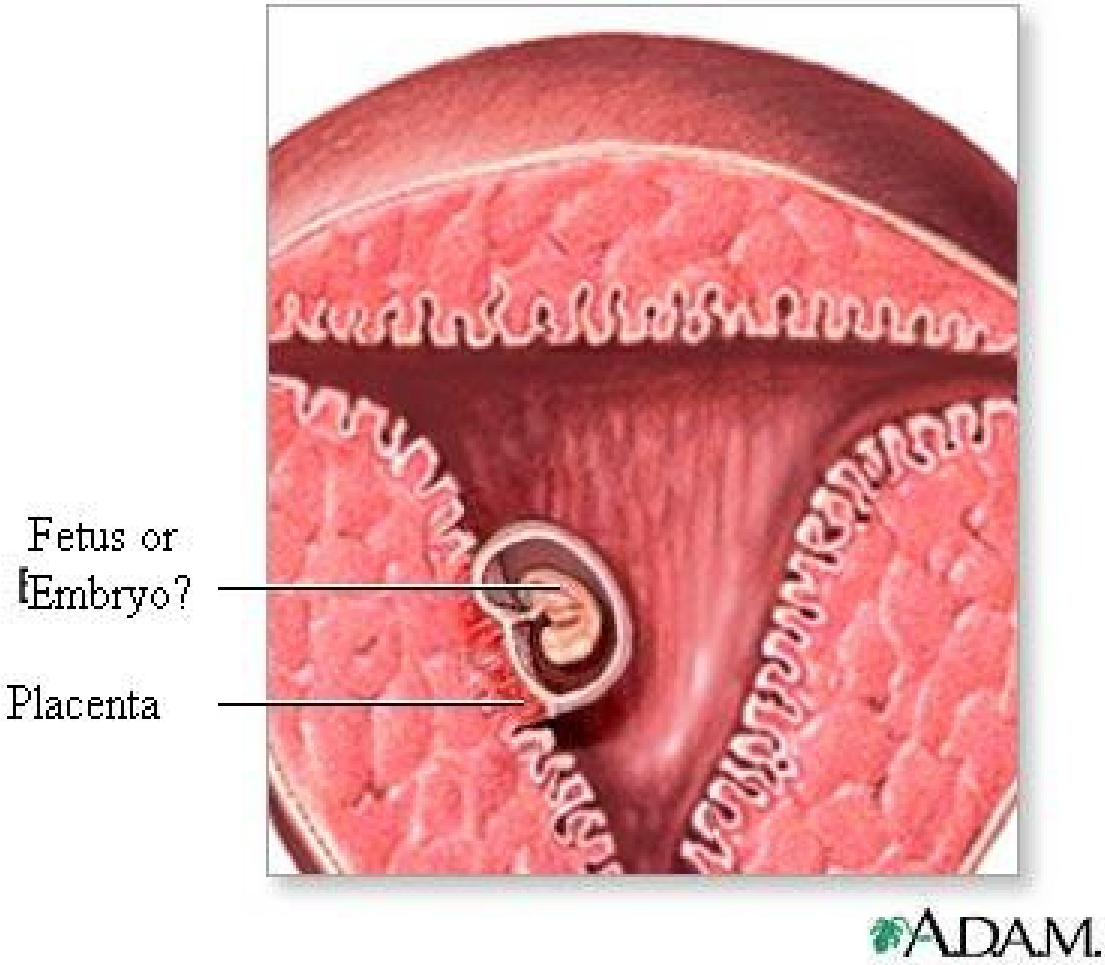
The Special Interest Group Journey
(backwards, by retracing the steps)

- “It is always a good thing to walk a mile in another man’s shoes”
- Nelson Mandela

Long Walk to Freedom The view from Robben Island Prison

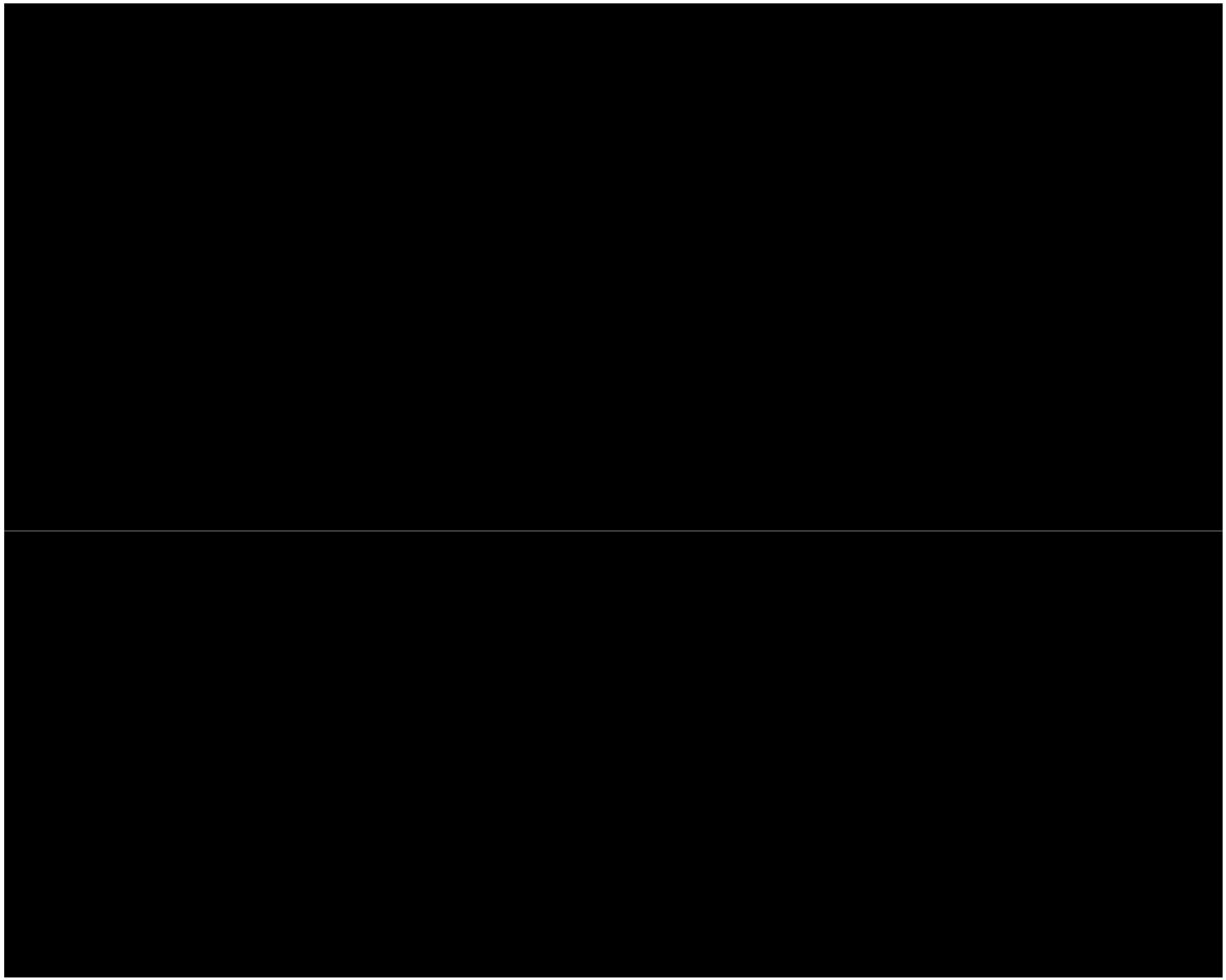
Nomenclature and Clarity

Updated and revised nomenclature for description of early pregnancy events (HR, 2005,20,3008-11)

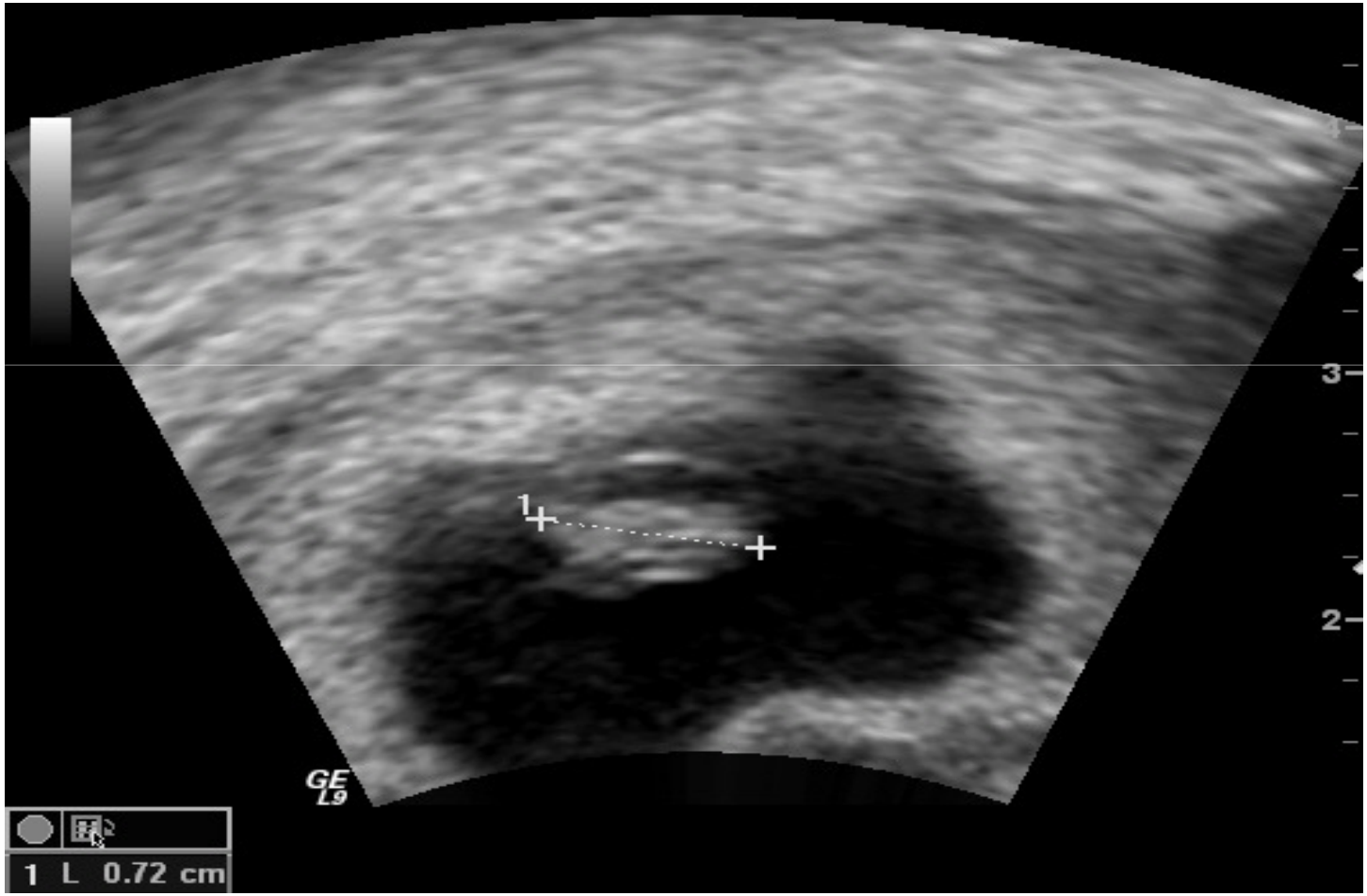


Predictive Modelling for Early Pregnancy

Area of Interest	**Best Diagnostic Utility**	**Parameter(s)**
Ovulation	Biomarker	D21 Progesterone
Pregnancy of Unknown Location (PUL)	Transvaginal (TVU) Scan and Biomarker	TVU Scan + HCG doubling time/ratio +/- Progesterone
Pregnancy of Uncertain Viability (PUV)	Scan Scan ExclusivelyScan	Fetal heart action plus Crown-rump length

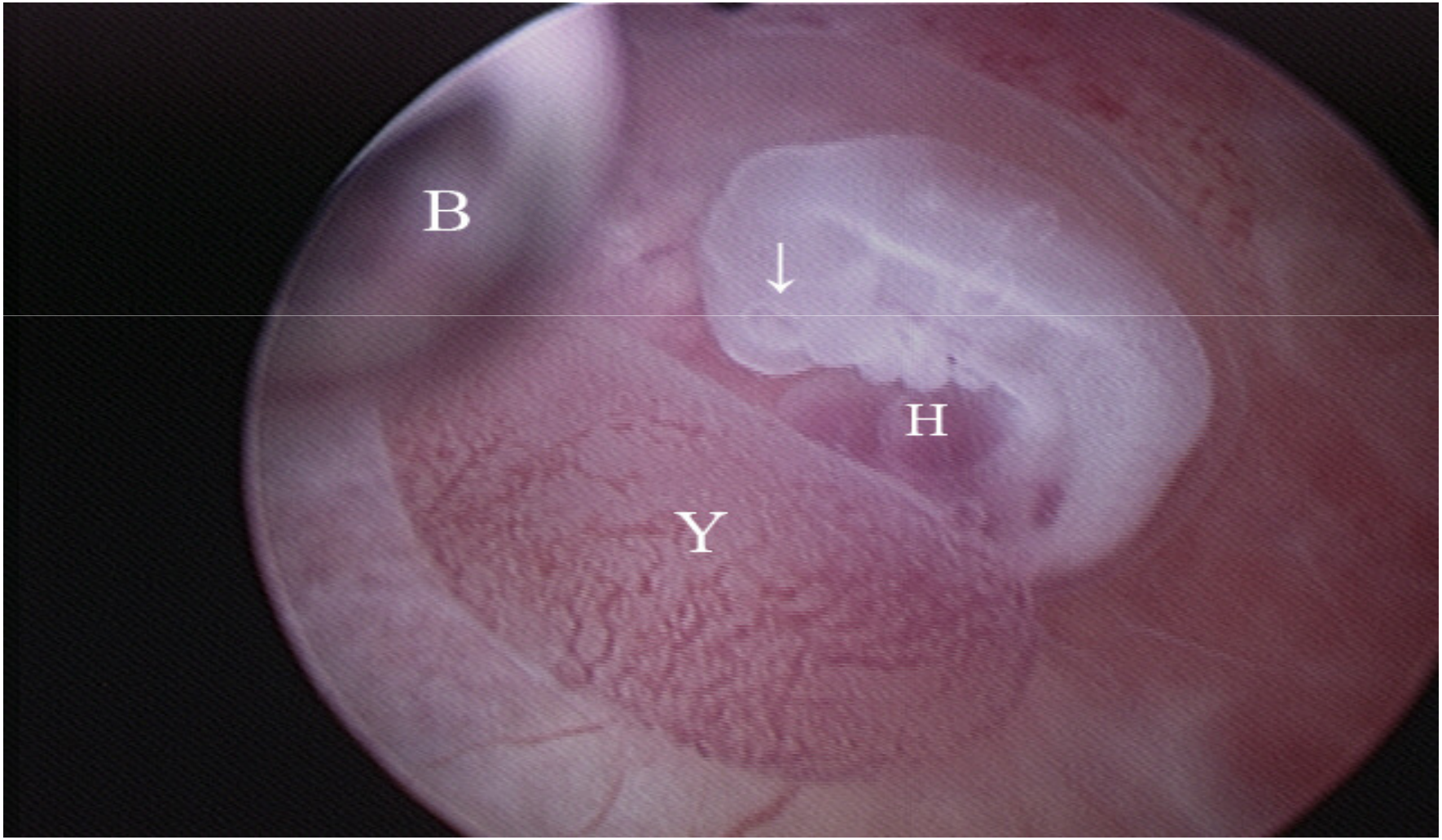


TV Ultrasound
Fetal loss with CRL =7mm

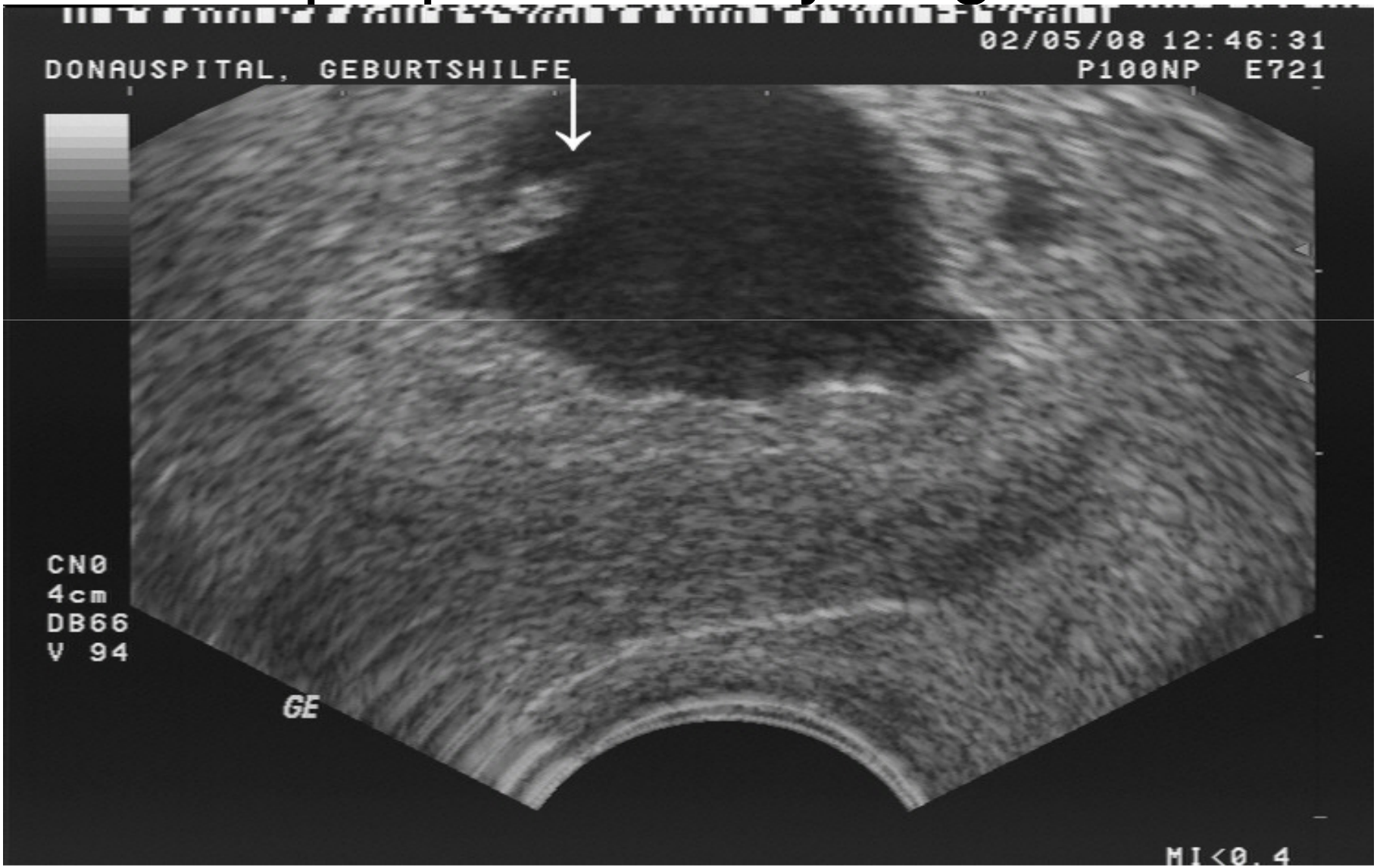


Embryoscopy – the close-up

H=head/heart prominence, Y=yolk sac, B=bubble

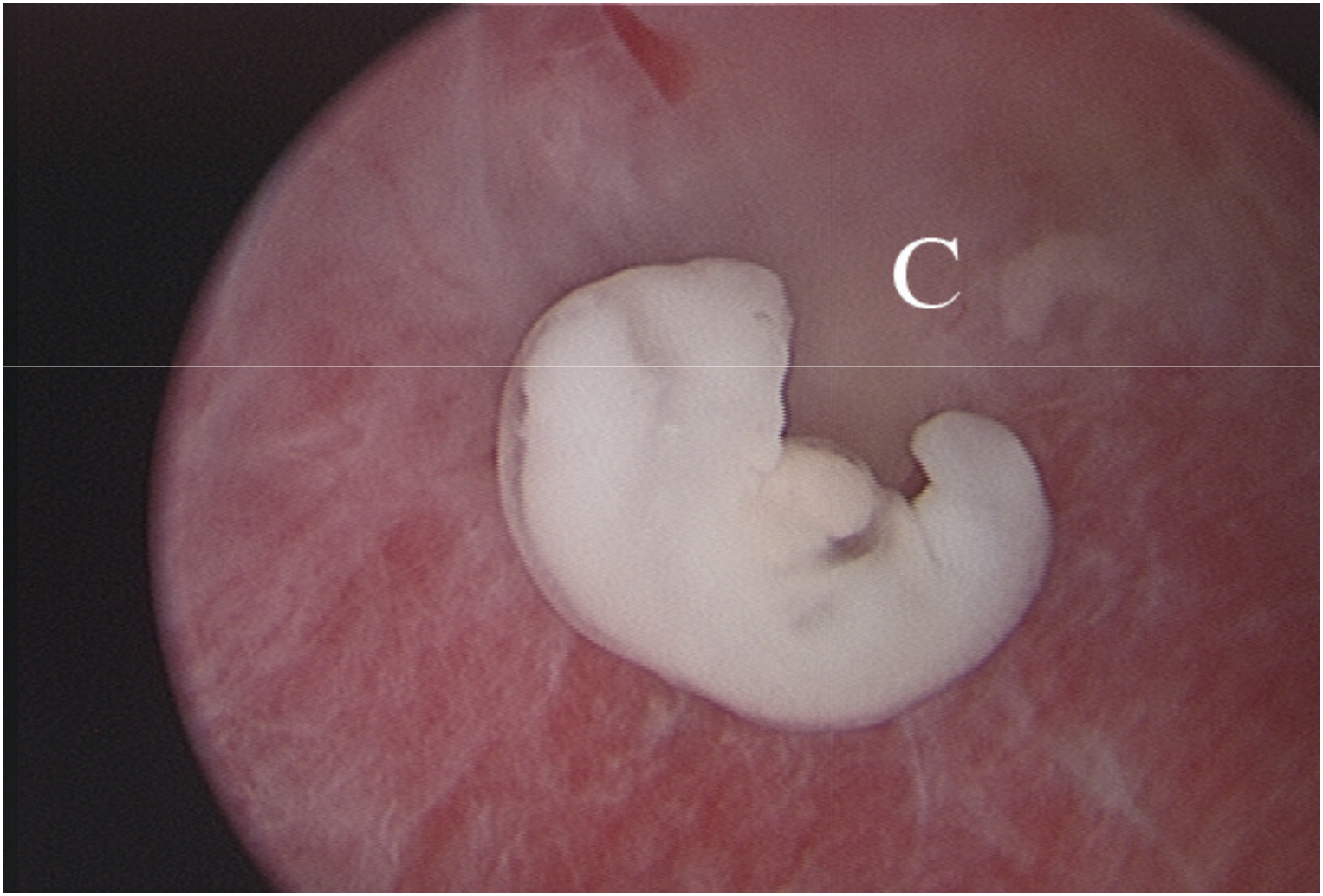


TVU – small embryonic structure in disproportionately large sac

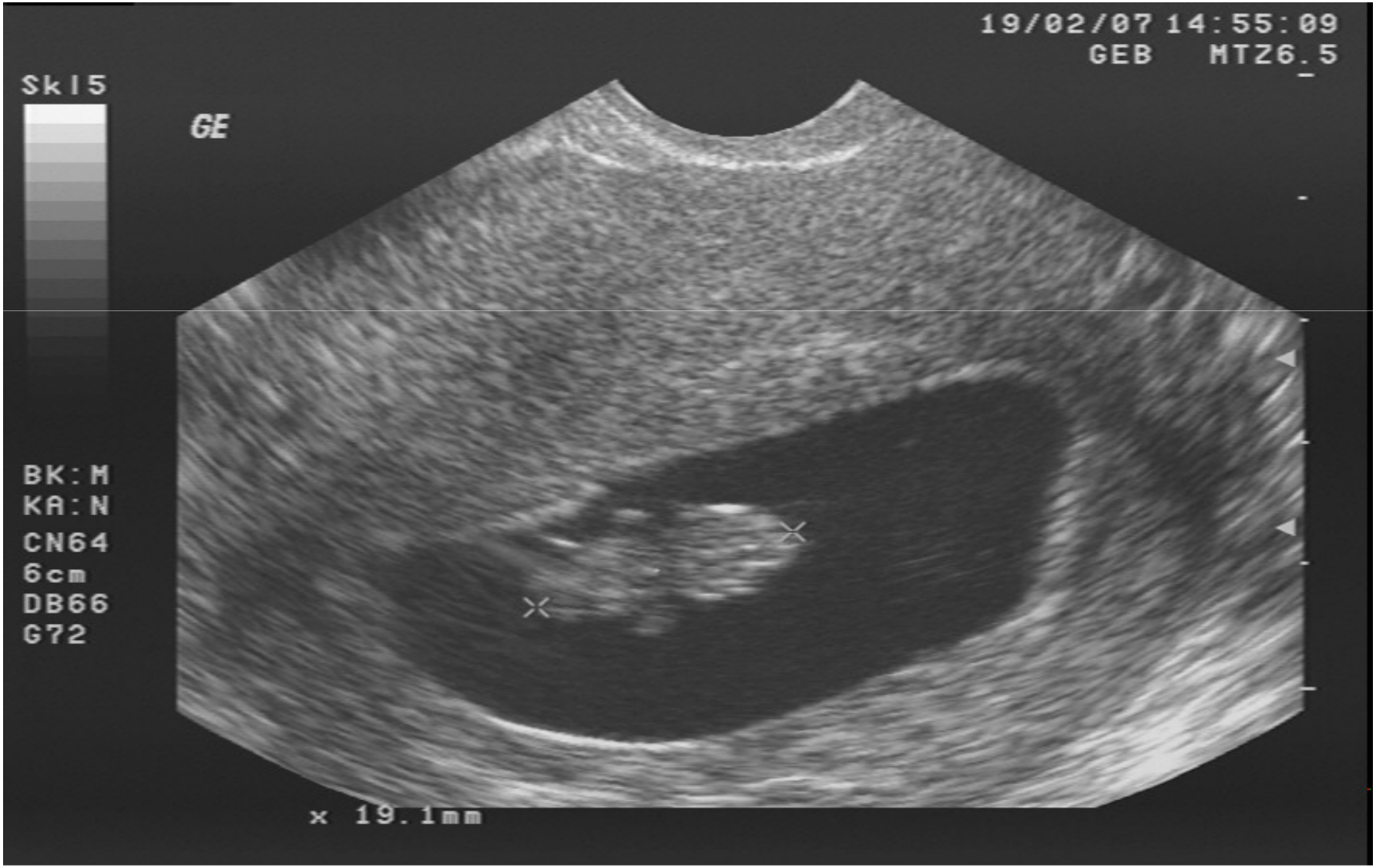


Embryoscopy – short body stalk with 6mm CRL

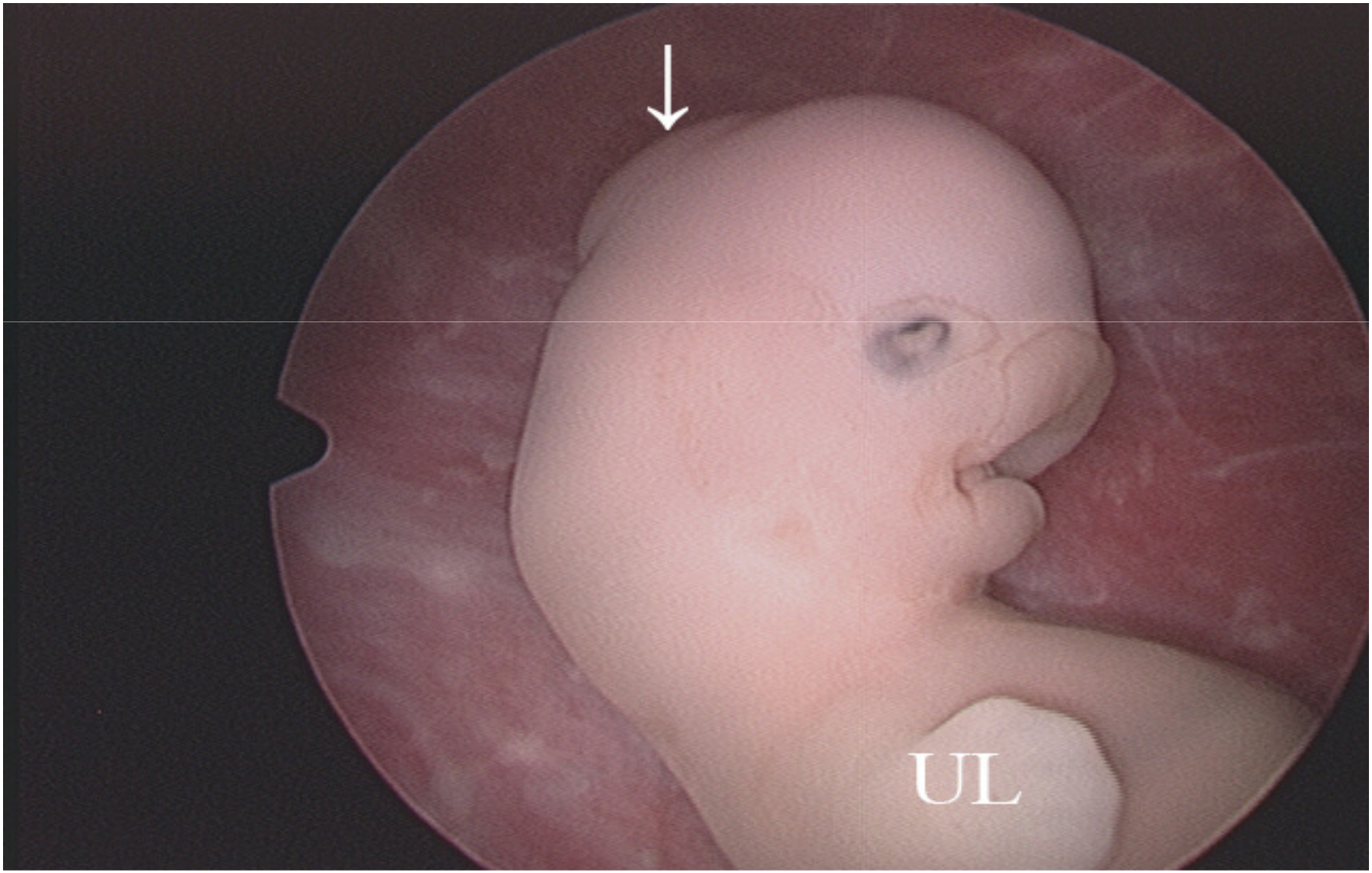
- cytogenetics = 47XY+7



Fetal loss at 7 weeks
CRL = 19mm



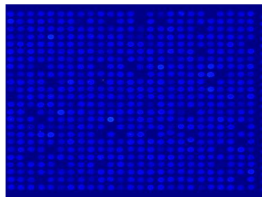
Cytogenetics = 47XY+15
Small head compared to CRL, dysplastic face, partial encephalocele



Is Treatment Failure in RM a valid concept? - Cytogenetic Analysis of Pregnancy Loss in RM

	Philipp et al, Hum Rep,2003 (n=221) Culture 70%+CGH	Stephenson et al, Hum Rep, 2002 (n=420) Culture 82%+CGH	Rubio et al, Hum Rep, 2003 (n=71) PGD+FISHProbe	Sullivan et al O&G, 2004 (n=122) Culture 85%
Trisomy Frequency in Descending Order	15	15	16	16
	16	16	21	15
	21	22	13	NA
	22	21	22	NA
	14	14	18	NA

Opportunityisnowhere

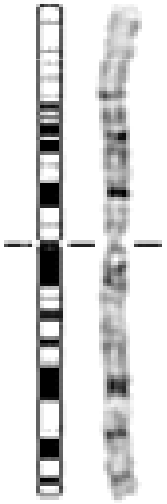


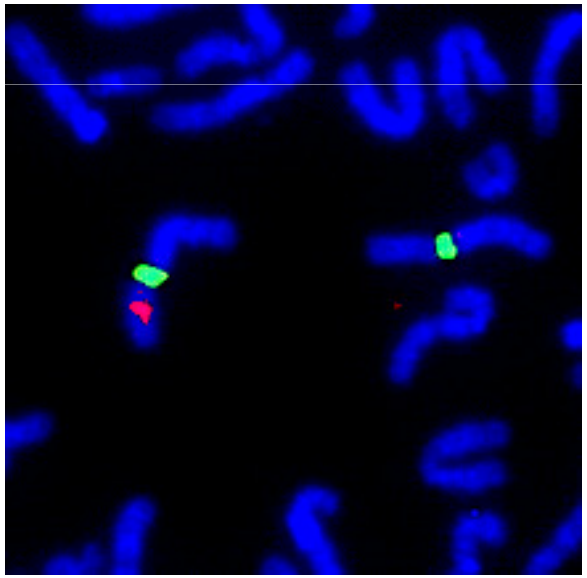
microarrays

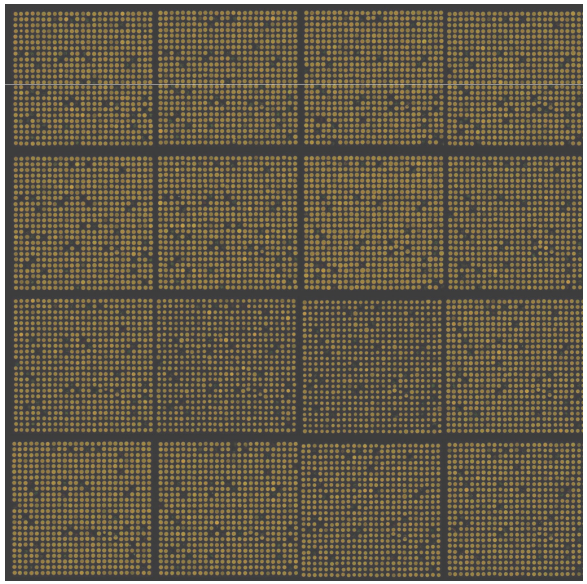
- **technique**

  high resolution WHOLE genome scan

cytogenetics FISH arrays







- **Microarray**

Advantages

- SINGLE test vs Karyotype + 5 FISH tests
- DNA extraction directly vs cell culture
- detect low level fetal cells vs maternal cell contamination
- higher resolution vs lower resolution

Disadvantages

- CANNOT detect ‘balanced’ rearrangements
- confirmatory follow up studies

- **Normal n=12**

  8 = ♀ 4 = ♂

  1 = Karyotype ♀ - MCC

- **Abnormal n=7**

  - X

  +13

  -13

  +16

  +15

  14q deletion

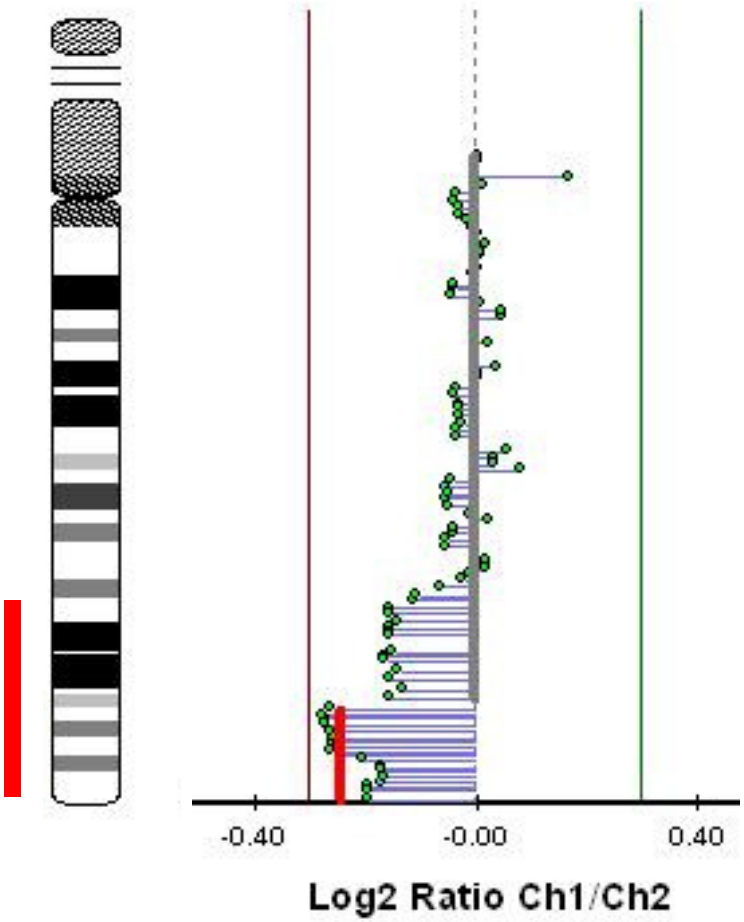
  +10

- 14q deletion - JS

Karyotype = Normal Female

Array = **Abnormal Female – deletion 14q**

FISH = confirm deletion in 11% of cells (89% MCC)

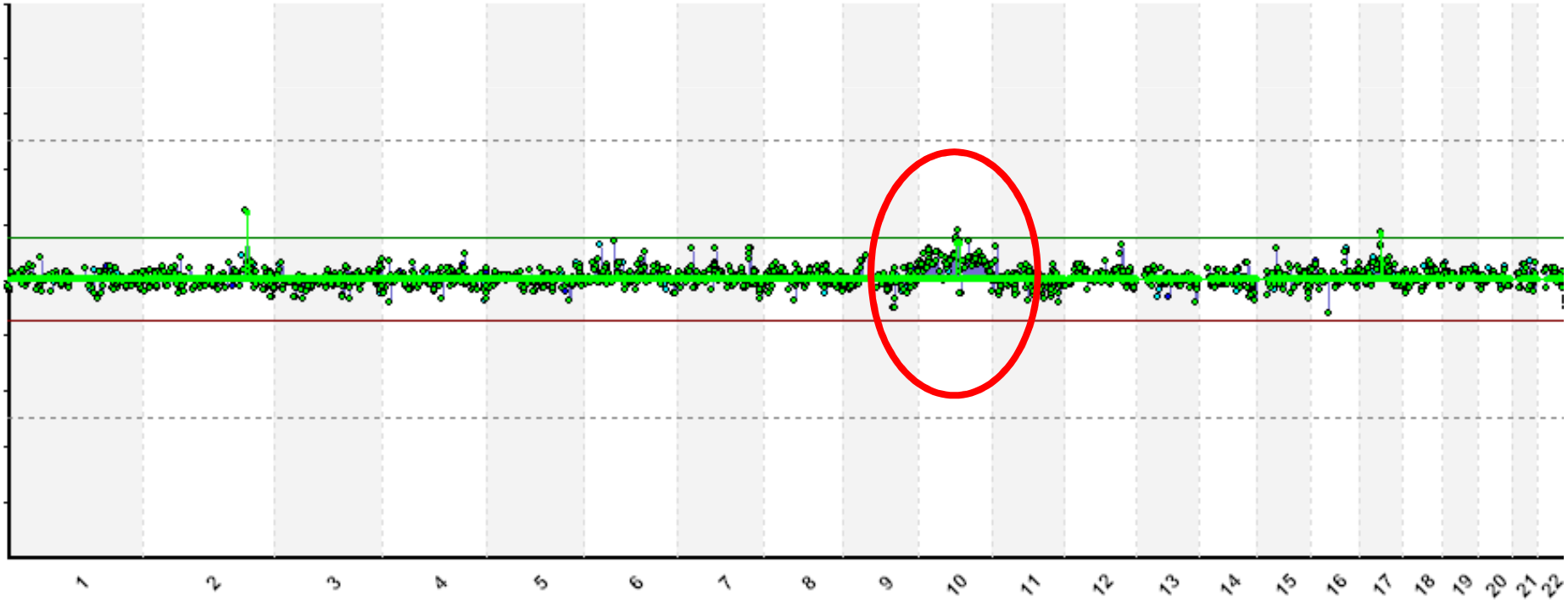


• Trisomy 10 - TR

Karyotype = Normal Female

Array = **Abnormal MALE result +10**

FISH = confirmed +10 (70% MCC)



Summary of CGH Application

- Developing a robust, efficient new protocol
- Less labour intensive
- Allows increased detection of fetal cells
  - Sampling and maternal cell contamination
  - Provides improved data on cause of EPL
- Impact on treatment intervention and patient management in future pregnancy

Never make predictions, especially about the future.
Casey Stengel

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; PROMISE Trial 2010

Spectral Disorder of Implantation

- Is RM a failure of Mother Nature's quality control? Can the endometrium be hyper-receptive? (Quenby et al, 2004)
- Endometrial receptivity seems unaltered by maternal age eg ED pregnancy(Paulson et al, 1997, 2002)
- WOI (window of implantation) assumes universal endometrial receptivity but MUC 1 disappears from SOI
- Is there an specific (?exaggerated) receptor response at SOI (site of implantation) that attracts embryos (normal 46 or abnormal)?

I never think of the future - it comes soon enough.
Albert Einstein

Chromosomal Mapping of Human Embryos – The Mosaic Hierarchy?

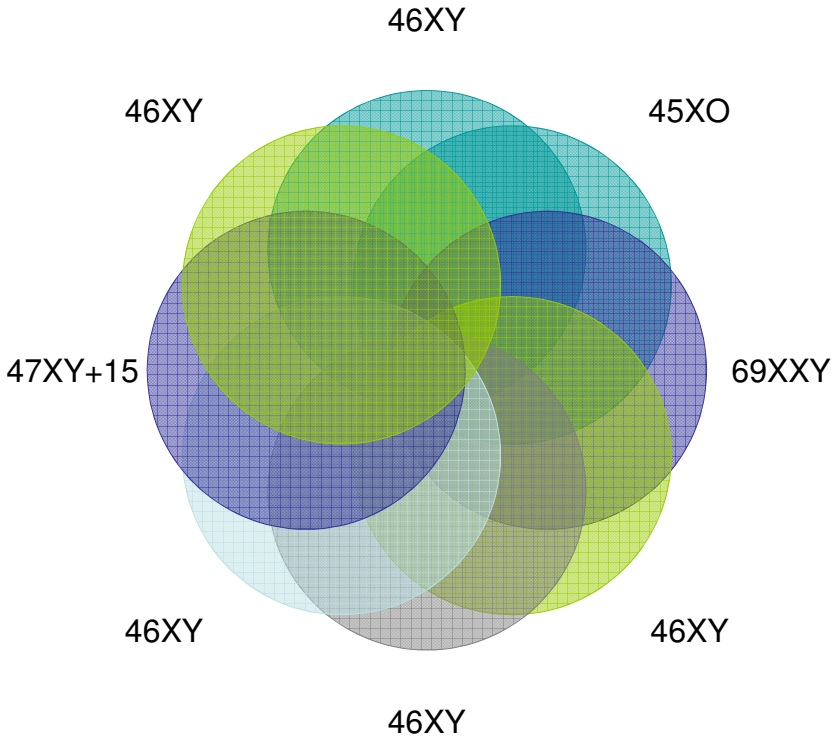
- It was not until the development of comprehensive chromosome screening methods for single cells, based upon the use of whole genome amplification and comparative genomic hybridization (CGH), that the true extent of human embryo aneuploidy was revealed (Wells *et al*., 1999). Using CGH, 51% of cleavage stage embryos were found to be aneuploid in every cell, while a further 24% contained a mixture of abnormal and normal cells. Only 25% of embryos were composed solely of normal cells (Voullaire *et al*., 2000 ; Wells and Delhanty, 2000).
- Recent study has identified 31 unselected (Group 3) good quality blastocysts carrying ***monosomies*** compared with 27 with ***trisomies***. This finding confirms that the morulla stage is not an insurmountable boundary to the further development of monosomic embryos (Fragouli et al, HR, 2008, 23, 2596-2608).
- Abnormal cells in diploid/aneuploid mosaic embryos decline in frequency from Days 3 to 6 and it is likely (BUT UNPROVEN) that this trend continues after implantation, resulting in a normal fetus in most cases.
- The fact that >70% of first trimester miscarriages are aneuploid emphasizes both the high incidence and lethality of this problem in humans (Menasha *et al*., 2005).

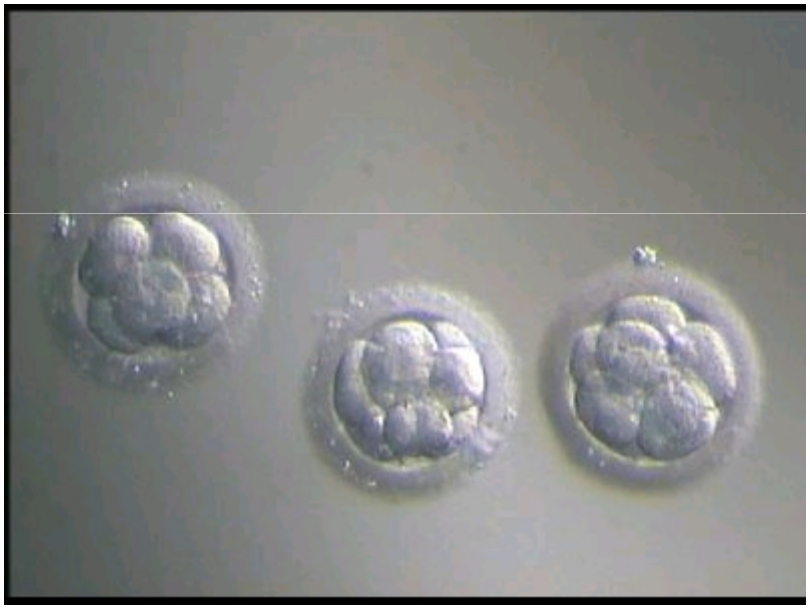
Karyotypic Concordance – is it time to dispel the embryo myth?

- Concordance studies in mammalian embryos are lacking
- Human study limited by legislation in many countries

- La Fleche de la Verite???

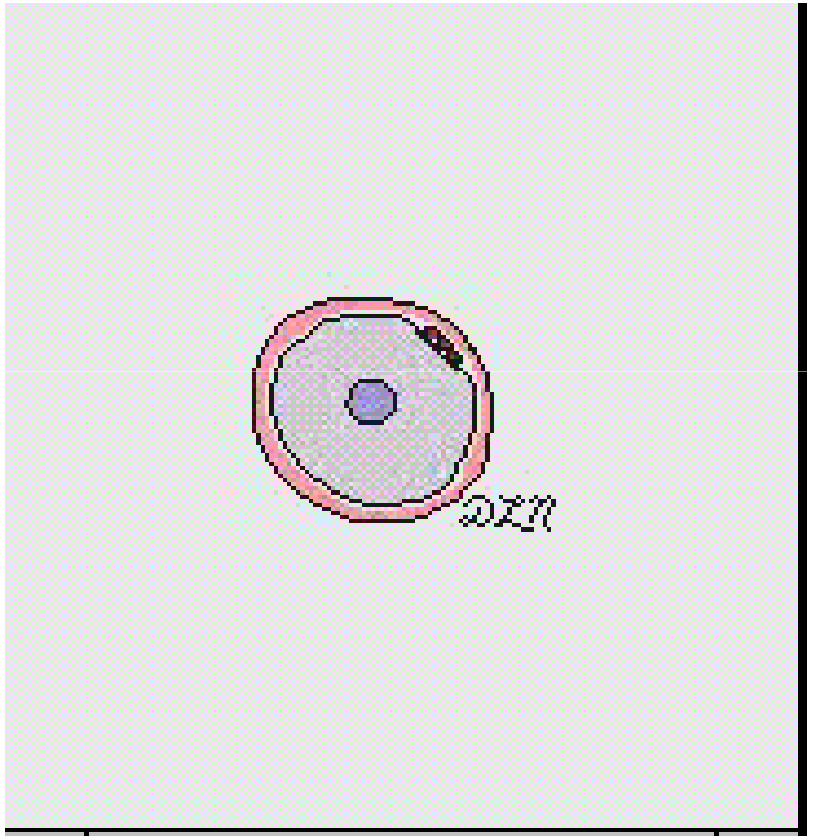
Chromosomal Mapping of Human Blastocyst – Discordance revealed?





Blastocyst at the SOI

- A. Apposition with unstable attachment to tips of pinopods
- B. Adhesion defined by resistance to dislocation by uterine flushing
- C. Penetration and entrance into maternal surface



Cell signalling

- Why does miscarriage occur so frequently?
- Sequences and Consequences – how does the site of implantation (SOI) attract the embryo?
- Non-specific apposition with abnormal cell ‘opsonised’ in preference to normal karyotype (euploid) cell
- Cause and effect through altered implantation effect which inevitably leads to aneuploid pregnancy loss

Conclusions

- Clear terminology that all can understand especially patients
- Chromosome testing of EPL should become mandatory prerequisite for RCT design and secondary outcome measure
- CGH sets best standard which should be employed
- Speaker declares no conflict of interest and receives no external financial support