Prenatal screening – aneuploidies and thyroid disorders

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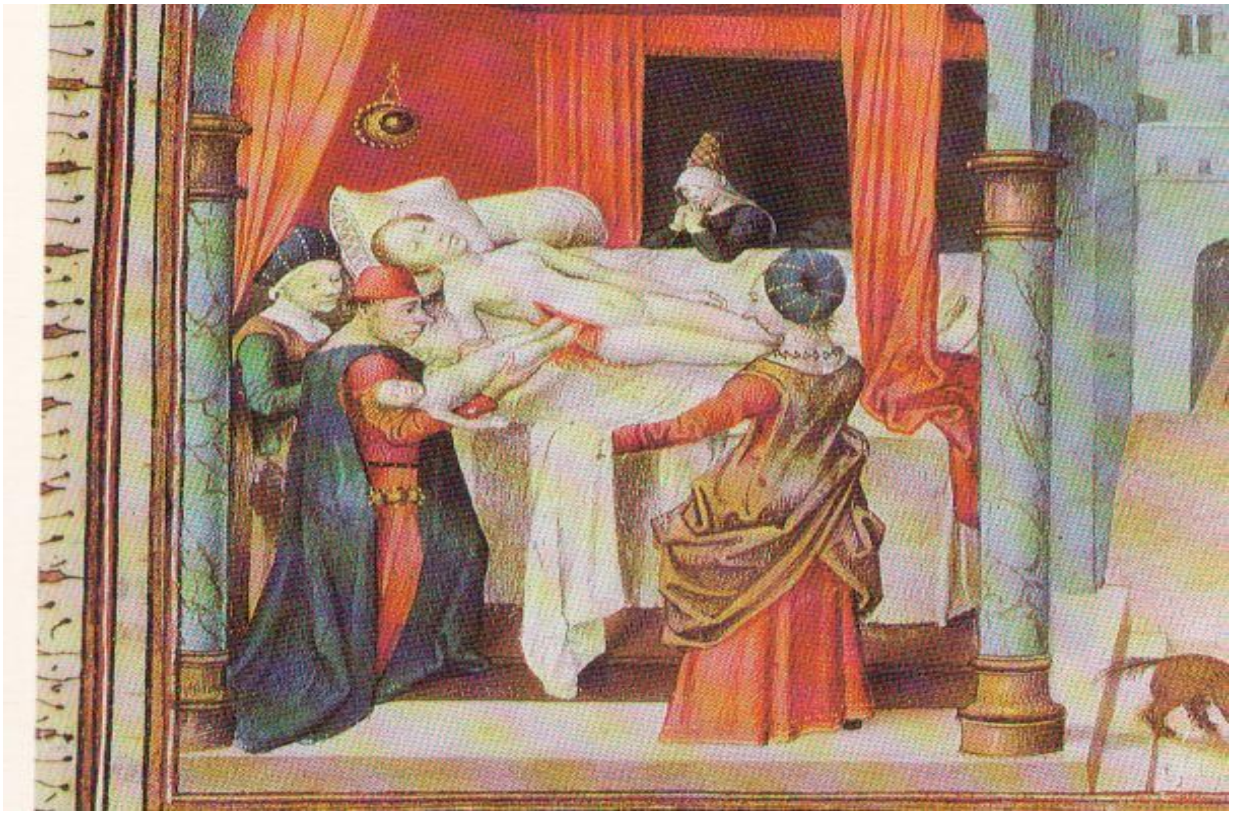
Aim of maternal-fetal care

- the uncomplicated birth of a healthy baby to a healthy mother at term

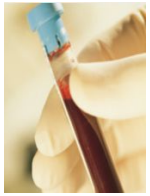


Paulus Orosius, Histoire du monde, 1460?

Screening tests in time

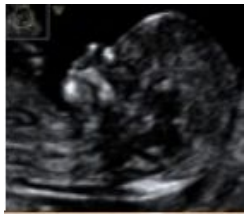
1980

Maternal age + AFP
DR 57 %
FPR 20 %



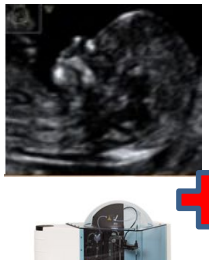
1990

2nd Trimester
AFP, hCG, uE3
DR 65 %
FPR 5 %



2000

1st Trimester
NT + βHCG, PAPP-A
DR 85 %
FPR 5 %



2000

Integrated test
NT + βHCG, PAPP-A
AFP, hCG, uE3
DR 90 - 95 %
FPR 2 %

2010

cffDNA
DR 95 – 99 %
FPR 0,1 %

????

Laboratory tests recommended during pregnancy

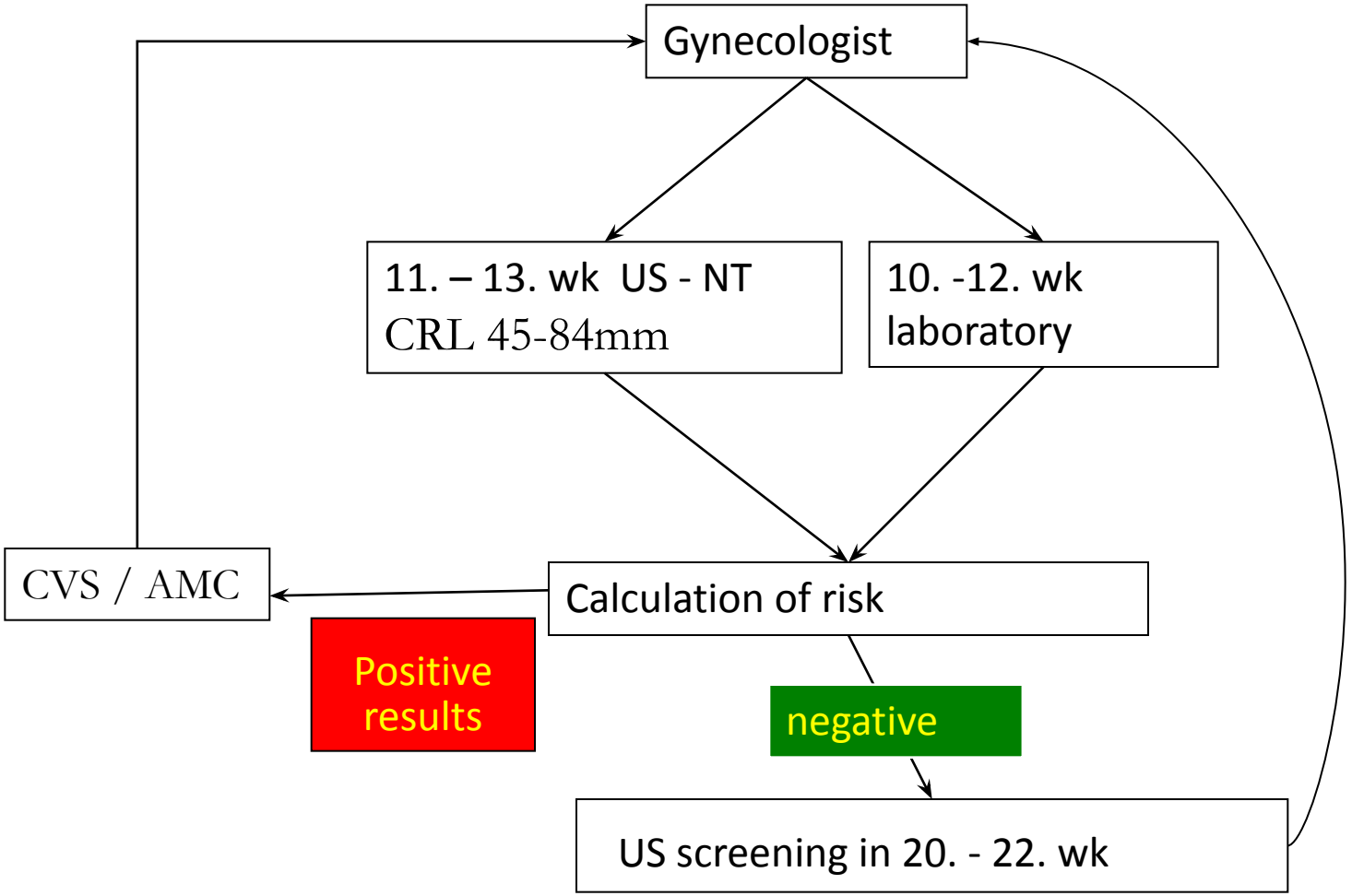
Week of pregnancy	Recommended test	New recommended screening test
8-10	Blood group, anaemia testing, rhesus-D status, hepatitis B, HIV, syphilis, rubeolla, toxoplasmosis	**Screening of thyreopathies** **TSH (FT4, anti TPO) screening**
11-13	Screening of DS - first trimester (PAPP-A, free b hCG, NT)	**NIPT - cffDNA** **Screening of Preeclampsia - PlGF**
16-18	Screening of DS - second trimester (AFP, hCG, uE3, inhibin A)	
24	Screening for GDM	**Screening Preeclampsia– sFlt-1/PlGF**
32 -38	anaemia testing, rhesus-D status, coagulation, vaginal infections, selective population – hepatitis B and HIV	

Springer Det al. Recommendations on prenatal screening and the connections. Clin Chem Lab Med 2012;50(7):1211–1220

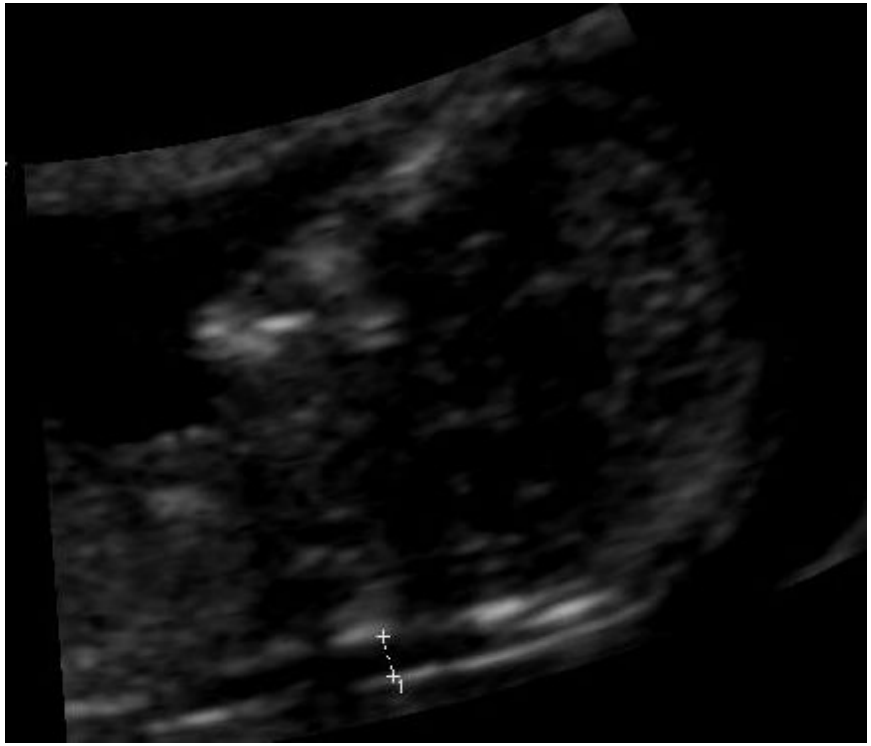
Procedure	Detection rate	Reported rate	Uptake	Process time (wk)
1st TM screening				
Maternal age	32%		80%	0
NT	74%	73%	80%	0
1st TM double test (PAPP-A, hCG)	63%	62%	80%	1
NT, PAPP-A, hCG	86%	80–85%	80%	1
2nd TM screening				
Maternal age	32%		80%	0
2nd TM double test (AFP, hCG)	60%	58–59%	80%	1
Triple test (AFP, hCG, uE3)	68%	67–69%	80%	1
Quadruple test (AFP, hCG, uE3, inhibin A)	79%	76–79%	80%	1
Integrated test (1st TM: NT, PAPP-A;) 2nd TM: quadruple test	95%	94%	80%	1

http://www.nacb.org

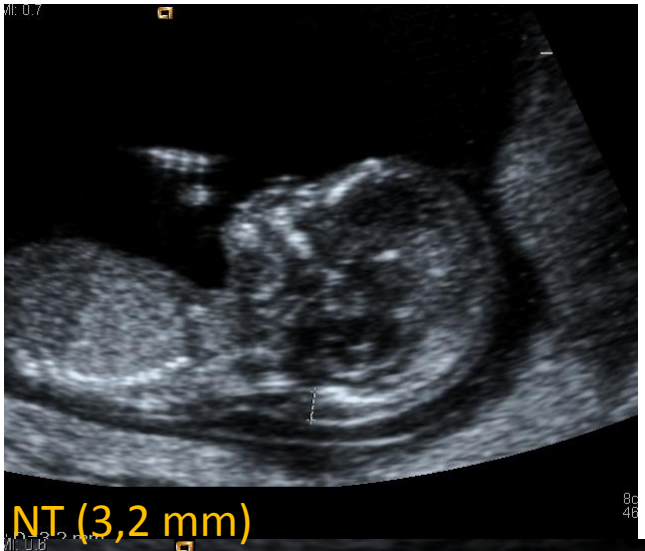
Scheme of 1st trimester screening

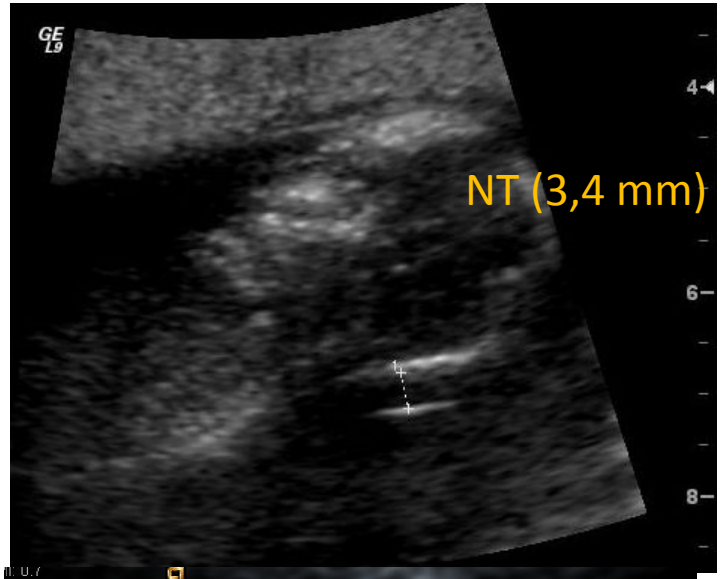


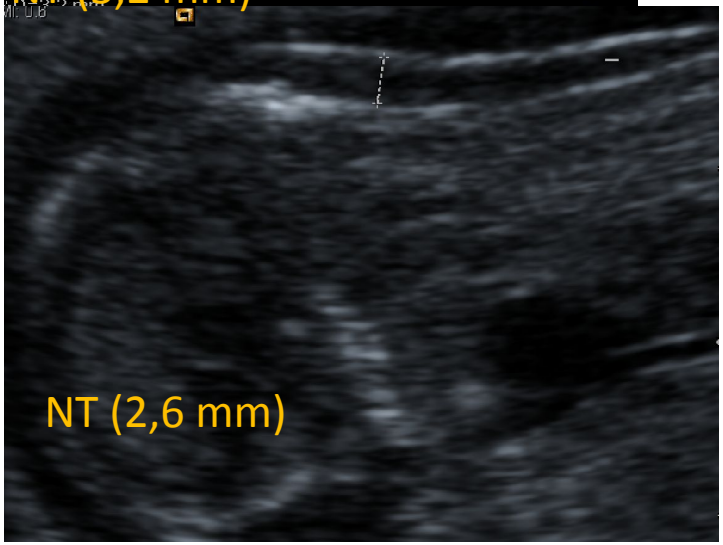
Measuring of NT
(1,5 mm)

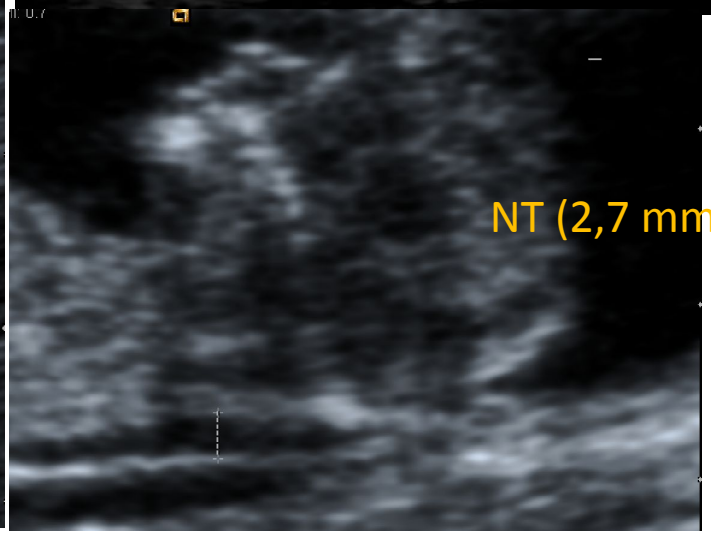


US of fetuses with DS

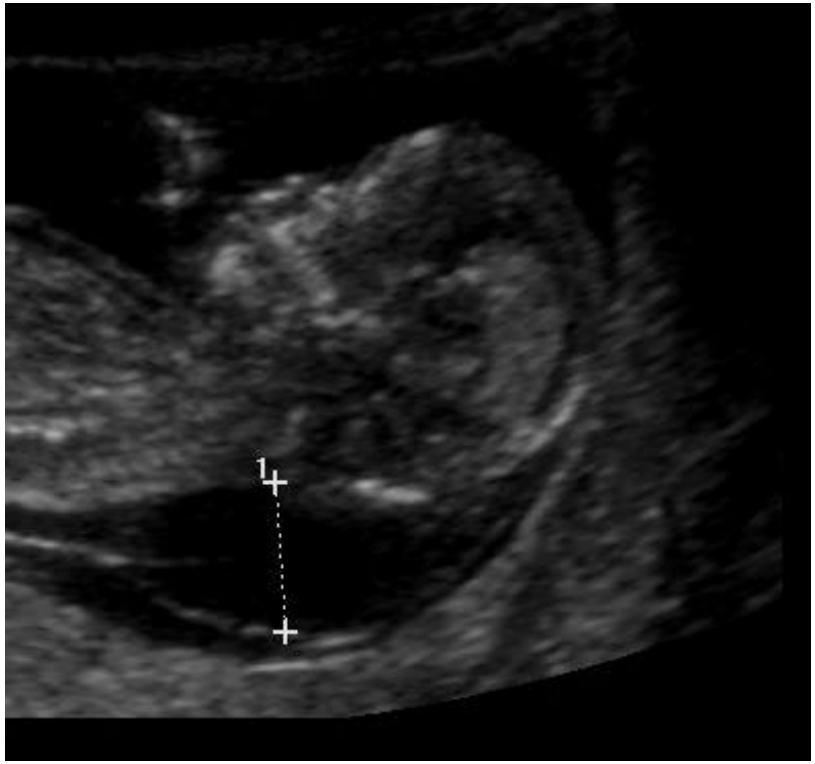




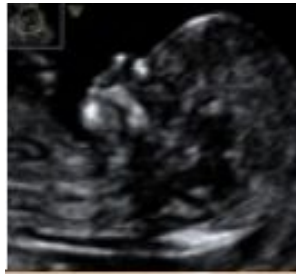


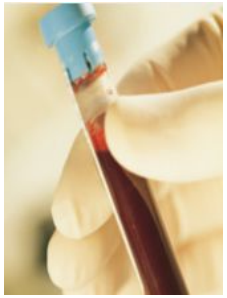


Fetus with Turner syndrome
NT (10 mm)



Integrated test





1st trimester

- determination of PAPP-A, optionally free bhCG
- determination of GA by US
- measuring of NT
- first evaluation by physician

2nd trimester

- determination of AFP and total hCG
- common evaluation with 1st trimester results

Markers of screening

	1st trimester	2nd trimester	Integrated test	Serum integrated test	cffDNA test
PAPP-A					
free b hCG					
Nuchal translucency					
AFP					
hCG					
inhibin					
uE3					
Sensitivity	**80 - 85%**	**65 - 75%**	**90-95%**	**80%**	**99%**

Prenatal screening in the Czech Republic

- In 1980 started investigation with AFP in combination with age – for women over 35 was automatically offered AMC
- In 1990 was prenatal testing in the second trimester obligatory for all pregnant women
- After 2000 started first trimester screening and the care shifted from biochemistry to gynecology
- More than 98% women with prenatal diagnosed DS choose a termination of pregnancy

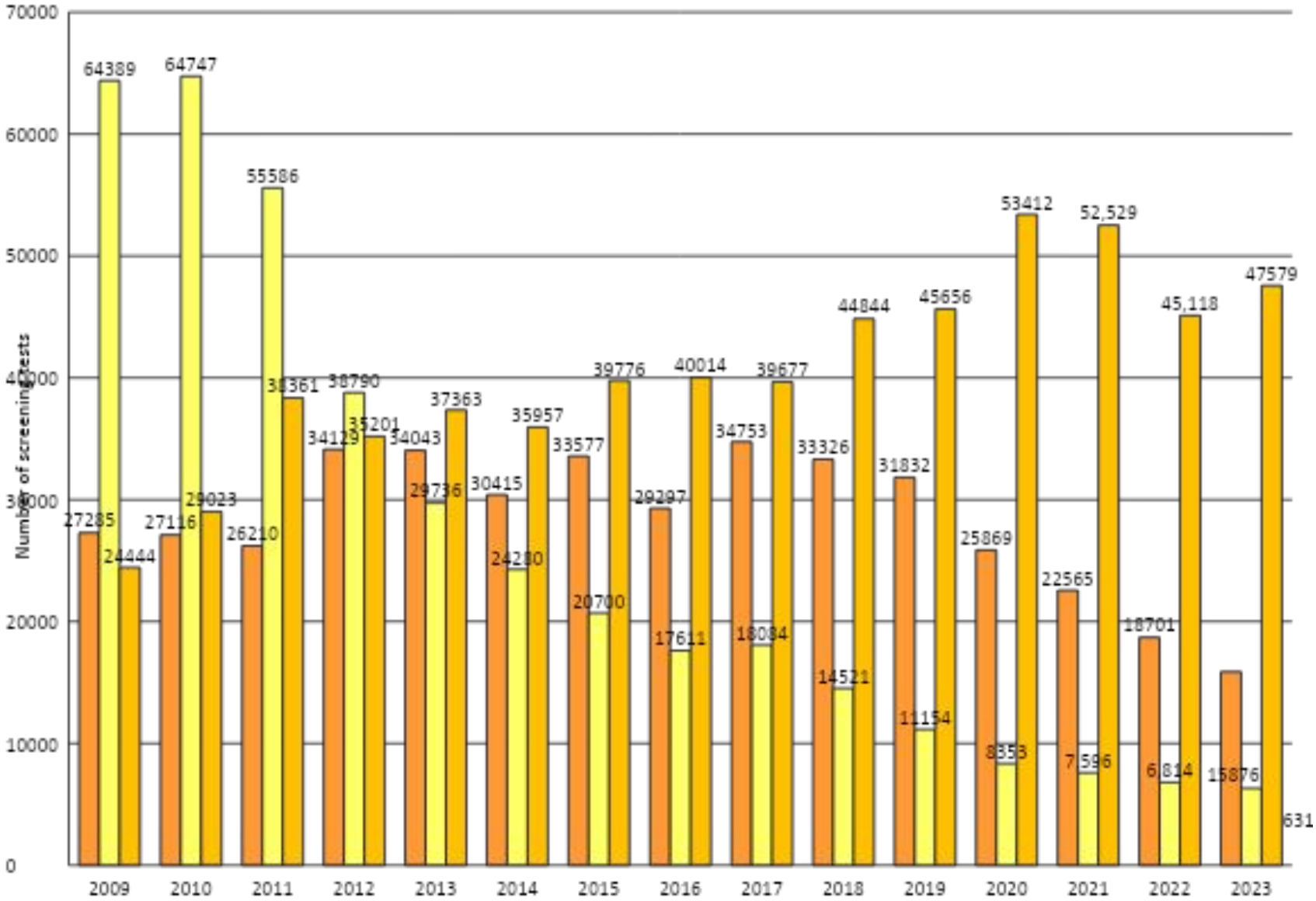
Screening of fetal aneuploidies in the CR

- List of laboratories performing laboratory screening of congenital malformations is kept at the Reference Laboratory for Clinical Chemistry in Prague since 2002.
  List of laboratories, lecture and links to external quality control are given on the website

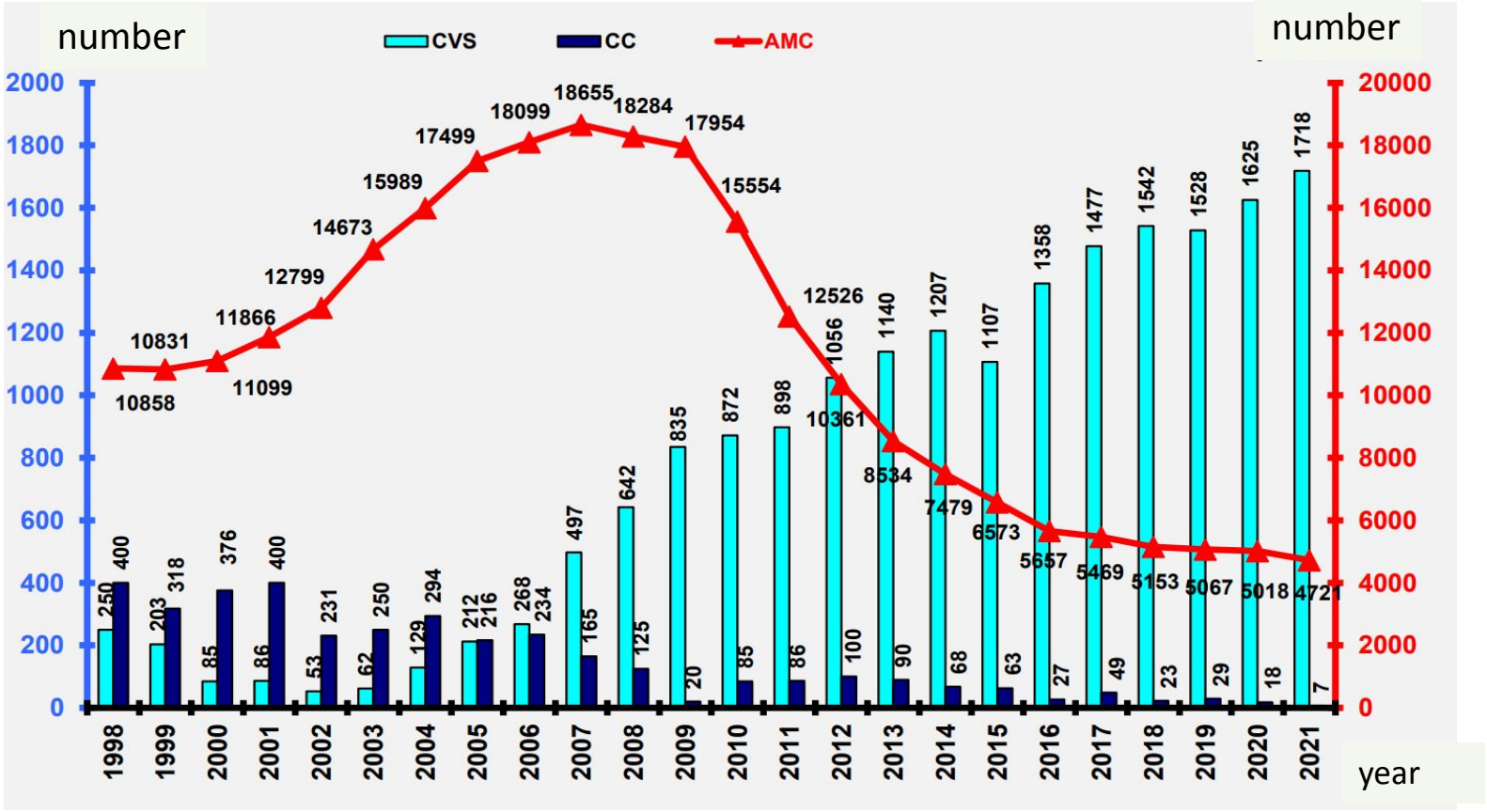
- In 2023 there were registered 40 laboratories.

- In the Czech Republic 91,200 children born in 2023

Screening in the Czech Republic



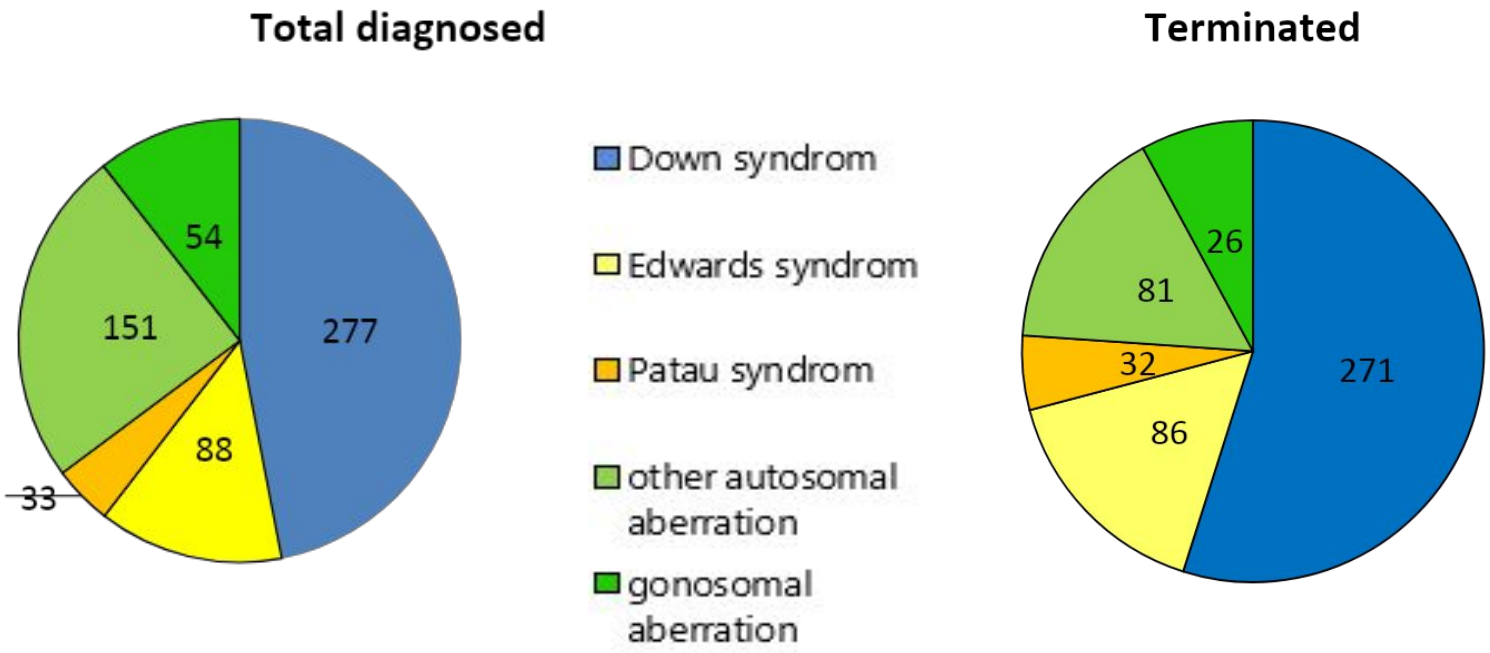
Development of invasive prenatal diagnosis of congenital malformations in the Czech Republic



CVS – Chorionic villus sampling, **CC** – cordocentesis, **AMC** - amniocentesis

http://www.vrozene-vady.cz

Congenital chromosomal aberrations in the CR 2022



http://www.vrozene-vady.cz

New phase of screening of chromosomal aberrations

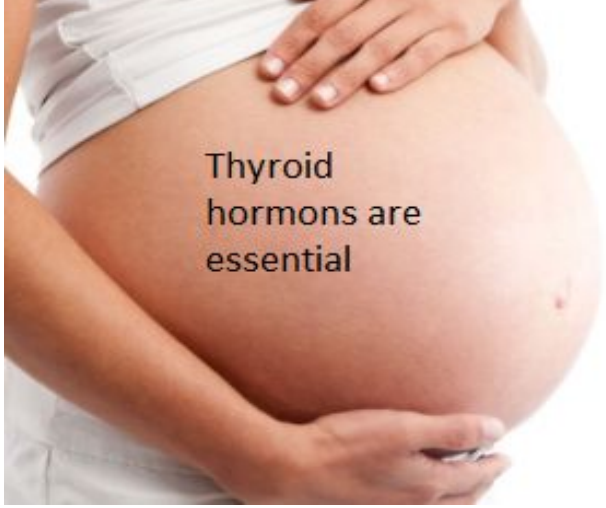
Madonna del Parto C. 1460
Fresco by Piero della Francesca

- New technologies detect developmental defects
- Non-invasive prenatal diagnosis - searching for fragments of fetal DNA in maternal blood

Summary I

- *Increasing numbers of chromosomal aneuploidies - Down, Edwards and Patau syndrome are mainly due to the increasing proportion of women giving birth over 35 years of age.*
- *Increasing the share of first trimester screening leads to improved prenatal detection of these diagnoses while reducing the number of procedures performed invasive prenatal diagnosis.*
- *Non-invasive prenatal diagnosis of fetal aneuploides or genetic disorders has become realistic goal in routine prenatal care.*
- *Recently fetal DNA was used to diagnose fetal aneuploides with a sensitivity of 90 % and price as cheap as any other relevant invasive procedure.*
- *The development of techniques for non-invasive prenatal diagnosis using cell-free DNA and fetal cells in maternal blood will contribute greatly to the field of perinatal medicine and result in safer antenatal care.*

Lorenzo Costa, 1490



Thyroid dysfunction

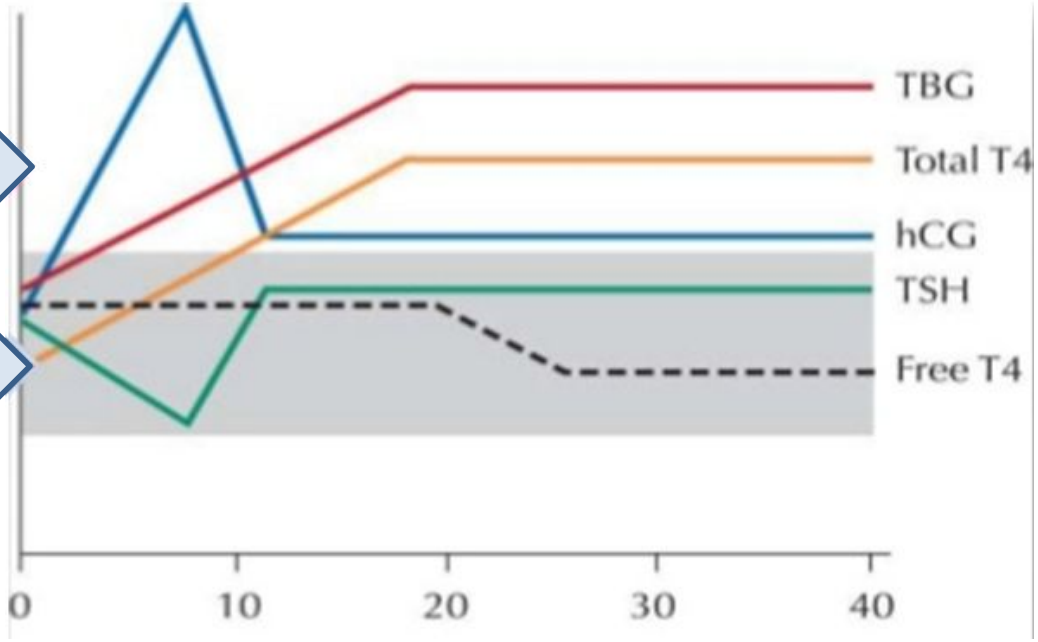
- Some diseases of the thyroid gland can affect both the pregnant woman and the fetus
- Maternal hypothyroxinemia results in the birth of children with decreased mental and psychomotor development
- Iodine status – important and regional differences
- Shift of TSH reference interval
- Important role of TPO Ab
- Pregnancy leads to increased demands on the production of thyroid hormones
- Until the time of fetus thyroid secretion at the end of the first trimester of pregnancy, the fetus is completely dependent on the mother's thyroxine

Physiological changes in pregnancy

- ***hCG and TSH have the same a subunit***

TBG increase caused by estrogens

TSH receptor stimulation caused by high levels of hCG

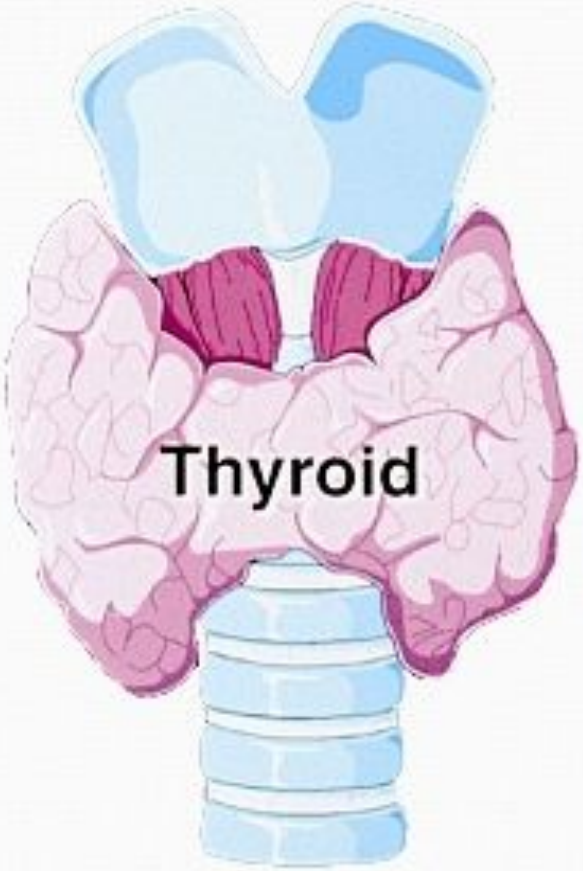


- The physiological adaptations or pathological changes
- the physiological adaptation may occur when a sufficient iodine supply and sufficient functional capacity thyroid gland
- decreased thyroid function, for example, with autoimmune chronic thyroiditis

Congenital hypothyroidism

- Screening of newborns in Czechoslovakia was established in 1985
- per year is captured about 20 children
- TSH is determined from dry blood drop by the screening of other congenital metabolic disorders

Functional thyroid disorders in pregnancy



HYPERTHYROIDISM

Less than 1% of pregnancies
autoimmune etiology
rather relapses
Diagnosis and treatment of sophisticated
Hyperemesis gravidarum is present only in 2/3 cases

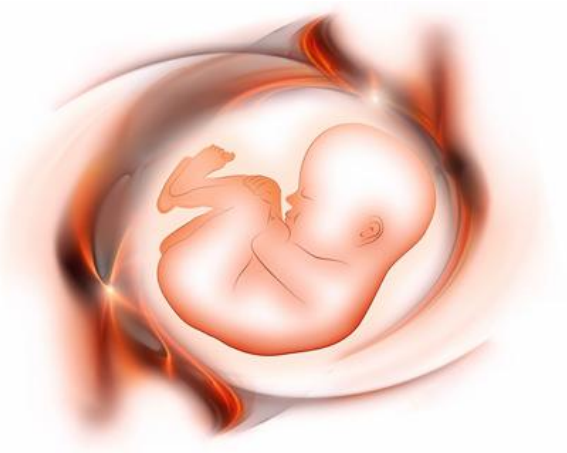
HYPOTHYROIDISM

The incidence of 1.5% - 2.5%
usually asymptomatic

Specific disorders associated with pregnancy

Transient gestational thyrotoxicosis
Postpartum autoimmune thyroiditis

The risk of hypothyroidism



for the mother

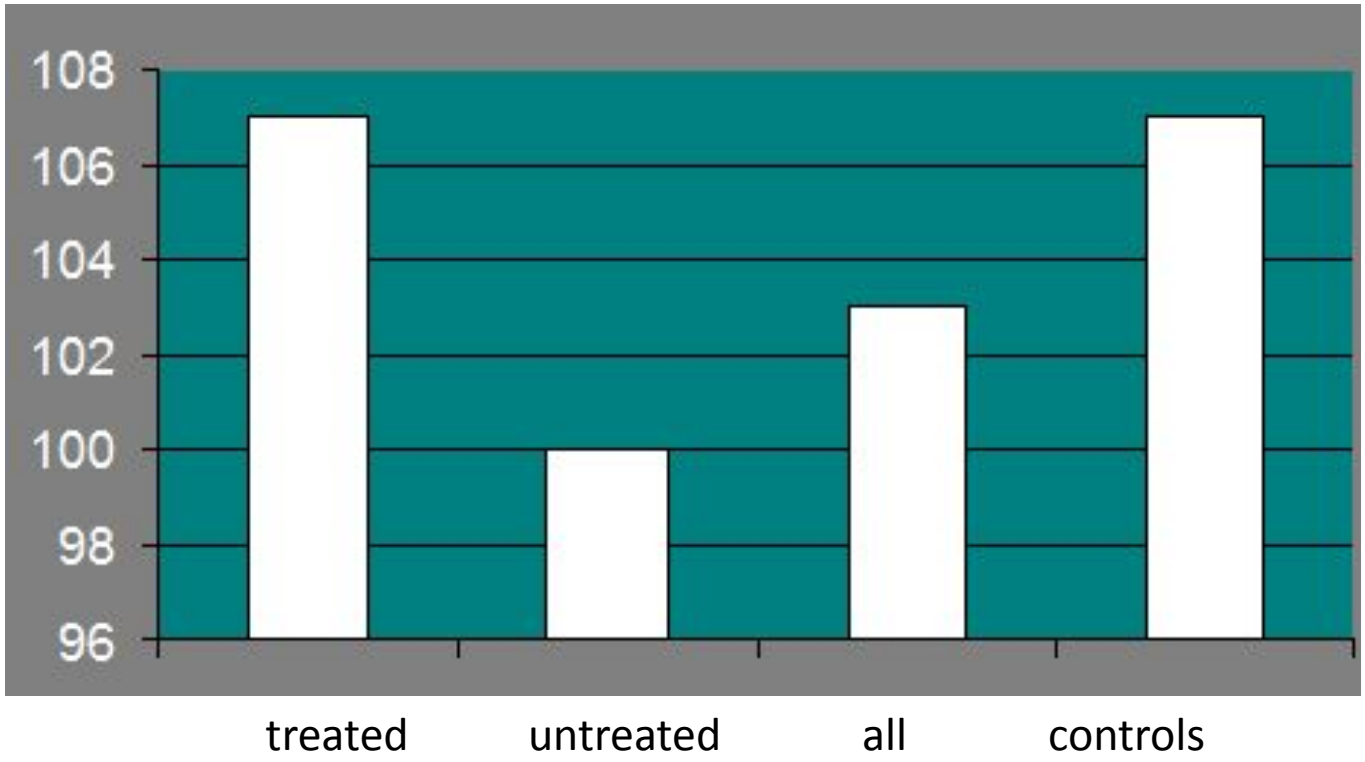
- Increased risk of spontaneous abortion, premature birth
- Anemia
- Preeclampsia
- Placental abruption
- Postpartum hemorrhage

for the fetus

- Increased risk of congenital anomalies
- Impaired postnatal adaptation
- Increased perinatal mortality
- Possible long-term neurological sequelae

Smallridge a Ladenson 2001, Pop et al 2003, Mitchell a Klein2004

The effect of substitution of thyroid hormones during pregnancy on children's IQ hypothyroid mothers



Haddow JE, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 1999; 341: 549-55

Iodine supplementation

- Investigations ioduria not completely generally available
- For all pregnant and lactating women are advised surface iodine supplementation at a dose of 100-150 mg of elemental iodine daily
- Overdose iodine is not described, the risk of activation in thyrotoxicosis

Women who are at increased risk of thyroid disease in pregnancy

- Thyreopathies in PA or FA
- Age over 30 years
- Symptoms of thyroid dysfunction and goiter
- Positive anti-TPO
- Type 1 diabetes or other autoimmune disease
- Miscarriage or premature birth history
- Irradiation of the head and / or neck history
- Obesity with a BMI ≥40 kg / m2
- Taking amiodarone, lithium, by cytokines, recent applications iodine ray contrast
- Infertility
- Woman living in areas with moderate or severe iodine deficiency

Study group – CR 2005-2008

- **7,530 pregnant women** (9^{th} – 11^{th} week of pregnancy, 99% Caucasian), undergoing their first trimester prenatal screening
- The average age was 31.3 (+/- 4.6) years.
- Serum were assayed for TSH, FT4 and anti-TPO

Springer D, Zima T, Limanova Z. Reference intervals in evaluation of maternal thyroid function during the first trimester of pregnancy. Eur J Endocrinol 2009;160:791-7.

Results

- Reference interval for TSH: 0.06 - 3.67 mIU/L
- Increased levels of TSH was found in 5.14% of women
- Decreased levels was found in 2.9% of women
- 11.5% of pregnant women were anti-TPO positive
- Less than half of the women with positive thyroid markers had a history of a risk factor.

Springer D, Zima T, Limanova Z. Reference intervals in evaluation of maternal thyroid function during the first trimester of pregnancy. Eur J Endocrinol 2009; 160:791-7

Thyroid function after delivery in positive screened pregnant women

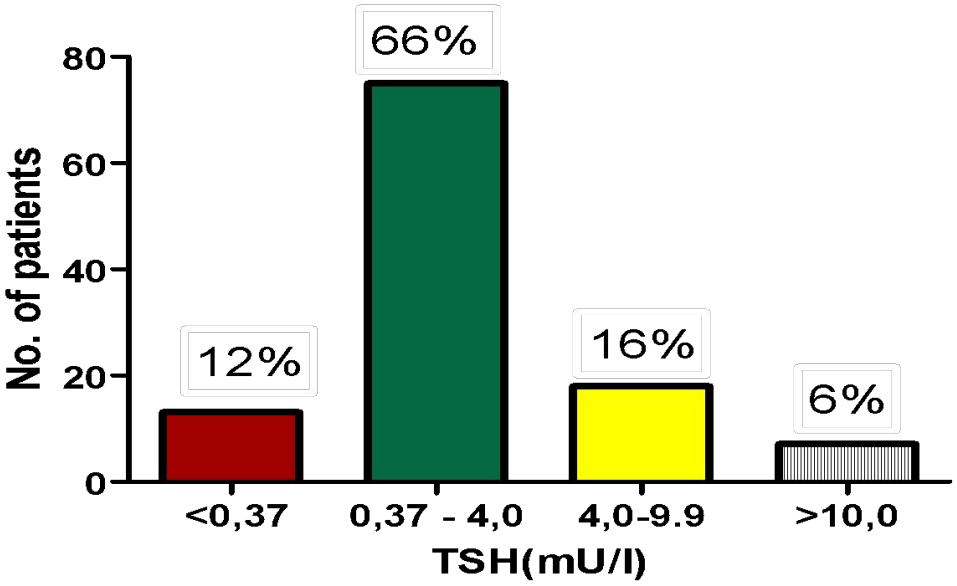
821 women positively screened for thyroid disorders (TSH, FT4 and TPOAb) in 9th – 11th week of pregnancy (in years 2006-2009, mean age 31 years) were follow-up one to three years after delivery (median 15 months; min. 2, max. 38) months.

Family history of thyroid disease, personal history of diabetes or previous treatment for thyroid disease were present only in 58 % of the positively screened pregnant women.

Only 67,5 % of all women had normal delivery

Springer Det al. Recommendations on prenatal screening and the connections. Clin Chem Lab Med 2012;50(7):1211–1220

Thyroid function after delivery in positive screened pregnant women



34% TPOAb-positive women who were euthyroid during pregnancy had a 1.5 year of childbirth TSH outside the normal range.

Springer Det al. Recommendations on prenatal screening and the connections. Clin Chem Lab Med 2012;50(7):1211–1220

Reference intervals of thyroid function tests in the first trimester of pregnancy

- Reference intervals for TSH and FT4, as well as the cut-off level for TPO Ab in the first trimester of pregnancy with 7 different analytical systems
- The reference intervals for TSH and FT4 were different according to the system used by each manufacturer.
- The established **cut-off limits for TPO-Ab** differ according to the system used and are similar to those recommended by their manufacturers for healthy population.
- **The first trimester specific reference intervals may help in a correct evaluation of the results.**

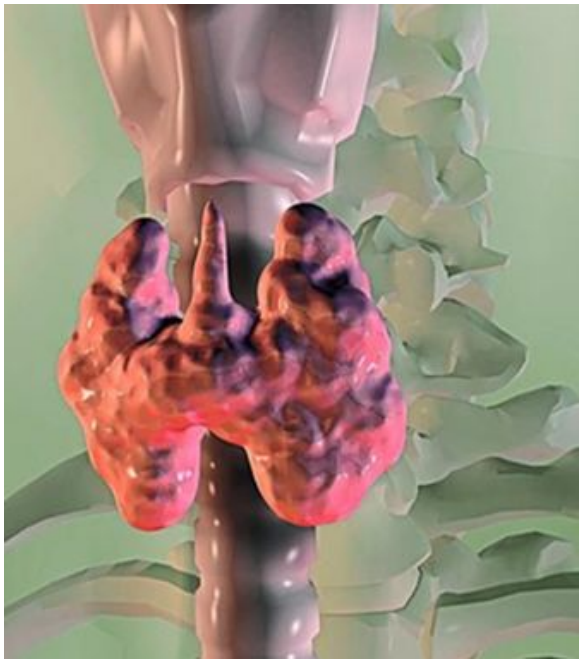
Reference intervals for TSH and FT4 in the first trimester of pregnancy

Analytical system Producer	TSH [mU/L]	FT4 [pmol/L]
Architect i2000$_{SR}$ Abbott Laboratories	0.22 - 3.31	11.81 - 18.38
UniCel DxI 800 Beckman Coulter	0.22 - 3.31	8.13 - 13.2
Immulite 2500 Siemens Healthcare Diagnostics	0.17 - 2.81	10.21 - 16.79
Advia Centaur Siemens Healthcare Diagnostics	0.22 - 3.31	11.81 - 18.38
Modular E170 Roche Diagnostics	0.25 - 3.86	11.81 - 18.38
AIA 2000 Tosoh Bioscience	0.17 - 2.81	10.21 - 16.79
RIA / IRMA Immunotech, Beckman Coulter	0.25 - 3.86	11.81 - 18.38

Springer D, Bartos V, Zima T: Reference intervals for thyroid markers in early pregnancy determined by 7 different analytical systems. Scand J Clin Lab Invest, 2014;74:95-101

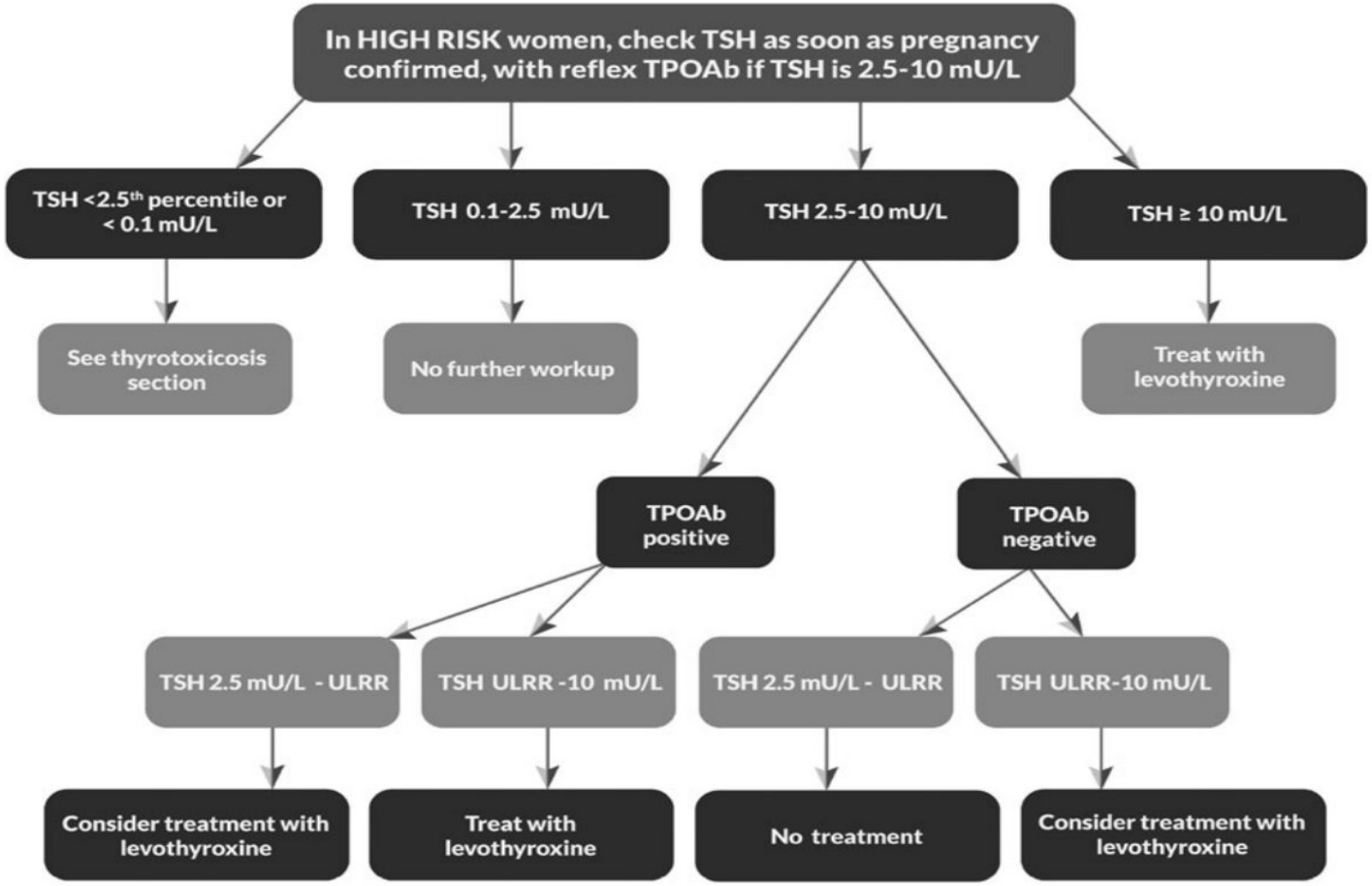
Summary

- 40% of the initially euthyroid pregnant women positive for TPOAb had a thyroid dysfunction more than one year after delivery
- Around 50 % of the positively screened pregnant women had a high-risk profile according to the medical history
- Our results support the implementation of not only general screening for AITD in pregnant women, but also a close follow-up for prolonged time period after the delivery



Screening of thyroid dysfunction during pregnancy is recommended

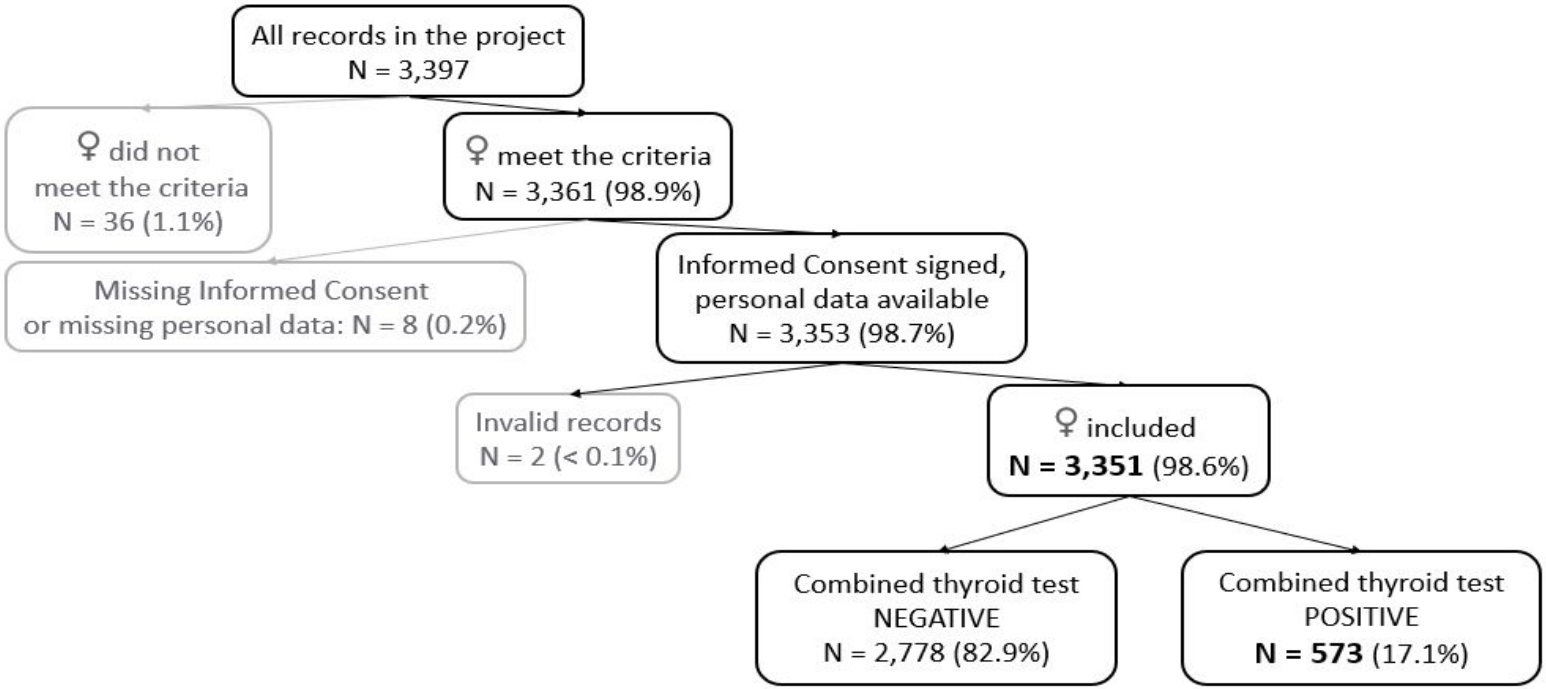
2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum



Erik K Alexander: 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid. 2017 Mar;27(3):315-389. doi: 10.1089/thy.2016.0457.17

- Despite guidelines for screening pregnant women with clinical risk factors for thyroid dysfunction, a significant number of cases with relevant thyroid dysfunction remain undetected
- Here we present the findings from a pilot project in the Czech Republic aimed at feasibility of implementation and modeling of various universal screening scenarios to identify the most practical approach for nationwide implementation

Flow diagram of women included in the pilot screening project (2020-2022)



At least one of the tested parameters (TSH, FT4 or TPOAb) was pathological in 573 (17.1%) of women.

Of these, 78 (2.3%) suffered from "clinically relevant" hypothyroidism expressed as hypothyroxinemia in combination with elevated TSH and/or TSH above 8 mU/l irrespective of FT4 .

Alternative hypothetical scenarios for testing selected parameters

An estimate of the size of the annual target population that would participate in screening

Considered scenario	Proportion of women with a positive result	Proportion of women with established levothyroxine treatment	Proportion of women with introduced levothyroxine who would have escaped diagnosis
Only TSH	7.2 %	72.9 %	30.0 %
Only FT4	3.6 %	35.0 %	84.3 %
Only TPOAb	9.6 %	37.0 %	54.8 %
TSH + FT4	9.5 %	64.1 %	20.9 %
TSH + TPOAb	15.1 %	47.9 %	6.5 %
FT4 + TPOAb	12.6 %	34.2 %	45.7 %
TSH + FT4 + TPOAb	17.1 %	45.5 %	0.0 %
Flex algorithm (TSH → TPOAb)	8.8 %	68.1 %	20.0 %

The share of women with a positive test result ranges from 3.6% to 17.1%, depending on the selected scenarios.

The highest proportion of women with a positive test result has only TSH testing, a high proportion also has the flex algorithm and the scenario of simultaneous TSH and FT4 testing.

At the same time, the mentioned scenarios have the lowest proportion of women who would escape treatment.

After assessing the financial complexity of testing, a flex test was chosen

- Based on the above stated considerations and data shown we decided to implement the two-phase flexible algorithm, using TSH as the first screening parameter, and if pathological, reflex measurement of FT4 and anti TPO.
- During an iterative process with repeated discussions between members of the Czech Society of Endocrinology, the screening-treatment algorithm depicted was decided for nationwide implementation beginning in 2024.

Selected flexible screening and treatment algorithm for nationwide implementation in the Czech Republic beginning in 2024

<table>
<tr><td colspan="4">TSH (mU/l)
morning fasting venous blood sampling at the gynaecologist as soon as possible (optimal gest. wk 11. no later than 14).</td></tr>
<tr><td colspan="2">TSH <LLRR[1]</td><td>TSH within RR for 1st trimester[1]</td><td>TSH> ULRR[1]</td></tr>
<tr><td colspan="2">Laboratory measures FT4</td><td rowspan="3">Negative result</td><td>Positive result (HYPOTHYROIDISM)</td></tr>
<tr><td>FT4 increased</td><td>FT4 normal</td><td>Laboratory measures FT4 and TPOAb</td></tr>
<tr><td>Positive result (HYPERTHYROIDISM)</td><td>Negative result</td><td rowspan="3">TSH <8 + normal FT4:
levothyroxine 50 µg/d
TSH <8 + ↓FT4:
levothyroxine 75 µg/d
TSH 8-10 + normal FT4:
levothyroxine 75 µg/d
TSH 8-10 + ↓FT4:
levothyroxine 100 µ/d
TSH >10 +normal FT4:
levothyroxine 125 µg/d
TSH >10 + ↓FT4:
levothyroxine 150 µg/d</td></tr>
<tr><td colspan="2" rowspan="2">Laboratory measures FT3. TPOAb a TRAb</td><td rowspan="2">Supplements with 150-200 µg iodine per day (started by gynecologist</td></tr>
<tr><td>Supplements with 150-200 µg iodine per day (started by gynecologist)</td></tr>
<tr><td colspan="2">Endocrinologist within 3 weeks</td><td></td><td>Endocrinologist within 3 weeks</td></tr>
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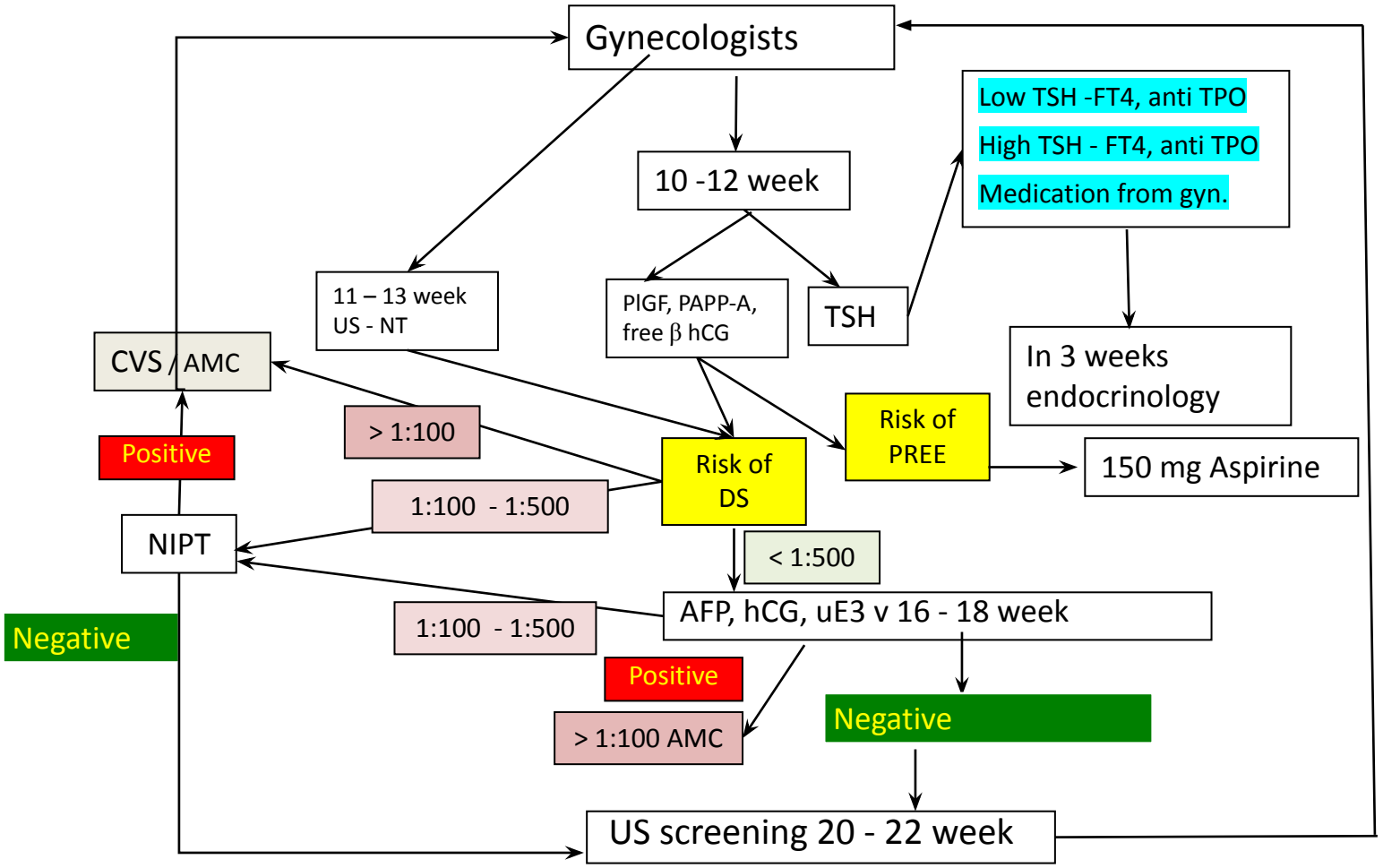
Recommended screening in pregnancy



Take home message

- The spectrum of genetic diseases that can be investigated by NIPT is still expanding (microdeletions/ microduplications, monogenic diseases)

- Preeclampsia screening would identify high-risk women for whom both prevention and treatment could be improved

- Finding thyroid disorders – flex testing by TSH, and in case of pathology determinations of FT4 and anti TPO

- Further follow-up of women with positive anti-TPO antibodies even after delivery.

Thanks to our collaborators and partners

- 3rd Dept. of Internal Medicine General University Hospital Prague
- Dept. of Gynecology and Obstetrics in all CR
- Team of lab
- Colleagues of working group – prenatal screening in Czech Republic
- Supports of grant of Ministry of Health, General Health Insurance Company Czech Republic, research project of Ministry of Education
- Pregnant women and mothers which cooperate on our projects

Thank you
Muchas gracias