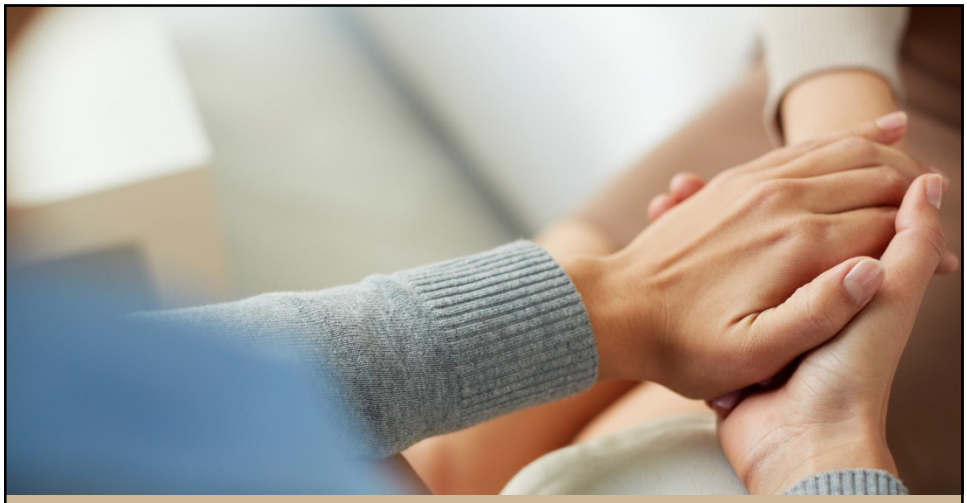


IS NIPT THE GREATEST THING SINCE SLICED BREAD?

Ken Seethram MD, FRCSC, FACOG, Co-Director

PCRM Pacific Centre for Reproductive Medicine

Disclosures

PCRM Pacific Centre for Reproductive Medicine

- No financial interest in the current NIPT providers – we receive no research grants, educational grants, or other initiatives from any NIPT provider
- We draw and send plasma on patients for NIPT at PCRM
- We perform first trimester screening (non-insured service) in our centre
- Current research affiliations are with:
  - UBC Cell biology
  - Igenomix (non-invasive ERA microbiome study)
- Lastly, I have not affiliations with Wonder Bread, or Dempster's Bakery of Canada

Objectives

PCRM Pacific Centre for Reproductive Medicine

01

Understand how **Non-Invasive Prenatal Testing** [NIPT] fits in with prenatal screening algorithms

02

Understand and differentiate current NIPT technologies

03

Review current advancements in NIPT

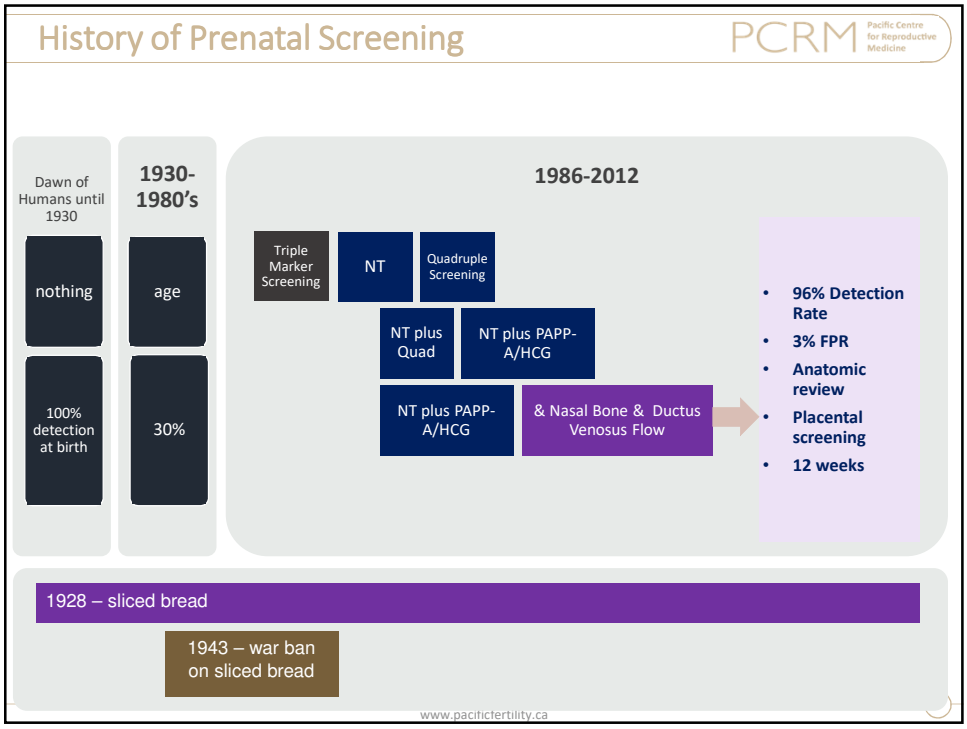
04

Review counseling considerations for prenatal screening and help you decide how to help your patients

Outline

PCRM Pacific Centre for Reproductive Medicine

1. History of prenatal screening and bread slicing
2. Prenatal Screening and the genetics behind it
3. NIPT and the Human Genome
4. Guideline based approaches to Screening
5. The future of prenatal screening and what to do today?


History of Prenatal Screening
PCRM
Pacific Centre for Reproductive Medicine
Dawn of Humans until 1930
nothing
100% detection at birth
1930-1980's
age
30%
1986-2012
Triple Marker Screening
NT
Quadruple Screening
NT plus Quad
NT plus PAPP-A/HCG
NT plus PAPP-A/HCG
& Nasal Bone & Ductus Venosus Flow
96% Detection Rate
3% FPR
Anatomic review
Placental screening
12 weeks
1928 – sliced bread
1943 – war ban on sliced bread
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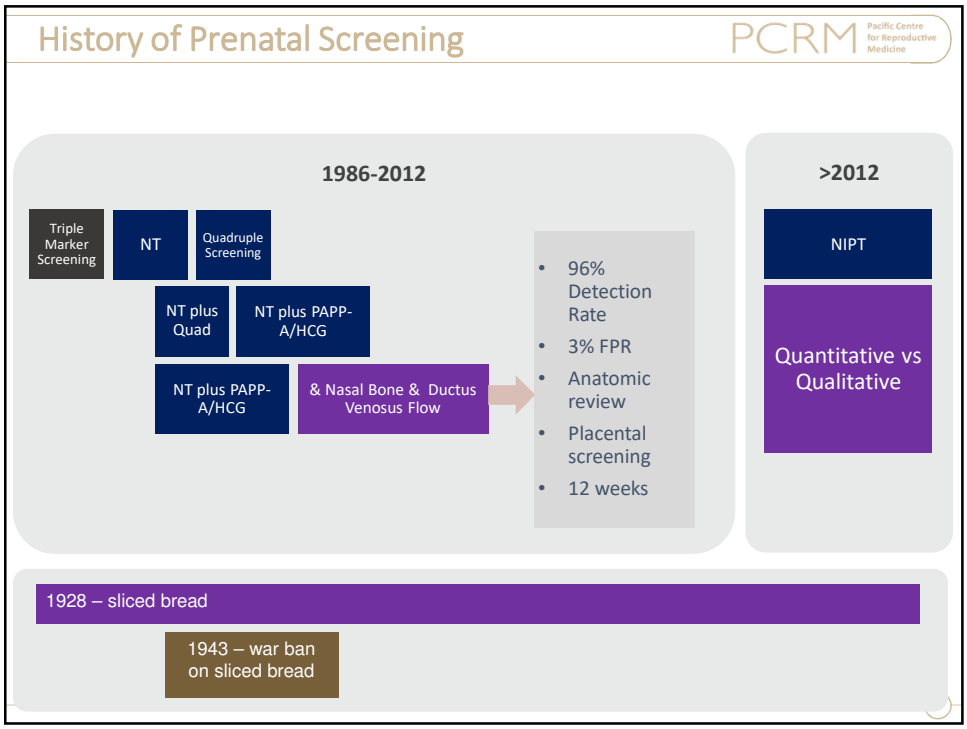
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96% Detection Rate
3% FPR
Anatomic review
Placental screening
12 weeks
>2012
NIPT
Quantitative vs Qualitative
1928 – sliced bread
1943 – war ban on sliced bread

Sliced bread saves time….

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One woman even wrote a letter to the New York Times admonishing the ban on sliced bread:

- "I should like to let you know how important sliced bread is to the morale and saneness of a household. My husband and four children are all in a rush during and after breakfast. Without ready-sliced bread I must do the slicing for toast—two pieces for each one—that's ten. For their lunches I must cut by hand at least twenty slices, for two sandwiches apiece. Afterward I make my own toast. Twenty-two slices of bread to be cut in a hurry!"
- The government lifted its ban in March (2 months later, March 1943)

Mental Floss article, Kaitlyn Boettcher, July 7, 2013

NIPT saves time also….

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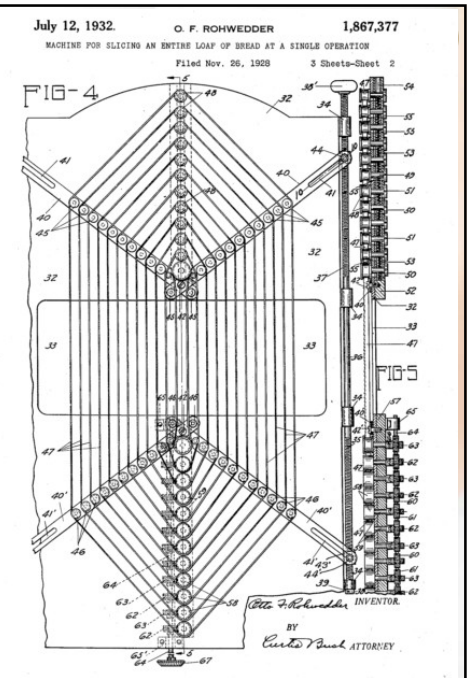
- By 2012, Prenatal screening had become very difficult
- What used to be a conversation about being 35 or older, or younger, and whether or not CVS/Amniocentesis is required, now became an extremely detailed, long conversation about the myriad of prenatal screening options, detection rates, risk, and counseling
- Suddenly, NIPT - **"Here's a risk-free blood test with over 99.9% accuracy"**

NIPT

- So the question is…

is NIPT the best thing since sliced bread?


July 12, 1932. O. F. ROHWEDDER 1,867,377
MACHINE FOR SLICING AN ENTIRE LOAF OF BREAD AT A SINGLE OPERATION
Filed Nov. 26, 1928 3 Sheets-Sheet 2
FIG-4
FIG-5
INVENTOR.
BY
ATTORNEY

Outline

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Prenatal Screening

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- What do we talk about when we talk about prenatal screening?
  - **Aneuploidy?**
  - Twins?
  - Neural tube defect screening?
  - Congenital anomaly screening?
  - Pre-eclampsia screening? (which affects 3-6% of pregnancies)
  - Preterm birth screening?
- Although one focus is aneuploidy, keep in mind that aneuploidy is simply one thing that can affect a pregnancy adversely

Prenatal Screening - Genetics

PCRM Pacific Centre for Reproductive Medicine

The biggest thing we are screening for is Trisomy 21 (also known as Down Syndrome)

- T21 is one of the most common aneuploidy to affect live-born children and has a background prevalence of 1:691, increasing with maternal age
- What causes T21 – hypo-methylation by the extra chromosome
- Prenatal **diagnosis** relies upon the procurement of fetal cells via amniocentesis (ACT) / trophoblast via chorionic villus sampling (CVS)

Prenatal Screening - Genetics

PCRM Pacific Centre for Reproductive Medicine

The biggest thing we are screening for is Trisomy 21 (also known as Down Syndrome)

- Back in the 1970-1980's - if your age based risk exceeded the risk of diagnostic testing, then do the test, which was roughly age 35-38
- So the only screening tool we had – was age

Quick Diversion: What is the inherent risk of Amniocentesis or CVS?

- For the last 40 years, it has been quoted at 1%

Prenatal Screening - Genetics

PCRM Pacific Centre for Reproductive Medicine

- **Akolekar, et al (2015)** ***Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis.*** **Ultrasound in Obstetrics and Gynecology 45(1):16):**
  - 42,000 women who underwent ACT and 138,000 who did not, data collected >1999
  - Attributable miscarriage risk: **0.11%**
  - 54,000 women who underwent CVS and 670,000 who did not, data collected >1999
  - Attributable miscarriage risk: **0.22%**
- **Odibo, et al (2008)** ***Revisiting the fetal loss rate after second-trimester genetic amniocentesis: a single center's 16-year experience.*** **Obstetrics and Gynecology 11(3):589**
  - 11 746 amniocenteses and 5243 CVS
  - **0.13%** loss rate in amnio and **0.7%** loss rate in CVS was no different than the loss rate in those without invasive procedures
- **Our previously agreed-upon 1% may not be so true**

Prenatal Screening - Genetics

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- The risks of invasive testing and resources should govern the decision to do the test
- And probably the risks of testing are ~1:1000 rather than 1:100
- However….end of the day – nobody wants invasive testing
- **This is fundamentally why we do prenatal screening – to figure out who needs the invasive test, and who does not**
- What are the major chromosomes we're looking for?.....

Prenatal Screening - Genetics

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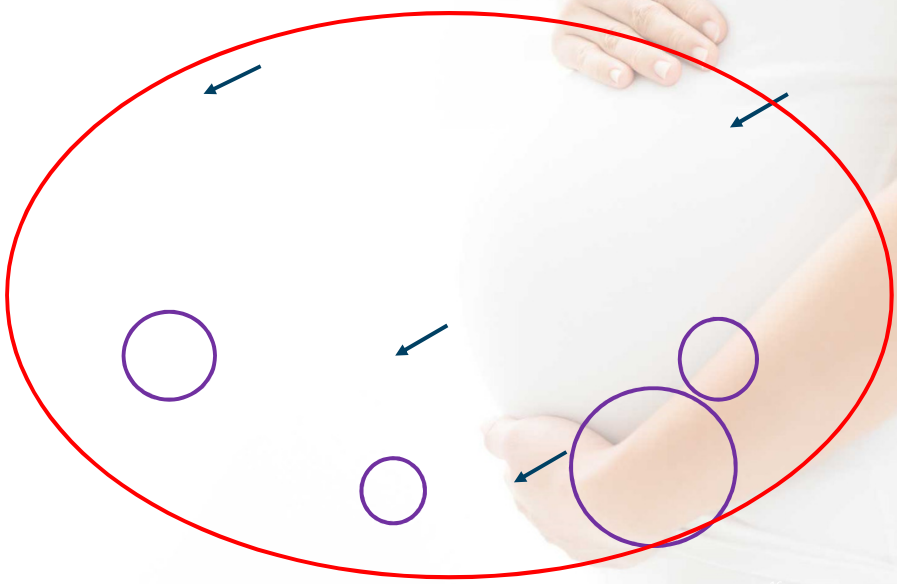

Prenatal Screening - Genetics

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- ✓Age
- **Double screening>Triple marker screening>Quad Screening**
  - MSAFP for Neural tube defect screening in the early 80's was then linked with hCG for T21 detection
  - 1991 (Cuckle and Wald) published in the BJOB – uE3, hCG for T21 detection
  - Combined this became triple marker screening
  - Modified in 1996 by the addition of dimeric Inhibin-A to become QUAD screen

Prenatal Screening - Genetics

PCRM Pacific Centre for Reproductive Medicine

- ✓Age
- ✓ Double screening>Triple marker screening>Quad Screening
- **Combined First Trimester Screening (NT+NB+DV+PAPP-A+bHCG)**
  - BMJ 1992 – K. Nicolaides published on the use of beta-hCG, PAPP-A and a single ultrasound measurement at 12w called nuchal translucency (NT)
  - Modified since 1992 to include nasal bone, FHR, ductus venosus flow (hepatic vein flow) using color flow Doppler measurements
  - Accreditation provided through single site – Fetal Medicine Foundation, UK
  - **Detection rate: 96% with screen positive rate = 3%**

Prenatal Screening - Genetics

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- ✓Age
- ✓ Double screening>Triple marker screening>Quad Screening
- ✓ Combined First Trimester Screening
  - (NT+NB+DV+PAPP-A+bHCG)

- And then people got fancy and started mixing first and second trimester screening to try and improve performance while lowering costs
  - **Combining PAPP-A with Quad (SIPS)**
  - **Combining NT, PAPP-A with QUAD (IPS)**
    - **DR 88% with screen positive rate of 3%**
- This is like mixing red and white wine – it feels like your doing the right thing, until later

Prenatal Screening - Genetics

PCRM Pacific Centre for Reproductive Medicine

- ✓Age
- ✓ Double screening>Triple marker screening>Quad Screening
- ✓ Combined First Trimester Screening
- ✓ mixing 1TM and 2TM screening
- **By 2011 however, globally, the focus was on the first trimester:**
  - NT
  - Ductus Venosus
  - Nasal Bone
  - PAPP-A and bhCG

Combined First Trimester Screening

- And then things got really complicated in 2011 with the introduction of NIPT
- Actually things got complicated long before that…..

Outline

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NIPT and the Human Genome

PCRM Pacific Centre for Reproductive Medicine

NIPT and the Human Genome

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- The first two drafts of human genome were published by the Human Genome Sequencing consortium and Celera Genomics (Venter) 2001.
- The HuRef Browser –online tool for browsing genome assemblies and studying individual human sequence variation
- And our understanding of how our genome was assembled led to the application of new discoveries such as NIPT

Axelrod, 2009 The HuRef Browser: a web resource for individual human genomics

NIPT and the Human Genome

PCRM Pacific Centre for Reproductive Medicine

Non-Invasive Prenatal Testing or NIPT

- Emerged in 2011, but first described in 1997
- "cell-free 'fetal' DNA (cffDNA)," actually **derived from the placenta,** comprises about 4% of all free-DNA in the maternal blood
- Can be detected as early as 4-5 weeks of gestation
- Usually does not exceed **150 base pairs** of length (very small fragments) but *the entire fetal genome is represented*
- Keep in mind:
  - Our entire genome - 6.5B base pairs
  - Chromosome 1 has 249M base pairs
  - Chromosome 21 has 48M base pairs
- So there's a lot of math required to re-construct part or all of the fetal genome from these bits of DNA

NIPT and the Human Genome

PCRM Pacific Centre for Reproductive Medicine

- And that's what NIPT is:
  - An assembly of the fetal genome through fragments of the placental free DNA circulating in maternal blood
  - 'non-invasive prenatal testing'
    - *Keep in mind – it's called fetal DNA, but it's not, it's placental*
    - *Keep in mind – it's called testing, but it's screening*
    - *Keep in mind – it's not non-invasive, but a blood test is pretty easy*

NIPT and the Human Genome

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Free DNA comes from apoptotic cells derived from:

- Maternal Circulation
  - Adipocytes
  - White Blood Cells
- Fetal
  - Placental cells (trophoblasts) in the maternal circulation
- Fetal Fraction – the percent ratio between placental:maternal DNA

NIPT and the Human Genome

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- Fragments of DNA in NIPT can then be compared against Venter's Human Genome Library (HuRef) to essentially either sequence the genome from those fragments, or use targeted gene analysis to count signals
- **Counting** - For example, if you know a certain gene resides on Chromosome 13, and you have three copies of that gene assembled from the NIPT fragments, then you can diagnose Trisomy 13 simply by counting (ex. Harmony)
- **Sequencing** - Or, if you can assemble the bits of DNA then sequence them together *or* compare single nucleotide polymorphisms (ex. Panorama)
- That is the big difference between NIPT products – counting and sequencing

NIPT and the Human Genome

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- How good is it?
- **As a screening tool, it is very very good**
- Most studies report detection rates exceeding 99%, with false positive rates of 0.1%
  - Study, Zhang – (Whole Genome sequencing) in over 140,000 pregnancies:
  - The overall sensitivity of NIPT was
  - T21 99.17% (specificity of 99.95%)
  - T18 98.24% (specificity of 99.95%)
  - T13 100% (specificity of 99.96%

Zhang, H, Ultrasound in Obstetrics and Gynecology January 2015

NIPT and the Human Genome

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Screening for Trisomy 21: NEXT clinical trial

15,841 women

	hCG + PAPP-A + NT		Cell-free DNA Screening
False Positives	854		9
True Positives	30		38
False Negatives	8		0

Norton M, et al. N Engl J Med. 2015 DOI: 10.1056/NEJMoa1407349

NIPT and the Human Genome

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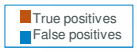
Traditional Screening



NIPT

PPV: 4-5%
Maternal serum screening would require **265** women to undergo invasive testing to discover **9** true positives.[1]

PPV: 91%
With NIPT, **10** women will undergo invasive testing to discover **9** true positives.[2]

[1]Norton et al, N Engl J Med 2015. [2]Dar et al. Am J Obstet Gynecol 2014 Nov;211(5):527

NIPT and the Human Genome

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- Bottom line – it's a great screening tool
  - It's super for T21
  - Performance and specificity for T13/T18 is less

NIPT and the Human Genome

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- Have you heard of fetal fraction?
  - Early gestation, high BMI, collection issues, aneuploidy
  - Can all cause a low proportion of placental DNA versus maternal
  - This can drop the accuracy of results
  - Minimum required: 4% for counting, 2.8% for SNP
- If low, you have to:
  - Redraw
  - Option of other screening methods
  - Up to 30% of people on redraw will still get non-reporting
  - **So although it's a great test, 3% of all patients will get non-reporting**

NIPT and the Human Genome

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- What about microdeletions
  - SNP sequencing is the only NIPT which can look for small deletions in the chromosomes
  - Syndrome from microdeletions can be quite severe
  - But those syndromes are RARE
  - Also, positive predictive values are low (20% maximum for 22q11.2 deletion syndrome)
- What about Twins
  - Sequencing with Single nucleotide polymorphisms (SNPs) provides one thing: **zygosity** (if in doubt)

NIPT and the Human Genome

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Microdeletion syndrome	Incidence (at birth)	Clinical features Babies born with this syndrome often have:
22q11.2 deletion syndrome/ DiGeorge	1 in 4,000-2,000	heart defects, immune system problems, and mild-to moderate intellectual disability. They may also have kidney problems, feeding problems, and/or seizures.
1p36 deletion syndrome	1 in 10,000-5,000	weak muscle tone, heart and other birth defects, intellectual disabilities, and behavior problems. About half will have seizures.
Angelman syndrome	1 in 12,000	delayed milestones (like sitting, crawling and walking), seizures, and problems with balance and walking. They also have severe intellectual disability and most do not develop speech.
Prader-Willi syndrome	1 in 25,000- 10,000	low muscle tone and problems with feeding and gaining weight. They also have intellectual disability. As children and adults, they have rapid weight gain and often develop obesity related medical problems.
Cri-du-chat syndrome (5p-)	1 in 50,000- 20,000	low birth weight, small head size, and decreased muscle tone. Feeding and breathing difficulties are also common. They have moderate-to-severe intellectual disability.

Risks

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- DiGeorge syndrome 22q11.2 deletion risk: 1:2000-4000
- Risk of dying in a car: 1:5000
- Risk of dying in pregnancy in Canada: 1:8800
- Risk of having a child with autism/spectrum: 1:68
- **What's my point?**
  - Microdeletion syndromes are rare
  - People read about these on the google and on the twitter, and they displace their baby concern with the concern for truly rare diseases

NIPT and the Human Genome

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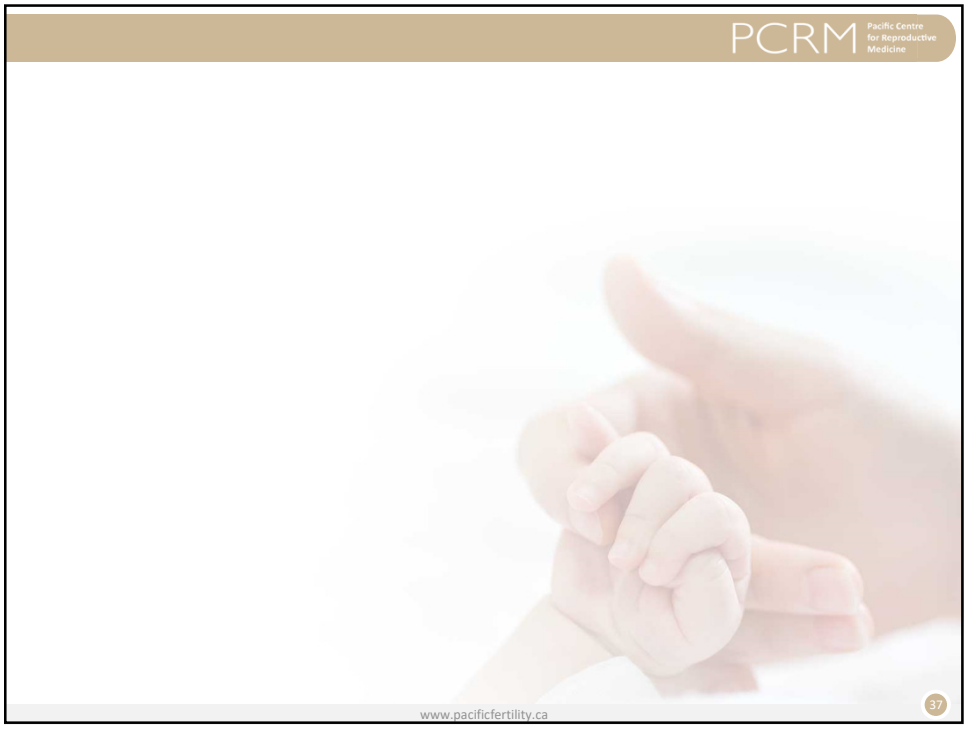
- **What are the major NIPTs available?**
  - **Targeted (counting method) – example - Harmony©**
    - Where we look for gene loci unique to certain chromosomes (like 21) and compare that number with the reads from other chromosomes (like 1)
    - If you get more reads from the locus on 21 in a ratio of 1.5X versus 1, then you have trisomy 21
    - Benefits – lower costs
  - **SNP based – example - Panorama©**
    - Where DNA sequence variations (normal in all of us) are compared between placental and maternal DNA
    - Variations of up to 1% are normal
    - This can then help segregate and compare maternal versus placental DNA
    - Benefits – microdeletions, zygosity, slightly lower fetal fraction threshold
  - **Whole Genome sequencing – example NIFTYpro©**
    - All chromosome numeric abnormalities
    - 84 microdeletions
    - March 2018



NIPT

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- So now y'all know:
  - What NIPT is
  - How it performs
  - The methods used
  - The variations like microdeletion assessment with SNP and Whole Genome sequencing
- So how does it fit into our prenatal screening world?

www.pacificfertility.ca
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Guideline based approaches to screening

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All women, regardless of maternal age, should be offered prenatal assessment for aneuploidy

Organization	Policy	Date
	"[cfDNA] appears to be the most effective screening test for **aneuploidy** in high risk women"	2012 - 2013

Guideline based approaches to screening

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- NIPT Funding in BC
  - has received a Positive Screen result from IPS, SIPS, or Quad;
  - has had a previous trisomy 13, 18, or 21 pregnancy; or
  - has a risk of Down syndrome greater than one in 300 based on results of screening and ultrasound marker(s) of aneuploidy (ie. Contingency model)

Guideline based approaches to screening

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- American College of ObGyns 2015
  - any patient may choose cell-free DNA analysis as a screening strategy for common aneuploidies regardless of her risks status
  - **Conventional screening methods remain the most appropriate choice** for first-line screening for most women in the general obstetric population
- International Society for Prenatal Diagnosis – all are acceptable:
  - Offer NIPT to all
  - Offer NIPT to high risk only
  - Use a contingency approach (high, moderate, low risk)
  - First trimester screening
  - Quad screen if after 14w
  - 2nd trimester scan

Guideline based approaches to screening

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- So why not just do NIPT on everyone?
  - Gives us no information about the baby – just about the placental DNA
  - Tells us nothing about anatomic problems
  - Tells us nothing about placental disorder risks (PIH, IUGR)

Why not do NIPT alone?

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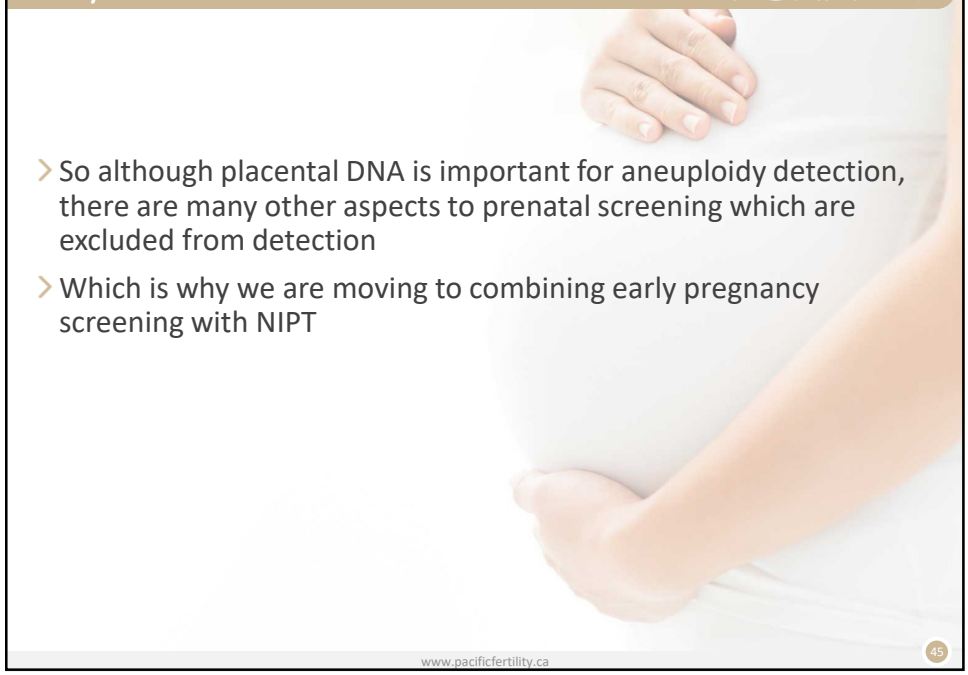
- Conventional screening, whether it's FTS, or sequential integrated screening provides other information including fetal structures, multiples etc.
  - Baer 2014 ACOG identified 9,051 FTS screen-positive and 30,928 screen-negative pregnancies
  - FTS screen positive women were
    - 1.7X more likely to be diagnosed with preeclampsia, placenta previa, or abruption
    - 3.5X more likely to experience fetal loss before 20 weeks
    - Women with positive results for more than one screened condition were at substantially greater risk (34-157X) relative risk of fetal and neonatal mortality
- PAPP-A screening with FTS – those under 10th percentile MoM have a 4.2X increased risk of several Pre-eclampsia

Am J Perinatol. 2015 Jun;32(7):703-12. doi: 10.1055/s-0034-1396697. Epub 2014 Dec 17

Why not do NIPT alone?



- So although placental DNA is important for aneuploidy detection, there are many other aspects to prenatal screening which are excluded from detection
- Which is why we are moving to combining early pregnancy screening with NIPT

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The future of Prenatal Screening

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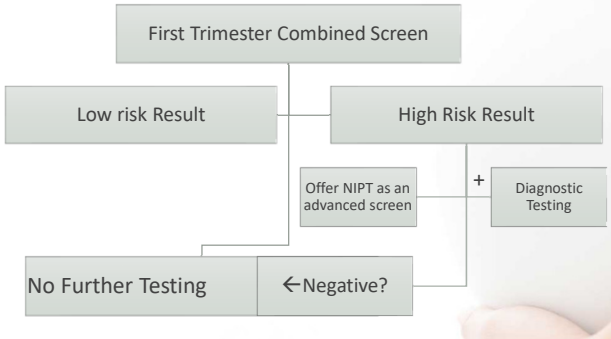
Advanced screening test



-reduction in invasive testing rates
-UK National Screening Committee recommends introduction of this model into NHS

Hui and Bianchi (2017) *Noninvasive Prenatal DNA Testing: The Vanguard of Genomic Medicine.* Annual Review of Medicine 68:21.1–21.14.

The future of Prenatal Screening

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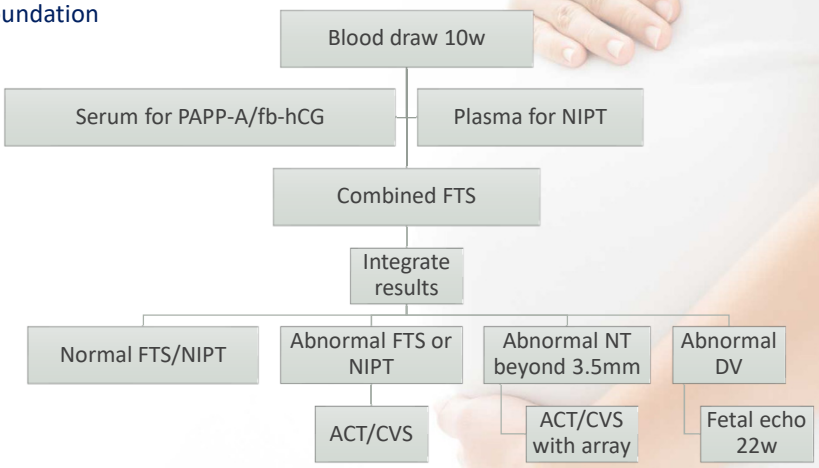
Fetal Medicine Foundation



The future of Prenatal Screening

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First Trimester Screening	Multiple markers History and Mean arterial pressure Placental Growth Factor
NIPT	Genomic information
Second Trimester	Ultrasound and medical screening
By 20w	We will be able to triage those at very low risk, and those at high risk – concentrating prenatal care on the people who need it the most: anomalies, PIH risk, Preterm birth risk, etc

The future of Prenatal Screening

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- Prenatal screening should always be presented and discussed as an available option, not as standard of care or required
- There is no right or wrong choice
- Many expectant parents have a difficult time deciding whether or not to have a prenatal screening test
- The benefits and limitations of prenatal screening should be discussed in reference to the patients current feelings, beliefs and wishes…. And resources

What do I do today?

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Regardless of the patient's age….

Wants to screen but not out of pocket	MSP covered serum screening with NT if over age 35	CVS/Amnio if screen positive or meets criteria
Wants to screen and okay to pay for non-insured services	First Trimester Screening with or without NIPT	CVS/Amnio if screen positive
Wants to screen and wants everything possible	First Trimester Screening with NIPT – Sequencing	CVS/Amnio if screen positive

The End

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- NIPT is a superior screening test for common aneuploidies but has limitations that are important to understand
- It is quick, and easy, but it is **not** the greatest thing since sliced bread
- The marketing power, and ease of results tempts patients and practitioners
- But it does so at the costs of ignoring the pregnancy as a whole
- FTS compliments NIPT and overcomes some of its limitations
- Pre-test counselling and informed decision making is KEY
- The future of prenatal screening will not be focusing on aneuploidy, but rather focusing on healthy outcomes for mother and baby

NIPT and the Human Genome

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	Detection Rate	False Positive Rate	In BC ~ 1/608 births is T21 (N=74)	Missed cases
NIPT	> 99%	< 0.1%	74 / 74	**0**
FTS	96%	< 3%	71 / 74	**3**
IPS (>35)	81%	3%	60/ 74	**14**
SIPS (<35)	71%	3%	53 / 74	**21**

Vital Statistics Canada. 45,000 births per annum BC, of which 25000 have screening

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