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CfDNA FMF contingent screening

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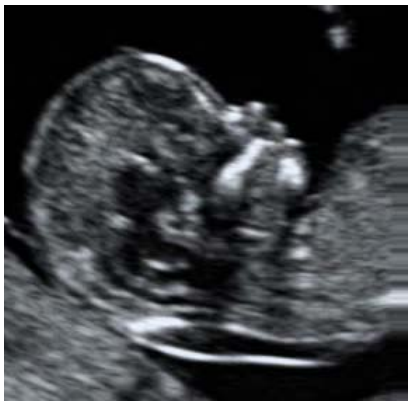
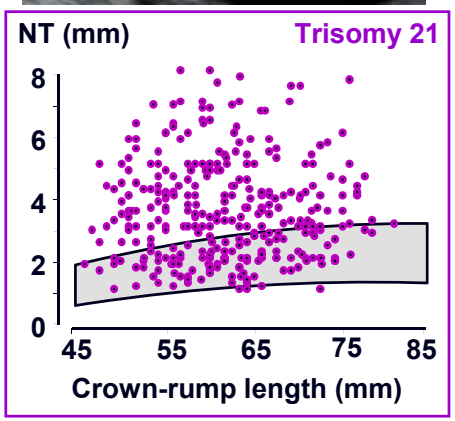
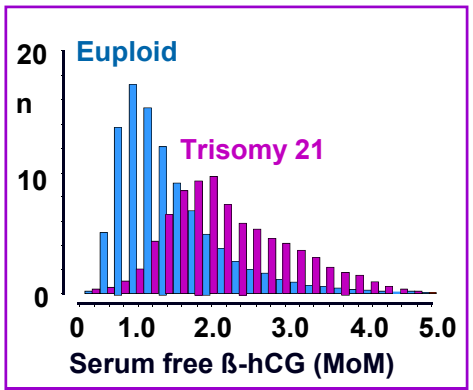
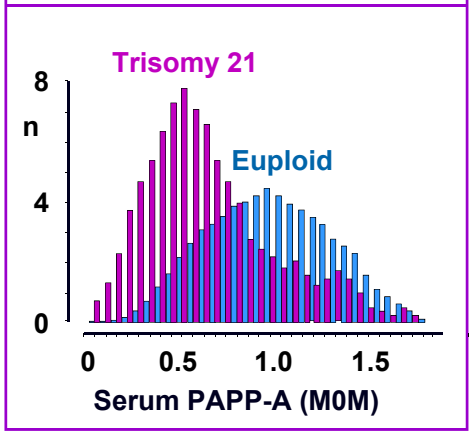
Screening for Down syndrome

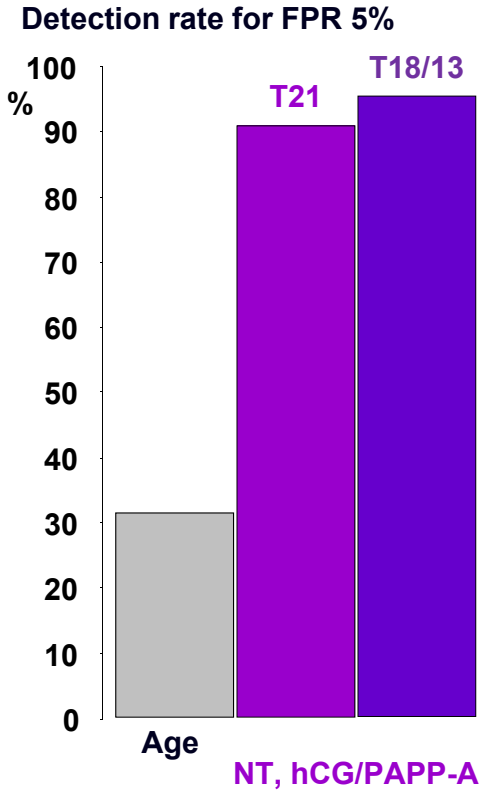
1st trimester combined test











- Nicolaides KH, Azar GB, Byrne D, Mansur CA, Marks K. Nuchal translucency: ultrasound screening for chromosomal defects in the first trimester of pregnancy. BMJ **1992**; 304:867
- Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Lancet **1998**; 352:343
- Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol **1999**; 13:231.
- Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free ß-hCG and pregnancy-associated plasma protein-A. Hum Reprod **2008**; 23:1968-75.

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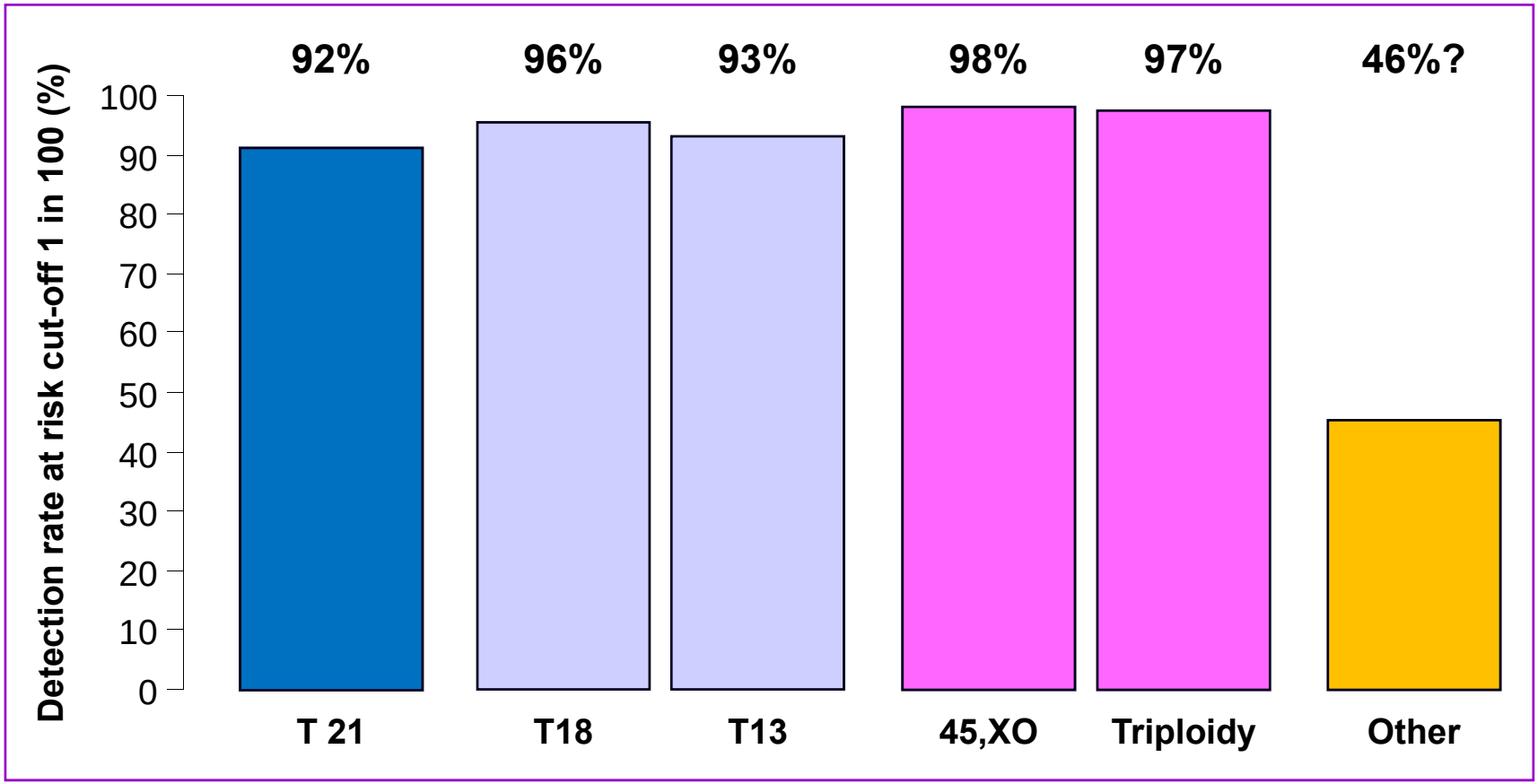
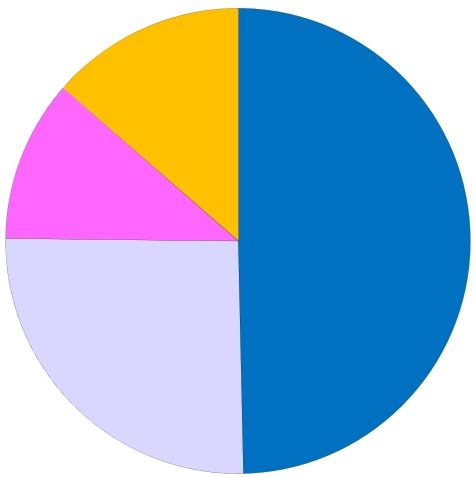
Screening for Down syndrome

1st trimester combined test





Prospective validation of 1st trimester combined screening for trisomy 21 (n=108,982); FPR 5.3%

A beneficial side-effect of screening for trisomy 21 is detection of many other aneuploidies

Santorum M, Wright D, Syngelaki A, Karagioti N, Nicolaides KH. Accuracy of first trimester combined test in screening for trisomies 21, 18 and 13. Ultrasound Obstet Gynecol **2016**; doi 10.1002/uog.17283

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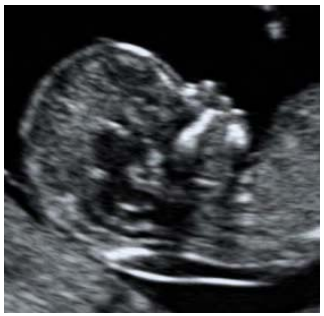
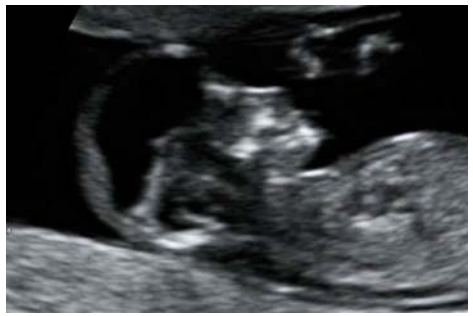
1st trimester combined test – validation n = 108,982

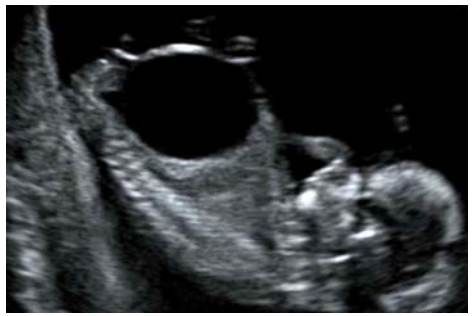
Holoprosencephaly, Exomphalos, Megacystis, NT ≥3.5 mm



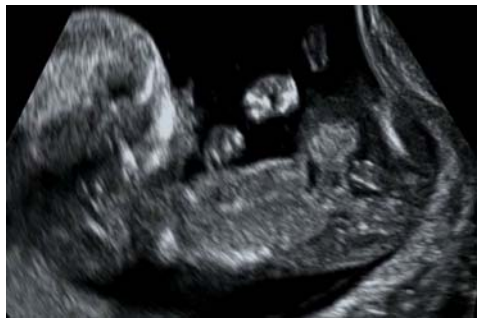
50% T21



80% T13



20% all



45% T18

Screen +ve rate 1.1%

This group contained 57% of all aneuploidies

Trisomy 21	53%
Trisomy 18	72%
Trisomy 13	88%
Triploidy	34%
Monosomy X	94%
Other	23%

Syngelaki A, Guerra L, Ceccacci I, Efeturk T, Nicolaides KH. Impact of holoprosencephaly, exomphalos, megacystis and high NT in first trimester screening for chromosomal abnormalities. Ultrasound Obstet Gynecol **2016;** doi 10.1002/uog.17283

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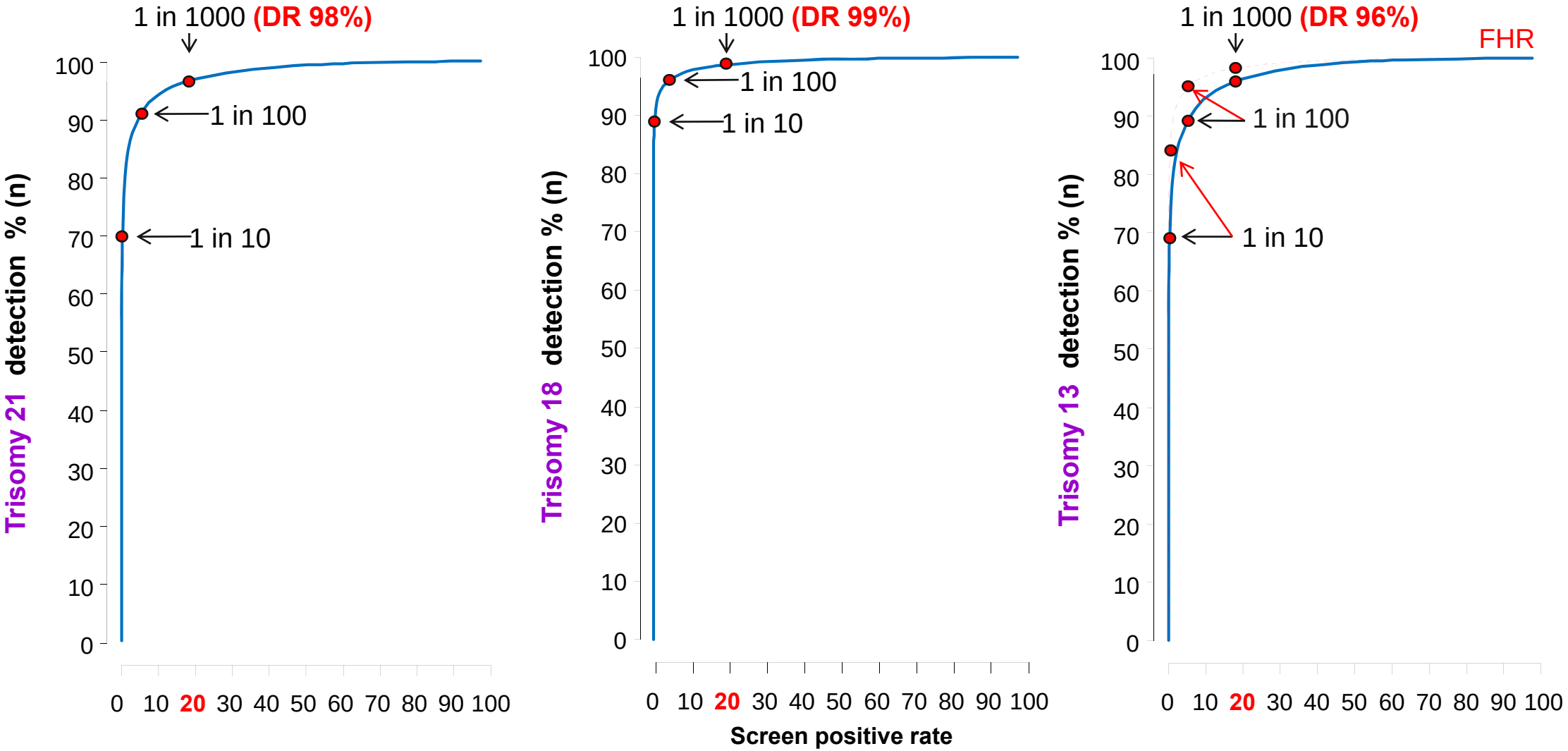
1st trimester combined test

Prospective validation n=108,982



Santorum M, Wright D, Syngelaki A, Karagioti N, Nicolaides KH. Accuracy of first trimester combined test in screening for trisomies 21, 18 and 13. Ultrasound Obstet Gynecol **2016**; doi 10.1002/uog.17283

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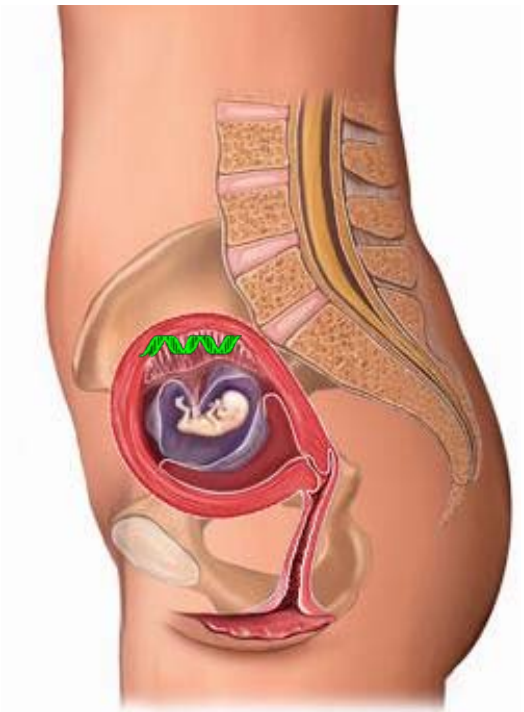
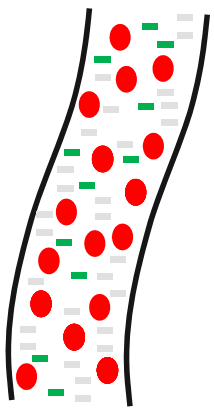
Screening for trisomies

Cell-free DNA in maternal blood





	DR	FPR
Trisomy 21 n=1,963	99.7%	0.04%
Trisomy 18 n= 560	98.2%	0.05%
Trisomy 13 n= 119	99.5%	0.05%

Updated meta-analysis 31 December 2016

Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. Lancet ***1997***; 350:485-7.
Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies Ultrasound Obstet Gynecol **2015**;45:249
Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies Ultrasound Obstet Gynecol **2017; in press**

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Screening for Down syndrome

Cell-free DNA in maternal blood

	100,000 pregnancies			
	Trisomy 21 N=200		99,800 normal	
METHOD OF SCREENING	DR	Detected	False positive	
Maternal age	30%	60	5%	4990
Serum biochemistry at 16 wks	70%	140	5%	4990
Combined test at 12 wks	90%	180	3%	2994
Cell-free DNA	>99%	199	0.04%	40

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Cell free DNA test

Points of discussion

- Which conditions to screen for
- Interpretation of results
- Importance of fetal fraction
- Test failure
- Screening in twins
- Models of implementation

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Cell free DNA test

Which aneuploidies?

Screening for sex chromosome aneuploidies

- Lethal Turners easily detectable by ultrasound
- Most cases are mild without intellectual disability
- 50% of sex chromosome aneuploidies are mosaics
- High incidence of maternal mosaicism
- Performance of screening is uncertain
  - Failure rate is higher than for T21
  - Detection rate is lower (about 90%) than for T21 (>99%)
  - False +ve rate is higher (about 0.3%) than for T21 (<0.1%)
  - Positive predictive value much lower than for T21
- Management of positive result: need for amniocentesis

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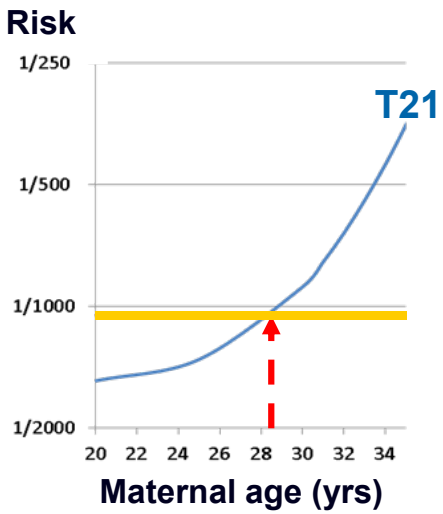
Cell free DNA test

Which aneuploidies?

Microdeletions	1/ births	≥3Mb
22q11.2 deletion	2-4,000	85%
Angelman / Prader Willi (15q11.2-q13 deletion)	20,000	70%
1p36 del	5,000	85%
Cri-du-chat (5p deletion)	50,000	99%



Di George - common features:

75% immune deficiencies
50% hypocalcemia
30% feeding problems
35% renal abnormalities
75% cardiac defects
95% intellectual deficits
25% schizophrenia in adulthood

- Detection rates *: 60-99%
- Total false positive rates: 1%
- Positive predictive value for 22q11.2 deletion: 5%
- Proportion of cfDNA panel of significant microdeletions: 10%

*** quoted by companies, but do not include those of <3 Mb**

Yaron Y *et al.* Current status of testing for microdeletion syndromes and rare autosomal trisomies using cell-free DNA technology. Obstet Gynecol 2015;126:1095

Wapner RJ *et al.* Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. Am J Obstet Gynecol 2015;212:332.e1-9.

Cell free DNA test

Which aneuploidies?

• Trisomy 21	YES
• Trisomies 18/13	Yes
• Sex chromosomes	No
• Triploidy	No
• Microdeletions	Not yet

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Cell free DNA test

Interpretation of results

		DR	FPR	LR +ve	LR -ve
Trisomy 21	n=1,963	99.7%	0.04%	2509	300
Trisomy 18	n= 560	98.2%	0.05%	2122	18
Trisomy 13	n= 119	99.5%	0.04%	2819	100

Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies Ultrasound Obstet Gynecol **2015**;45:249
Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies Ultrasound Obstet Gynecol **2017; in press**

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Cell free DNA test

Interpretation of results

Trisomy 21

- **DR** 99.7%
- **FPR** 0.05%
- **LR +ve** 2509
- **1/(LR -ve)** 300

a priori risk	*Odds of being affected*	
	cfDNA +ve	cfDNA -ve
1:100,000	1 in 42 (2.4%)	1 in 30,000,000
1:10,000	1 in 6 (20.1%)	1 in 3,000,000
1:1,000	1 in 1.4 (71.5%)	1 in 300,000
1:500	1 in 1.2 (83.4%)	1 in 150,000
1:100	1 in 1.03 (93.2%)	1 in 30,000
1:10	1 in 1.004 (99.6%)	1 in 3,000
1:2	1 in 1.001 (99.9%)	1 in 600

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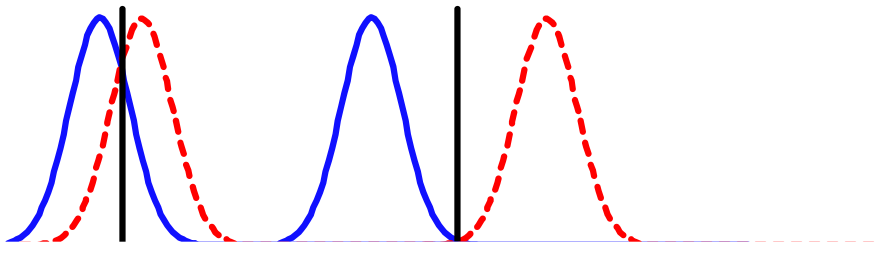
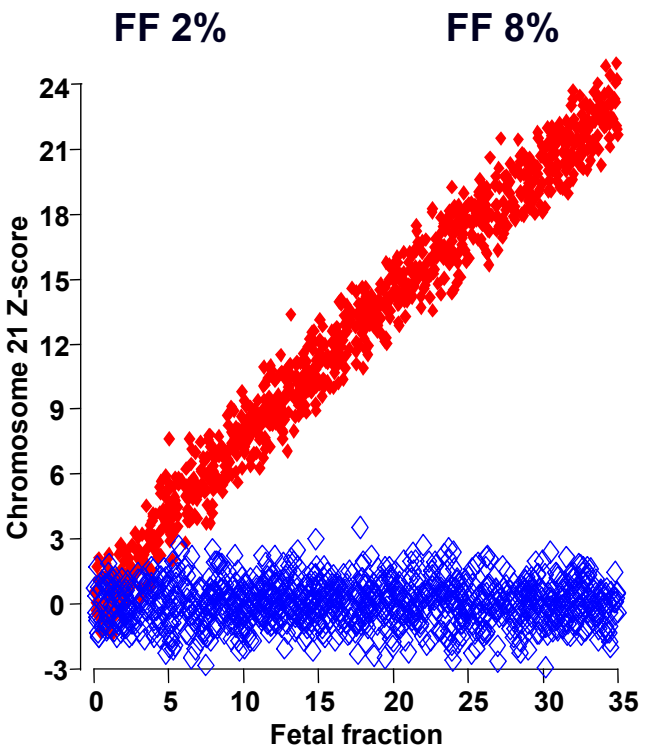
Cell free DNA test

Importance of fetal fraction





Fetal fraction	cfDNA test	
	FPR (%)	DR (%)
4%	0.1	62.1
5%	0.1	87.4
6%	0.1	97.6
7%	0.1	99.8
8%	0.1	100
9%	0.1	100
≥10%	0.1	100
All	0.1	99.0

Wright D, Wright A, Nicolaides KH: A unified approach to risk assessment for fetal aneuploidies. Ultrasound Obstet Gynecol 2015; 45: 48-54

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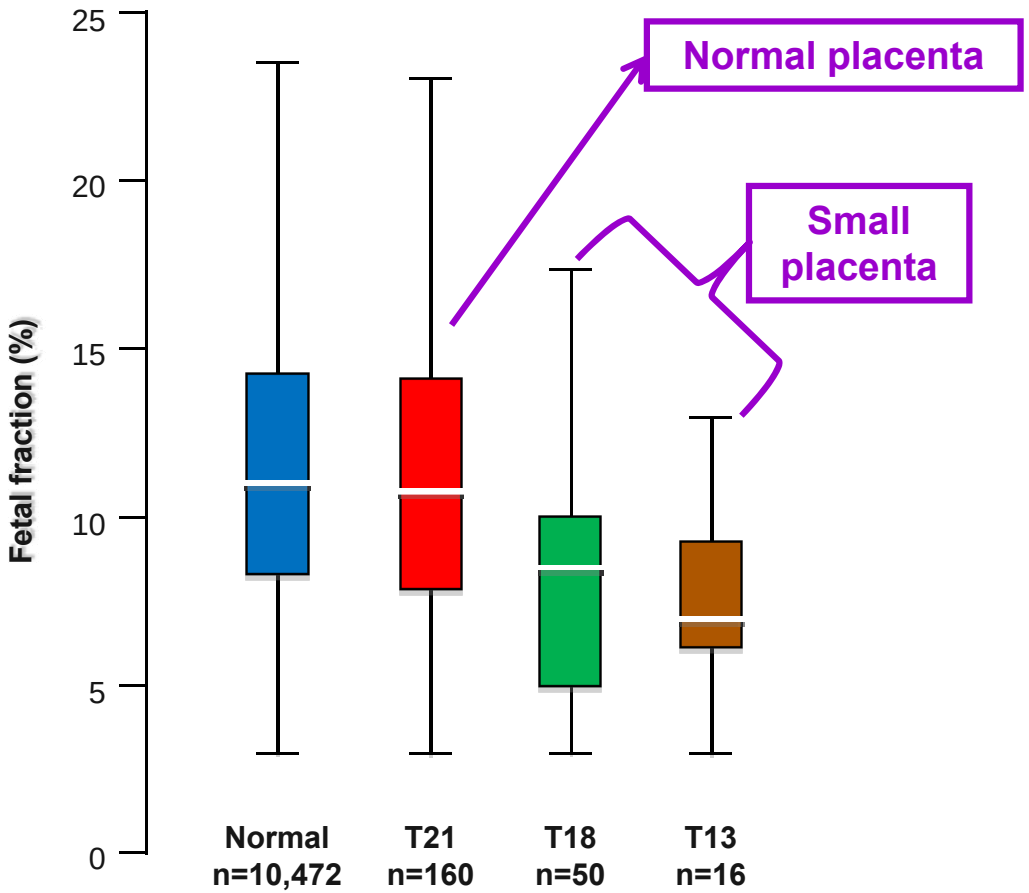
Cell free DNA test

Test failure

Singleton pregnancies (n=10,698)



Failed result:

Normal	304 (2.9%)
Trisomy 21	3 (1.9%)
Trisomy 18	4 (8.0%)
Trisomy 13	1 (6.2%)

Combined risk >1 in 5
Ultrasound defects

Revello R, Sarno L, Ispas A, Akolekar R, Nicolaides KH. Cell-free DNA testing for trisomies: management of failed result. Ultrasound Obstet Gynecol **2016**; 47: 698-704.

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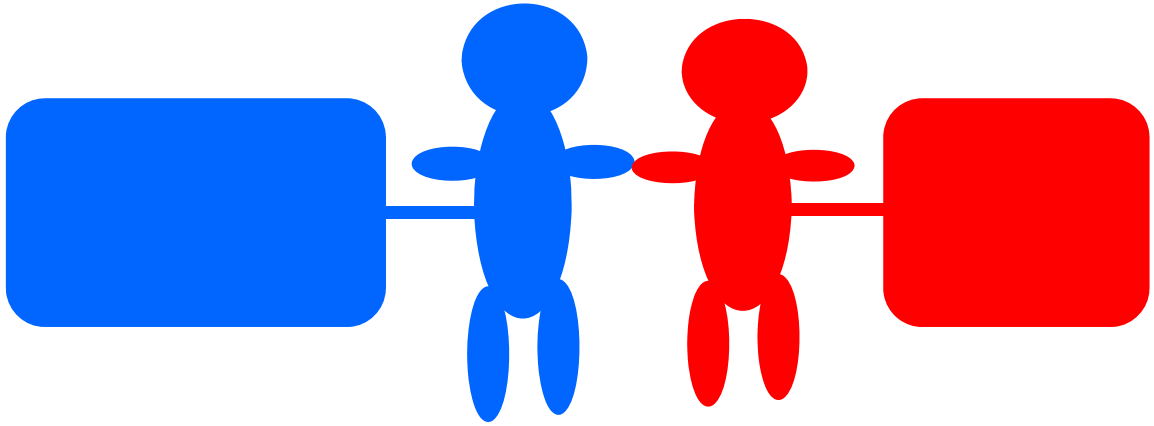
Cell free DNA test

Screening in twins



DC twins:

- **The placental products of the normal fetus may mask the abnormality of the co-twin.**
- **Measure fetal fraction in both.**

- **The test is not offered by all companies**
- **Only some companies measure fetal fraction**

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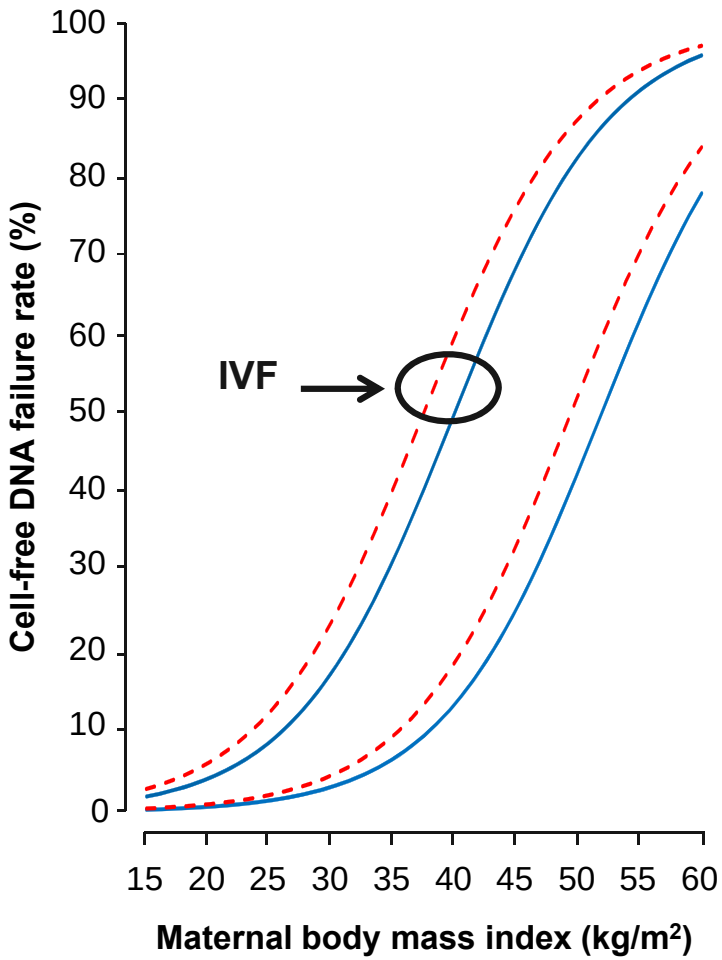
Cell free DNA test

Failure in singletons and twins

Harmony test in 10,698 singletons and 438 twins
Significant predictors of fetal fraction & cfDNA test failure: increasing BMI, IVF conception and twins

BMI (kg/m²)	Rate of failure of cell free DNA testing (%)			
	Singleton pregnancy		Twin pregnancy	
	Spontaneous	IVF	Spontaneous	IVF
20	0.6	4	1	6
25	1.4	9	2	12
30	3	17	5	24
35	7	32	10	41
40	14	51	19	61
45	26	70	35	77
50	44	84	54	88

High failure rate in twins, compared to singletons (9% vs. 3%) mainly because many twins are IVF conceptions (56% in twins vs. 10% in singletons)



Sarno L, Revello R, Hanson E, Akolekar R, Nicolaides KH. Prospective screening for trisomies by cell-free DNA testing of maternal blood in first trimester twin pregnancies. Ultrasound Obstet Gynecol **2016**; 47: 705-11.

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Cell free DNA test

Validation / implementation in twins

Stored samples from pregnancies with known outcome

Trisomy 21:	DR 23/24	(96%)
Trisomy 18:	DR 1/1	(100%)
Trisomy 13:	DR 2/2	(100%)
FPR:	0/321	(0%)

Canick *et al*: Prenat Diagn 2012; 32: 730

Gil *et al*: Fetal Diagn Ther 2014; 35 : 204

Gromminger *et al*: J Clin Med 2014; 3: 679

Fosler *et al*: Ultrasound Obstet Gynecol 2016;doi10.1002/uog

Prospective studies with complete outcome

Trisomy 21:	DR 25/25	(100%)
Trisomy 18:	DR 4/6	(67%)
Trisomy 13:	DR 0/1	(0%)
FPR:	1/1098	(0.2%)

Lau *et al*: J Matern Fetal Neonatal Med 2013; 26: 434

Huang *et al*: Prenat Diagn 2014; 34: 335

Benachi *et al*: Obstet Gynecol 2015; 125: 1330

Papageorghiou *et a*l: Ultrasound Obstet Gynecol 2016; 47: 188

Sarno *et al*: Ultrasound Obstet Gynecol 2016; 47: 705

Tan *et al*: Prenat Diagn 2016; 36: 672

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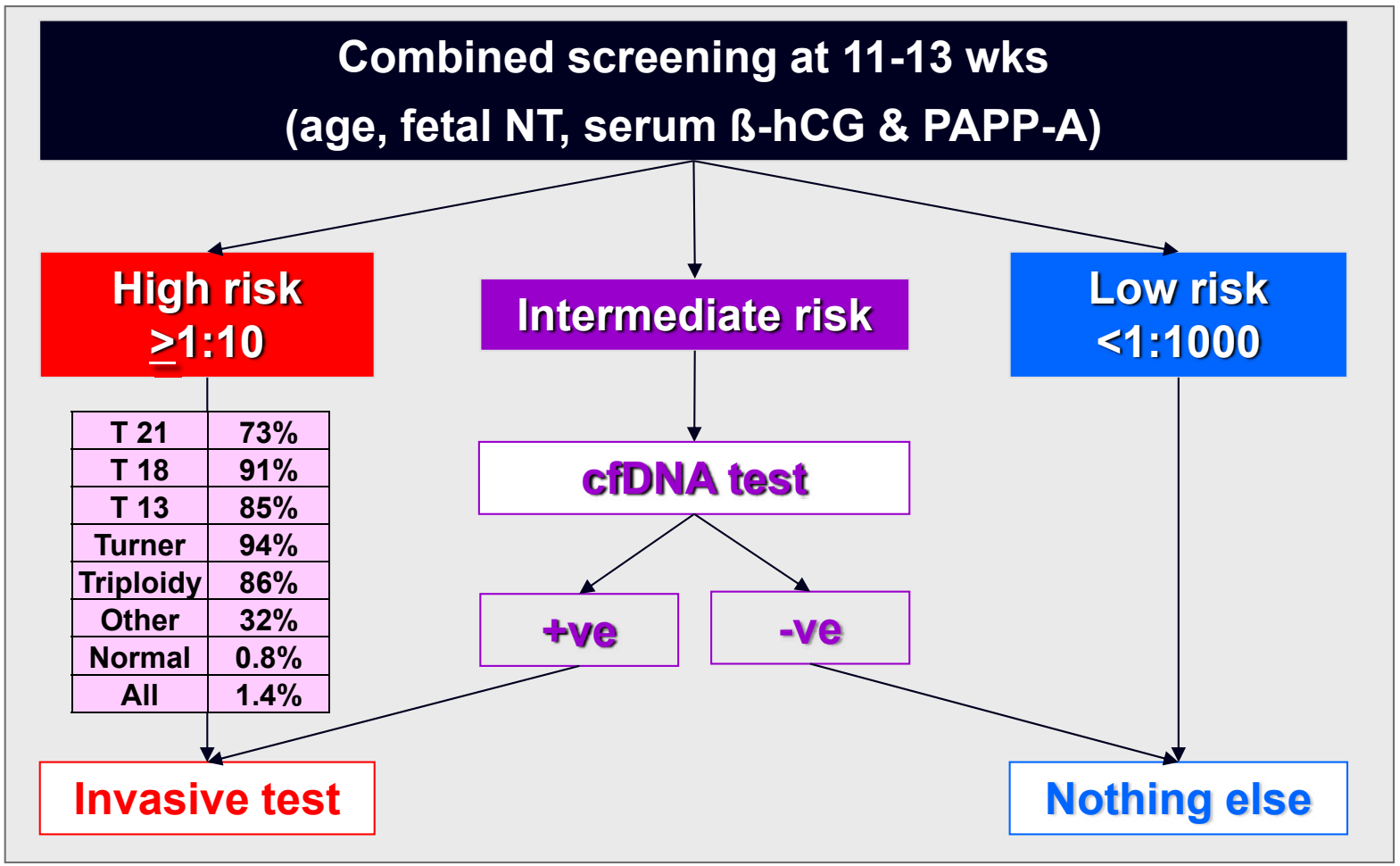
Cell free DNA test

Model of clinical implementation



Cut off	FPR (%)	DR T21 (%)
100	2.2	87.0
200	3.9	90.4
300	5.4	92.1
400	6.7	93.2
500	7.9	94.0
1000	13.0	96.1
1500	17.2	97.0
2000	20.8	97.6
2500	23.9	98.0
3000	26.6	98.3
3500	29.0	98.5
4000	31.3	98.7
5000	35.2	98.9
6000	38.7	99.1

Santorum M, Wright D, Syngelaki A, Karagioti N, Nicolaides KH. Accuracy of first trimester combined test in screening for trisomies 21, 18 and 13. Ultrasound Obstet Gynecol **2016**; doi 10.1002/uog.17283

Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D. First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing. 2014; 35: 185-92.

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Cell free DNA test

Miscarriage from amnio / CVS

Royal College of Obstetricians & Gynaecologists	AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS	THE SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA	Sociedad Española de Ginecología y Obstetricia
Amnio 1%	Amnio 0.3-0.5%	Amnio 0.2-1.5%	Amnio 0.2-1.5%
BC 1-2%	BC 0.3-0.5%	BC 0.2-1.5%	BC 0.7-1%

RCOG: *Amniocentesis and Chorionic Villus Sampling. Green Top Guideline No.8. London: RCOG, 2010.*

ACOG: *Invasive prenatal testing for aneuploidy. ACOG Practice Bulletin No. 88. Obstet Gynecol 2007; 110: 1459-1467.*

SOGC: *Mid-trimester amniocentesis fetal loss rate. J Obstet Gynaecol Can 2007; 29: 586-595.*

SEGO*: Diagnóstico prenatal de los defectos congénitos. Cribado de las anomalías cromosómicas. 2010.*

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Cell free DNA test

Miscarriage from amnio / CVS

King's College hospital study

Singleton pregnancies with combined screening at 11-13 w

- Expectant management
- Livebirth n = 33,310; Miscarriage n = 404 (1.2%)
- Regression model to predict miscarriage

Variable	OR	95% CI
Age (per year)	0.870	0.766-0.988
Delta nuchal translucency	1.778	1.496-2.114
Ductus venosus: reversed a-wave	2.208	1.508-3.232
Log_{10} PAPP-A MoM	0.356	0.233-0.543

CVS n = 2,396

Miscarriage

Observed: 44 (1.8%)

Expected: 45 (95% CI 32-58)

Akolekar R, Bower S, Flack N, Bilardo K, Nicolaides KH: Prediction of miscarriage and stillbirth at 11-13 weeks and the contribution of chorionic villus sampling. Prenat Diagn 2011:31:38.

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Cell free DNA test

Miscarriage from amnio / CVS

Danish study

- 147,987 singleton pregnancies
- All had first trimester combined screening
- Propensity score stratification

Miscarriage risk difference:	CVS	**-0.2%**
	Amniocentesis	**0.6%**
Stillbirth risk difference:	CVS	**-0.3%**
	Amniocentesis	**0.1%**

Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB, **Tabor A**: Risk of fetal loss with invasive testing following first trimester screening: national cohort of 147,987 singleton pregnancies. Ultrasound Obstet Gynecol **2016**; 47: 38-44.

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Cell free DNA test

Conclusions

- Which conditions to screen for: T21 and perhaps 18 / 13
- Interpretation of results: Modify *prior* risk with +ve and –ve LRs
- Importance of fetal fraction: Depends on the company
- Test failure: Does not increase the risk for trisomy 21
- Screening in twins: Need for more data on accuracy
- Models of implementation: Intermediate risk from combined test
- Risk of miscarriage from invasive test: 1 in 1000

Thank you

The Fetal Medicine Foundation

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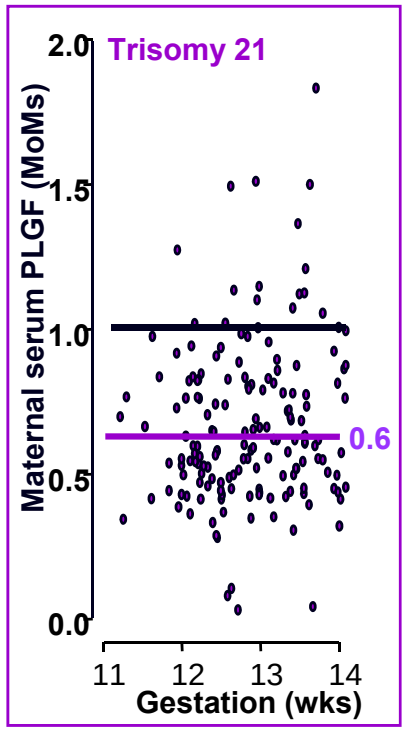
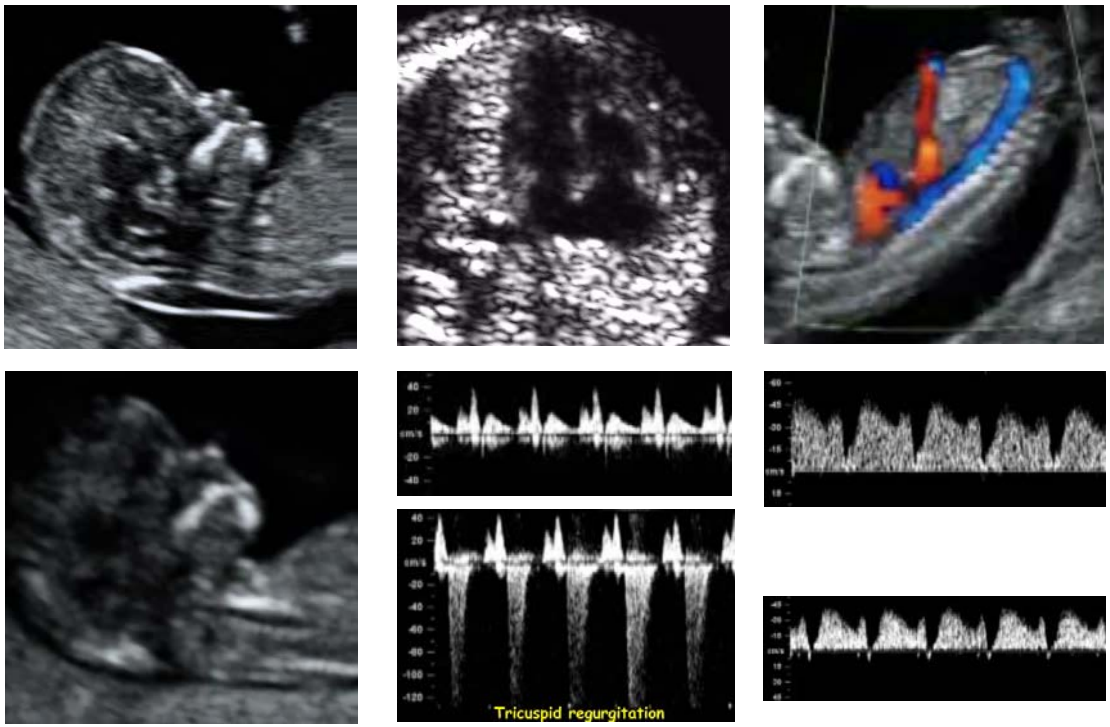
The Fetal Medicine Foundation

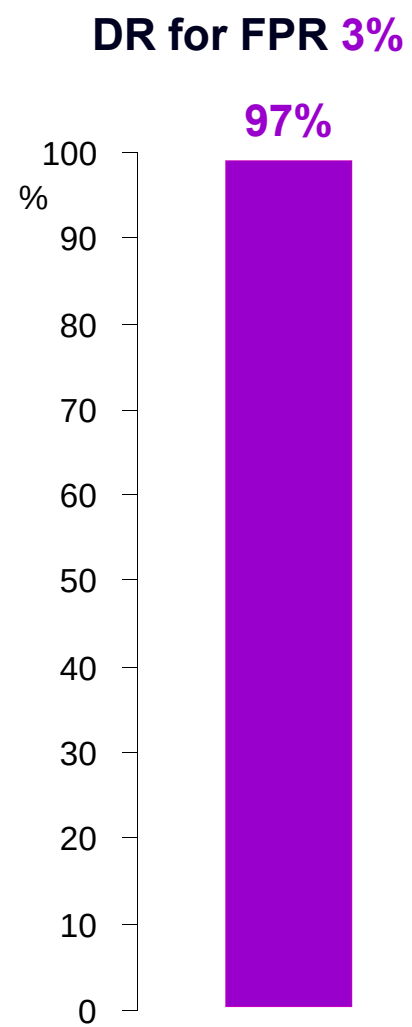
2000-10

Screening for Down syndrome

1st trimester combined test PLUS







- Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: an observational study. Lancet 2001;358:1665-1667.
- Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. Fetal **nasal bone** in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol **2009**; 33:259-64.
- Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. **Tricuspid regurgitation** in screening for trisomies 21, 18 and 13 and Turner syndrome at 11 + 0 to 13 + 6 weeks of gestation. Ultrasound Obstet Gynecol **2009**; 33:18-22.
- Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. **Ductus venosus** Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol **2009**; 33:512-7.