Thrombophilia and Reproductive Failure

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Declaration of Interests

- Chair elect, European Society of Human Reproduction and Embryology (ESHRE) (2015 -2017)
- NICE Guideline Development Group (CG 154, 2010-2013) NICE Evidence Update Advisory Group, 2014
- Chair, Association of Early Pregnancy Units, UK (2006-2011)
- ESHRE Co-ordinator, Special Interest Group for Early Pregnancy (2007-2010), Executive Committee (2011 -2015)
- Associate Editor, Human Reproduction Update (2010-2014)

Contents of Talk

- Historical perspective of EPL and RPL
- Definition and pregnancy loss type
- Investigating cause and thrombophilia testing
- Quality of care and the patient perspective
- Analysis of treatment interventions
- Where we stand
- What next?

Historical perspective of RPL

- **It's all to do with Percy Malpas** (Liverpool,1938), Whitehouse (London, 1929) and Mall (USA,1917)
- **It uses** 'statistics, damn statistics and theoretical projection'
- **It starts** with a figure of spontaneous loss in general population then works theoretically forward to define expected numbers of recurrent and non-recurrent causes

A study of Abortion Sequences
Percy Malpas, Liverpool
BJOG, 1938, 45, 932-949

men is [illegible] the aetiology of some cases of abortion must [illegible]

General Conclusions.

Abortion sequences have a varied aetiology. In any population the total number of instances of sequential abortion and stillbirth is made up of two groups of patients. The first comprises those cases in which successive pregnancies fail because of the chance succession of random and casual factors.

From a statistical standpoint, the presence of a recurrent factor may be inferred with reasonable certainty after the occurrence of three successive abortions, so that a 3-abortion sequence is almost certainly due to a recurrent factor. The total number of pregnant women in whom such recurrent factors occur is about 1 per cent, as opposed to about 17 per cent of pregnant women who abort from random and accidental causes.

A cause can be discerned in about one-half of the segregated recurrent aborters. These causes fall into the following three groups:—

(1) Early lesions of the developing embryo, probably resulting from a defective nutrition of the early chorion producing lesions at the successive critical phases of development.[3] In some of these cases familial causes may be demonstrated and the problem is closely linked with that of the recurrent factors which have been demonstrated by the geneticists as occurring in lower animals.

Talking of numbers....

- **Non-recurrent causes have a high frequency eg chromosome errors found in 50% of RPL cohorts and 70% in random spontaneous single loss**
- Self cure rate is high
- Recurrent causes are low
- Lots of associations but not always directly causal

Chromosomal Mapping of Human Cleavage Embryo/Blastocyst – the ENIGMA of Euploid/ Aneuploid Mosaicism

46XY

47XY
+15

45XO

46XY

69XXY

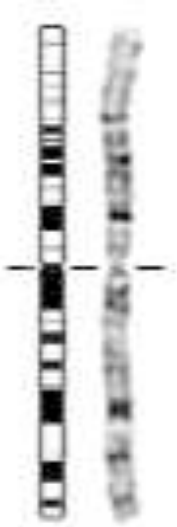
46XY

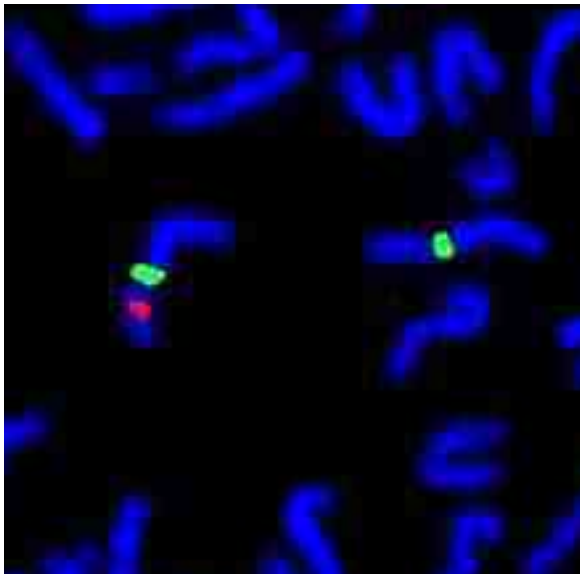
46XY

microarrays

- **technique**
  high resolution WHOLE genome scan NOW with NGS

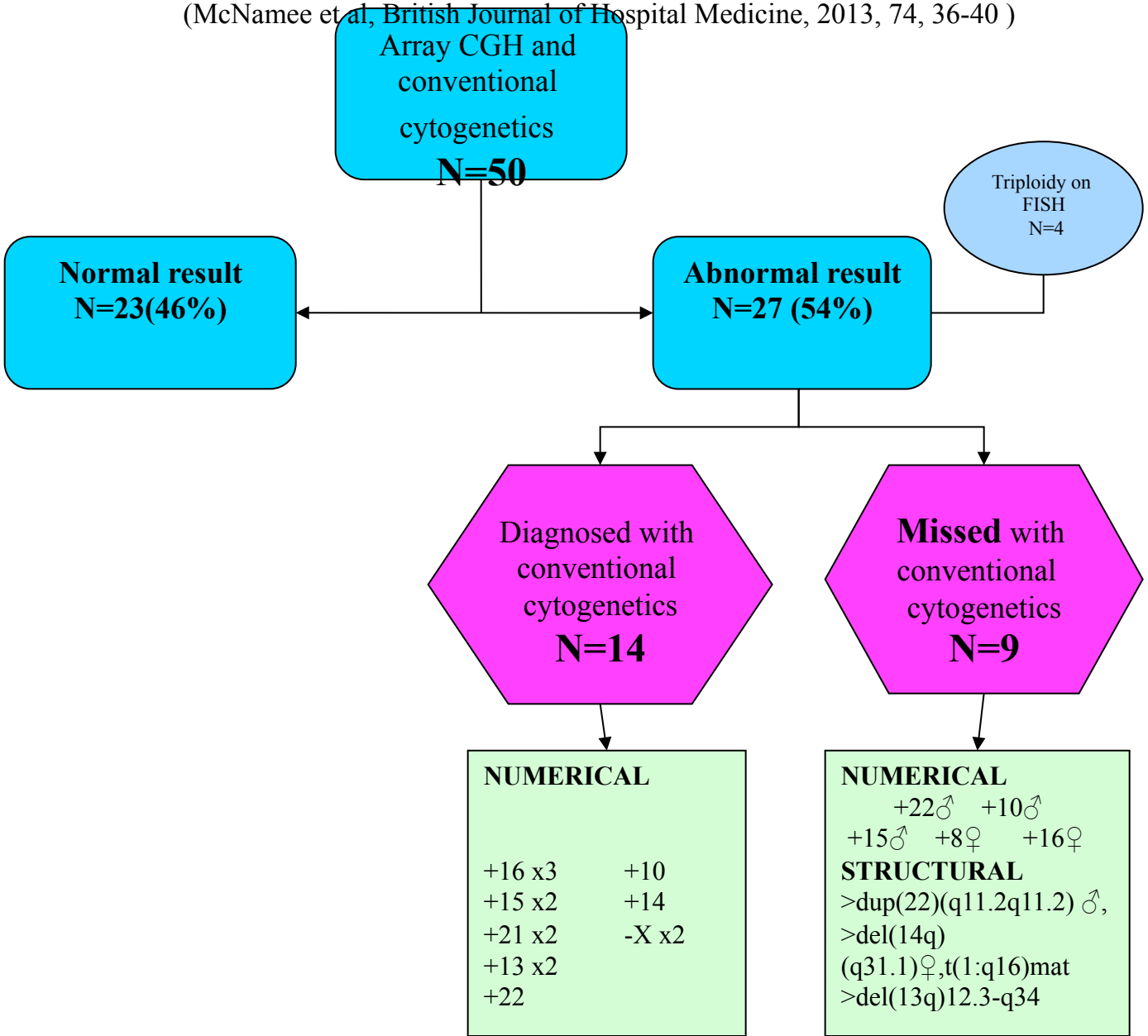
cytogenetics FISH arrays





RM – Evaluation of Array CGH v Conventional Cytogenetics

(McNamee et al, British Journal of Hospital Medicine, 2013, 74, 36-40)



Talking of numbers....

- Self cure rate is high
- Non-recurrent causes have a high frequency eg spontaneous chromosome errors
- **Recurrent causes are low;**
  - >50% have a negative RPL screen
  - APS positive rate 5-10% in early RPL
- Lots of associations but not always directly causal

RPL Investigation screen 2015

ALL NEW PATIENTS

- Thrombophilia Screen: Antiphospholipid Syndrome (DRVVT,ACA IgG/IgM) ;Activated Protein C resistance (APCR/APCRV (acquired), Factor V Leiden (inherited): Protein C/S level
- Autoimmune screen (AntiNuclear Antibody and double stranded DNA)
- ABO grouping: RH grouping/ Antibody
- FBC
- FSH/LH/E2/Test/Progesterone/PRL
- Thyroid Function and Antibodies
- Glycosuria testing
- Uterine anomaly screening using 3-D scan optional

NEXT PREGNANCY LOSS KARYOTYPE (array CGH/NGS)

SECOND TRIMESTER LOSS

3D US/HYSTEROSCOPY
Cervical Length (CLM)
Uterine Anomaly (CUA)

+

SWABS X2 preconceptual and T1

BACTERIAL VAGINOSIS

Standards for Testing

Guidelines on the investigation and management of antiphospholipid syndrome
Keeling D, et al, British Journal of Haematology, 2012, 157, 47-58

- APS Testing – *best practice*

  - Arm sample to Laboratory transit time ASAP (as LAC activity disappears after 4 hours)

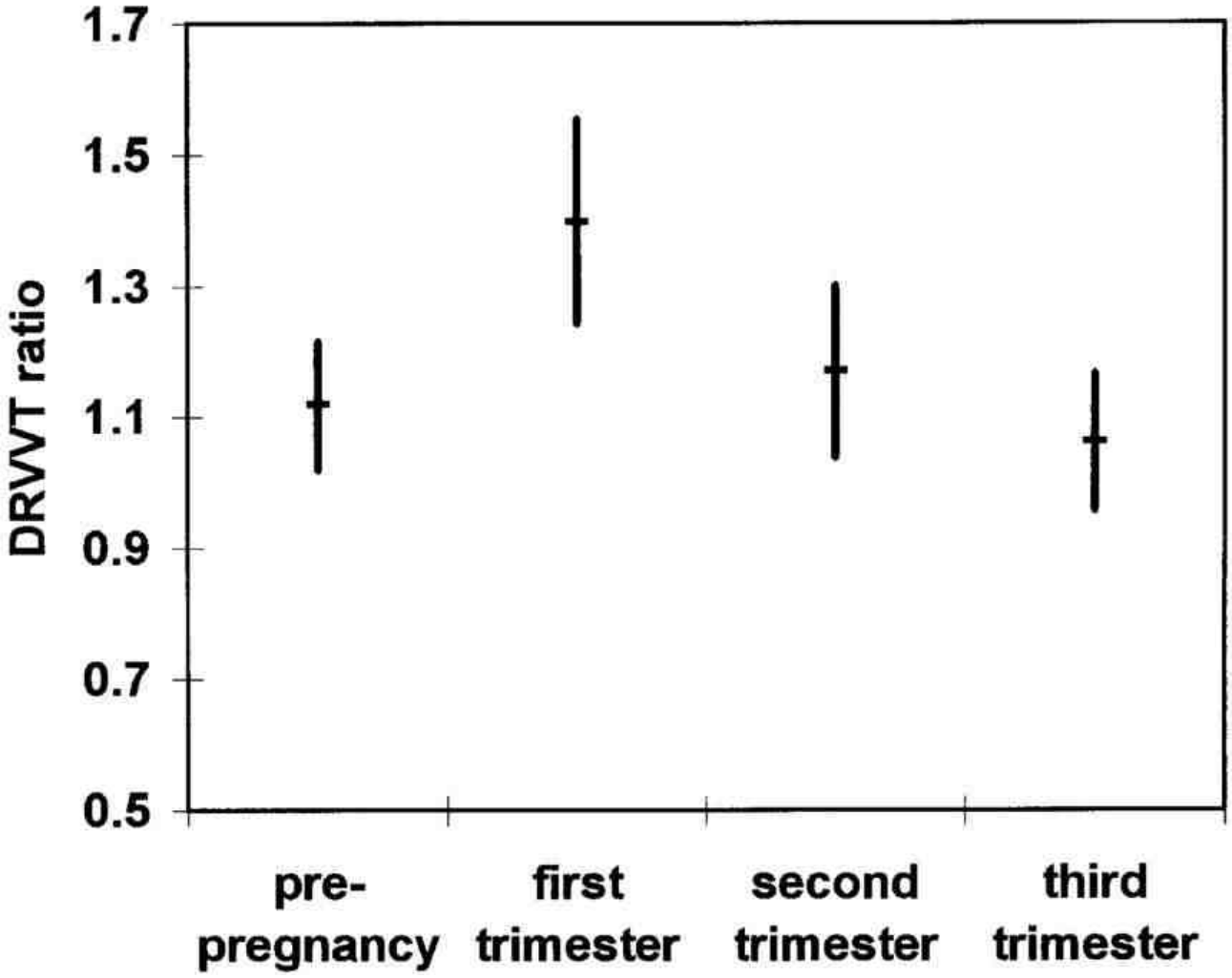
  - Sample spun then stored frozen at -70C

  - established Laboratory Quality control

- between 5 and 15 % of RM cohorts have positive APS
- does your lab under-report?
- if <2% positive, you have a detection problem

Variation in the dilute Russell's viper venom test (DRVVT) ratio for the antiphospholipid syndrome (APS) group (n=16) during pregnancy

mean ± 95% confidence intervals.



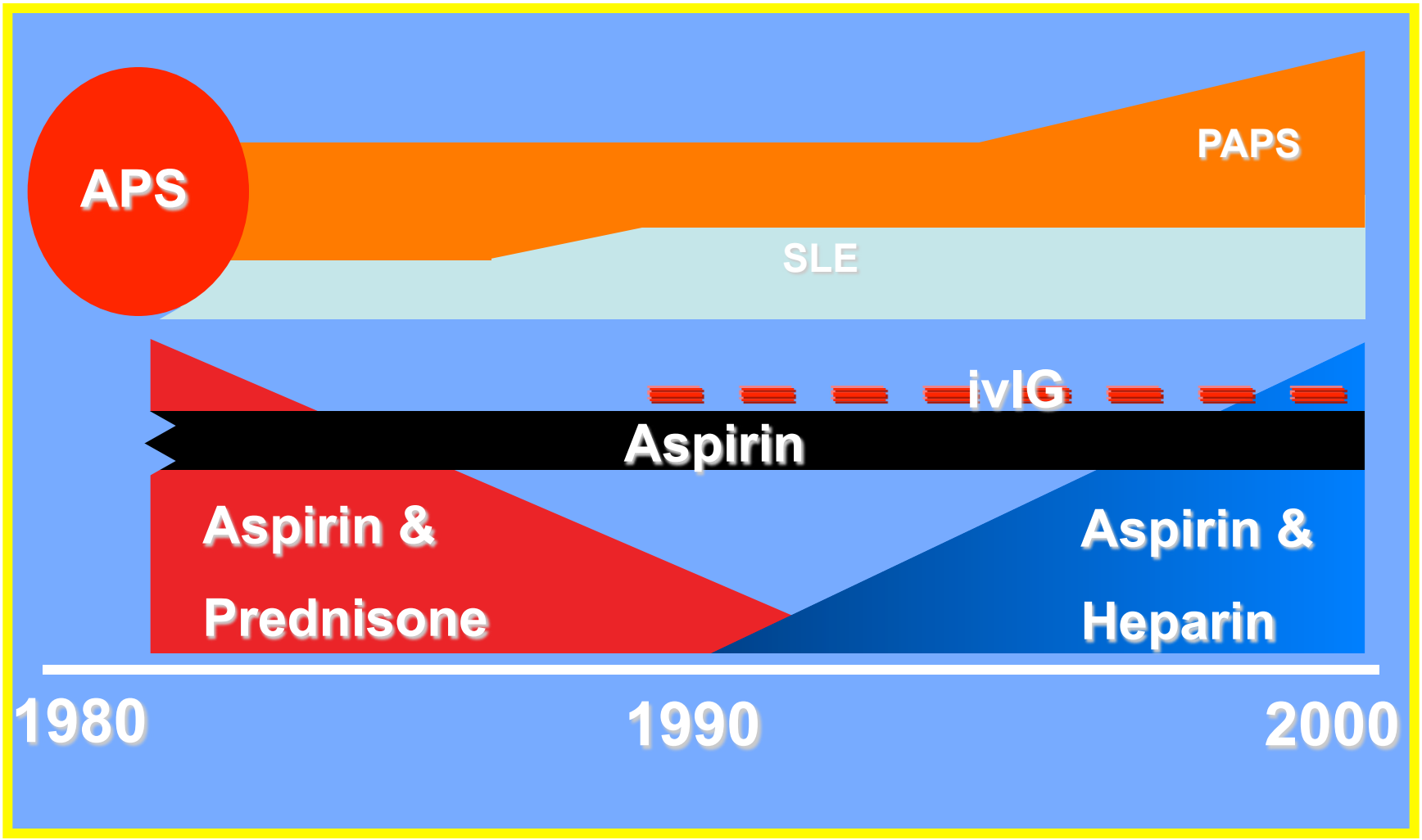
Topping J et al. Hum. Reprod. 1999;14:224-228

human reproduction

Longitudinal APS positivity in women positive for APS pre-conceptually (n=16) and negative controls (n=16)

Gestation	APS	Control
Preconceptual	16/16	0/16
First Trimester	15/16	5/16
Second Trimester	7/14 2 losses in T1	1/16
Third Trimester	3/12 2 losses in T2	1/16
Live birth Outcome	12	16

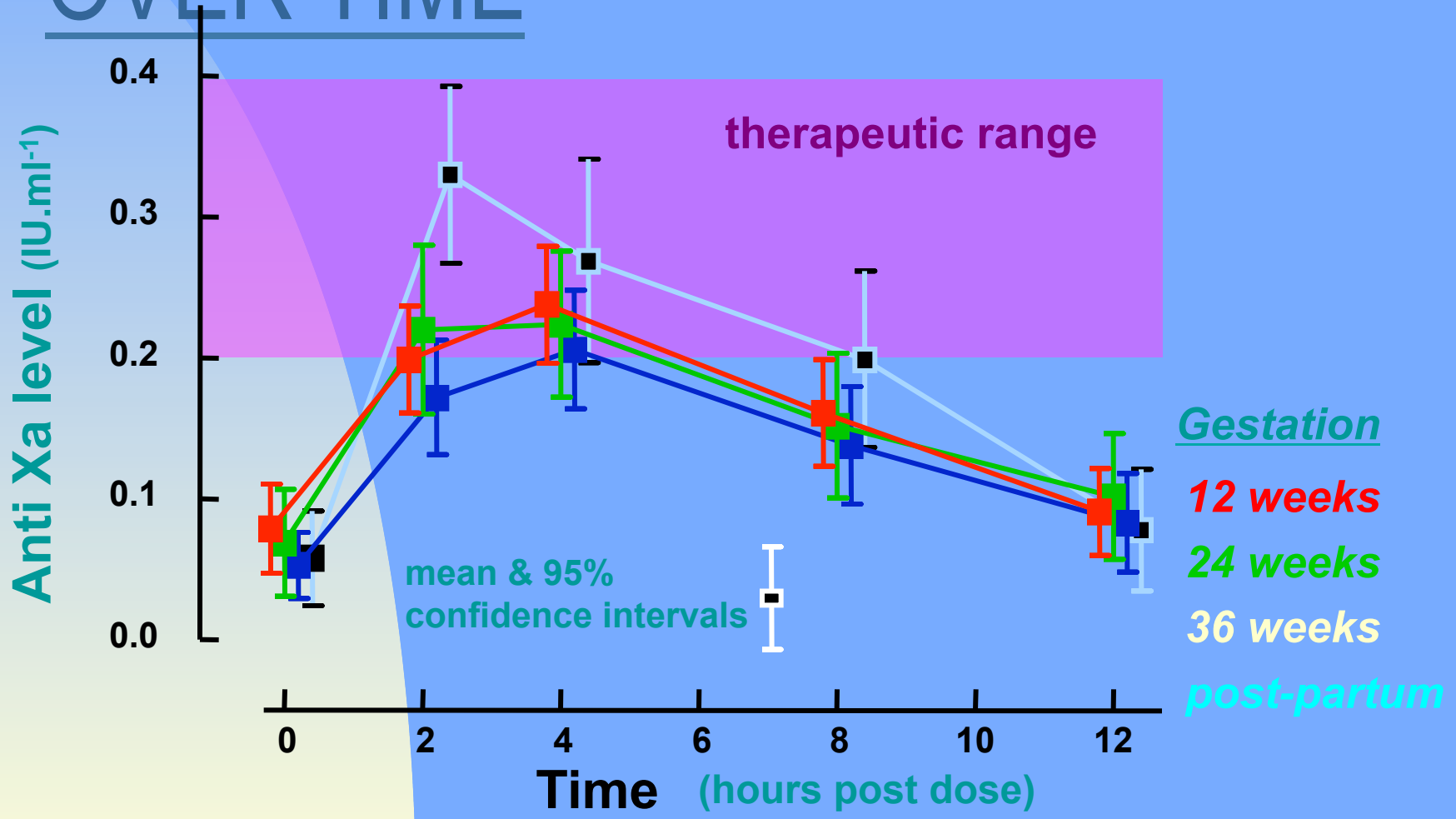
APS Timeline



METHODS

- 29 IN Prospective STUDY GROUP
- 5000 IU DALTEPARIN SC. OD started at 6 weeks gestation.
- SERIAL PLASMA ANTI-Xa LEVELS at 0, 2 , 4, 8, 12 hours post injection
- Standardised Gestation Intervals at 12, 24, 36 weeks and non-pregnant (8 weeks postnatal)

GROUP ANTI-Xa ACTVITY OVER TIME



Thrombophilia defect	Sporadic miscarriage OR	Recurrent miscarriage OR	Intra-uterine fetal death OR
Lupus anticoagulant	3.0	7.8	2.4
Anticardiolipin antibodies	3.4	3.6 – 5.1	3.3
AT deficiency	1.5	0.9	7.6 (0.3-196)
PC deficiency	1.4	1.6	3.1
PS deficiency	Heterogeneous data	14.7 (1.0-218.0)	7.4 (1.3-42.8) 20.1 (3.7-109.2)
Factor V Leiden	1.7	2.0	2.1 – 3.3
Prothrombin 20210A	2.1	2.3 – 2.7	2.3 – 2.7
Homozygous / combined defects	2.7	-	-
Hyperhomocysteinemia	6.3	2.7 – 4.2	1.0

Rey, Lancet 2003; Robertson, Br J Haematol 2006; Nelen et al, Fertil Steril 2000

Inherited Thrombophilia Tests
UK National EPU Survey 2008

(Norrie et al, Brit J Haem, 2009, 144, 241-4)

- 70% response rate (115/164 EPU's) in UK
- Heritable Thrombophilias (eg FVL, Prot C, S) tested for late miscarriage (80%), recurrent miscarriage (76%) and placental abruption (88%)
- Highly variable range of tests between EPU's which frequently led to heparin/aspirin administration in next pregnancy
- Evidence based practice for testing and intervention inconsistent across UK

Risk of ART (assisted reproductive technique) failure with thrombophilia – a systematic review

Di Nisio M, Rutjes AW, Ferrante N, Tiboni GM, Cuccurullo F, Porreca E.
Blood. 2011 Sep 8;118(10):2670-8.

Thirty-two studies (23 evaluating antiphospholipid antibodies, 4 inherited thrombophilia, and 5 both) involving 5891 patients were included. Overall, methodological quality of the studies was poor.

Combined results from case-control studies showed that factor-V Leiden was significantly more prevalent among women with ART failure compared to healthy parous women or those successfully undergoing ART (OR 3.08;95%CI: 1.77-5.36).

The prothrombin mutation, methylenetetrahydrofolate reductase mutation, deficiency of protein S, protein C, or antithrombin were all not predictive of ART failure.

Women with an unsuccessful ART tested more frequently positive for antiphospholipids antibodies (OR 3.33;95%CI:1.77-6.26) with evidence of high degree of between-study heterogeneity (I2=75%;p<0.00001).

***Prospective** cohort studies did not show any significant effects of thrombophilia on ART outcomes*

Talking of numbers....

- **Self cure rate is high (Malpas, 1938)**
- Non-recurrent causes have a high frequency eg spontaneous chromosome errors
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Pregnancy Success Prediction Matrix

Following idiopathic RM, the predicted probability (%) of successful pregnancy is determined by age and previous miscarriage history (95% confidence interval <20% in bold).

Age	Number of Previous Miscarriages			
(yrs)	**2**	**3**	**4**	**5**
20	**92**	**90**	**88**	85
25	**89**	**86**	**82**	79
30	**84**	**80**	**76**	71
35	**77**	**73**	**68**	62
40	69	64	58	52
45	60	54	48	42

Brigham et al, Hum Rep, 1999, 14, 2868-2871; Lund et al, O&G, 2012, 119, 43-47

Who's doing what in 2015?

- Historically, the presence of 3 consecutive pregnancy losses has constituted an accepted definition (RCOG/ ESHRE). A clinically identifiable pregnancy loss is defined by presence of ultrasound verified intrauterine pregnancy or histological confirmation of chorionic villi.
- ***Recently, both the number and consecutive components of the historical definition have become questionable*** .

What's the reasoning behind this?

- Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors in 1020 women with two versus three or more recurrent pregnancy losses. Fertility and Sterility, 2010, 193, 1234-43.
- Boogard E, Cohn D, Korevaar JC, Dawood F, Vissenberg R, Middeldorp S, Goddijn M and Farquharson RG. Number and sequence of preceding miscarriages and maternal age for the prediction of antiphospholipid syndrome in women with recurrent miscarriage, Fertility and Sterility, 2013, 99, 188-92.

RIF and failed PUL – are they prognostically linked?

- Kolte AM, van Oppenraaij RH, Quenby S, Farquharson RG, Stephenson M, Goddijn M, Christiansen OB; ESHRE Special Interest Group Early Pregnancy.
  Human Reproduction 2014, 29, 931-7

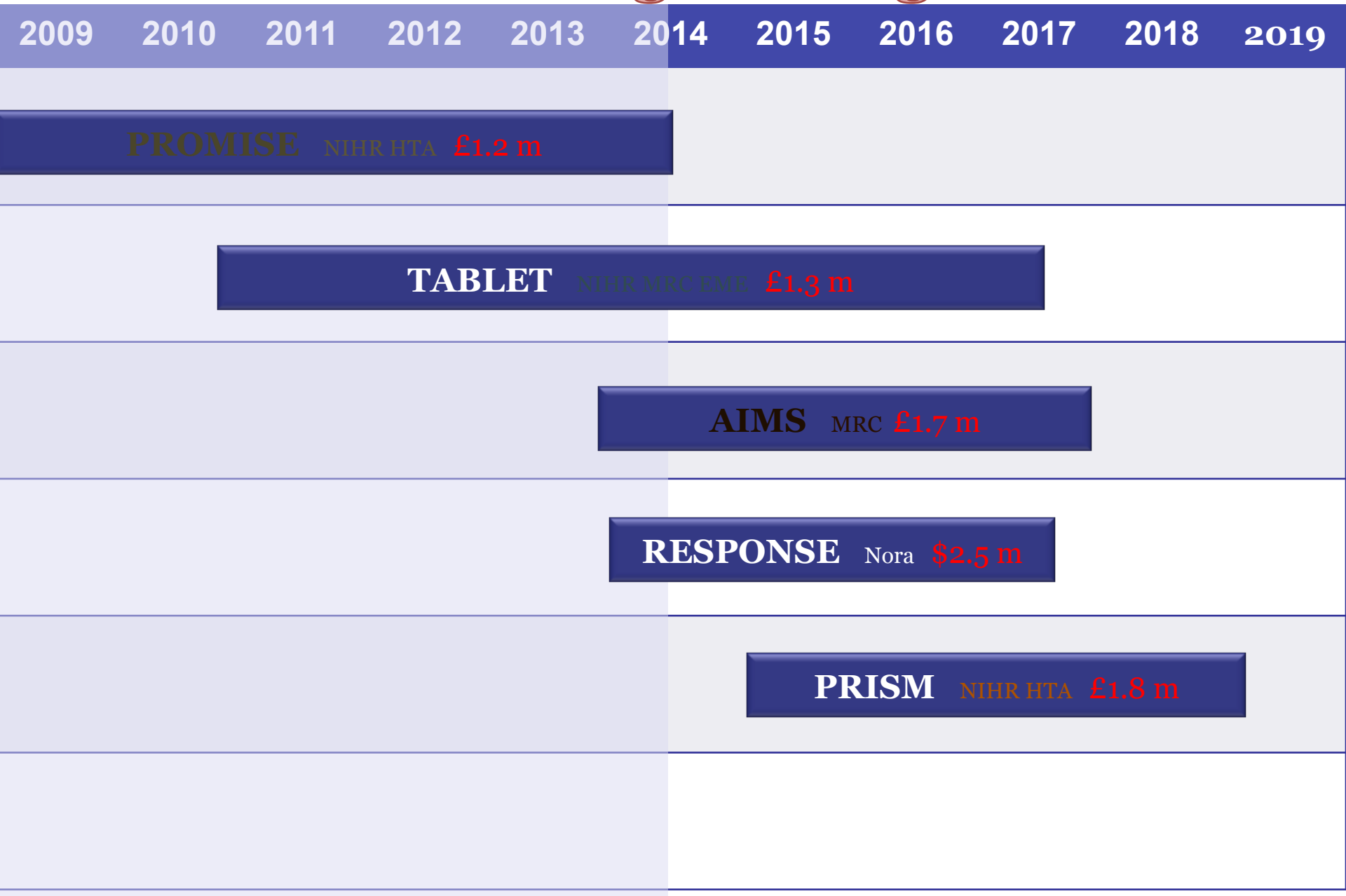
- **Are non-visualized pregnancy losses (biochemical pregnancy loss and failed pregnancy of unknown location combined) in the reproductive history of women with unexplained recurrent miscarriage (RM) negatively associated with the chance of live birth in a subsequent pregnancy?**

- **SUMMARY ANSWER:** ***Non-visualized pregnancy losses contribute negatively to the chance for live birth: each non-visualized pregnancy loss confers a relative risk (RR) for live birth of 0.90 (95% CI 0.83; 0.97), equivalent to the RR conferred by each additional clinical miscarriage.***

What's on the horizon?

- RCTS are back in fashion with double-blind, placebo-controlled studies
- PROMISE (idiopathic RPL & Progesterone)
- TABLET (RPL SCH screen & T3 intervention)
- PRISM (Thr EPL and Prog support)
- RESPONSE (idio RPL & rhG-CSF)
- AIMS (antibiotics v placebo in miscarriage)
- HYPATIA (APS +/- HCQ with LDA/LMWH)

Funded RCT's – gathering evidence



Consensus but not Unanimity

- Following recurring pregnancy loss, it is important to offer the couple **appropriate preconceptual investigation** and then **early pregnancy support** and **empathic care** in a subsequent pregnancy.

- The **aetiology remains unknown in more than 50% of couples** with RPL despite a thorough evaluation and is therefore classified as idiopathic.

- Couples with **idiopathic recurrent miscarriage** have a **high chance of a successful outcome without intervention**.

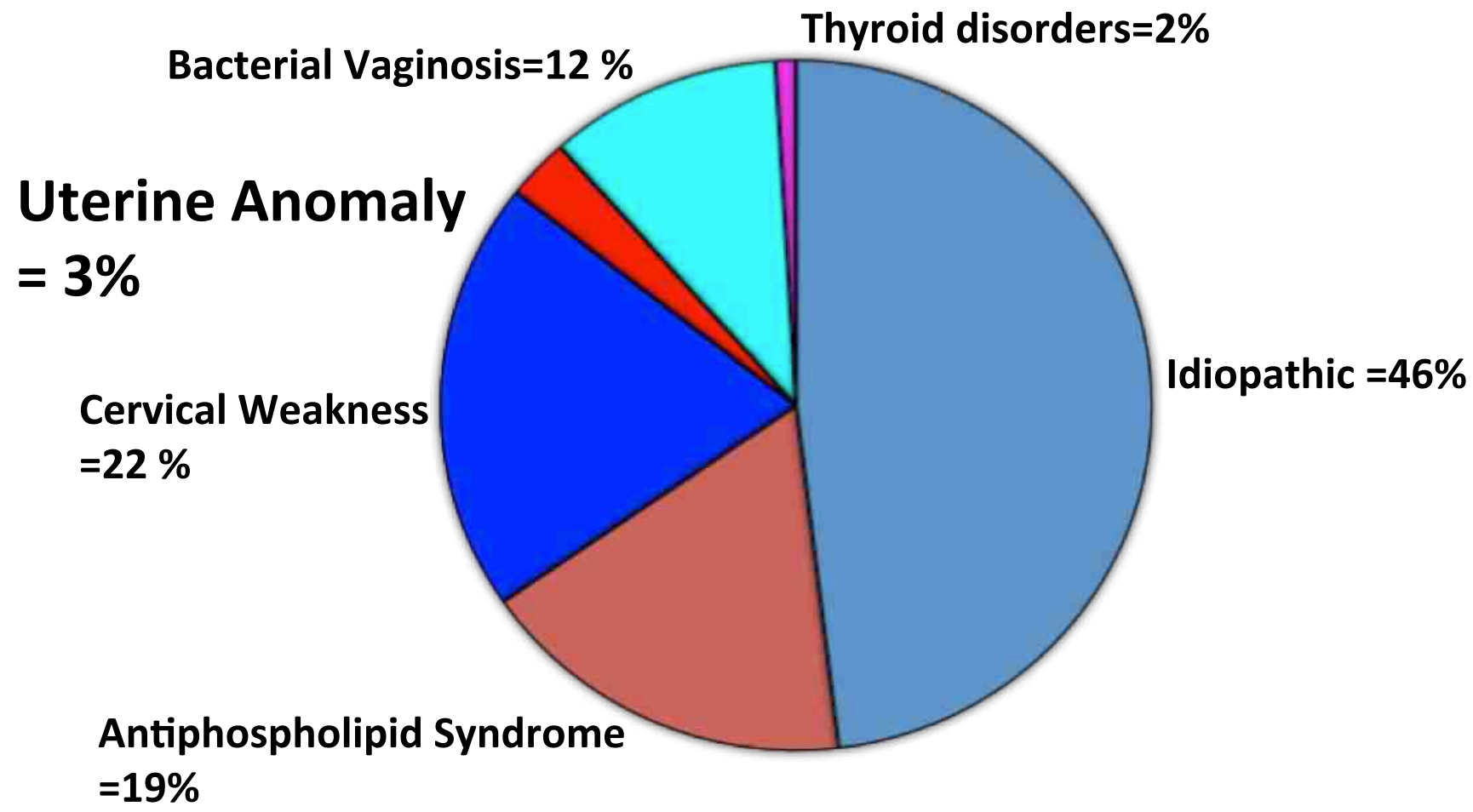
Midtrimester Loss (MTL)

- Pregnancy loss between 12 and 23 weeks gestation inclusive
- Deserves investigation as rate doubles after ART (1-2%)
- Particular attention paid to Clinical Event Sequence (CES) & symptom history
- Screen for Thrombophilia and BV essential plus imaging for Uterine Anomaly

Clinical Event Sequence (CES)

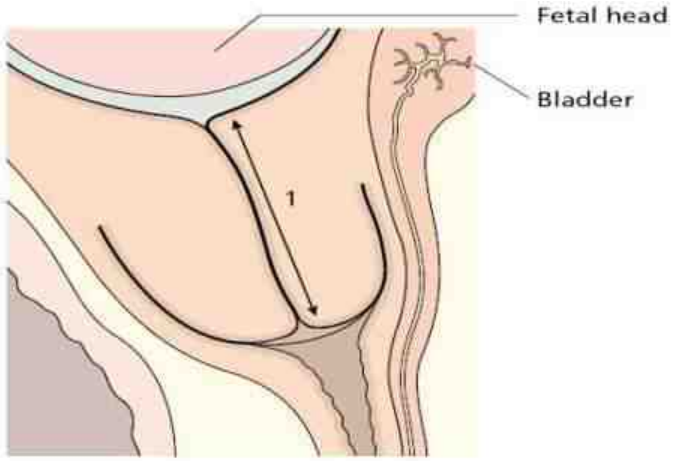
EVENT versus CAUSE	Pinkish Discharge PV	SROM with FLUID PV	FETAL HEART ACTION
Cervical Weakness	OPEN CERVIX	Absent until expulsion of sac	Present
Maternal Thrombophilia Eg APS	Closed Cervix	Absent	ABSENT (Intrauterine death)
Bacterial Vaginosis	Closed Cervix	PRESENT	Present ?until sac expulsion

Mid-trimester Loss consecutive cohort at Liverpool Women's 1988 -2010 (n=504)

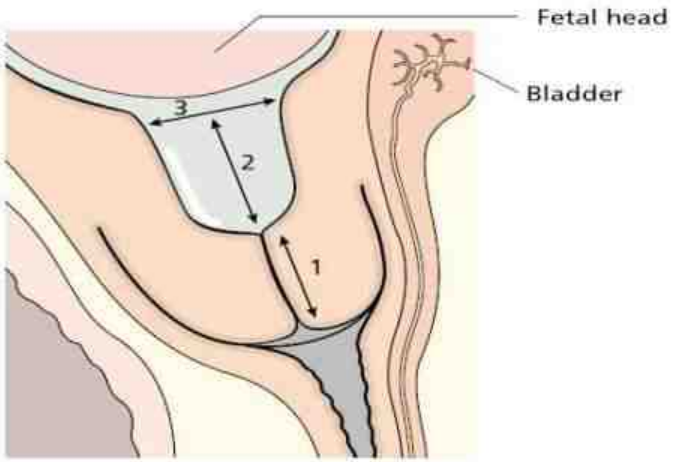


Cervical Length Measurement (CLM) and Funnelling

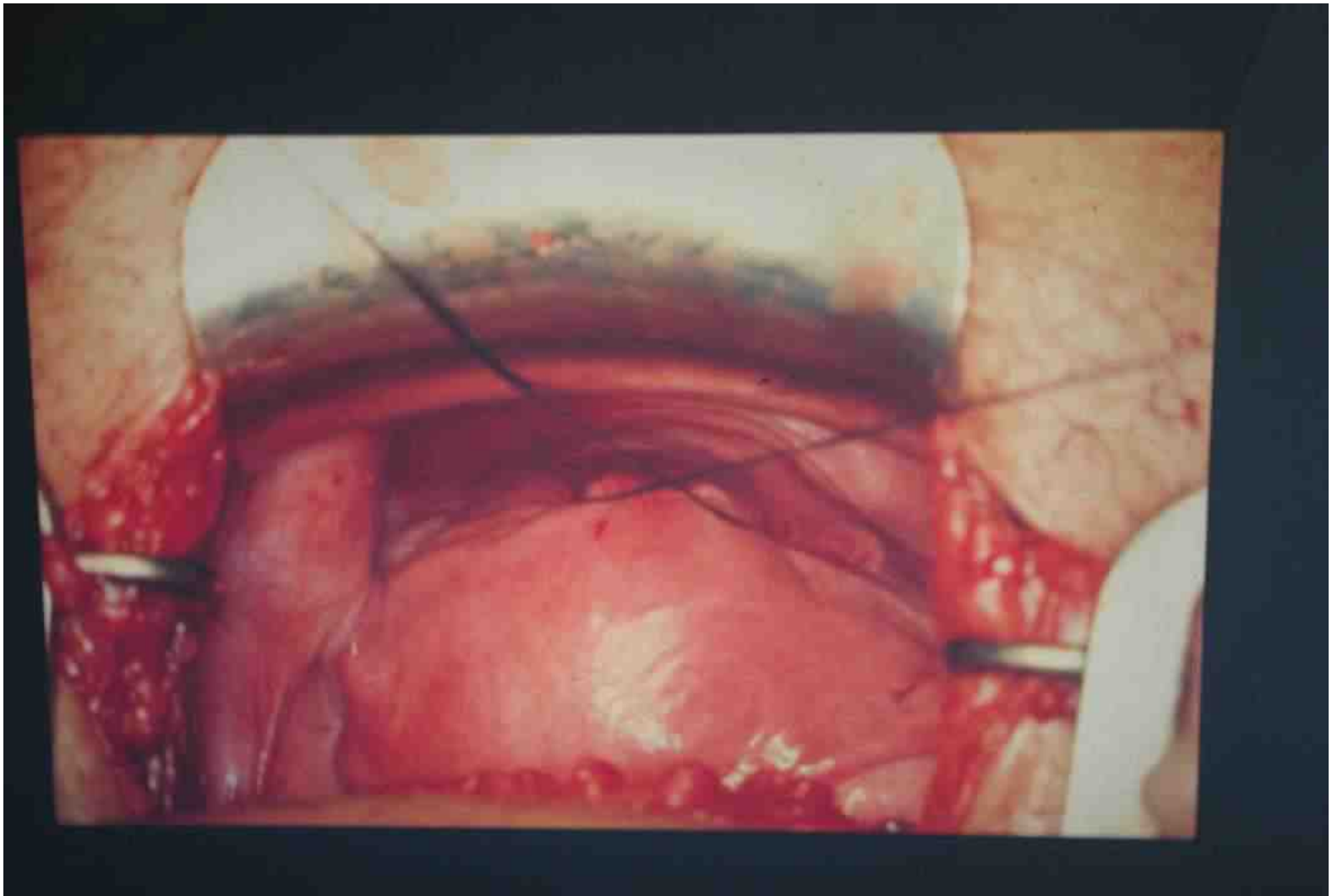
- Normal CLM circa 50mm



- Funnelling appears after 16 weeks, sometimes before



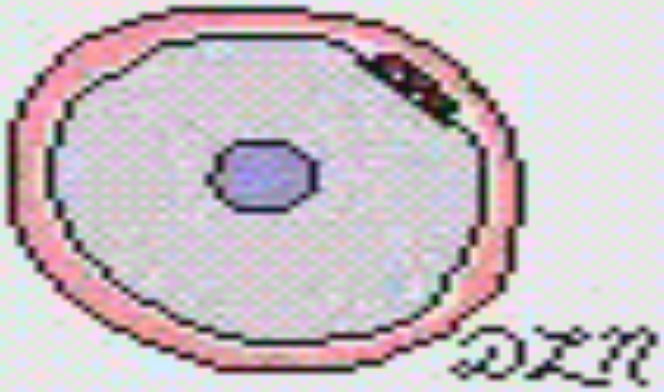
Transabdominal Cerclage -- tying the knot anterior



Comparison of vaginal (TVS) and abdominal (TAC) cerclage for treatment of cervical weakness for Midtrimester Loss based on consecutive cohort data from Liverpool Women's Hospital (2001-2008)

	Vaginal (TVS) (n=58)	Abdominal (TAC) (n=78)
Success Rate	**75%**	**90%**
Preterm Delivery (PTD <34 weeks)	25%	30% (60% if dual pathology)
Failure Rate after 14 weeks	Virtually all failures assoc. with Thrombophilia/BV	>90% assoc. with Thrombophilia/BV
Insertion	**12 weeks gestation**	**10 weeks gestation or Preconceptual with less morbidity**
Morbidity	**Minimal**	**Haemorrhage Trauma to bladder/bowel**
Long Term	**Removal at 36 weeks**	**Permanent**
Delivery	**Option of vaginal**	**Mandatory Caesarean Section**

It’s all about Quality of Care


DNA

(2) Poor condition of the mother, chronic diseases, obstetric trauma.

(3) Evidence of chronic nephritis and the occurrence of pregnancy toxaemia and accidental haemorrhage. Evidence is presented which shows that the onset of late toxaemia in cases of recurrent abortion in which treatment has been successful is not the unmasking of a new lesion, but a manifestation of the same renal lesion which led to the former abortions. All cases of pregnancy toxaemia cannot be ascribed solely to a specific pregnancy toxin.

About one-half of the habitual aborters do not show any abnormality beyond occasional evidences of endocrine or metabolic disturbance. The efficacy of vitamin E therapy in this group is open to criticism. At the moment progestin therapy represents the best method of treatment.

The process of normal development is essentially the continued readjustment of the environment to the changing needs of the foetus. Concerned in this continual adaptation are vitamins, hormones, minerals and probably the essential foodstuffs, including the special amino-acids such as lysine and arginine, an aspect of the problem which has not yet received much study.

Embryos die for two reasons, either from maldevelopment or from interference with their early nutrition, and to advance our knowledge, further knowledge is necessary of the normal metabolism of the early human embryo.

I am indebted to Dr. R. A. Morton for his help with the statistical aspects of the problem, and to Dr. A. A. Gemmell for some helpful criticism.

REFERENCES.

1. Whitehouse, B. *Proc. Roy. Soc. Med.*, 1929–30; xxiii, 1, 248.
2. Mall, F. P. "The frequency of localized anomalies in the human embryo