Polni hormoni i hemostaza

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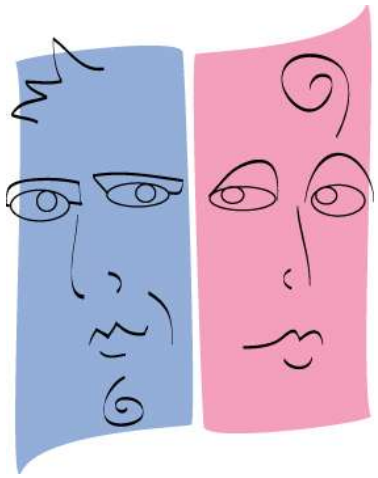
Does gender matter?

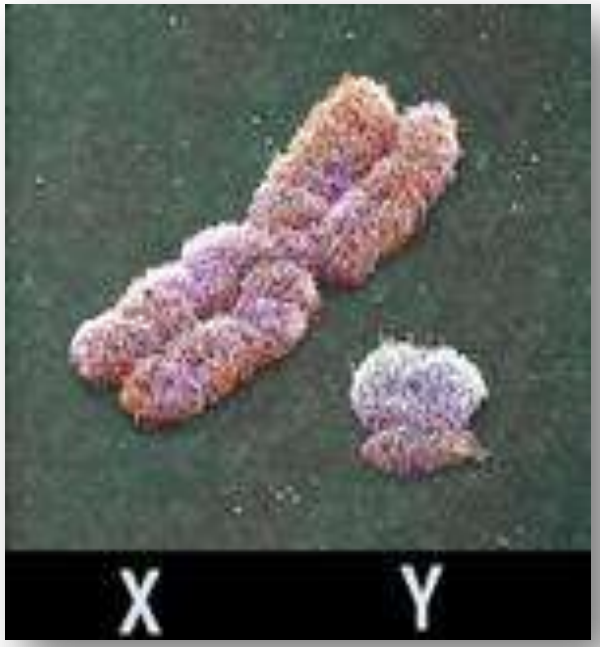
”The more we learn and the more we look, sex is important and important in ways and to an extent we never even dreamed would be the case”

Citation from Institute of Medicine,

Swedish National Academy of Sciences



U svim životnim razdobljima muskarci umiru pre žena …



Ali žene imaju lošiji kvalitet života…

- **Č**ešci kontakt sa zdravstvenim slu**ž**bama
- **Češć**e i du**ž**e odsustvo s posla, raniji odlazak u penziju
- **Češć**e i du**ž**e su na terapiji antidepresivima i analgeticima

”The health paradox”

3

CILj:

- Predstaviti osnovne informacije o uticaju muških i **ž**enskih polnih hormona i uticaju hormonske terapije na parametre hemostaze

- Predstaviti osnovne informacije o rizicima hormonske terapije za razvoj trombotskih komplikacija

Rezultat:

- Razumeti važnost pravilnog planiranja kliničkih studija koje se bave proučavanjem uticaja polnih hormona na parametre hemostaze

- Kritički posmatrati rezultate već postojećih studija

Normalna hemostaza

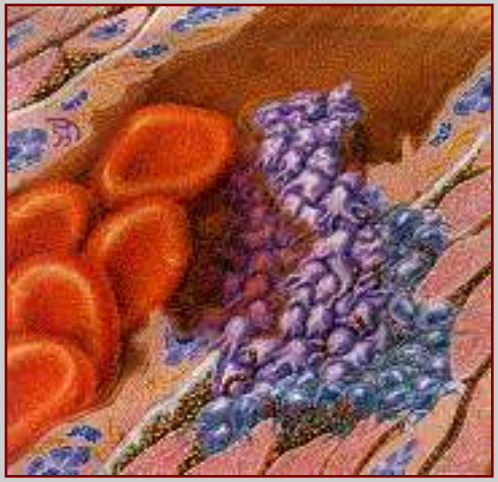
- **omogućava normalan tok krvi u očuvanim krvnim sudovima**
- **sprečava i zaustavlja krvarenje nakon oštećenja krvnog suda**

Fiziologija normalne hemostaze

primarna hemostaza:

krvni sudovi

- vazokonstrikcija
- oslobađanje tkivnog faktora (TF) i prostaciklina



trombociti

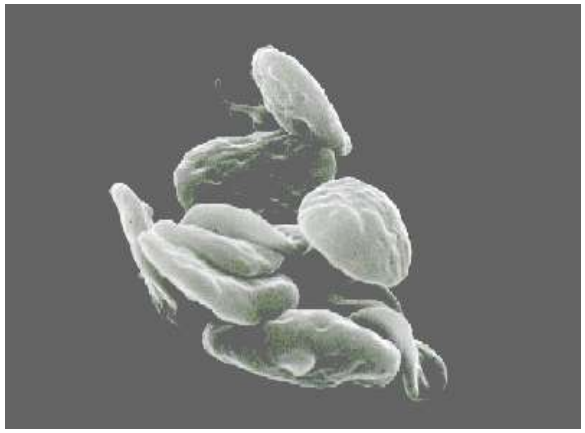
adhezija

agregacija i aktivacija

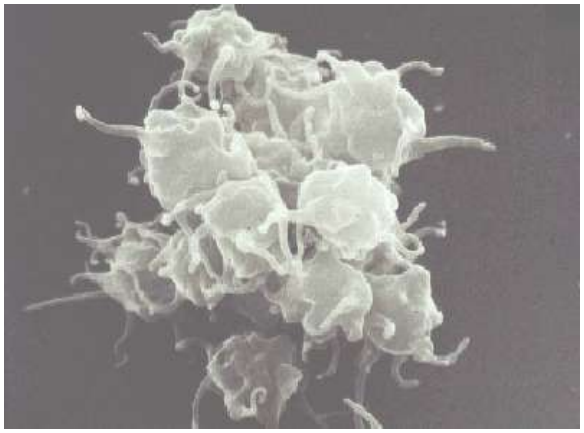
Karolinska Institutet

Danderyds Sjukhus

primarna hemostaza – aktivacija trombocita:

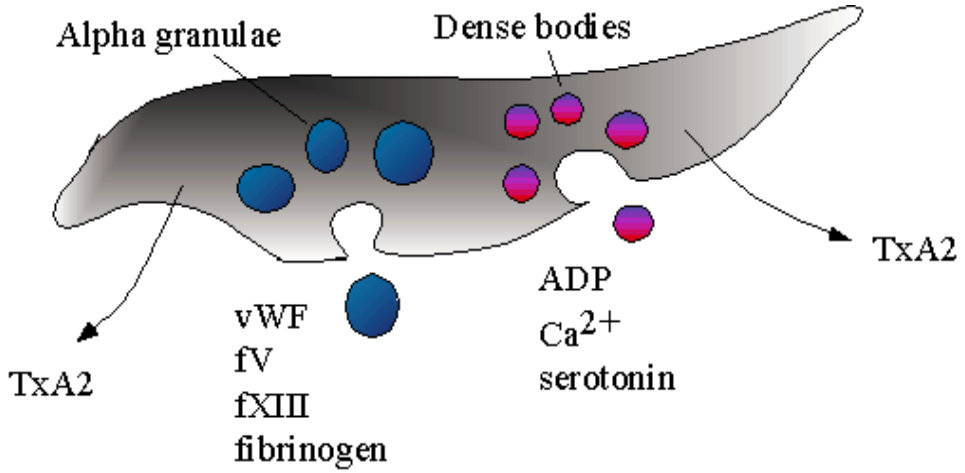


neaktivni trombociti



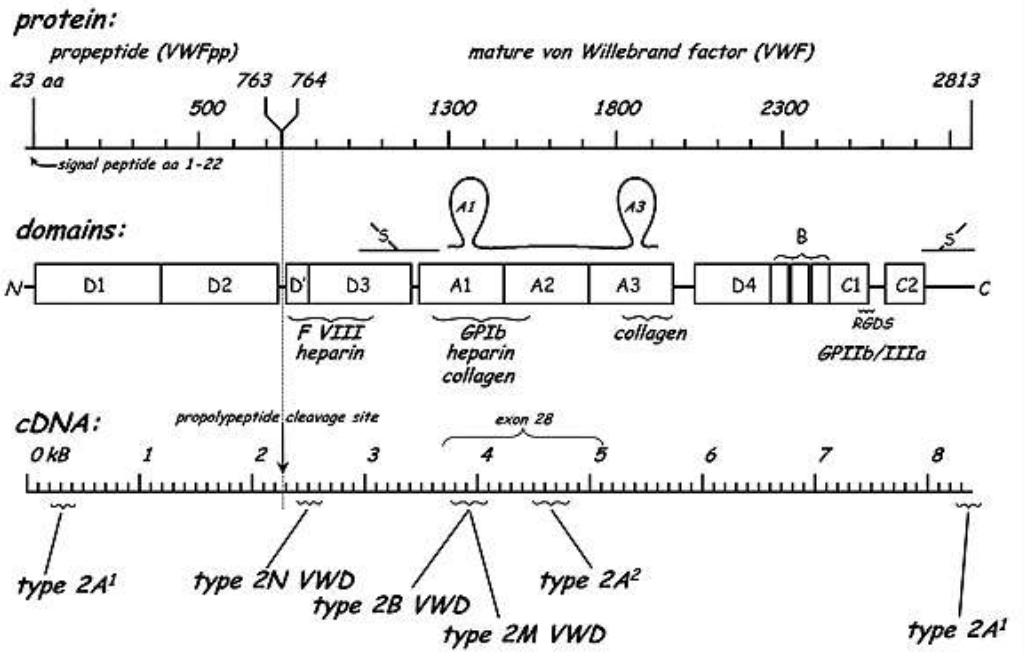
aktivirani trombociti

oslobadjanje vazoaktivnih supstanci iz α-granula i “dense bodies”



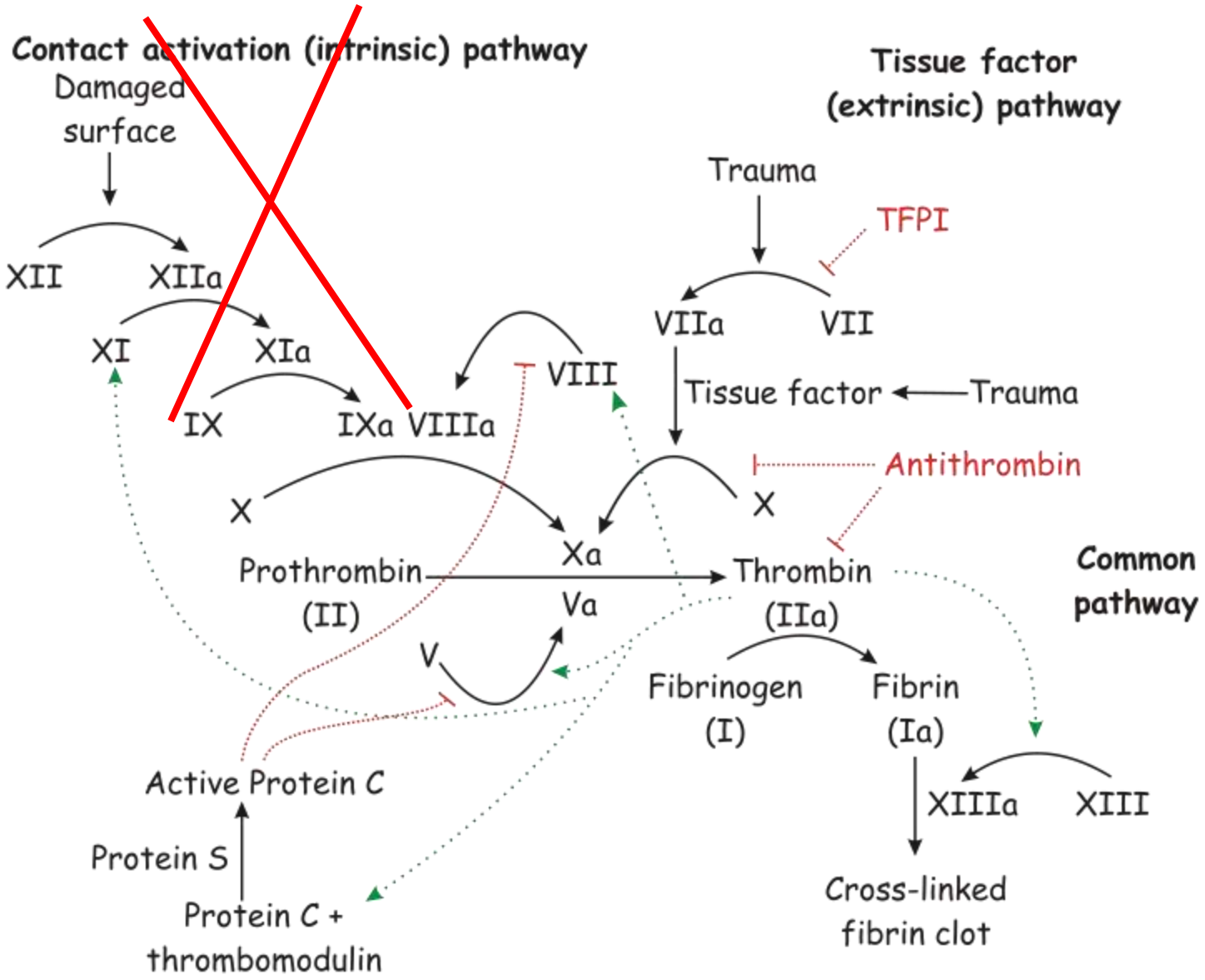
Von Willebrand Factor (VWF)

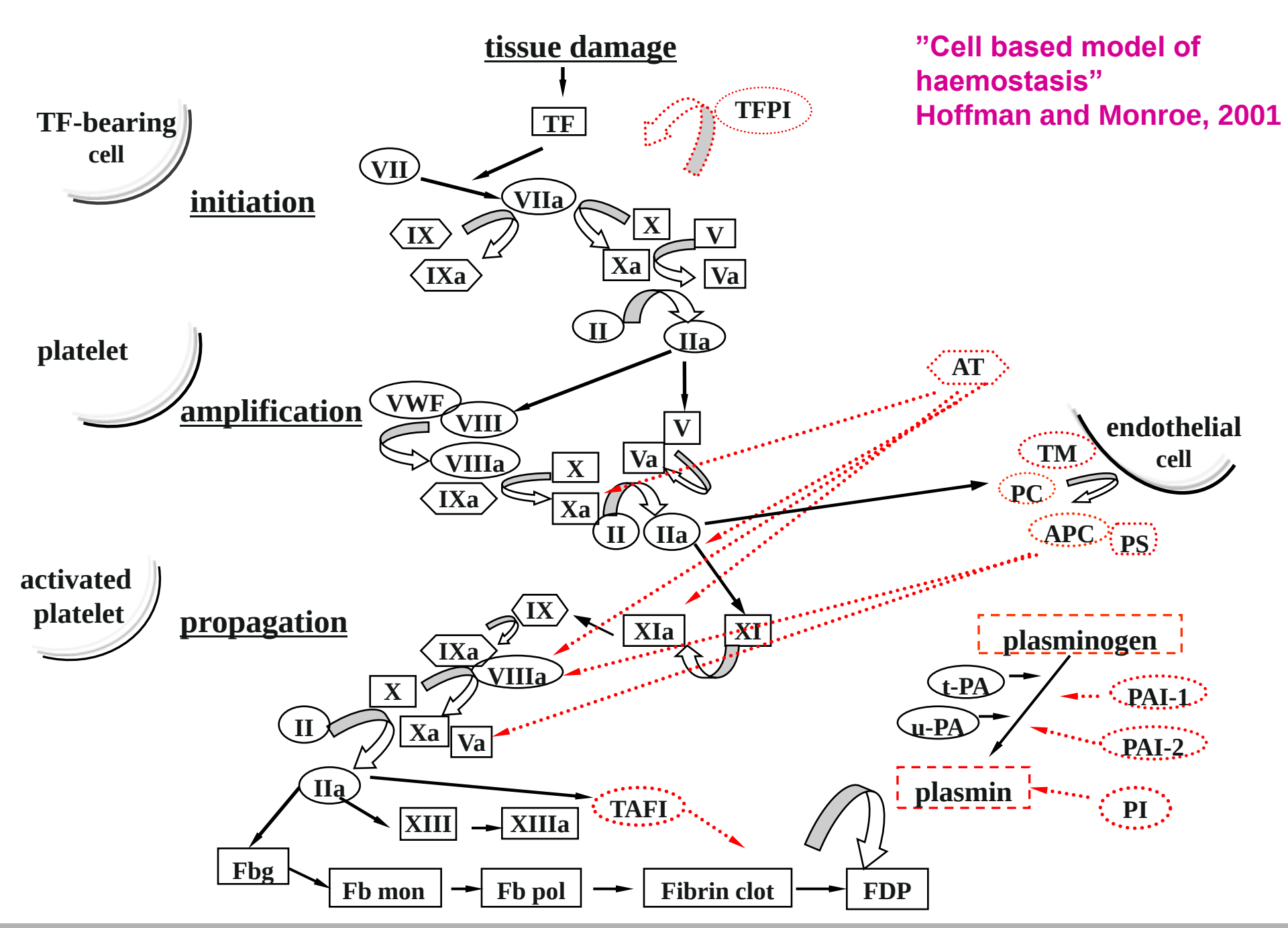
- VWF je sastavljen od identičnih ≈250 kDa polimera koji su disulfidnim vezama povezani u multimere od >200,000 kDa
- VWF omogucava adheziju trombocita za ostećeni zid krvnog suda preko specifičnih glikoproteinskih receptora
- VWF je nosač FVIII
- Osobe sa 0-om krvnom grupom imaju oko 30% nižu konc. VWF-a
- VWD je najčešći nasledni hemoragijski poremećaj (prevalencа ~ 1%)



Protein akutne faze: povećava se u stresu

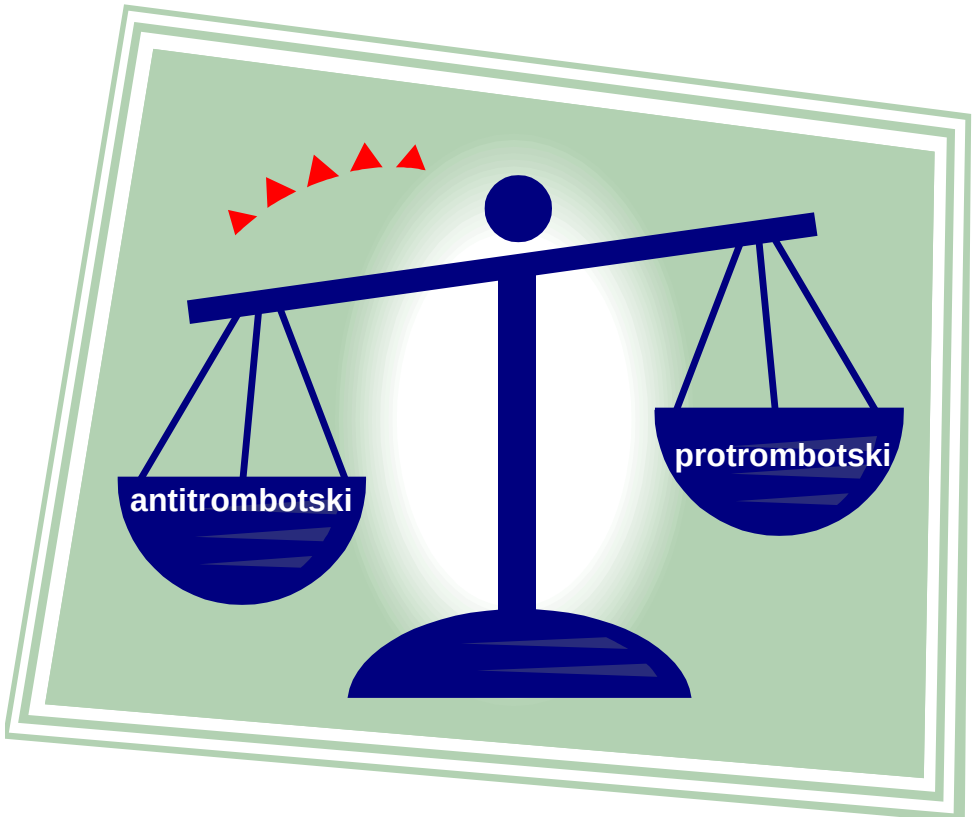
Sekundarna hemostaza – aktivacija koagulacije





”Cell based model of haemostasis”
Hoffman and Monroe, 2001

Prevencija tromboze i zaustavljanje krvarenja su posledica dejstva antitrombotskih i protrombotskih mehanizama hemostaze



The Biology of Human Sex Differences

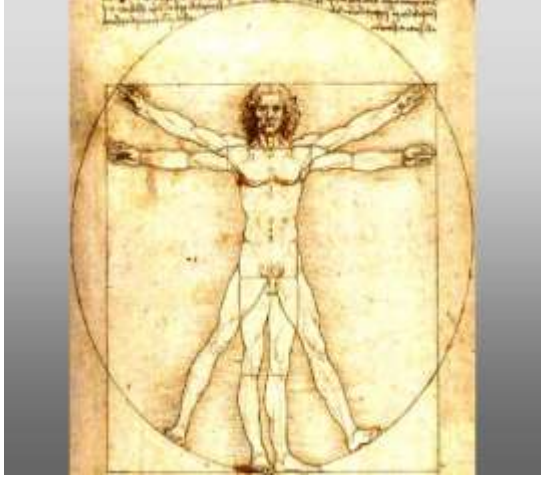


Table 1. Differences between the Sexes in the Reproductive Strategy.

Variable	Female	Male
Timing of fertility	12 hr/mo	Constant
Germ cells	Fixed supply plus disappearance by apoptosis	Mitotic replacement
Endocrine pattern	Cyclic	Acyclic
Control	Hypothalamus Corpus luteum	Hypothalamus

N Engl J Med 2006;354:1507-14.

The Biology of Human Sex Differences

Celokupna količina estrogena se sintetiše od androgena

Fiziološke razlike su **KVANTITATIVNE**

Razlike izmedju muškaraca i žena u količini polnih-steroidnih hormona su regulisane preko dva mehanizma:

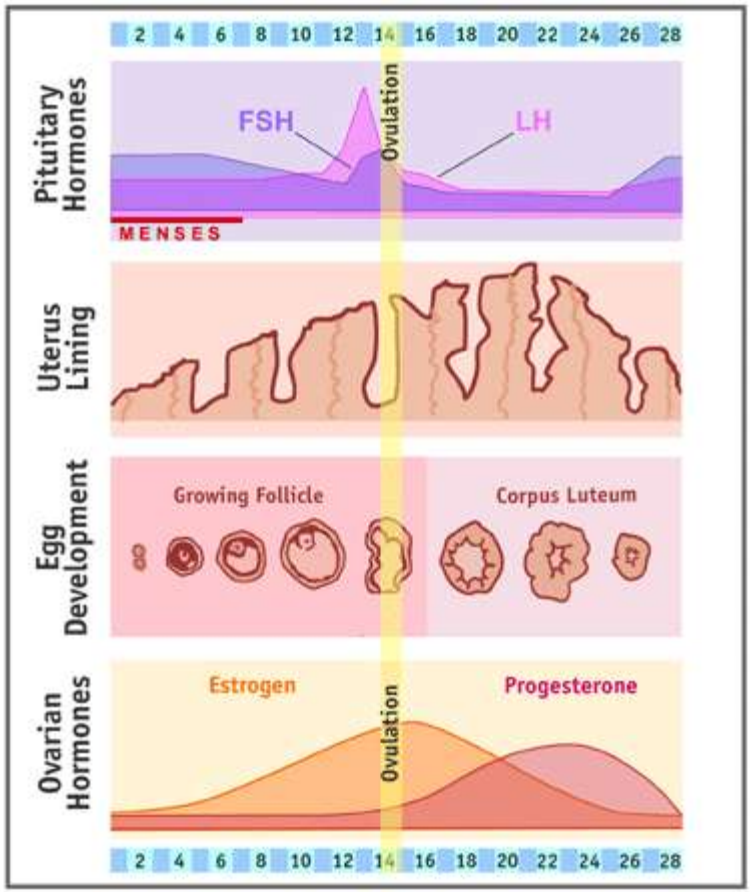
1. koliko androgena se sintetiše
2. koliki % od ukupne količine androgena se transformiše u estrogen

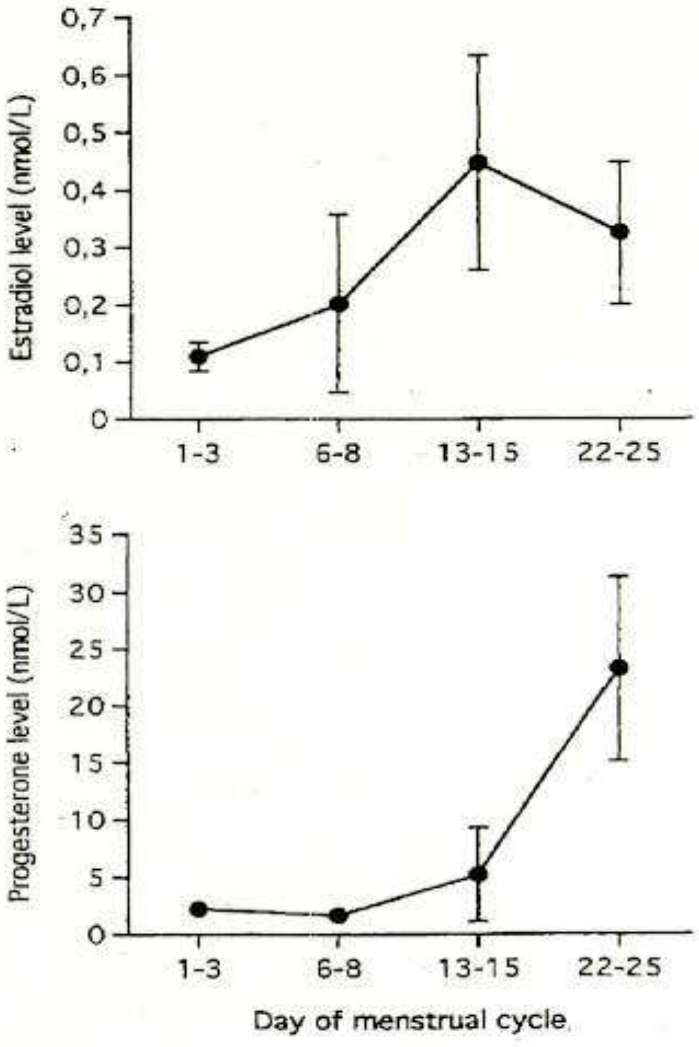
♂ Testis proizvede oko 7000 µg testosterona dnevno od čega se 1/4 od 1% transformiše u estradiol.

♀ Jajnik proizvede 300 µg testosterona dnevno od čega se polovina transformiše u estradiol.

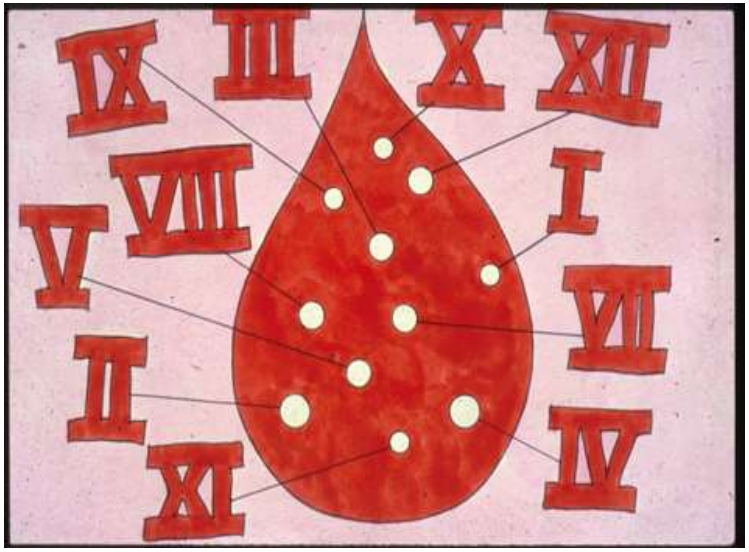
N Engl J Med 2006;354:1507-14

Regulacija lučenja polnih hormona – estrogena i progesterona u toku različitih faza menstrualnog ciklusa





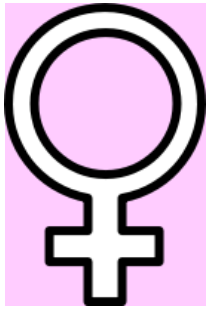
Uzimanje uzoraka krvi za laboratorijsko odredjivanje hemostatskih parametara:



Uzimanje uzoraka krvi za laboratorijsko odredjivanje hemostatskih parametara:

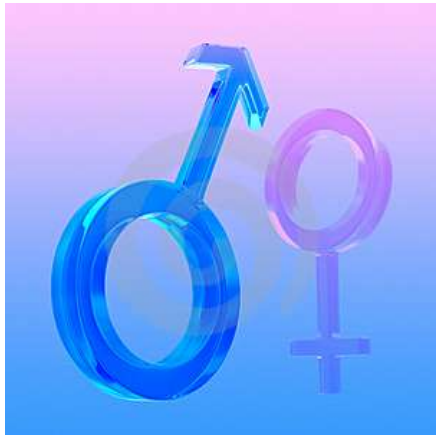
Žene u premenopauzi:

- dan menstrualnog ciklusa
- trudnoća
- terapija oralnim kontraceptivima



Žene u menopauzi:

- HRT ?
- Komorbiditet – CVD, dijabetes, gojaznost



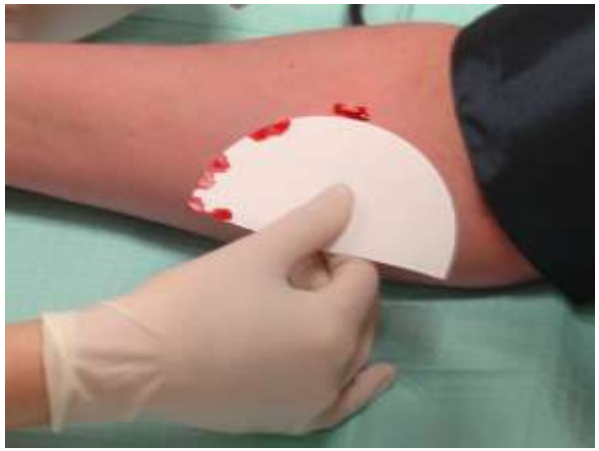
- **Izbegavati:**
- fizički stres (koncentracija faktora koagulacije se povećava u stresu, naročito FVIII i VWF)
- psihički stres
- pušenje (2 sata pre uzimanja uzorka?)

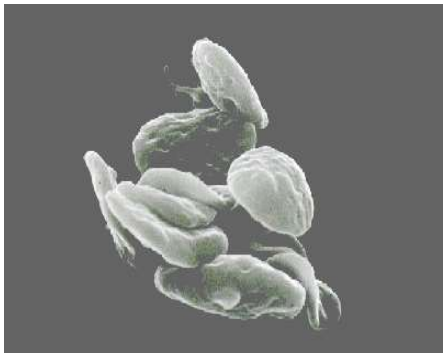
♦♦♦ UZIMANJE UZORAKA: nakon mirovanja od 15-20 min

PRIMARNA HEMOSTAZA: VREME KRVARENJA, BROJ I FUNKCIJA TROMBOCITA

Početak ciklusa (najniži nivo estradiola)

Produzeno vreme krvarenja - Jacob&Selle 1974
Broj trombocita najniži - Ygge 1969
Najniži nivo VWF i FVIII





Nivo faktora i inhibitora koagulacije i fibrinolize u toku menstrualnog ciklusa

Bez značajnih promena:

– FX, fibrinogen

– Antithrombin, APC

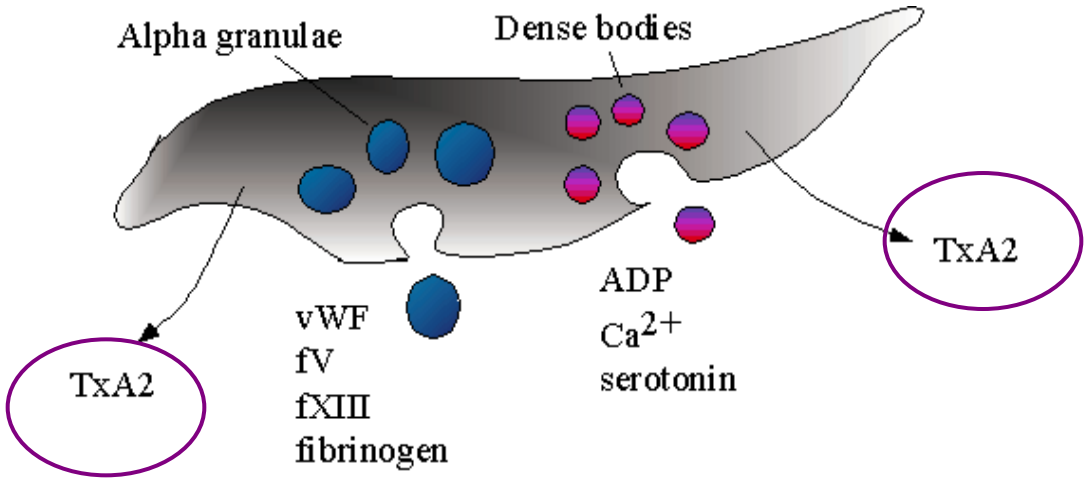
– Plasminogen

– Plasminogen aktivator inhibitor – 1 (PAI -1)

– Plasmin-plasmin inhibitor complex

– F1+2, D-dimer

Uzorak uzimati u toku iste faze menstrualnog ciklusa kod svih žena uključenih u jednu kliničku studiju



TROMBOCITI MUŠKARACA PROIZVODE VIŠE TxA_2

Pinto et al, Prost Leukot Essent Fatty Acids 1990

TESTOSTERON STIMULIŠE EKSPRESIJU TxA_2

Ajayi et al, Circulation 1995

Izraženo smanjenje in vivo sinteze tromboksana kod muškaraca u toku terapije estrogenom

- 10 pacijenata sa karcinomom prostate. Uporedjivani efekti terapije parenteralnim estrogenom (n = 5) i terapije hirurškom kastracijom (n = 5).

- Terapija estrogenom: **smanjenje nivoa tromboksana in vivo (~40%)**
- Hirurška kastracija: **udvostručenje in vivo nivoa tromboxana**.

- **Odnos prostaciklina i thromboksana povećan ~** 50% (p = 0.023) u toku terapije estrogenom.

Henriksson et al, Eur J Clin Invest. 1996

Efekat testosterona na pojedinačne parametre koagulacije i fibrinolize

TESTOSTERON SMANjUJE SINTEZU FVII

Bonithon-Kopp et al Atherosclerosis 1988

TESTOSTERON KORELIŠE NEGATIVNO SA FIBRINOGENOM

Glueck et al J Lab Clin Med 1993

TESTOSTERON SMANjUJE SINTEZU PAI-1

Anderson et al Thromb Haemost 1995

Efekat menopauze na markere aktivacije trombocita

(markeri aktivacije trombocita su odredjivani na fow citometru)

TABLE I. Comparison of Two Study Groups (42 Pre- and 49 Postmenopausal Women)*

Variable	Premenopausal group mean (SD)	Postmenopausal group mean (SD)	*t* Stat. (*df*)	*P* value[a]
Age (year)	39.38 (7.07)	56.16 (33.51)	−12.44 (89)	<0.001
BMI (kg/m^2)	25.09 (4.2)	25.35 (4.37)	−0.28 (89)	0.775
Cholesterol (mmol/L)	5.53 (0.85)	6.41 (1.26)	−3.81 (78)	<0.001
Estradiol (pmol/L)	381.15 (181.89)	36.38 (13.36)	13.28 (89)	<0.001
CD 62P (% positive cells)	0.52 (2.71)	2.66 (4.26)	6.28 (85)	<0.001
PAC-1 (% positive cells)	3.70 (2.31)	21.54 (2.48)	9.48 (89)	<0.001

*Abbreviations: SD, standard deviation; BMI, body mass index.
[a]Independent *t*-test.

Roshan et al, Am J Hematol 2005

Parametri koagulacije kod žena različite starosti u poredjenju sa muškarcima istih starostnih grupa (MONICA study)

n = 747muškaraca & 817 žena

	25-34	35-44	45-54	55-64	65-74
Fibrinogen	↑		↑		↑
FVII			↑	↑	↑
FVIII	↑			↑	↑
PS	↓	↓	↓	↓	↓
F 1+2	↑	↑			↑
TAT	↑	↑			↑

Lowe et al, Br J Haematol 1997

MONICA study

- **Žene na terapiji oralnim kontraceptivima u poredjenju sa ženama bez terapije**
  - povisen nivo FVIII i FIX
  - snizen nivo PS
- **Žene u menopauzi u poredjenju sa ženama u reproduktivnom dobu:**
  - povisen nivo fibrinogena, FVII i FIX
- **Žene u menopauzi na terapiji HRT u poredjenju sa ženama bez terpije:**
  - nizi nivo FVII
  - nizi nivo PS

Lowe et al, Br J Haematol 1997

Hemostatski faktori i estrogen u toku perimenopauzalnog perioda

3302 žena (42–52 godina) na ukljucenju u studiju

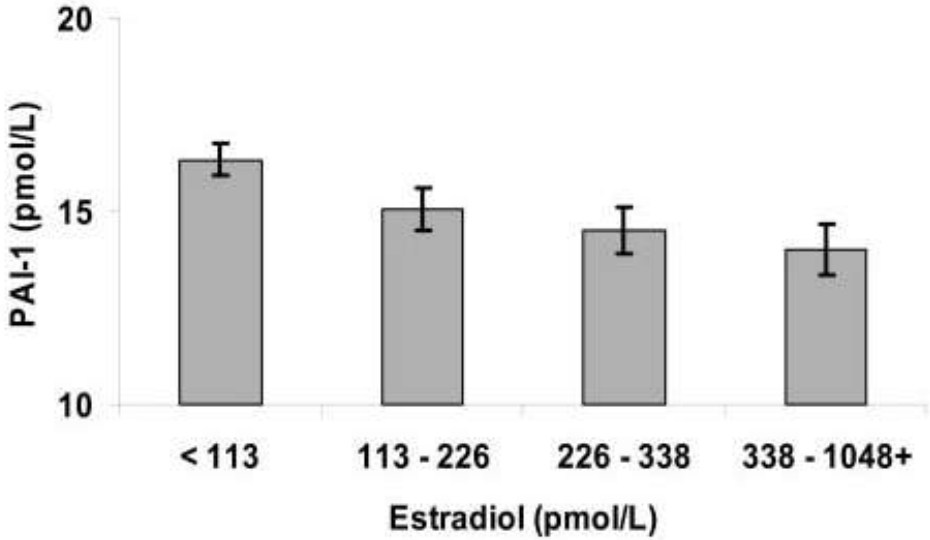
– Nivo hormona – estradiola i FSH je odredjivan pri ukljucenju u studiju i 2001, 2003 i 2005-e godine.

– Koncentracija hormona je korelisana sa markerima koronarne bolesti – fibrinogen, FVII, PAI-1, t-PA, CRP.

– **Rezultati:**

Nizak nivo estradiola
je bio udružen
sa **visokim nivoom PAI-1 i t-PA**

Nije bilo signifikantne koerelacije sa fibrinogenom, FVII, ili CRP.



Sowers et al, J Clin Endocrinol Metab, 2005

Coagulation factors and the protein C system as determinants of thrombin generation in a normal population

Dielis et al.

Study population:

Blood samples were collected from 140 healthy volunteers (67 males, 47.9%, and 73 females, 52.1%). Mean age was 54 years (range 22–90 years).

Women using oral contraceptives and individuals on anticoagulation therapy were not included. Six out of the 140 individuals (4.3%, four males and two females) were found to carry the factor V Leiden mutation and were subsequently excluded from the analysis.

J Thromb Haemost 2008; 6: 125–131

Table 2 Coagulation factor and inhibitor levels

	All samples (n = 134) Mean ± SD	Males (n = 63) Mean ± SD	Females (n = 71) Mean ± SD
Fibrinogen (mg mL^{-1})	3.23 ± 0.54	3.10 ± 0.55	3.34 ± 0.49*
Prothrombin (U dL^{-1})	119 ± 18.2	117 ± 16.5	120.5 ± 19.5
FV (U dL^{-1})	108 ± 18.6	105 ± 19.3	110.7 ± 17.7
FVII (U dL^{-1})	122 ± 22.6	116 ± 21.3	126.8 ± 22.7*
FVIII (U dL^{-1})	96.7 ± 20.0	93.6 ± 19.2	99.6 ± 20.4
FIX (U dL^{-1})	101 ± 11.5	100 ± 11.0	102.2 ± 11.9
FX (U dL^{-1})	126 ± 15.5	123 ± 16.6	129.3 ± 13.9*
FXI (U dL^{-1})	94.1 ± 12.2	90.7 ± 13.2	96.9 ± 10.6*
FXII (U dL^{-1})	90.9 ± 20.2	89.3 ± 19.0	92.3 ± 21.3
AT (U dL^{-1})	118 ± 10.1	116 ± 8.81	119.5 ± 10.9*
Protein C (U dL^{-1})	109 ± 19.8	103 ± 13.5	114.4 ± 22.9*
Free PS (U dL^{-1})	95.9 ± 17.4	101 ± 17.6	91.0 ± 15.9*
Total PS (U dL^{-1})	114 ± 14.9	113 ± 14.9	114.4 ± 15.1
TFPI activity (ng mL^{-1})	49.4 ± 22.6	50.4 ± 21.3	48.5 ± 23.8
Free TFPI antigen (ng mL^{-1})	11.2 ± 3.05	11.5 ± 2.51	10.9 ± 3.45

TFPI, tissue factor pathway inhibitor; PS, protein S. *$P < 0.05$ compared with males.

Dielis et al. **J Thromb Haemost 2008; 6: 125–131**

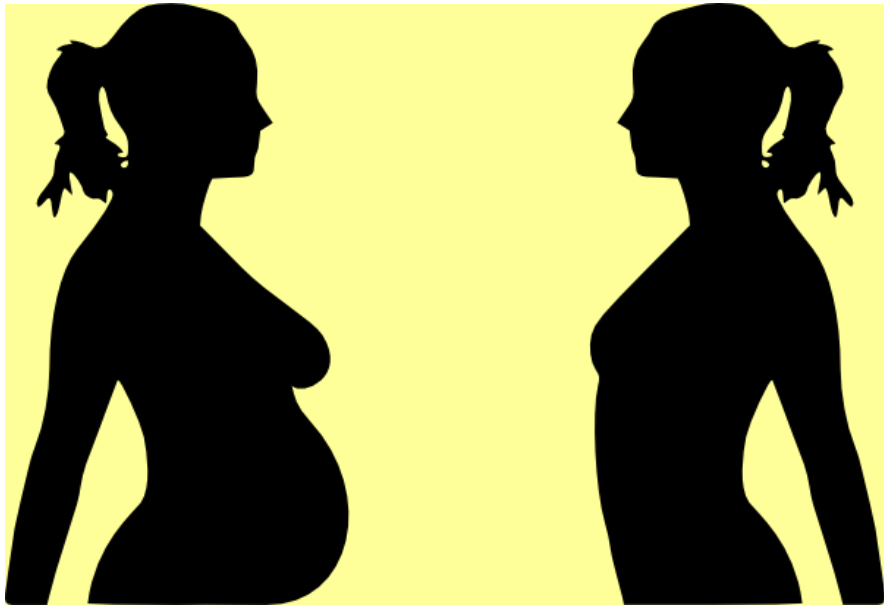
TRUDNOĆA

↑ sinteza estrogena i progesterona (najmanje 5 puta)

↑ prokoagulantna aktivnost sa maksimumom pred porodjaj

↓ sinteza inhibitora koagulacije

↓ celokupne fibrinolize



↑ rizik za DVT (4 - 10 puta)

↑ rizik za AMI (3 - 4 puta)

Faktori rizika: starost, hipertenzija, pušenje, nasledje

Nivoi nekih faktora koagulacije u toku trudnoće

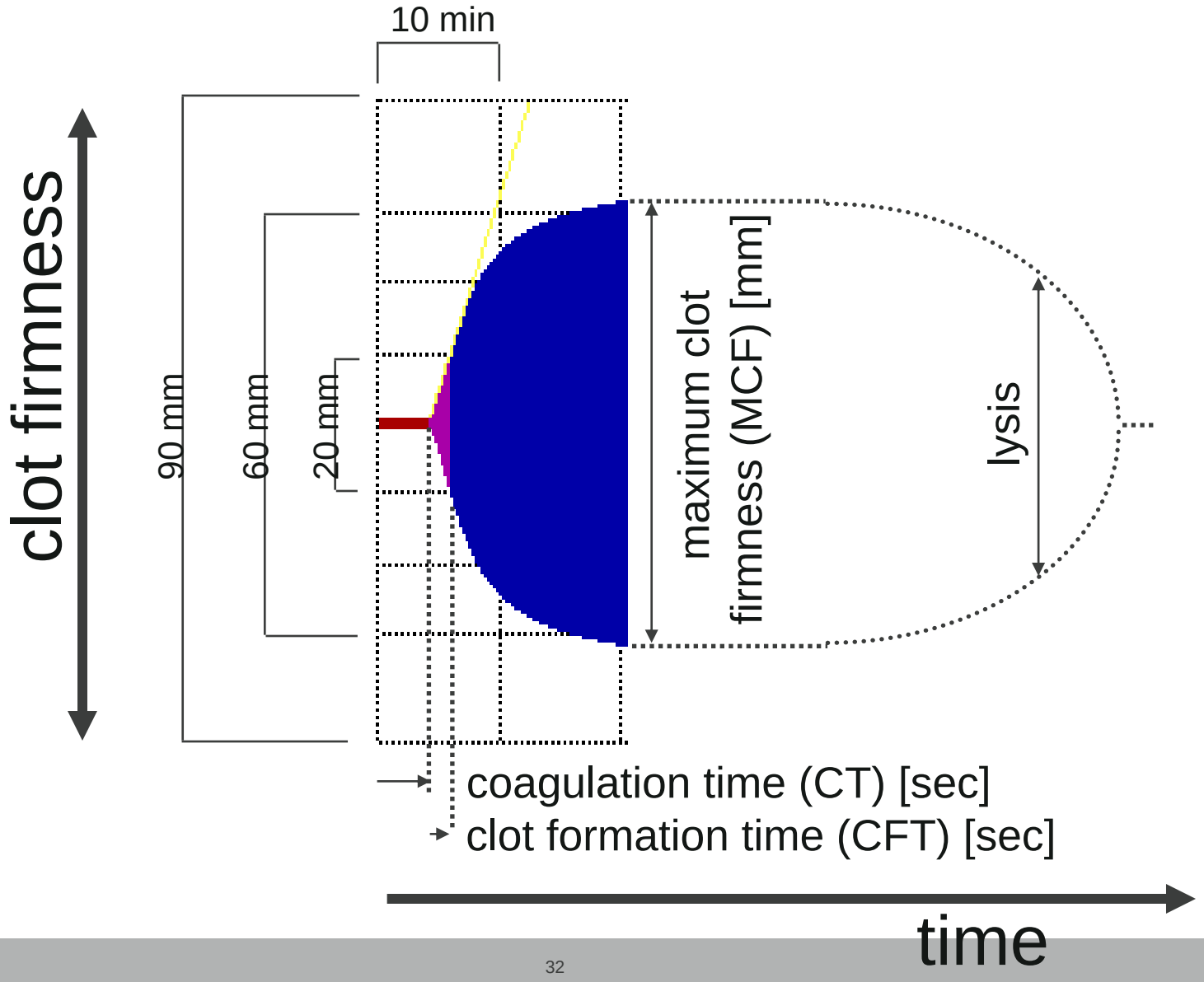
Variables % of normal	11 – 15	21 – 25	31 – 35	36 – 40	1 w	8 w	>12
F VII	111	150	162	**171**	104	94	91
F X	103	115	123	**127**	101	91	92
F V	93	82	82	85	98	80	84
F II	**125**	125	115	115	110	106	107
F VIII	122	141	185	**212**	**213**	86	109
VWF	133	167	262	**376**	**351**	93	78

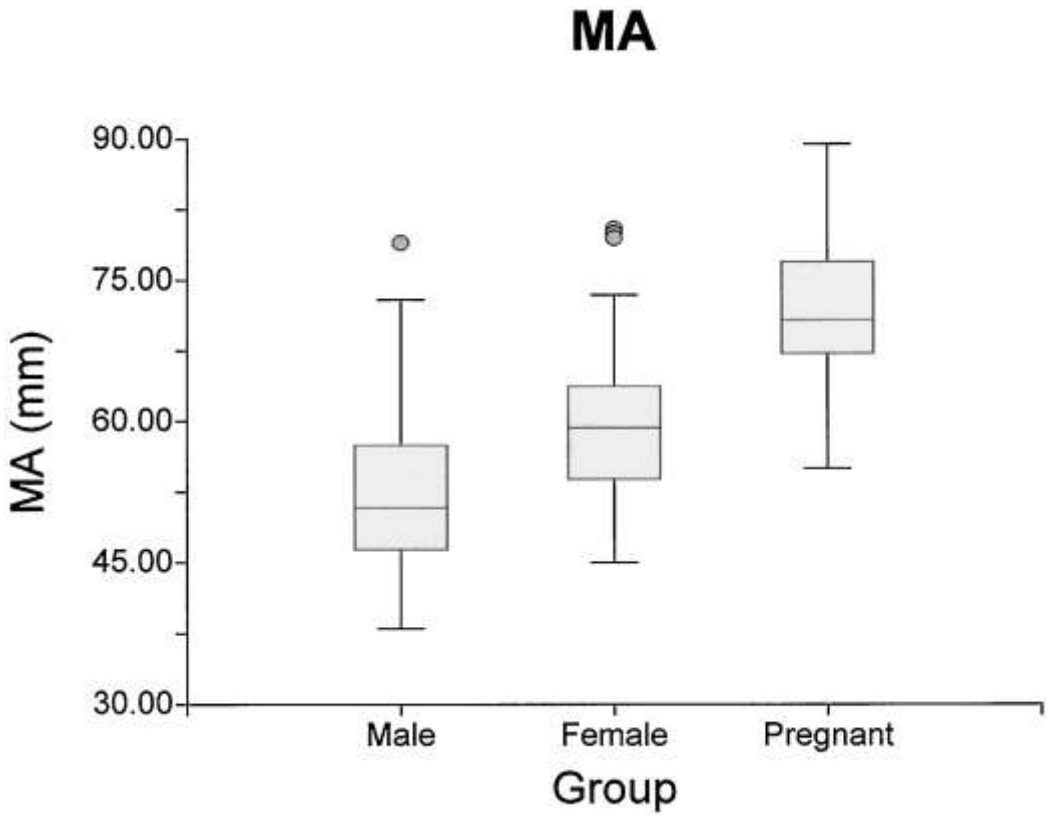
Table 1 Haemostatic changes during pregnancy

	Increased	Decreased	No change
Systemic changes			
Procoagulant factors	I, V, VII, VIII, IX, X	XI	
Anticoagulant factors	Soluble TM	PS	PC
Adhesive proteins	vWF		
Fibrinolytic proteins	PAI-1, PAI-2	t-PA	TAFI
Microparticles and antiphospholipid antibodies	MP		APLA
Local placental changes	TF	TFPI	

Brenner B, Thromb Res 2004

roTEG analysis: parameters





Maximum amplitude (MA) predstavlja čvrstinu fibrinskog klota i zavisi od funkcije trombocita i koncentracije fibrinogena. **MA je najmanji kod muškaraca a najveći kod trudnica**, što govori u prilog da je **fibrinski klot najčvrsći kod trudnica**.

Gorton et al, Anesthes Analges 2000

Circulating microparticles: a marker of procoagulant state in normal pregnancy

	Non pregnant controls N = 19	Healthy pregnant women N = 15	Statistics
Endothelial MP (/µl)	7 [1-21]	13 [1-32]	p=0.04
Platelet MP (/µl)	39 [17-674]	193 [14-773]	p = 0.0006
Total Annexin V positive MP (/µl)	122 [18-447]	429 [260-1598]	p = 0.0005
Procoagulant activity Eq nM PS	8 [1.8-11.6]	11.9[3.9-25]	p=0.018

Bretelle et al, **Thromb Haemost 2003; 89: 486–92**

Oralni kontraceptivi:

Niska doza kombinovanih oralnih kontraceptiva **nije udružena sa klinički povećanim rizikom od MI ili CVI**, uključujući pušače mladje od 35 godina.

Upotreba 50 µg ethinyl estradiol-a povezana je sa 2 od 100,000 slučajeva godišnje.

Ovaj rizik je **čak niži za žene mladje od 35 godina koje su pri tom nepušaci i nemaju hipertenziju.**

Ženama pušačima starijim od 35 god. ne preporučuje se upotreba bilo koje kombinacije oralnih kontraceptiva

Carr et al, Contraception 1997

ORALNI KONTRACEPTIVI I RIZIK ZA VENSKU TROMBOZU

TABLE 1. CURRENT BEST EVIDENCE OF THE RISK OF VENOUS THROMBOSIS AMONG APPARENTLY HEALTHY USERS OF AVAILABLE LOW-DOSE COMBINED ORAL CONTRACEPTIVES.*

STUDY	YEARS INCLUDED	AGE RANGE	STUDY DESIGN	EVENTS	NO. IN WHOM VENOUS THROMBOSIS DEVELOPED	RELATIVE RISK (95% CI)
		yr				
Helmrich et al.[12]	1976–1983	18–49	Case–control	Nonfatal deep venous thromboembolism and pulmonary embolism	5	11.0 (3.7–32.0)
Vessey et al.[13]	1968–1985	25–56	Cohort	Fatal and nonfatal superficial venous thrombophlebitis, deep venous thromboembolism, and pulmonary embolism	3	3.3 (0.9–11.4)
World Health Organization[14]	1989–1993	15–49	Case–control	Nonfatal deep venous thromboembolism and pulmonary embolism	132 from Europe, <35 yr old 42 from Europe, ≥35 yr old 93 from developing country, <35 yr old 28 from developing country, ≥35 yr old	4.3 (2.9–6.5) 3.9 (2.3–6.6) 3.2 (2.3–4.5) 2.5 (1.5–4.3)
Jick et al.[15]	1991–1994	<40	Cohort	Nonfatal deep venous thromboembolism and pulmonary embolism	75	6.1 (2.5–15.1)
Lewis et al.[16]	1993–1995	16–44	Case–control	Fatal and nonfatal deep venous thromboembolism and pulmonary embolism	334	4.4 (3.4–5.8)

*Adapted from Hannaford and Owen-Smith.[11] CI denotes confidence interval.

Vandenbroucke et al NEJM 2001

ORALNI KONTRACEPTIVI I RIZIK ZA VENSKU TROMBOZU

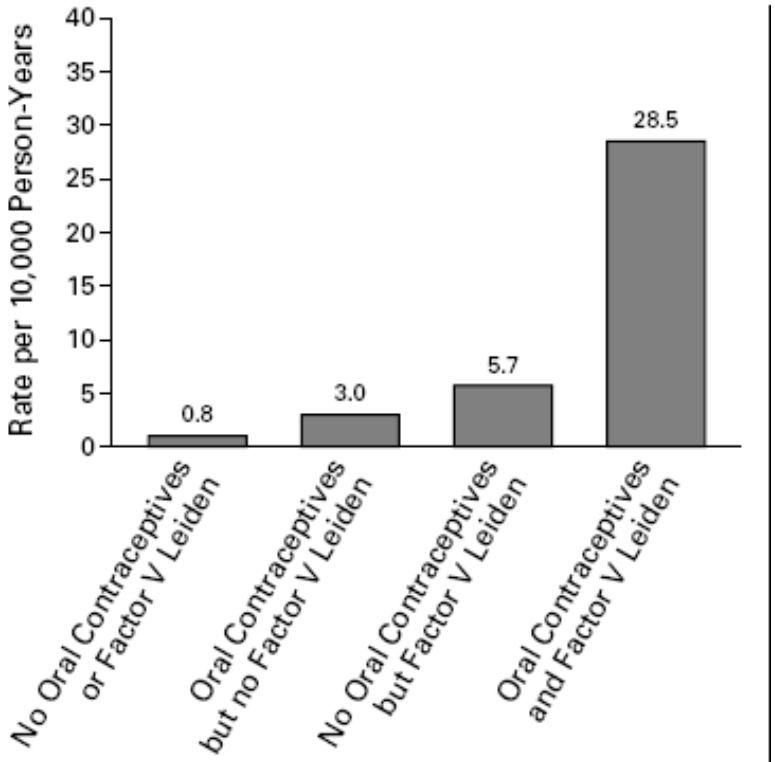


Figure 1. Cases of Deep-Vein Thrombosis per 10,000 Person-Years, According to the Use of Oral Contraceptives and the Presence of Factor V Leiden.

Vandenbroucke et al NEJM 2001

ORALNI KONTRACEPTIVI I RIZIK ZA VENSKU TROMBOZU

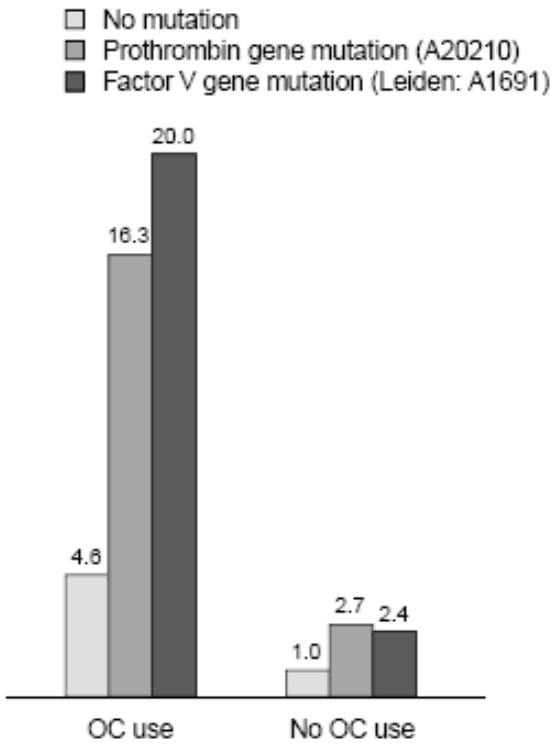
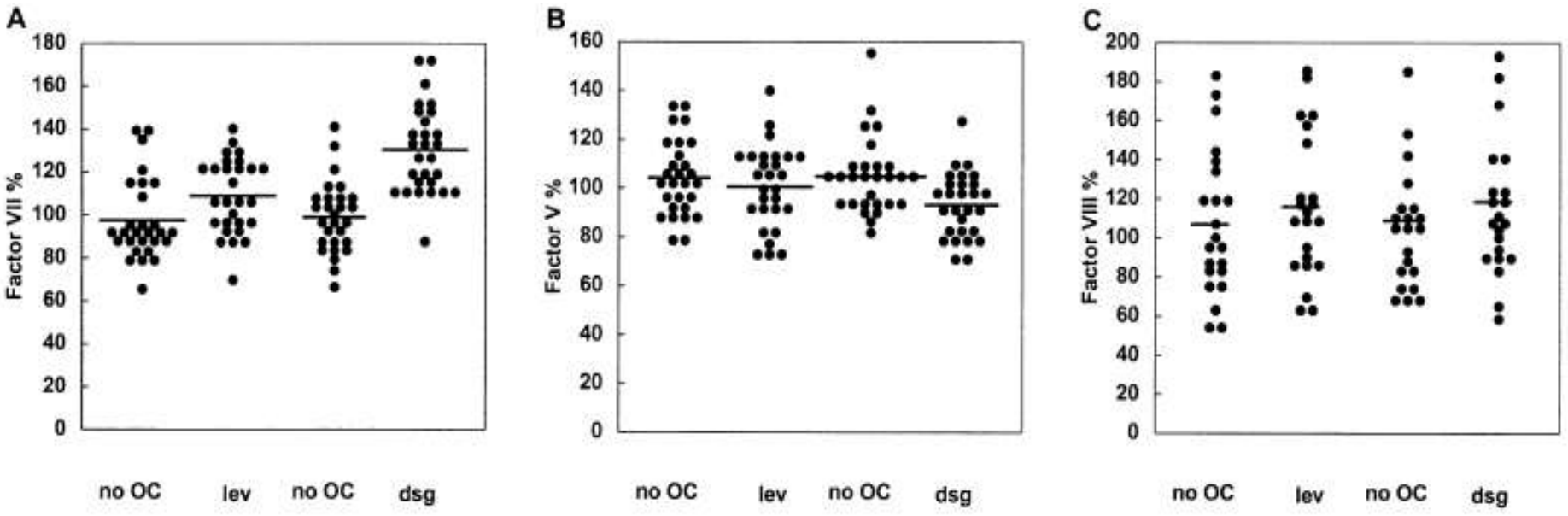


Fig. 3. Risks of venous thromboembolism (shown as odds ratios on each column) according to oral contraceptive use (OC) and the presence or absence of mutations in common factors of the haemostatic system known to affect thrombosis risk.[71]

Godsland et al, Drugs 2000

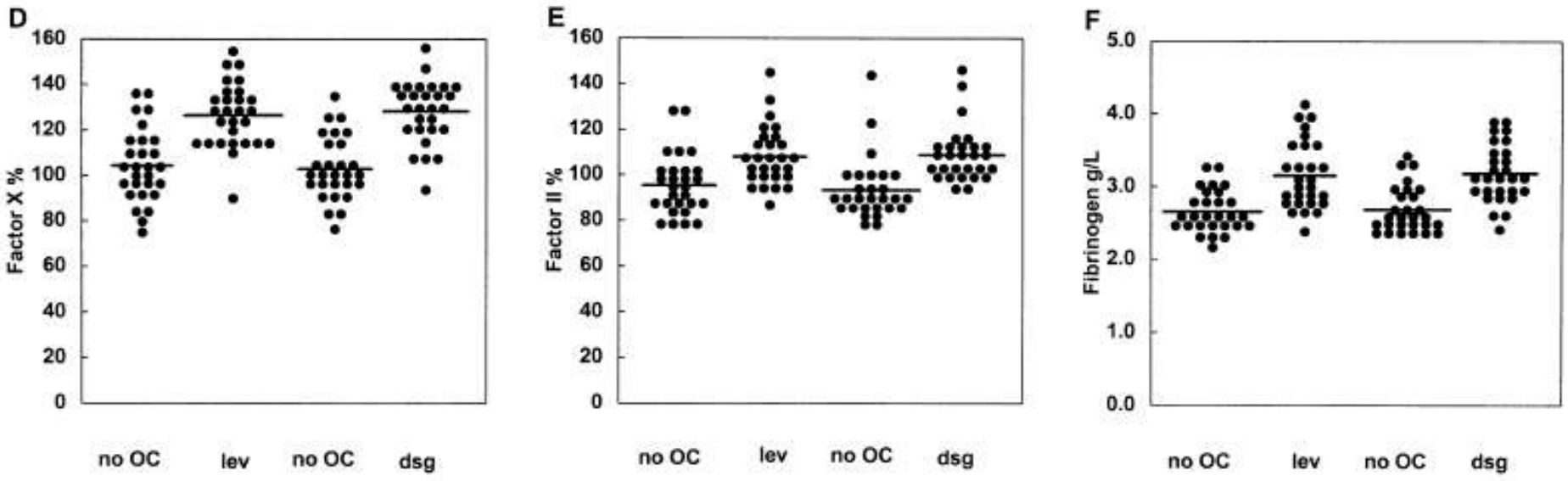
Uticaj niske doze levonorgestrela and desogestrela na koagulaciju



N=33 women, cross-over design

Middeldorp et al, Thromb Haemost, 2000

Uticaj niske doze levonorgestrela and desogestrela na koagulaciju



Koncentracija F II, VII, X i fibrinogena signifikantno je povećana u toku terapije i levonorgestrelom i desogestrelom

Middeldorp S et al, Thromb Haemost, 2000

Increased Fibrinolytic Activity during Use of Oral Contraceptives

A Randomized Cross-over Study of Two Low-dose Oral Contraceptives

↑ koncentracija plazminogena

↓ PAI-1 Ag i aktivnosti

↑ F1+2 (aktivacija koagulacije)

↑TAFI Ag

↓ celokupne fibrinolize

OC-induced increase in endogenous fibrinolytic activity is counteracted by an increased capacity of the coagulation system to down regulate fibrinolysis via TAFI.

Meijers et al Thromb Haemost 2000

THE LANCET

Volume 362, Number 9391

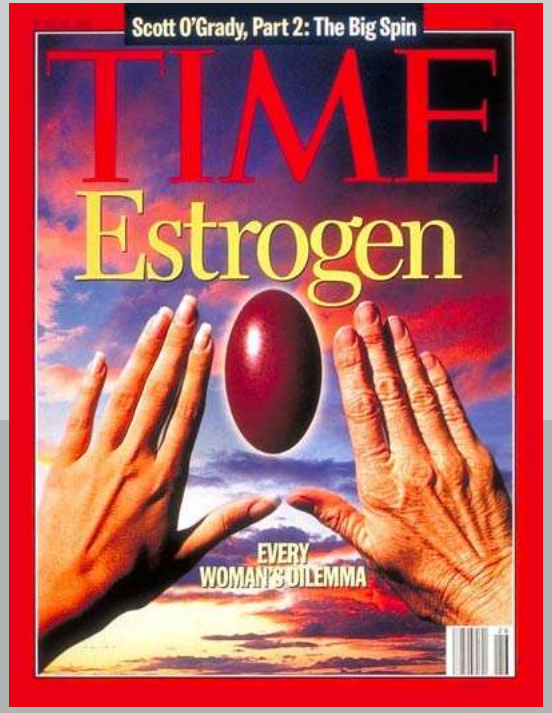
The greatest threat to women's health

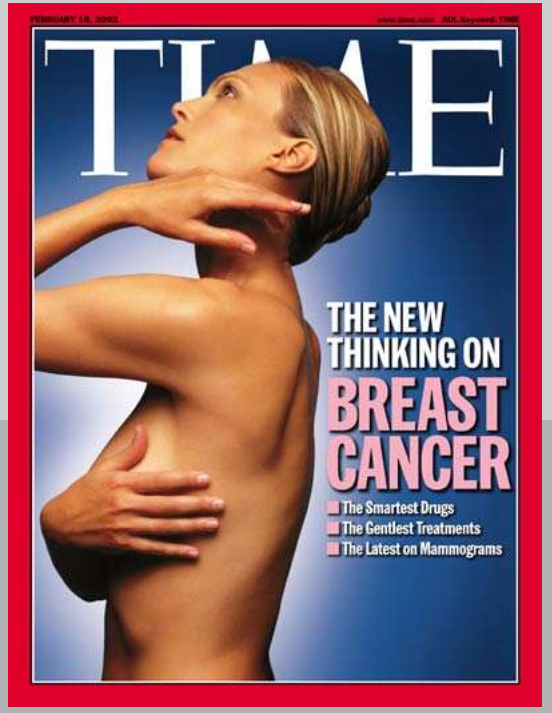
Which disease kills the greatest number of women worldwide? Judging by the attention given to the disease in newspaper headlines and media campaigns, many women and men in developed nations might answer breast cancer. Others might hazard a guess at malaria, tuberculosis, or HIV/AIDS. They would all be wrong. In fact, heart attacks and stroke kill twice as many women as all cancers combined. Women are four times more likely to die from coronary heart disease than from breast cancer. Moreover, contradicting conventional wisdom, women are more likely to die from cardiovascular disease than men.

disease in women will take national campaigns to include antenatal care, schools, and the mass media. Promoting images of women with cardiovascular disease in television dramas (men are almost always portrayed as the victims of heart disease), newspaper articles, and magazines would help.

It is too easy, though, to blame others and to ask others to change. Breast cancer researchers are very successful at promoting public awareness of their field as the media hype around the launch of a 10-year trial of anastrozole demonstrated last week. Cardiovascular disease researchers, who have largely ignored

Hormone Replacement Treatment (HRT)





Karolinska Institutet

Danderyds Sjukhus

Randomized Trial of Estrogen Plus Progestin for Secondary Prevention of Coronary Heart Disease in Postmenopausal Women (HERS)

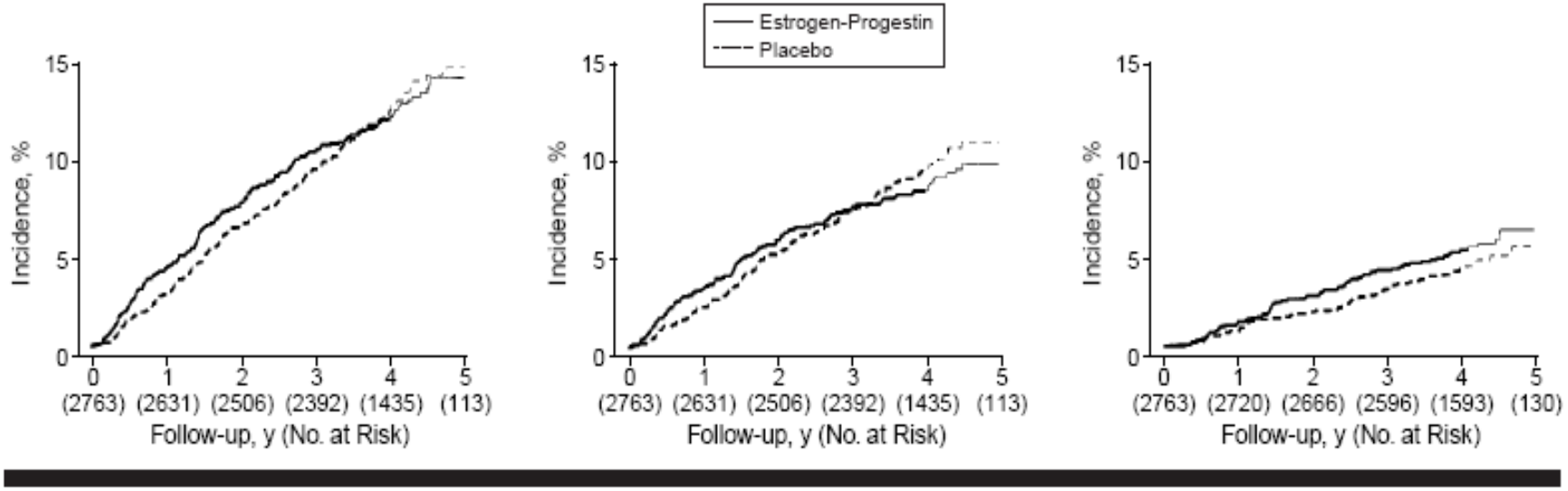


Figure 3.—Kaplan-Meier estimates of the cumulative incidence of primary coronary heart disease (CHD) events (left) and to its constituents: nonfatal myocardial infarction (MI) (center) and CHD death (right). The number of women observed at each year of follow-up and still free of an event are provided in parentheses, and the curves become fainter when this number drops below half of the cohort. Log rank *P* values are .91 for primary CHD events, .46 for nonfatal MI, and .23 for CHD death.

Hulley et al JAMA 1998

Table. Outcomes by Treatment Groups and Year Since Randomization in the Heart and Estrogen/progestin Replacement Study*

Outcome	Women with Events Who Received Estrogen plus Progestin	Events per 1000 Woman-Years	Women with Events Who Received Placebo	Events per 1000 Woman-Years	Relative Hazard Ratio (95% CI)	*P* Value for Trend†
	n		*n*			
Primary coronary heart disease event‡						
Year 1	57	42.5	38	28.0	1.52 (1.01–2.29)	0.03
Year 2	47	36.9	49	37.8	0.98 (0.66–1.46)	–
Year 3	35	28.7	42	33.9	0.85 (0.54–1.33)	–
Years 4 and 5	40	28.0	53	37.4	0.75 (0.50–1.13)	–

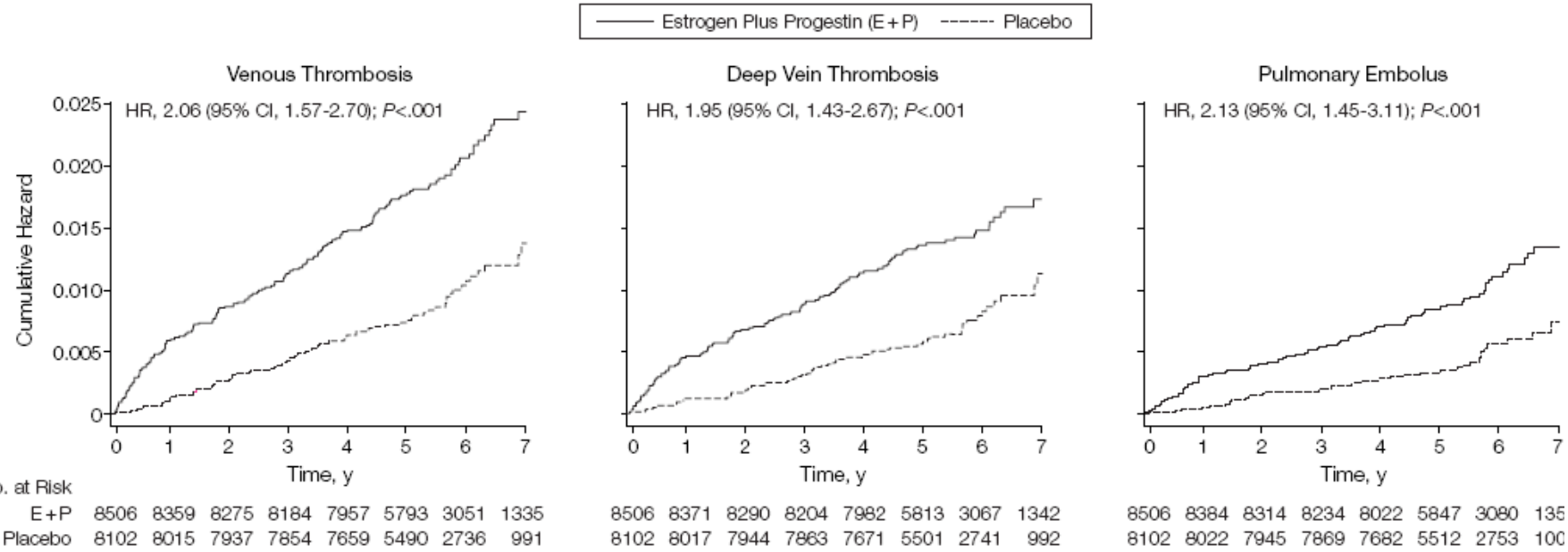
* A more detailed version of this table, as well as updated versions of all the tables from the original Heart and Estrogen/progestin Replacement Study report (1), is available on the World Wide Web at http://www.epibiostat.ucsf.edu/HERS/ (posted 24 February 1999). This table reflects the final results after inclusion of 13 additional primary events that were not yet adjudicated at the time of the original publication.
† Nominal test for trend in Ln (hazard ratio).
‡ Includes nonfatal myocardial infarction and death from coronary heart disease.

Outcomes by Treatment Groups and Year Since Randomization in the Heart and Estrogen/Progestin Replacement Study

Herrington Ann Intern Med 1999

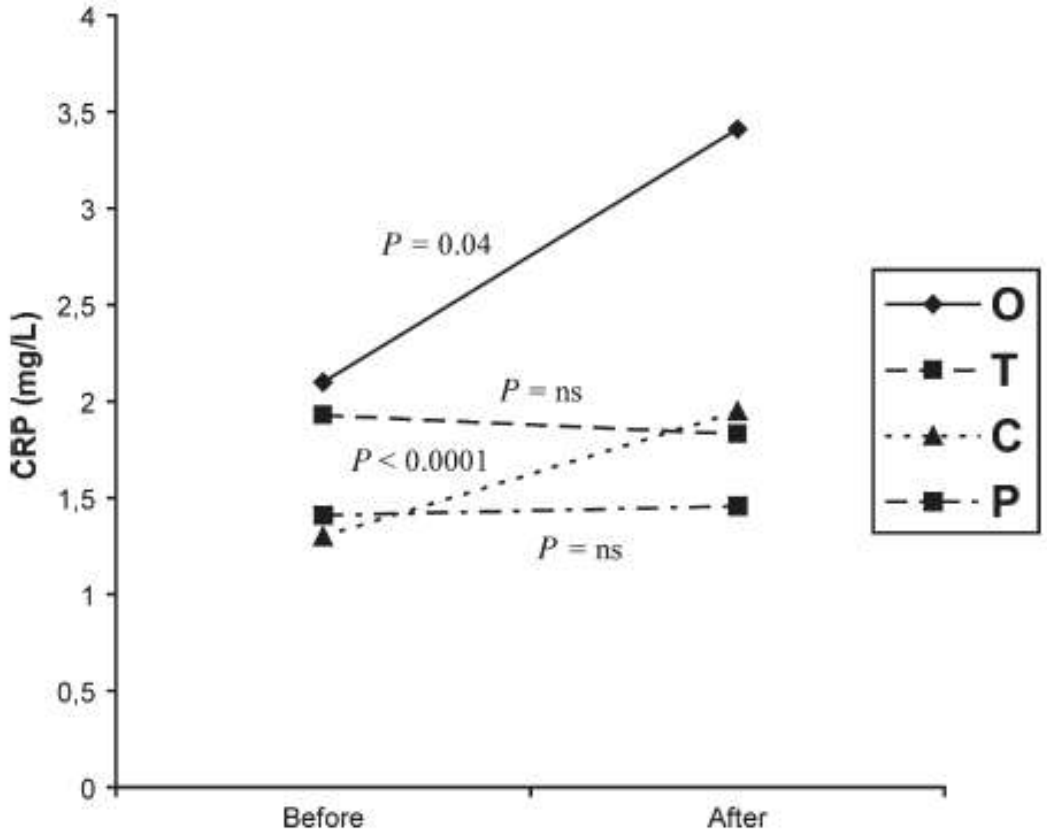
Estrogen plus Progestin i rizik od venske tromboze

Figure 1. Cumulative Hazard of Venous Thrombosis, Deep Vein Thrombosis, and Pulmonary Embolus



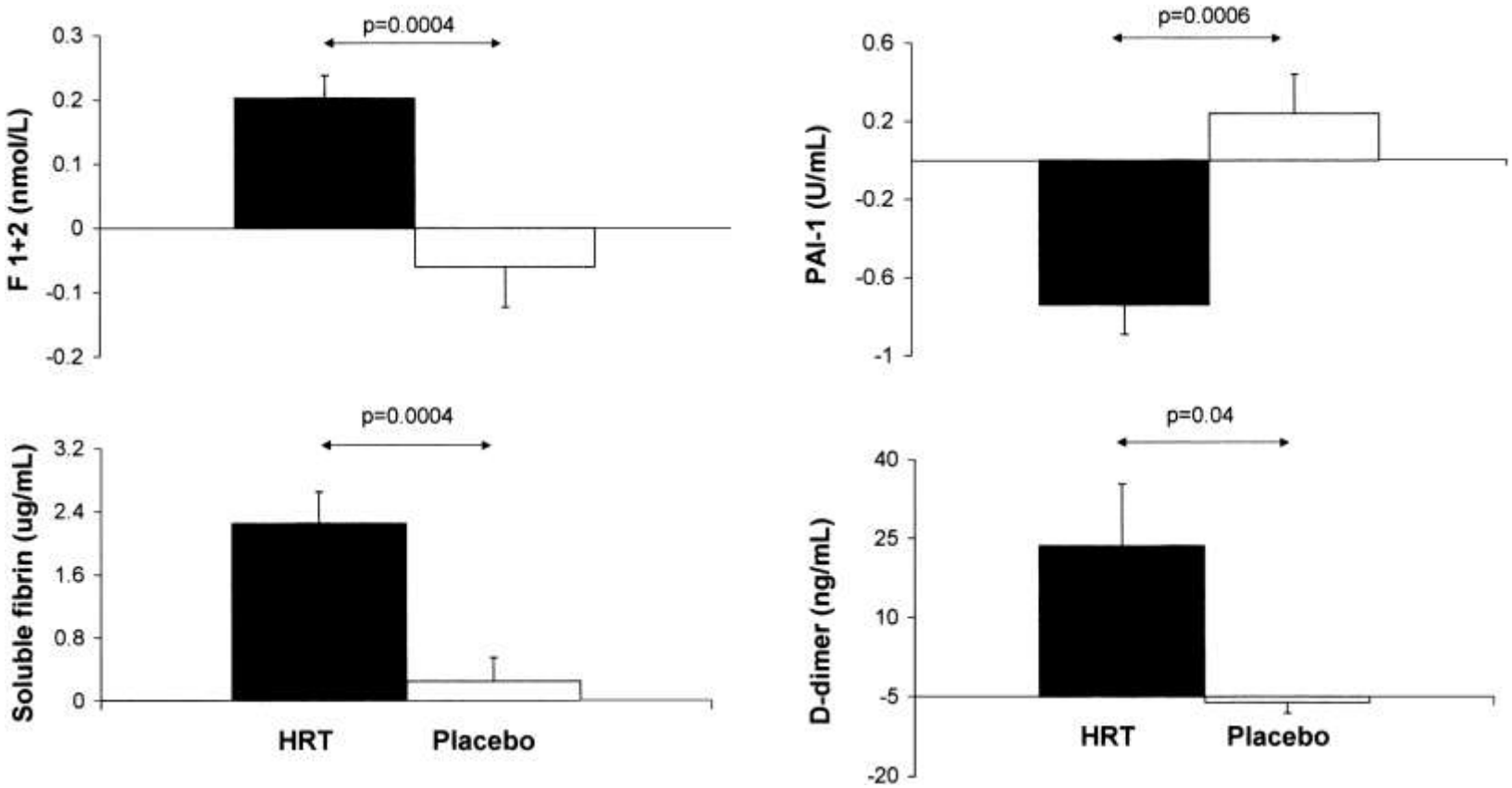
CI indicates confidence interval; HR, hazard ratio.

Cushman JAMA. 2004;292:1573-1580



Plasma C-reactive protein (CPR) level at baseline and after 6 months of oral (O) and transdermal (T) estradiol therapy, combined (C) menopause-related hormone therapy, and placebo (P).

Zegura et al Menopause 2006



Promene prothrombin fragmenta (F 1+2), solublnog fibrina, PAI-1 i D-dimera posle 6 nedelja terapije placebom ili HRT

Teede et al. Arterioscler Thromb Vasc Biol 2000

Kombinovana oralna estradiol valerate-norethisterone terapija u trajanju više od 3 godine kod žena u menopauzi

	Pre-treatment	Year 3	*P* (*t*)
Platelets	260 (52.8)	258 (54)	0.63
Fibrinogen	3.42 (0.69)	4.05 (0.83)	<0.0001
Protein C	1.44 (0.30)	1.05 (0.22)	<0.0001
Anti-thrombin III	1.15 (0.14)	0.99 (0.16)	<0.0001
Total protein S	1.28 (0.34)	1.05 (0.19)	<0.0001
Free protein	0.36 (0.16)	0.45 (0.11)	<0.0001
Prothrombin time	12.2 (0.70)	12.53 (0.94)	<0.0001
KPTT	32.3 (3.6)	31.9 (3.3)	<0.13
Thrombin	14.7 (1.1)	16.0 (1.4)	<0.0001

Perry & Wiseman, Maturitas 2002

Da li kratkotrajna hormonska terapija ima drugačije efekte na hemostazu u poredjenju sa dugotrajnom hormonskom terapijom?

A randomized, placebo-controlled trial of the effects of continuous combined HRT; N=61

Plasma levels of markers of coagulation at baseline and after 3 and 6 months of treatment with HRT or placebo.

Variable	Group	Baseline value	After 3 months	After 6 months	*P* value[a]
Antithrombin III activity (% NPP)	HRT	96 ± 9	87 ± 10[b]	88 ± 9[b]	<.001
	Placebo	95 ± 11	95 ± 11	99 ± 12	
Protein C activity (% NPP)	HRT	130 ± 29	117 ± 25[b]	117 ± 21[b]	<.001
	Placebo	126 ± 21	125 ± 20	126 ± 20	
Prothrombin fragments 1 and 2 level (nmol/L)	HRT	0.91 (0.69, 1.50)	0.98 (0.76, 1.23)	1.02 (0.73, 1.40)	NS
	Placebo	0.94 (0.70, 1.42)	0.93 (0.70, 1.16)	0.84 (0.64, 1.03)	
Thrombin–antithrombin complex level (μg/L)	HRT	1.7 (1.4, 4.0)	1.9 (1.5, 2.7)	2.1 (1.4, 3.0)	–[c]
	Placebo	1.7 (1.4, 2.3)	1.6 (1.4, 3.0)	1.6 (1.4, 2.7)	

Plasma levels of markers of fibrinolysis at baseline and after 3 and 6 months of treatment with HRT or placebo.

Variable	Group	Baseline	After 3 months	After 6 months	*P* value[a]
Plasminogen activator inhibitor-1 antigen level (ng/mL)	HRT	12.1 (6.7, 21.5)	5.9[c] (3.8, 10.0)	5.8[c] (3.7, 14.3)	<.001
	Placebo	9.1 (6.6, 12.9)	11.3 (7.4, 19.6)	13.3 (6.4, 19.6)	
Tissue-type plasminogen antigen level (ng/mL)	HRT	9.1 ± 4.1	6.7 ± 2.4[b]	6.8 ± 3.5[b]	<.05
	Placebo	8.9 ± 4.2	9.3 ± 3.8	8.8 ± 3.8	
D-Dimer level (ng/mL)	HRT	19 (14, 25)	30[c] (15, 75)	29[c] (16, 64)	<.001
	Placebo	21 (14, 50)	23 (14, 46)	24 (12, 44)	
Euglobulin clot lysis time (min)	HRT	288 ± 53	228 ± 73[c]	230 ± 62[c]	<.01
	Placebo	287 ± 71	280 ± 87	276 ± 73	

Nakon 6 meseci HRT, signifikantno sniženje aktivnosti AT, PC i nivoa PAI-1- Ag, t-PA-Ag i euglobulin-CLT i signifikantno povećanje nivoa D-dimera u poredjenju sa placebo tretmanom.

Guruzicic-Salobir et al, Fertil Steril 2002

Efekat visoke doze estradiola aplikovanog vaginalnim putem na hemostazu kod žena u menopauzi

Pilot studija. N=8 tretiranih 17 beta-estradiolom putem vaginalnih prstenova koji oslobadjaju ukupno 22.5 µg / 24 h. Prstenovi su menjani svakog jutra u toku 14 dana.

	Before			Day 8			Day 15		
Hemostatic variable	25 thp	50 thp	75 thp	25 thp	50 thp	75 thp	25 thp	50 thp	75 thp
Fibrinogen g/L	2.59	3.14	3.89	2.70	3.41	3.80	2.70	3.33	3.80
VWF, IU/mL	0.79	1.01	1.18	0.60	1.11	1.19	0.81	1.02	1.15
APC ratio	0.87	1.03	1.09	1.03	1.19	1.31	0.98	1.10	1.30
Factor VIIa, IU/mL	39.00	43.50	52.00	36.50	47.50	57.00	40.50	49.00	54.50
Protein C, IU/mL	1.01	1.08	1.19	1.01	1.12	1.14	0.96	1.10	1.22
Protein S free U/mL	0.25	0.27	0.32	0.26	0.27	0.28	0.25	0.27	0.29
Fr1+2, nmol/L	0.61	0.84	1.08	0.62	0.79	0.95	0.55	0.77	1.63
Soluble fibrin nmol/L	8.00	9.50	13.00	9.00	11.00	16.50	11.00	12.00	17.50
D-dimer ng/mL	11.00	15.50	21.50	11.50	14.50	34.50	12.30	22.00	28.80
PAI-1 IU/mL	3.00	3.00	10.00	1.00	3.00	15.00	2.00	3.50	6.00

Nema značajnih promena u hemostatskim parametrima u toku vaginalne primene estradiola u dozi ekvivalentnoj transdermalnoj dozi.

Hall et al, Fertil Steril, 2002

ŠTA SE ZNA?

Polni hormoni utiču na hemostazu

Trudnoća je protrombotsko stanje

HRT i oralni kontraceptivi multipliciraju rizik za trombozu kod žena sa trombofilijama

Smatra se da:

Estrogen smanjuje aktivnost trombocita

Estrogen pojačava globalnu hemostazu (ali i fibrinolizu)

Testosteron smanjuje globalnu hemostazu

Efekat na hemostazu je različit u zavisnosti od načina pimene estrogena (oralni u poredjenju sa transdermalnim)

Metabolizam u jetri svih hormonskih preparata utiče na koagulaciju

Šta se ne zna?

Uticaj endogenih polnih hormona na svaki hemostatski parametar

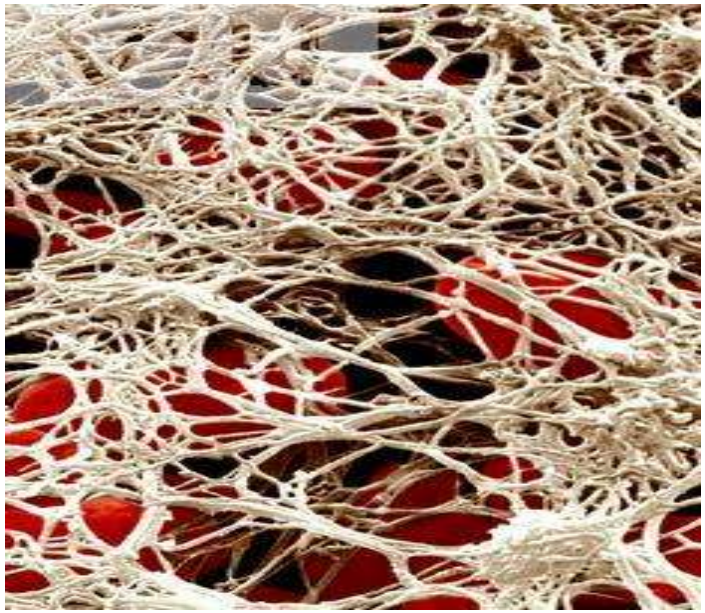
Razlika izmedju prirodnih i sintetskih hormona na hemostazu

Meta och Birger Blombäck i sitt laboratorium på Karolinska Institutet någon gång under femtiotalet med en av sina första flaskor Fraktion 1-0.

In the light of recent data, there is compelling evidence to suggest that transdermal estrogens should be considered when choosing a hormone therapy regimen, especially in women at high risk for cardiovascular disease. The understanding of the molecular mechanisms constitute the basis for new pharmacological developments allowing the prevention of deleterious effects and preserving the beneficial ones [52,68]. The effects of selective estrogen receptor modulators (SERMs) on the different actors of the atheroma plaque formation have now to be analyzed on the basis of their specific regulation of the ERα but also of the ERβ, which may mediate some anti-inflammatory actions *in vivo*. Various classes of estrogens and SERMs have been described according to their molecular actions through ERα [69–71]. Due to the complexity of the mechanisms of action of estrogens and SERMs, their effect on various cell types and tissues cannot be predicted from their structure. Hence, integrated models that allow the screening of present and future SERMs in terms of beneficial and deleterious effects will be valuable, important tools. Theoretically, it is conceivable to design a SERM (or a combination of molecules) which would retain most (if not all) of the desired effects of estradiol (on the central nervous system to prevent vasomotor flushes, on bone, on endothelium), but which should be devoid of the undesirable effects of estradiol (mainly breast cancer, thromboembolism and probably pro-inflammatory effects). SERMs currently available (tamoxifen, raloxifen) prevent breast cancer, but are devoid of effects on menopause symptoms and on cardiovascular risk. Prevention of both breast cancer and cardiovascular diseases by novel SERMs thus represents the major challenge in the future treatment of menopause.

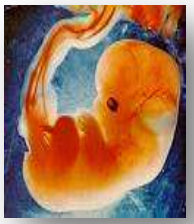
Fibrinogen:

Glikoprotein, sintetiše se u jetri, 2-4 g/L; ¾ u plazmi, ima ga i u trombocitima, limfnim žlezdama i intersticijalnoj tečnosti, poluživot 3-5 dana.



- substrat za trombin u završnom stadijumu koagulacije
- substrat za plazmin u početnoj fazi fibrinolize
- neophodan za agregaciju trombocita
- utiče na funkciju endotelnih celija
- pomaže proliferaciju i migraciju glatkih mišićnih ćelija
- utiče na viskozitet plazme, viskozitet krvi i agregaciju crvenih kvnih zrnaca
- **protein akutne faze**

Male vulnerability…
Shortened lifespan



- Intrauterine: more misscarriages of male fetuses
- Neonatal: higher death rate and complications at birth, birth defects
- Teenage to age 40: suicide and murder
- Adults >40: heart disease

59

Gender Research

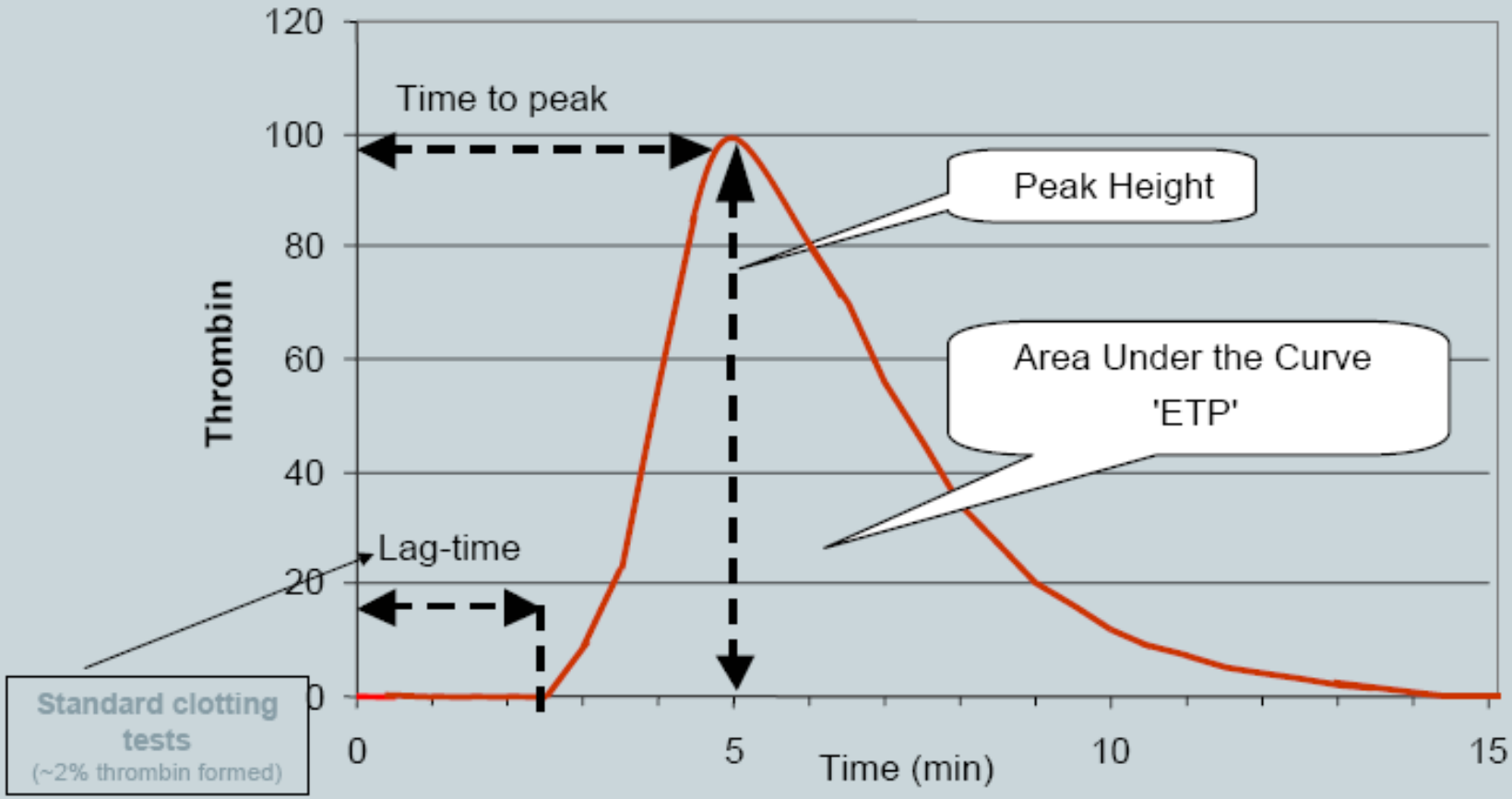
Legislature

- 1970 FDA -exclusion of fertile women from drug trials
- 1980's womens health movement made demands for change driven by lack of new therapies for breast cancer
- 1993 Revitalization Act-
  - Women must be included in clinical trials"……

60

Analyzing the Thrombin Generation Curve

A schematic representation of a thrombin generation curve



The thrombin generation (TG) curve provides information on Lag-time, Time to peak, Peak height (PH) and area under the curve or endogenous thrombin potential (ETP).

Table 1 Parameters of thrombin generation determined at 1 pM tissue factor (TF) in the absence and presence of thrombomodulin (TM), and at 13.6 pM TF in the absence and presence of activated protein C (APC)

	Lag time (min)	ETP (nM.min)	Peak height (nM)
All samples (n = 134)			
1 pM TF	5.84 ± 1.65	995 ± 289	116 ± 50.8
1 pM TF + TM	5.57 ± 1.85	299 ± 143	59.6 ± 34.2
13.6 pM TF	2.67 ± 0.38	1490 ± 224	335 ± 40.7
13.6 pM TF + APC	4.34 ± 0.88	152 ± 107	31.8 ± 27.1
Males (n = 63)			
1 pM TF	6.06 ± 1.52	948 ± 300	113 ± 55.2
1 pM TF + TM	5.81 ± 1.79	280 ± 150	56.1 ± 36.4
13.6 pM TF	2.72 ± 0.39	1480 ± 239	328 ± 38.0
13.6 pM TF + APC	4.62 ± 1.01	99.9 ± 73.9	18.9 ± 17.8
Females (n = 71)			
1 pM TF	5.66 ± 1.74	1030 ± 277	119 ± 47.3
1 pM TF + TM	5.38 ± 1.89	315 ± 137	62.5 ± 32.2
13.6 pM TF	2.62 ± 0.36	1490 ± 212	342 ± 42.1*
13.6 pM TF + APC	4.11 ± 0.69*	194 ± 111*	42.2 ± 28.9*

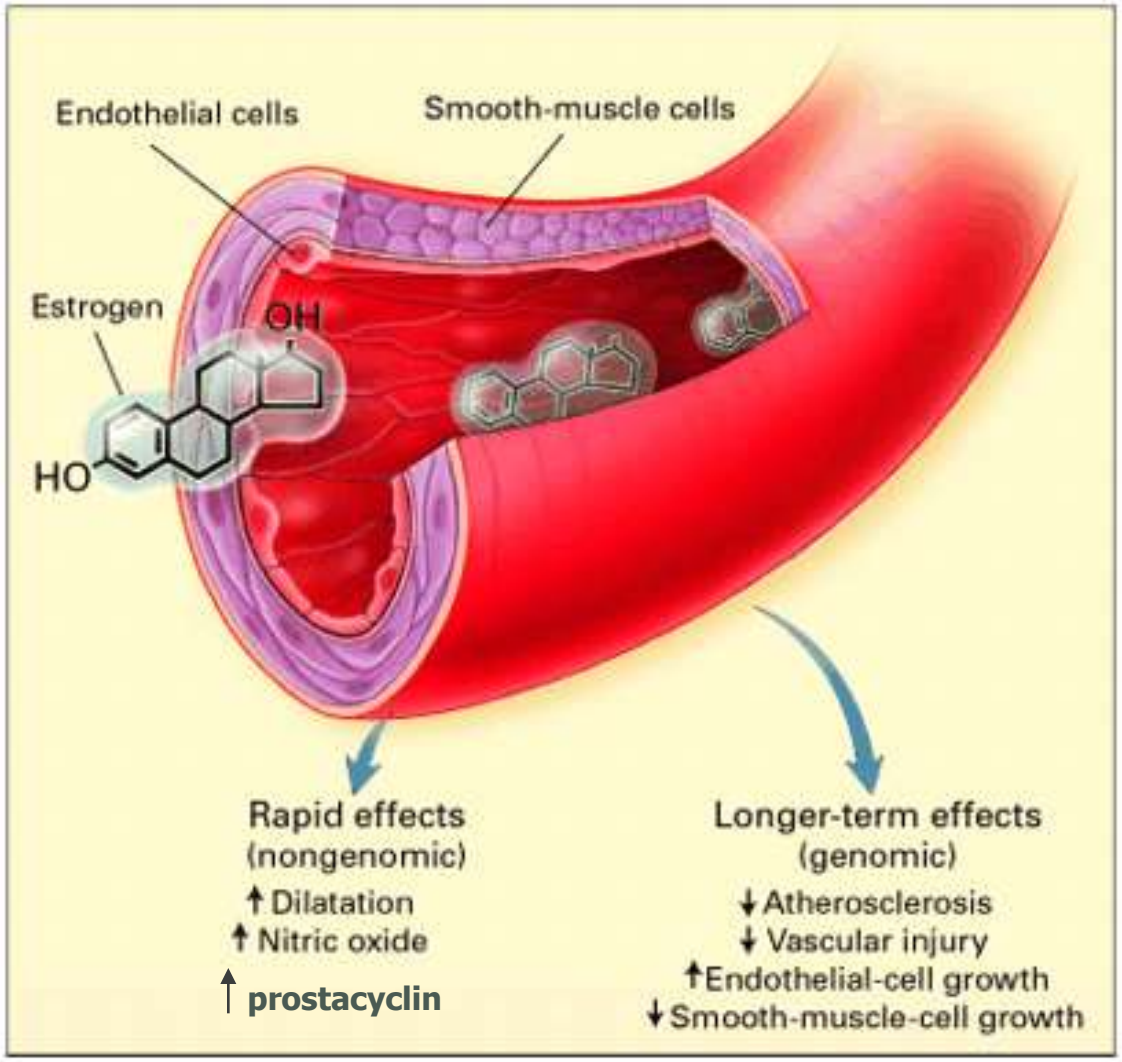
Data are expressed as mean ± SD. *P < 0.05 compared with males.

Dielis et al. **J Thromb Haemost 2008; 6: 125–131**

U kojoj fazi mestrualnog ciklusa treba uzimati uzorak za dijagnostiku hemoragijskih poremećaja uzrokovanih disfunkcijom trombocita & VWD?

- **Edlund et al,** 1996: ***dan 5-7*** dobar CV izmedju uzoraka i izmedju dana
- **Kadir,** 1999: najniži nivoi FVIII / VWF ***(dan 5-7)***
- **Miller et al 2002-** najniži nivoi FVIII, VWF i fibinogena ***dan 1-4***
- ***Zaključak:*** **U POČETNOJ FAZI MENSTRUALNOG CIKLUSA (cd 1-4)**

Uticaj estrogena na endotel



Koncentraija markera koagulacije i fibrinolize u plazmi

Parameter	Group	Baseline	After 6 mo	*P*
PAI-1 antigen (ng/mL)	O	8.25 (5.10-11.3)	4.20 (2.0-8.10)	ns
	C	12.1 (6.7-21.5)	5.8 (3.7-14.5)	<0.001
	T	10.30 (3.7-14.3)	8.20 (3.35-17.1)	ns
	P	9.1 (6.6-12.9)	13.3 (6.4-19.6)	ns
PAI-1 activity (IU/mL)	O	4.30 (0.00-9.05)	1.60 (0.00-6.10)	<0.05
	C	9.9 (0.0-13.9)	4.3 (0.0-11.5)	ns
	T	8.02 (0.80-12.10)	8.50 (1.60-14.70)	ns
	P	8.9 (0.0-16.7)	5.1 (0.0-23.9)	ns
t-PA antigen (ng/mL)	O	5.15 (3.50-7.60)	3.9 (2.80-5.7)	0.01
	C	9.0 (5.6-11.4)	6.2 (4.9-8.1)	<0.05
	T	6.00 (5.50-6.70)	5.85 (4.70-7.35)	<0.05
	P	8.8 (5.8-11.3)	8.1 (6.2-10.6)	ns
ECLT (min)	O	240 (170-285)	180 (125-285)	<0.05
	C	290 (255-330)	230 (195-270)	<0.01
	T	285 (175-297)	275 (220-310)	ns
	P	300 (245-330)	285 (225-330)	ns
Fibrinogen (g/L)	O	3.58 (2.81-3.77)	2.58 (2.47-2.80)	0.002
	C	3.36 (2.79-3.98)	3.04 (2.44-3.57)	ns
	T	3.62 (3.17-3.95)	3.20 (2.65-3.61)	0.007
	P	3.36 (2.82-3.85)	3.26 (2.62-3.55)	ns
AT III activity (% NPP)	O	1.03 (0.89-1.08)	0.97 (0.83-1.02)	ns
	C	0.98 (0.89-1.03)	0.90 (0.82-0.94)	ns
	T	0.99 (0.89-1.02)	0.96 (0.83-1.04)	ns
	P	0.95 (0.91-1.01)	0.98 (0.90-1.05)	ns
Protein C activity (% NPP)	O	1.25 ± 0.20	1.30 ± 0.25	ns
	C	1.30 ± 0.29	1.17 ± 0.21	<0.001
	T	1.19 ± 0.17	1.15 ± 0.15	ns
	P	1.26 ± 0.21	1.25 ± 0.20	ns

Variables are shown as medians and interquartile ranges or as means ± SDs.
PAI-1, plasminogen activator inhibitor-1; ns, not significant; t-PA, tissue plasminogen activator; ECLT, euglobulin clot lysis time; AT III, antithrombin III; NPP, normal pooled plasma.

At baseline and after 6 months of combined (C) hormone therapy, oral (O) and transdermal (T) estradiol therapy, and placebo (P). N=112. Samples taken at baseline and 28 weeks after.

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