

*An Investigation in to a New
Protocol for the Processing of
Solid Tissue Samples Resulting
From Pregnancy Loss*

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Outline of Talk

- Background of pregnancy loss
- Issues with current protocol
- Investigations into new protocol and results
- Future developments
- Summary

Pregnancy Loss

- 15% of all pregnancies result in loss
- 50-60% cytogenetically abnormal
- Many other causes...

Therefore cytogenetic analysis plays an important role in patient management...

- *Abnormal* – counselling and reassurance
- *Normal* – commence/alter therapy and consider clinical trials

Current Protocols

- LWH receives on average **565** tissue samples per year
- Cord insertion and fetal tissue
 - culture and conventional karyotyping
- Products of conception
 - X;Y;18 FISH prelim
 - culture and conventional karyotyping

Issues with Current Protocol

- Low abnormality rate
- Higher than expected level of normal female results
 - Skewed female to male ratio of 70% : 30%
 - Maternal cell contamination
 - NOT maximising the detection of the fetal cell line

Previous Work . . .

Collaborative trial undertaken with North West Recurrent Miscarriage Clinic

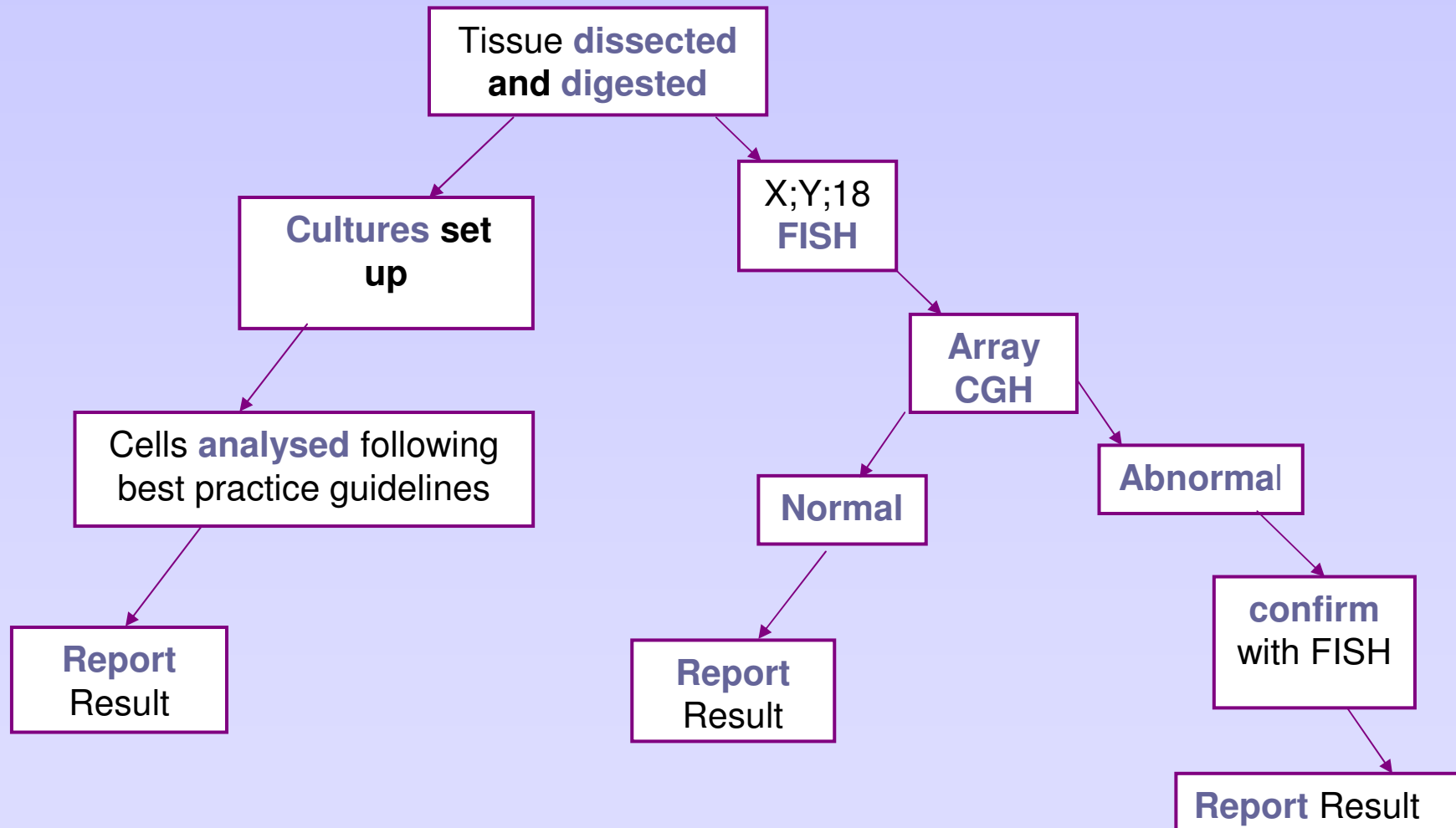
Using ArrayCGH to analyse products of conception from recurrent early miscarriage patients...

...Showed many advantages over current protocol

Advantages of Microarray

- Direct DNA extraction
 - No cell culture
- Detect low level fetal cells
 - Despite maternal cell contamination
- Higher resolution analysis (2Mb)
 - Often only short chromosomes available for conventional analysis



Workflow for Samples in Trial



Aims of this Project

- Assess sensitivity of the CytoChip FOCUS array used for solid tissue samples.
- Calculate and compare costs of the different tests available for solid tissue samples.
- Design workflow for solid tissues that maximises fetal cell detection
 - therefore improve abnormality rate whilst maintaining a cost effective service

Assessing FOCUS Array Sensitivity

- Data review – set a baseline for investigating smaller abnormalities 
- 4 retrospective cases were chosen and used to create 'artificial' positive controls
 - The DNA from these cases was diluted with the same sex reference DNA to give abnormal DNA concentrations ranging from 100% to 5% 

Imbalances Detected Previously

- Smallest :
 - 27.54- 28.75Mb DELETION of 14q31.1-q32.33
 - 11% fetal cell population

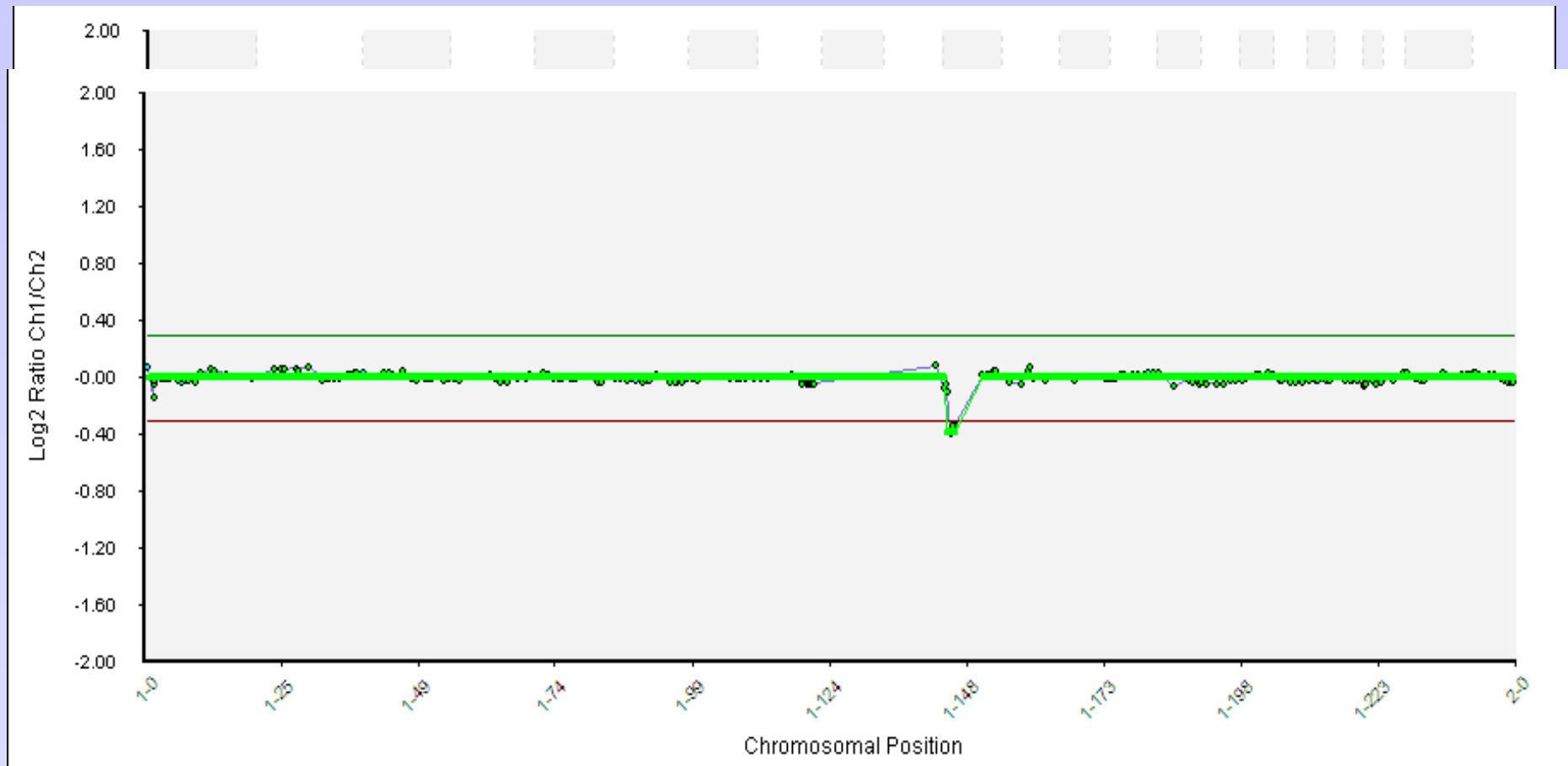
- Lowest Level:
 - Trisomy 22 (49.7Mb)
 - 5% fetal cell population

Artificial Positive Controls

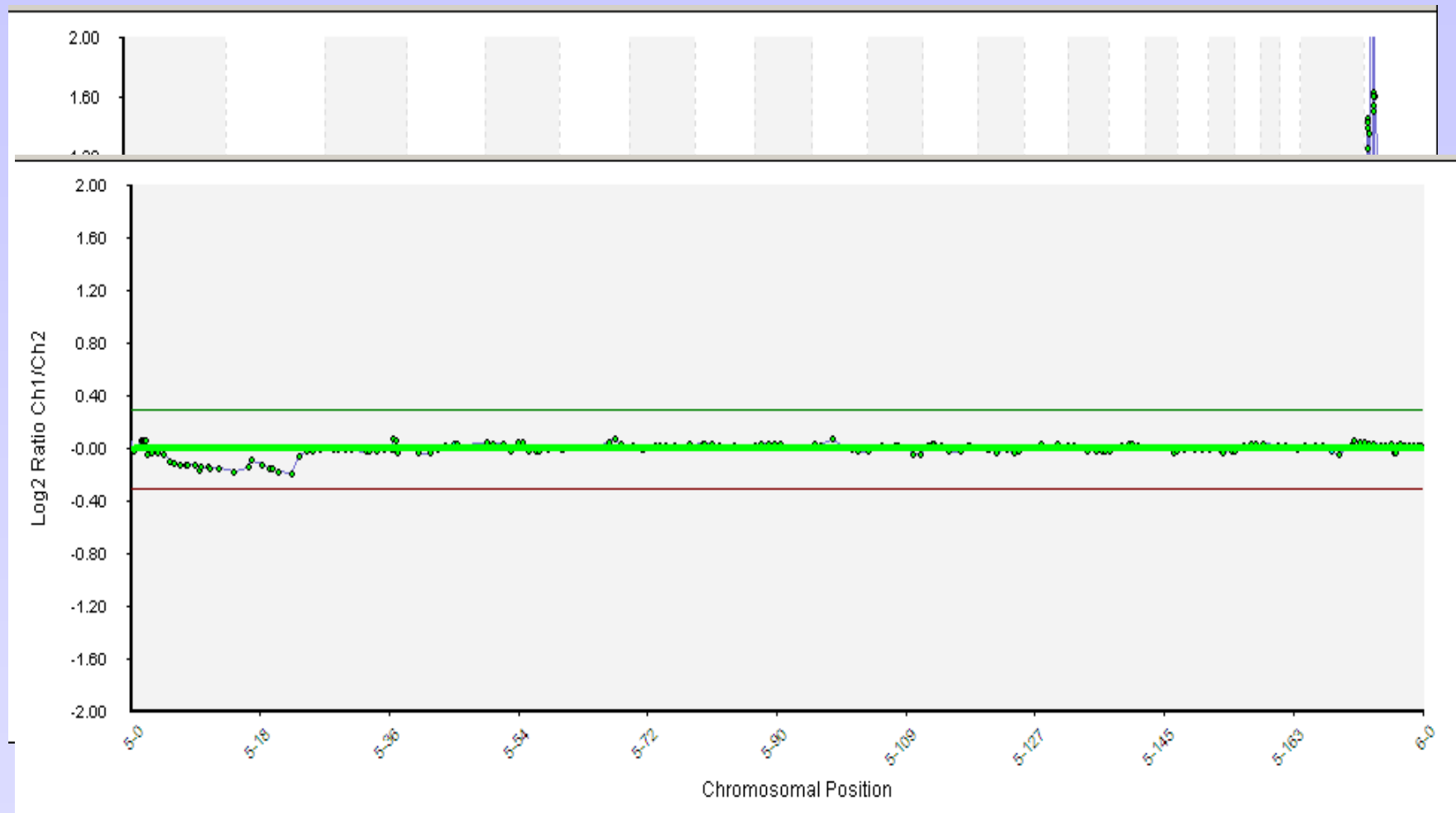
Imbalance	Chromosomal position
20Mb deletion	5p14.2-p15.32
10Mb duplication	12q21.33-q23.2
2Mb deletion	1q21.1
500Kb duplication	10q11.21

Array Results...

2Mb deletion at 1q in 50%



20Mb deletion at 5p in 25%



Result Summary

Abnormality	100%	75%	50%	25%	15%	5%	
~20Mb DELETION	Y	Y	Y	Y	Y	N	N
~10Mb DUPLICATION	Y	Y	Y	Y	Y	N	N
~2Mb DELETION	Y	Y	Y	Y	N	N	N
~500Kb DUPLICATION	N	N	N	N	N	N	N

Array Sensitivity

Can detect...

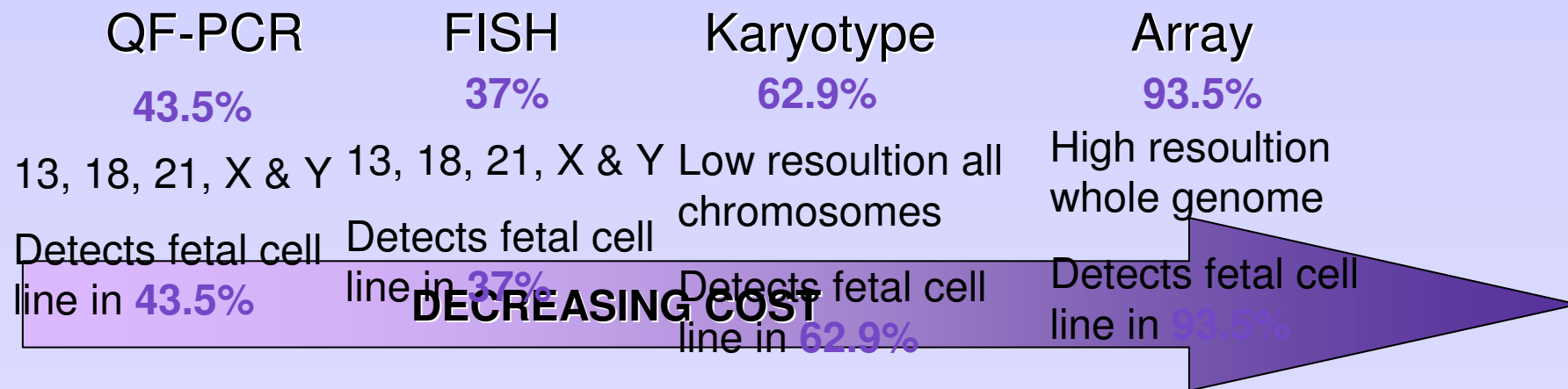
- Trisomy/monosomy
 - 5% fetal cells
- As low as 2Mb
 - 50% fetal cells

Cost Comparison

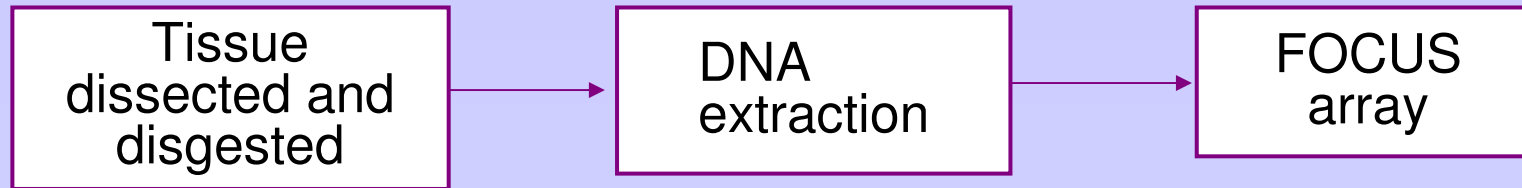
Cost per fetal cell line identified

Based on results from samples in trial

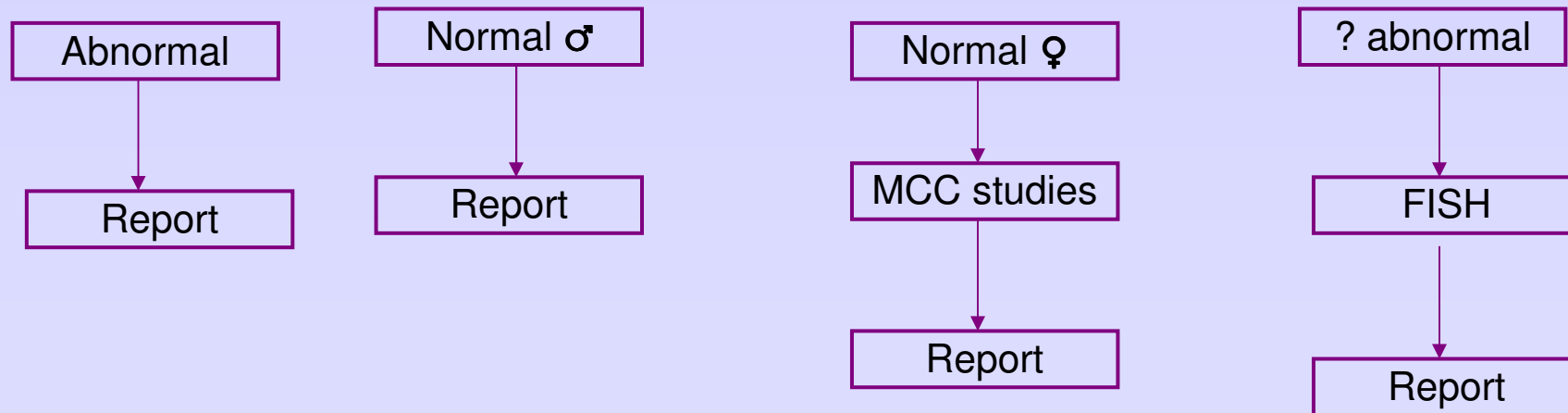
Cost calculated on workload units



Proposed Workflow



Outcomes



Future Developments

- Already have established array protocol and equipment ∴ begin implementing new protocol later in year
- Monitor results for later pregnancy losses
 - What size imbalance will cause a miscarriage?

Thank you...

Una Maye & Julia Kilender

Array section

Joan Getty

Tissue section

Any Questions?