestogen for preventing miscarriage (Oates-Whitehead et al.)

Background...It has been suggested that a causative factor in many cases of miscarriage may be inadequate secretion of progestogens.

Selection criteria Randomised or quasi-randomised controlled trials comparing progestogens with placebo or no treatment given in an effort to prevent miscarriage.

Data collection and analysis. Thirty trials were identified in the initial search...

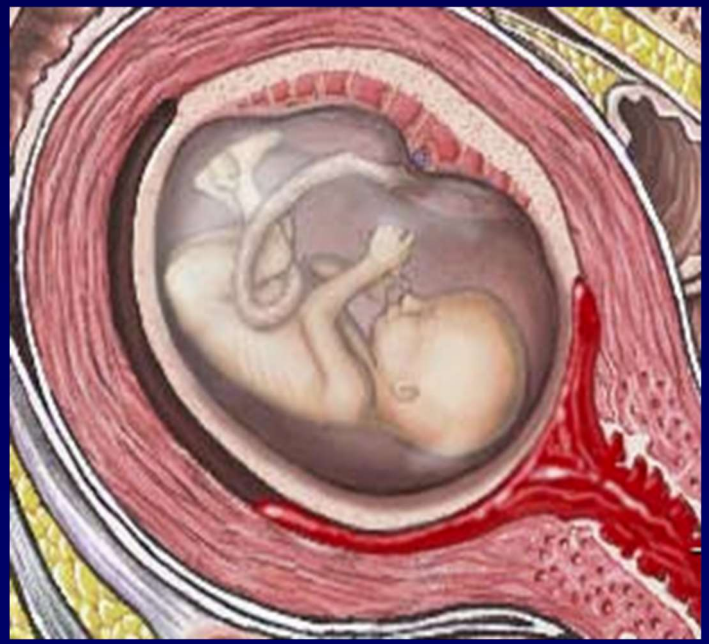
Main results. Fourteen trials (1988 women) met the inclusion criteria... No statistically significant ...in the risk of miscarriage between progestogen and placebo or no treatment groups (odds ratio (OR) 1.05, 95% confidence interval (CI) 0.83 to 1.34) **and no statistically significant difference in the incidence of adverse effect in either mother or baby.**

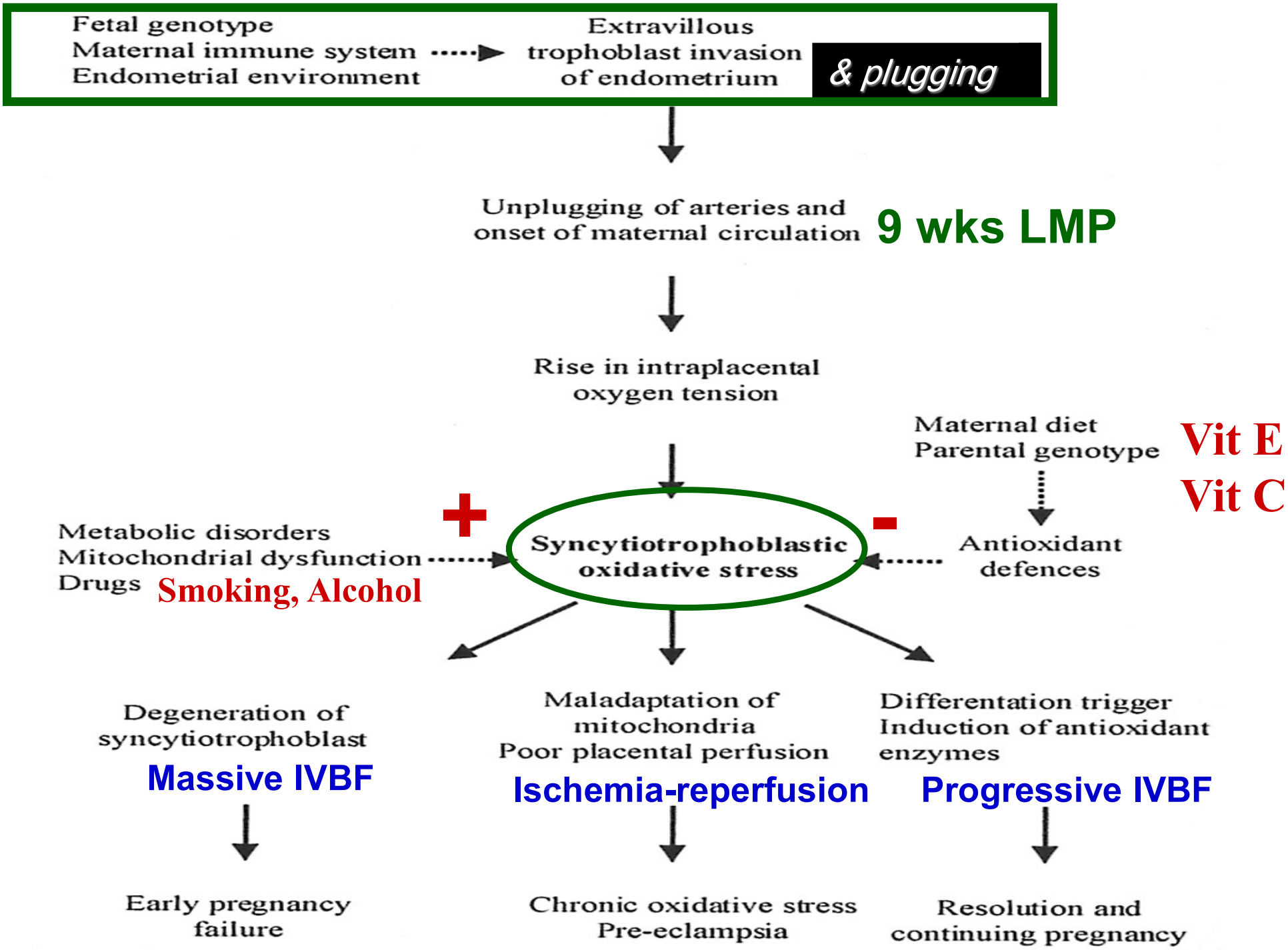
...subgroup analysis of three trials involving women who had recurrent miscarriages ...progestogen treatment showed a statistically significant decrease in miscarriage rate compared to placebo or no treatment (OR 0.39, 95% CI 0.17 to 0.91). **No statistically significant differences were found between the route of administration of progestogen (oral, intramuscular, vaginal) versus placebo or no treatment.**

Authors' conclusions. There is no evidence to support the routine use of progestogen to prevent miscarriage in early to mid pregnancy. However, further trials in women with a history of recurrent miscarriage may be warranted, given the trend for improved live birth rates in these women and the finding of no statistically significant difference between treatment and control groups in rates of adverse effects suffered by either mother or baby in the available evidence.

CONCLUSIONS

?



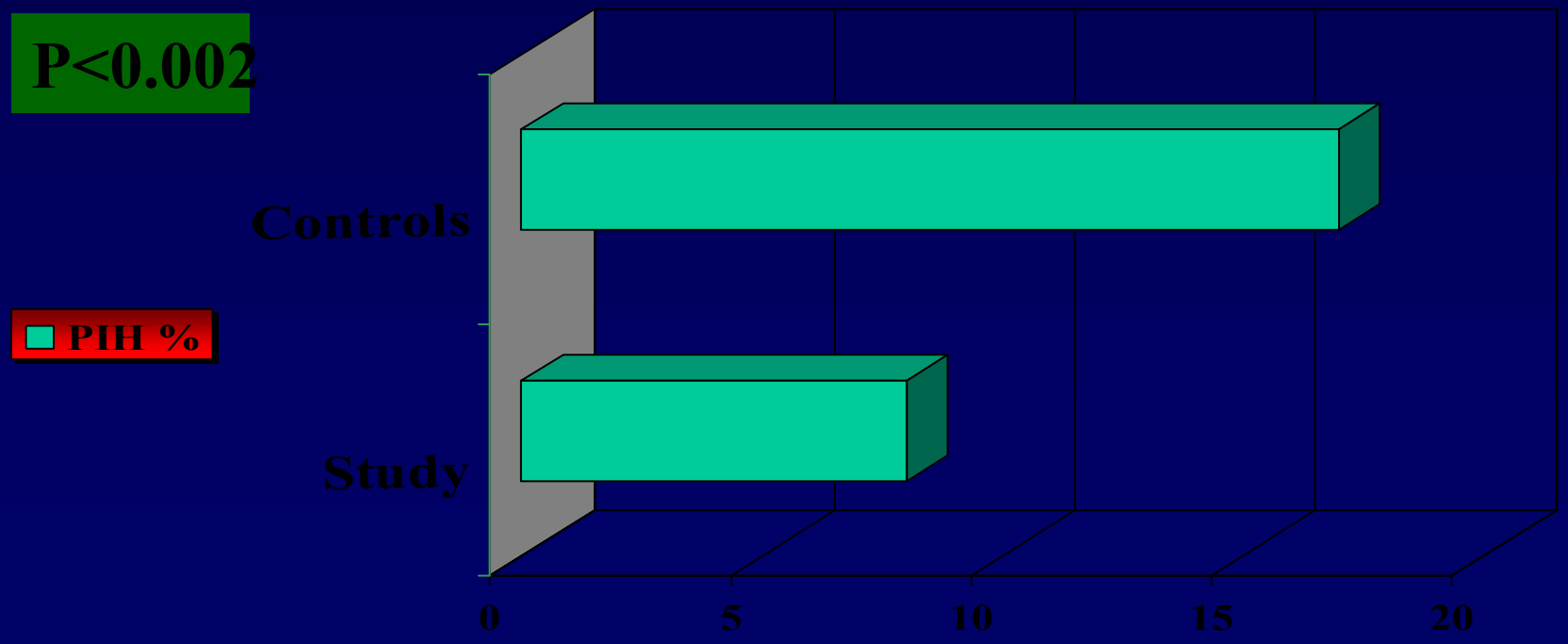

Fetal genotype
Maternal immune system
Endometrial environment
Extravillous trophoblast invasion of endometrium
& plugging
Unplugging of arteries and onset of maternal circulation
9 wks LMP
Rise in intraplacental oxygen tension
Maternal diet
Parental genotype
Vit E
Vit C
+
-
Metabolic disorders
Mitochondrial dysfunction
Drugs
Smoking, Alcohol
Syncytiotrophoblastic oxidative stress
Antioxidant defences
Degeneration of syncytiotrophoblast
Massive IVBF
Maladaptation of mitochondria
Poor placental perfusion
Ischemia-reperfusion
Differentation trigger
Induction of antioxidant enzymes
Progressive IVBF
Early pregnancy failure
Chronic oxidative stress
Pre-eclampsia
Resolution and continuing pregnancy

ANTIOXIDANTS TRIAL

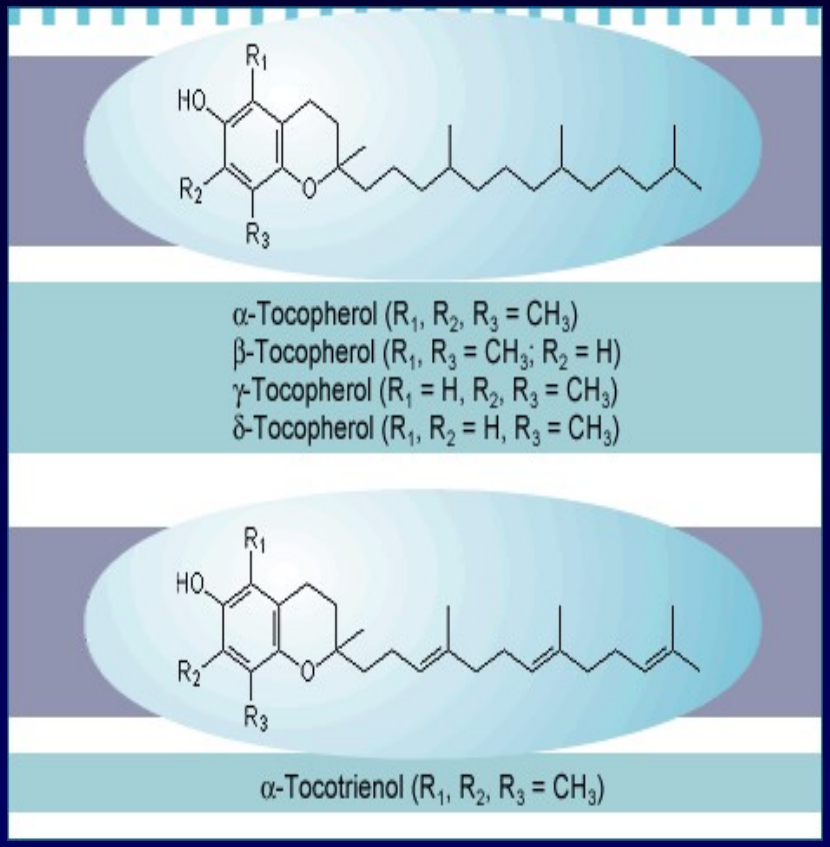
Chappell et al 1999 Lancet

283 women (London) with increased risk of PIH (history or abnormal UA Doppler)=> Vit C (1000mg/d) + Vit E (400 IU/d) from 16-22 wks vs placebo (Random & Prospective)

P<0.002



VITAMIN E (Tocopherol)



Vit E are fat-soluble vitamins with slow placental transfer rate via the TTP.

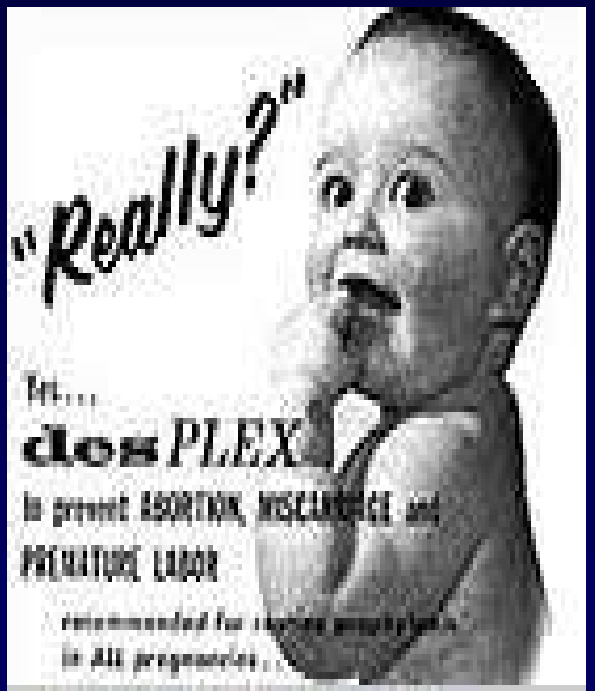
Low concentrations in coelomic fluid & undetectable in AF (JCEM 2003).

Vit E has an anti-teratogenic and infertility effect in rats (1922)

Pincheira et al, Clin Genet 199
chromosomal
aberrations in patients

Sivan et al., AJOG 1996 and Siman et al, Diabetologia 1997 &Teratology 2000: Vit E reduces the severity of fetal malformation in the diabetic rat.

PARENTAL PRESSURE vs MEDICAL (IR)RESPONSIBILITY: THE DES STORY



This ad from a 1957 medical journal promoted the use of DES to prevent miscarriage.

 # DES was the first synthetic estrogen (USA).

 # Prescribed to nearly 5 millions women to prevent miscarriage between 1938 and 1970.

 # DES children (female) are at higher risk of vaginal and cervical dysplasia (Cancer ?) and uterine anomalies (Infertility, ectopic pregnancy, late miscarriage & Premature labour).

REMEMBER THALIDOMIDE!!!!!

The fruit of the date palm (*): its possible use as the best food for the future?

Al-Shahib W, Marshall RJ.

London Metropolitan University, Department of Health & Human Sciences, London, UK.

Int J Food Sci Nutr. 2003 Jul;54(4):247-59

(*)= Mg + Selenium + Vit C, B, A…

A combination treatment of prednisone, aspirin, folate, and progesterone in women with idiopathic recurrent miscarriage: a matched-pair study

Clemens B. Tempfer, M.D., Christine Kurz, M.D., Eva-Katrin Bentz, M.D., Gertrud Unfried, M.D., Katharina Walch, M.D., Ullrike Czizek, and Johannes C. Huber, M.D., Ph.D.

Department of Gynecologic Endocrinology and Reproductive Medicine, University of Vienna School of Medicine, Vienna, Austria

Conclusion(s): A combination treatment of prednisone, aspirin, folate, and progesterone is associated with a higher live birth rate compared with no treatment in women with IRM. (Fertil Steril® 2006;xx:xxx. ©2006 by American Society for Reproductive Medicine.)

THANKS…

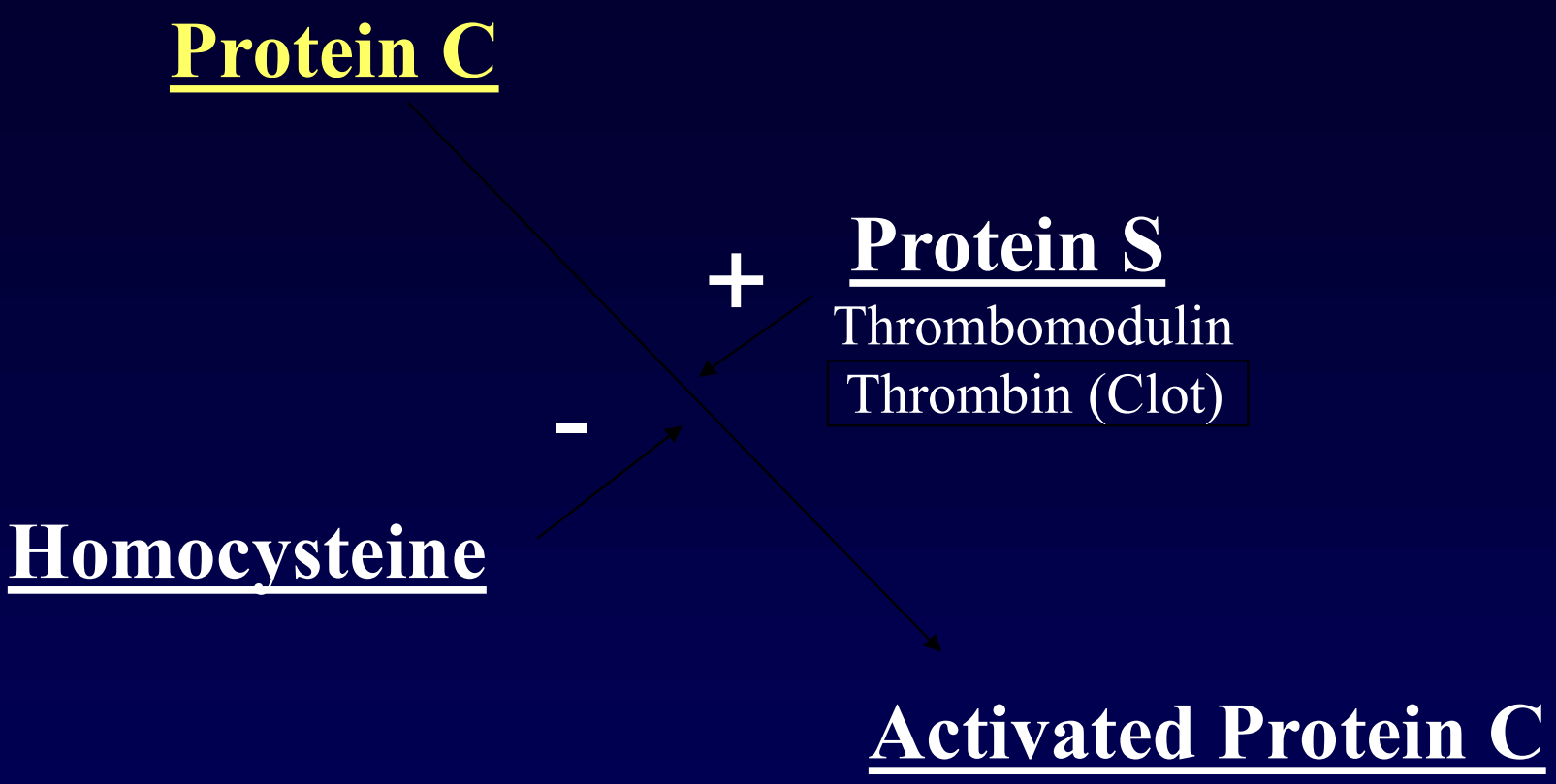
- “Do nothing”.
- No routine therapy but once or twice weekly measurement of progesterone levels to suggest whether pregnancy support with progestogens is or is not necessary. The lower cut-off level is usually taken to be about 300 nmol/l. Therapy is usually continued to about 16 weeks’ gestation.
- Routine pharmacological “support of the pregnancy”. This is frequently advocated empirically in all pregnancies following recurrent losses because “it can do no harm”.
- Treat only within properly designed prospective trials (with adequate follow-up).

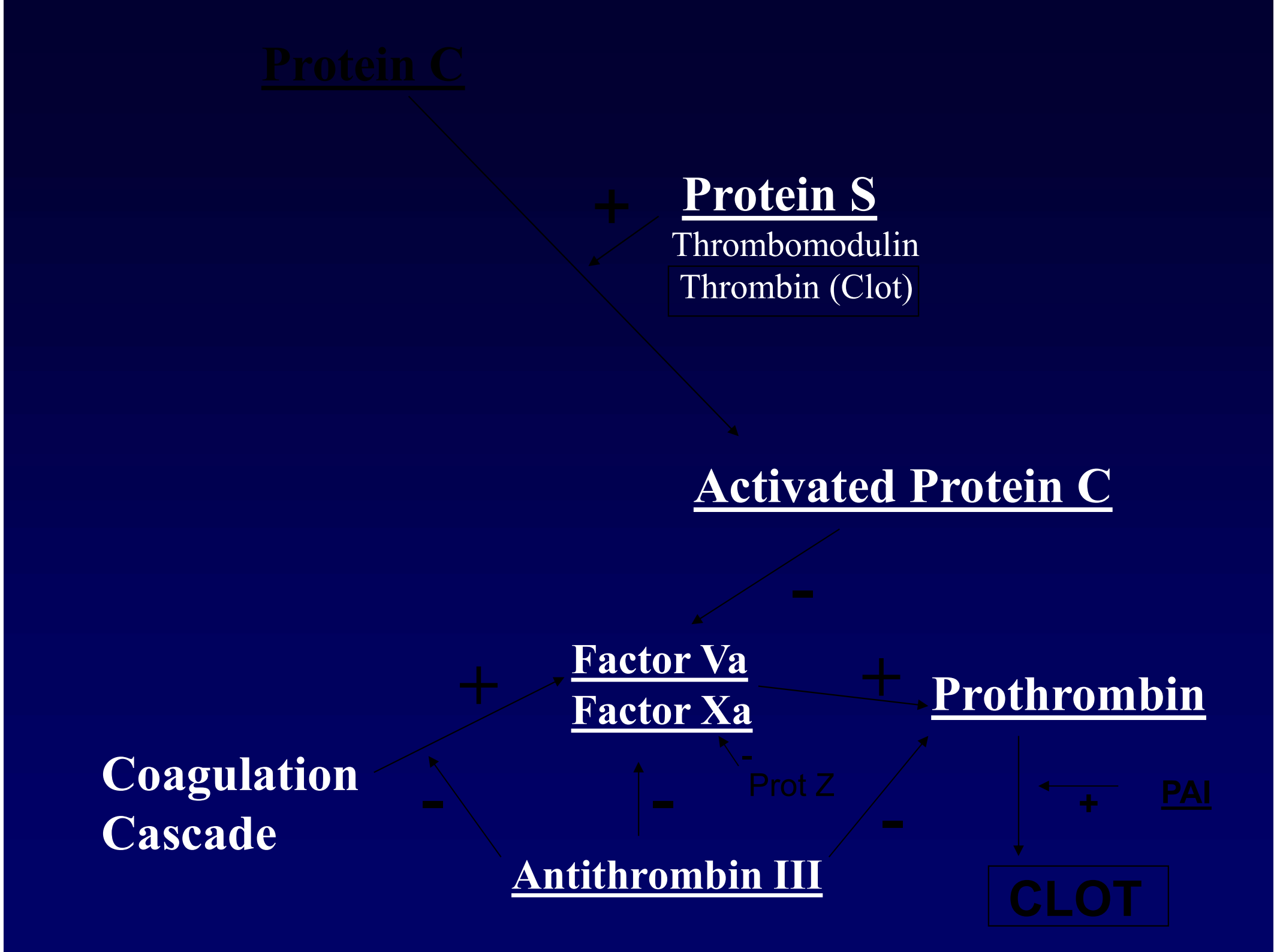
- There is no evidence that progestogens reduce the risk of sporadic miscarriage, stillbirth or neonatal death.

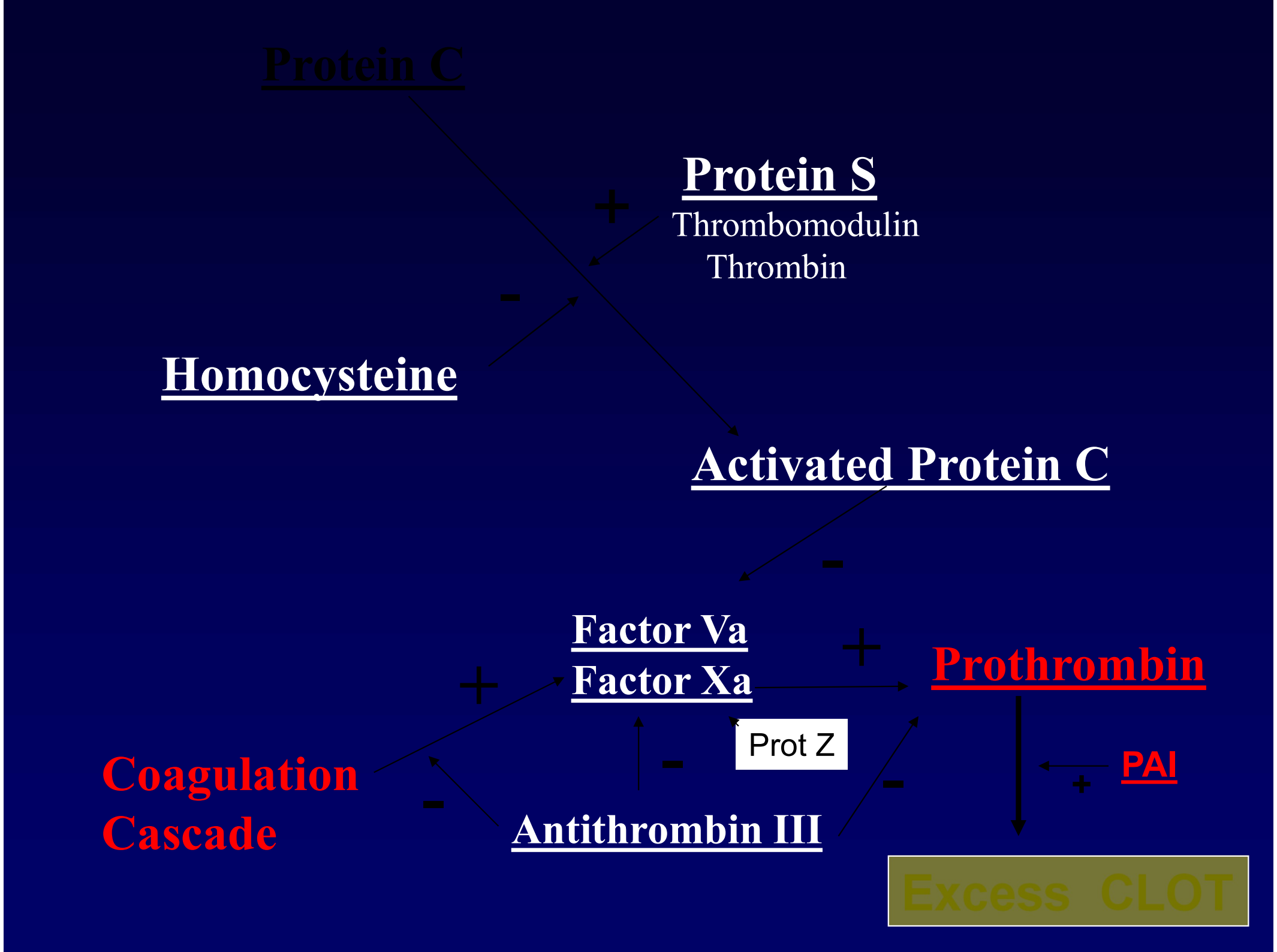
- Present data are suggestive of protective effect of progesterone in idiopathic recurrent miscarriage.

Congenital Thrombophilias

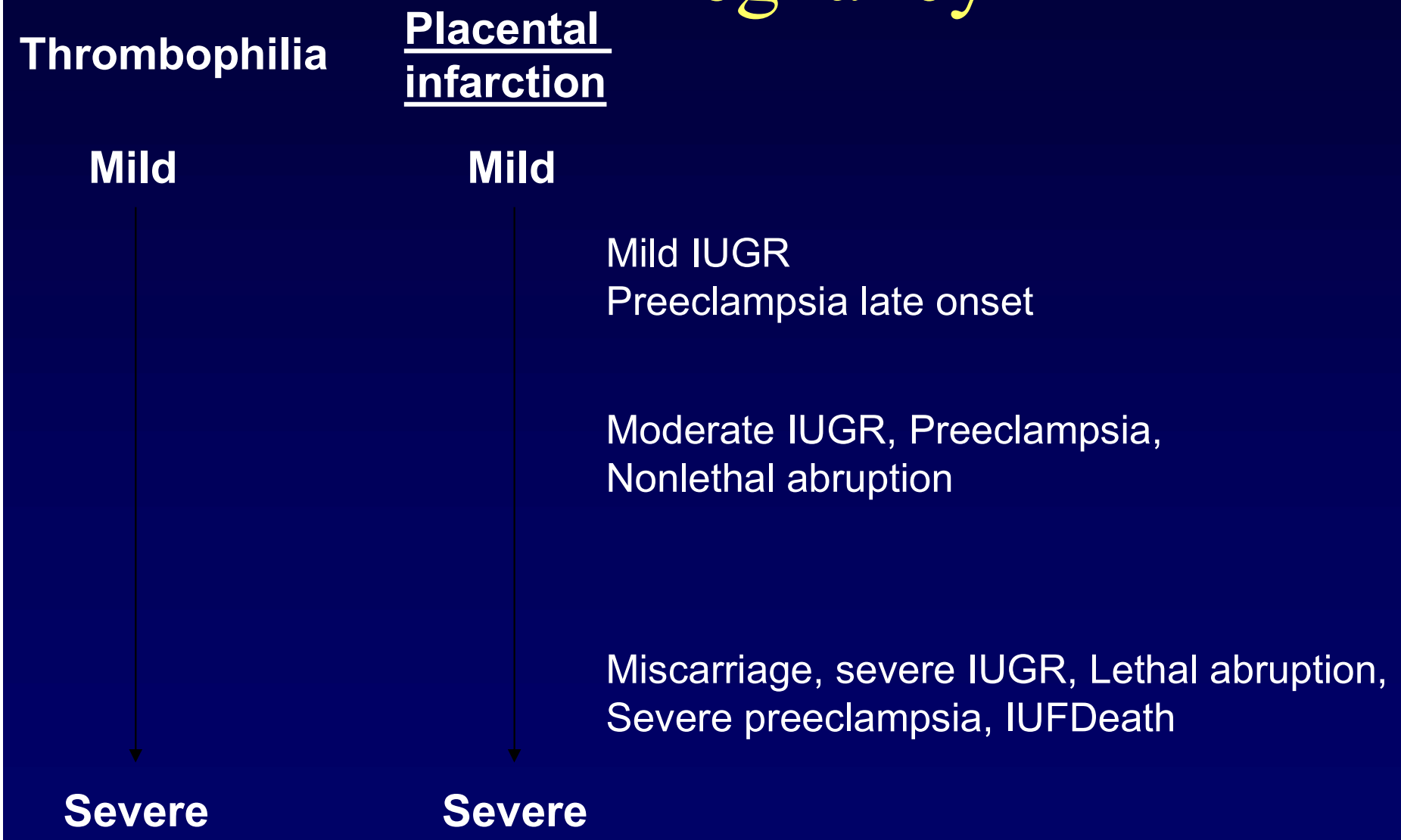
- How do they cause pathology?


Protein C
+
Protein S
Thrombomodulin
Thrombin (Clot)
-
Homocysteine
Activated Protein C


Protein C
+
Protein S
Thrombomodulin
Thrombin (Clot)
Activated Protein C
-
Factor Va
Factor Xa
+
+
Prothrombin
Coagulation
Cascade
-
-
-
Prot Z
+
PAI
Antithrombin III
CLOT


Protein C
Protein S
Thrombomodulin
Thrombin
+
-
Homocysteine
Activated Protein C
-
Factor Va
Factor Xa
+
+
Prothrombin
Prot Z
Coagulation
Cascade
-
-
-
Antithrombin III
PAI
+
Excess CLOT

Congenital Thrombophilias and Pregnancy



Congenital Thrombophilias

- Do they affect pregnancy?
- Literature 10 years old
  - Outcomes in pregnancy not consistent

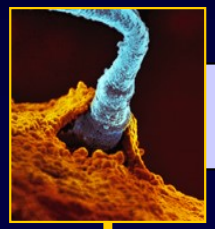
Recurrent miscarriage

US is important in the management of early pregnancy by confirming or predicting viability and, when fetal heart activity is detected, providing maternal reassurance. TV-US shows a gestation sack at 5 weeks, a yolk sack at 5,5 wees and fetal heart activity in embryos less than 4 to 5 mm at 6 weeks.

A scan to show fetal heart activity may be obtained every week or every 2 weeks until the end of the first trimester. These scans with the finding of normal fetal growth and activity, may be reassuring.

Women with APS should be offered aspirin and heparin treatment. Heparin should be initiated when intrauterine pregnancy is confirmed by ultrasound (aspirin when urinary pregnancy test results positive).

MEDICINA PERICONCEZIONALE



*d*0
w+d

27
3+6

153
21+
6

MEDICINA PERINATALE

ABORTIONS AND DIABETIC CONTROL

Initial Glycosilated Hemoglobin (S.D. Control Mean)	N	Fetal Losses
< 2	137	
9.5%		
2- 4	131	
14.5%		
> 4	112	21.4%

*Six losses before measurement

Mills 1988

Recurrent miscarriage

Despite significant improvement in live birth rates, pregnants with APLs and treated with aspirin and heparin until 34 weeks of gestations are at risk for late complications (preeclampsia, IUGR, placental abruption, preterm delivery).