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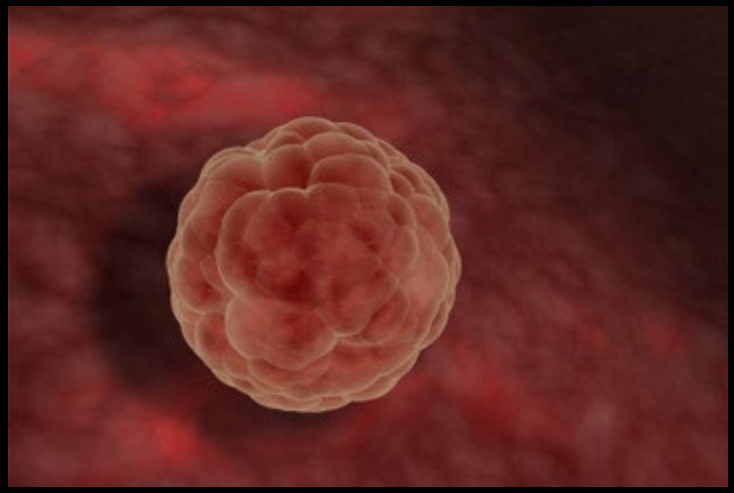


RECURRENT IMPLANTATION FAILURE

Disclosures

- I have no financial conflicts of interest
- I have no research conflicts of interest

Outline

- Recall important facets of study design
- Review of normal implantation
- Define RIF
- Identify the components of implantation / evaluation of RIF
- Describe and analyze some of the treatments for RIF

Sackett's Criteria for Causation

Decreasing Importance

1. Is there evidence from true experiments in the Human
2. Is the association strong?
3. Is the association consistent from study to study?
4. Is the temporal relationship correct?
5. Is there a dose response gradient?
6. Does the association make epidemiologic sense?
7. Does the association make biologic sense?
8. Is the association specific
9. Is the association analogous to a previously proved causal association?

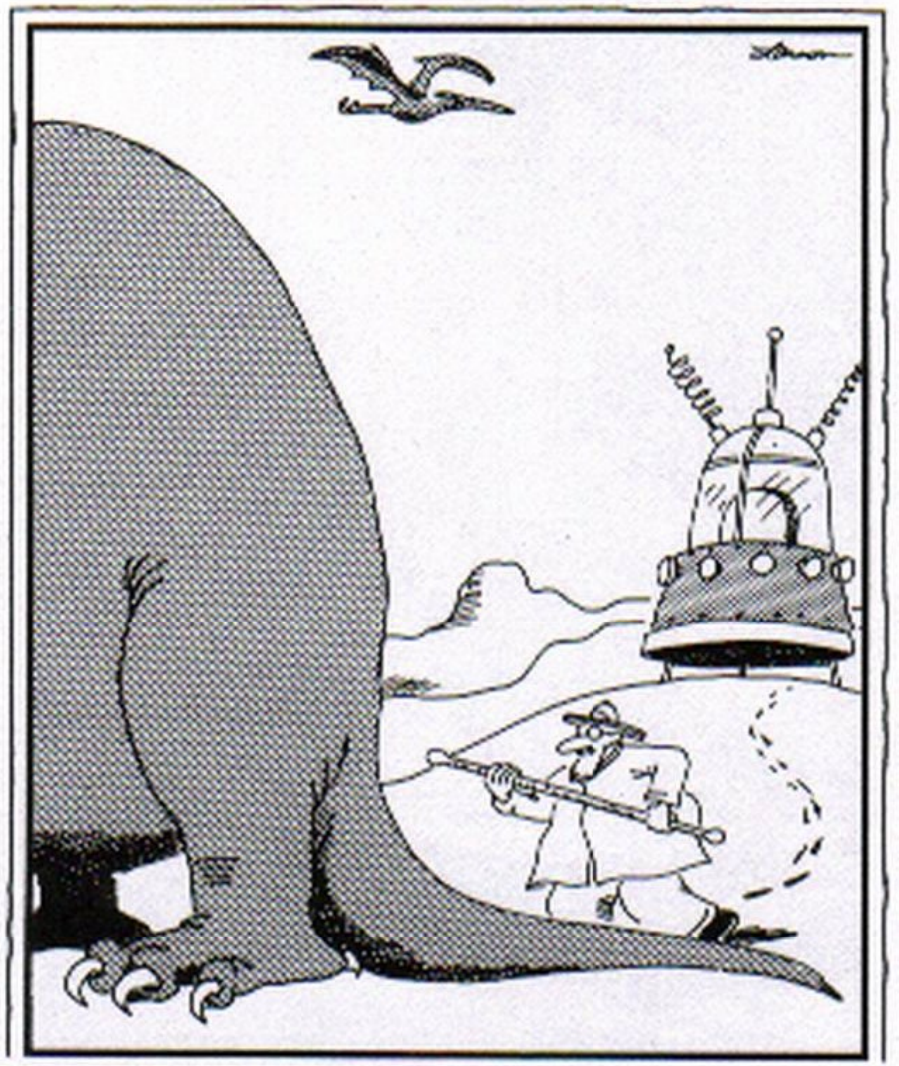
Sacket et al. Clinical Epidemiology:
Basic Science for Clinical Medicine, Little Brown, 1985

Biochemical Levels of Proof

- Is the gene present?
- Is the transcript present?
  - Are the amounts reasonable?
- Is the protein present?
  - Does the amount make biologic sense?
  - Is the product functional?
- Is the expected biological response found?
- Is there an appropriate dose-response?
- If an antagonist available, dose it block the response?

Data Sources

- What question are they asking?
  - Is study designed to answer the question?
- Patient populations
  - What definition of RIF used?
  - Control group?
  - Use euploid embryos?
- Laboratory Conditions / Protocols
  - Appropriate cut-offs
  - What are they based on?
- Treatment protocols
- Outcome measures
  - Measured correctly?
  - Surrogate endpoint used?
- Statistical Analysis
- Does nature recognize ratios?



An instant later, both Professor Waxman and his time machine are obliterated, leaving the cold-blooded/warm-blooded dinosaur debate still unresolved.

Gary Larson , 1983

Relative Risk / Cause and Effect

- Smoking and lung cancer
  - RR= 103 in men
  - RR= 62 in women
- Estrogen therapy and endometrial cancer
  - RR= 10-20
- Long term elevated cholesterol and CHD
  - RR = 7-10

Blot WJ, Fraumeni JF Jr. Cancers of lung and pleura. In: Schottenfeld D, Fraumeni J Jr eds. Cancer Epidemiology and Prevention. Oxford University Press, NY, p 637-65, 1995
Sjogren LL et al Maturitas Sept:25-35, 2016
Anderson KM et al JAMA 257:2176-2180, 1987

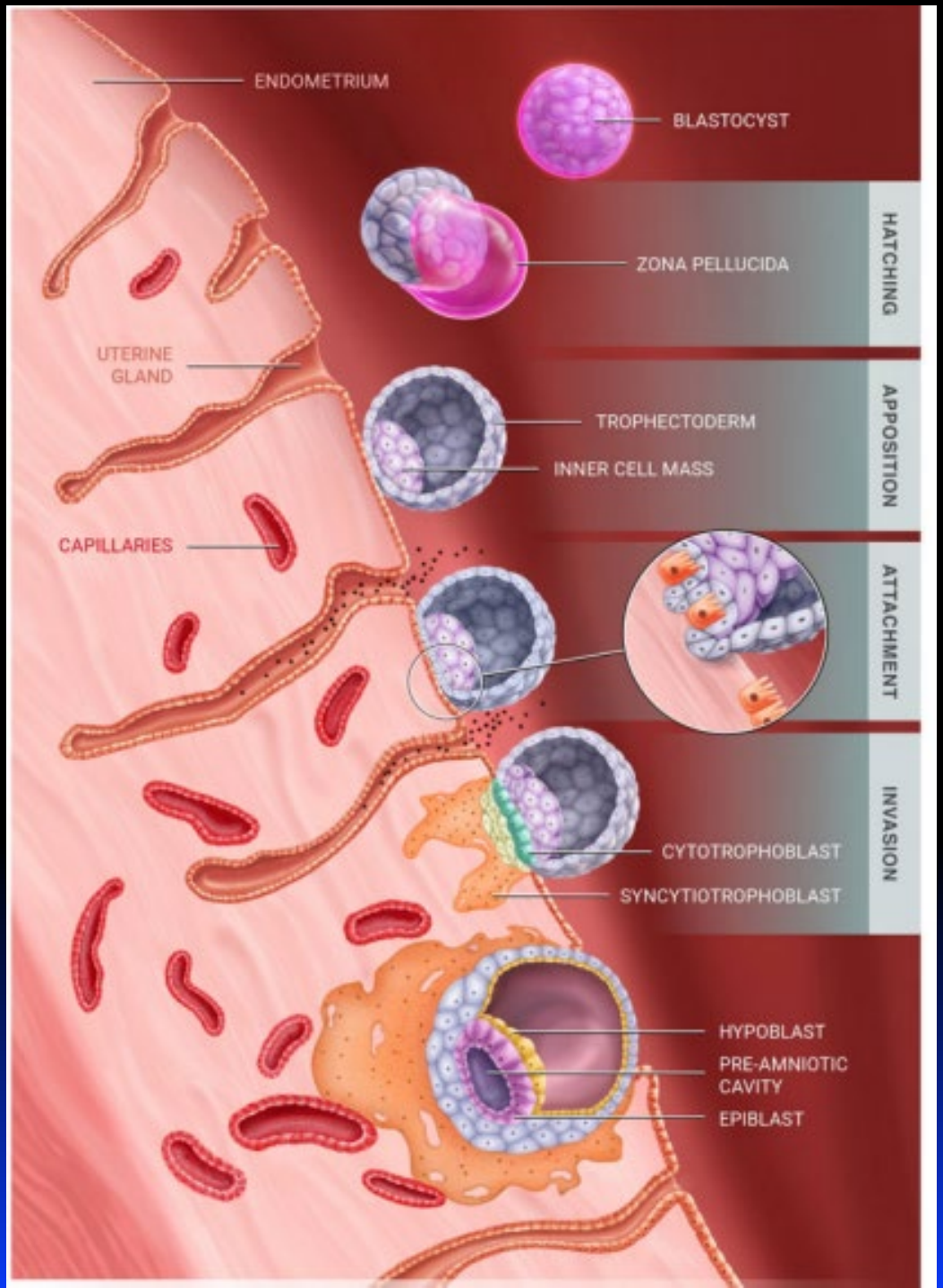
Normal Implantation

- Human Reproduction is inefficient.
- Successful Pregnancy Requires
  - Normal embryo
    - Genetically
    - Metabolically
    - Functionally
  - Morphologically normal uterus
  - Receptive endometrium
  - Appropriate synchronization between embryo and endometrium
  - Ability of embryo to attach and invade endometrium but not go too far
- Is not a single event
  - Cascade of interactions between embryo and endometrium

Kliman HG Frankfurter D. Fertil Steril 111:618-28, 2019
Moustafa S, Young SL F1000 Research 9:208, 2020
Franasiak JM et al Fertil Steril 116:1436-48, 2021

Normal Implantation

- Window of implantation
  - Thought to be ~ 48h, 7-10d after ovulation
  - Studies standardized for ovulation CD 14
- Natural Window
  - CD 18.5-21 (Hertig AJ et al Am J Anatomy 98:435-93, 1956)
- IVF
  - CD 19-23 (Bergh PA Navot D. Fertil Steril 58:537-42,1992)
- Is probably tighter
  - In Vitro- trophoblasts only adhere to CD 19 endometrium (Kliman HJ et al Placcenta 1990:349-67)

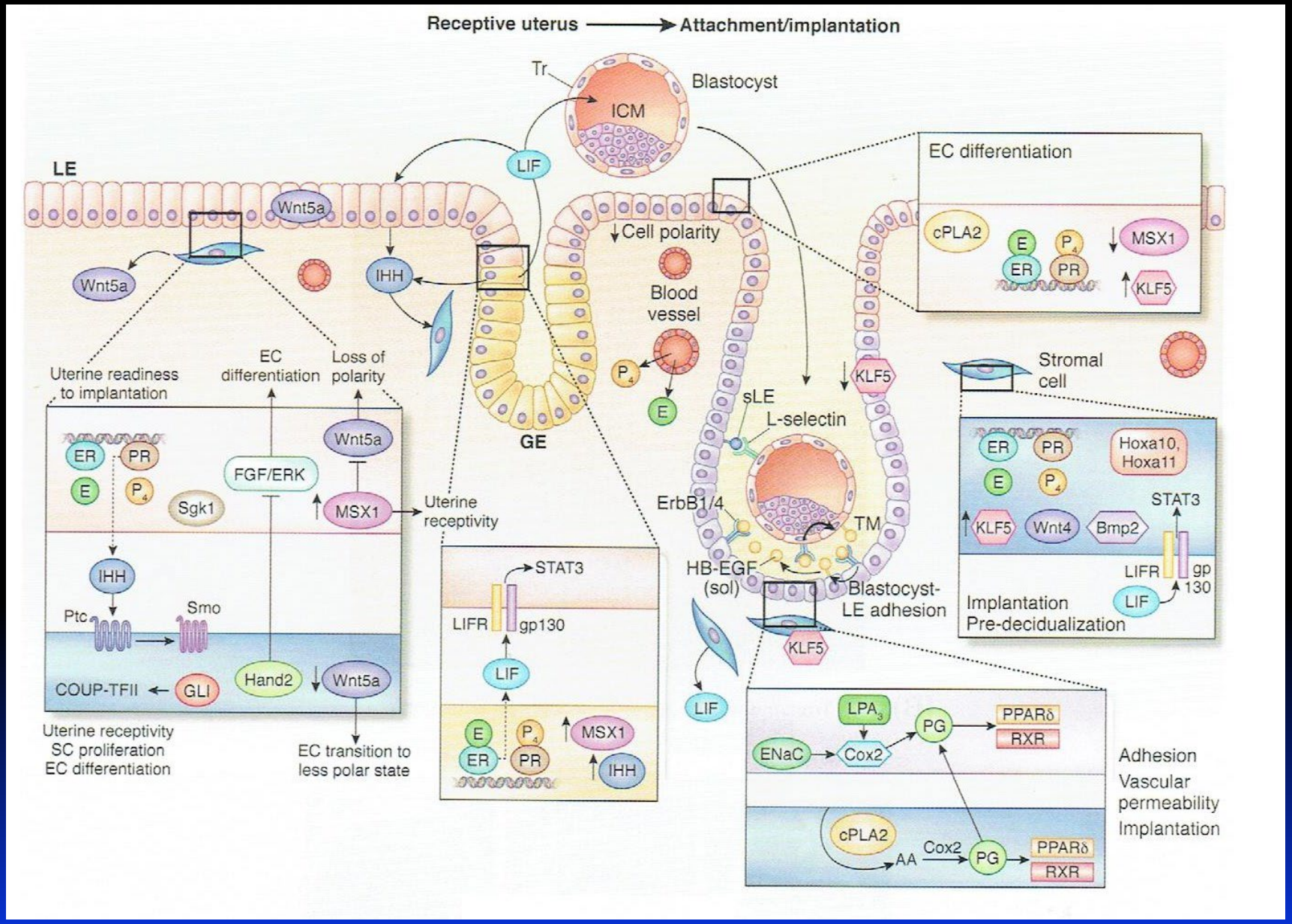


Normal Implantation

- Highly coordinated
  - Apposition
  - Adhesion and attachment
  - Invasion

Moustafa S, Young SL F1000 Research 9:208, 2020
Ojosnegros S et al Hum Reprod Update 27:501-530, 2021

Normal Implantation



Cha et al Nat Med 18:1754-67, 2012

Definition

- RIF≠ RPL
  - But they are part of the continuum of reproductive failure
- RIF is not a diagnosis but a clinical presentation
  - The underlying problem causing failure is the diagnosis
- No agreed upon definition until recently
  - Number of unsuccessful cycles
    - Fresh, Frozen, both?
    - 2-6; 3 most common
  - Number of embryos
    - 3-10
    - Quality and stage not always accounted for
  - ESHRE: >3 failed ETs with high quality embryos or failed transfer of 10 or more embryos in multiple transfers

Cimadoma D et al Hum Reprod 36:305-17, 2021

The True Rate of RIF is Low

Pirtea P et al Fertil Steril 115:45-52, 2020

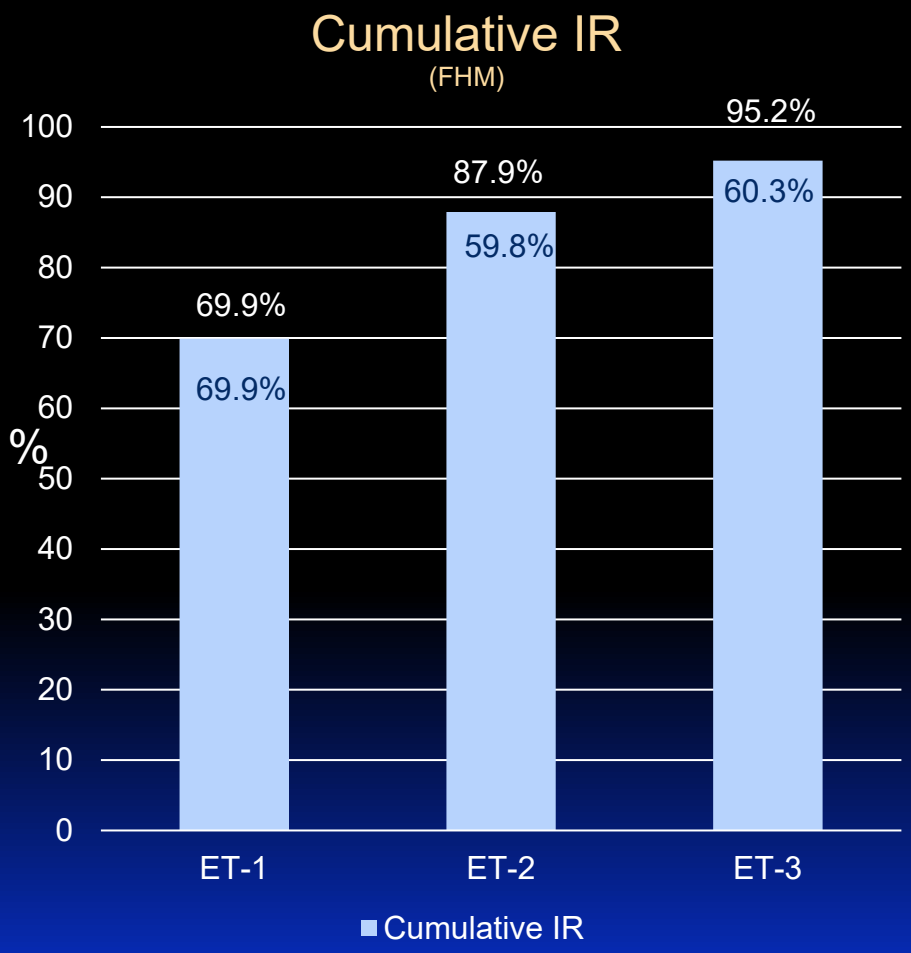
- Design: Retrospective Cohort
- Dates: 1/2012-7/2018
- Patients
  - n= 4229
  - 18-45 yo
  - Up to 3 FETs
  - All SET with euploid embryo
  - No OD or GC
- PGT-A: q PCR or NGS
- Evaluation: Normal uterus / Endometrium ≥ 7 mm

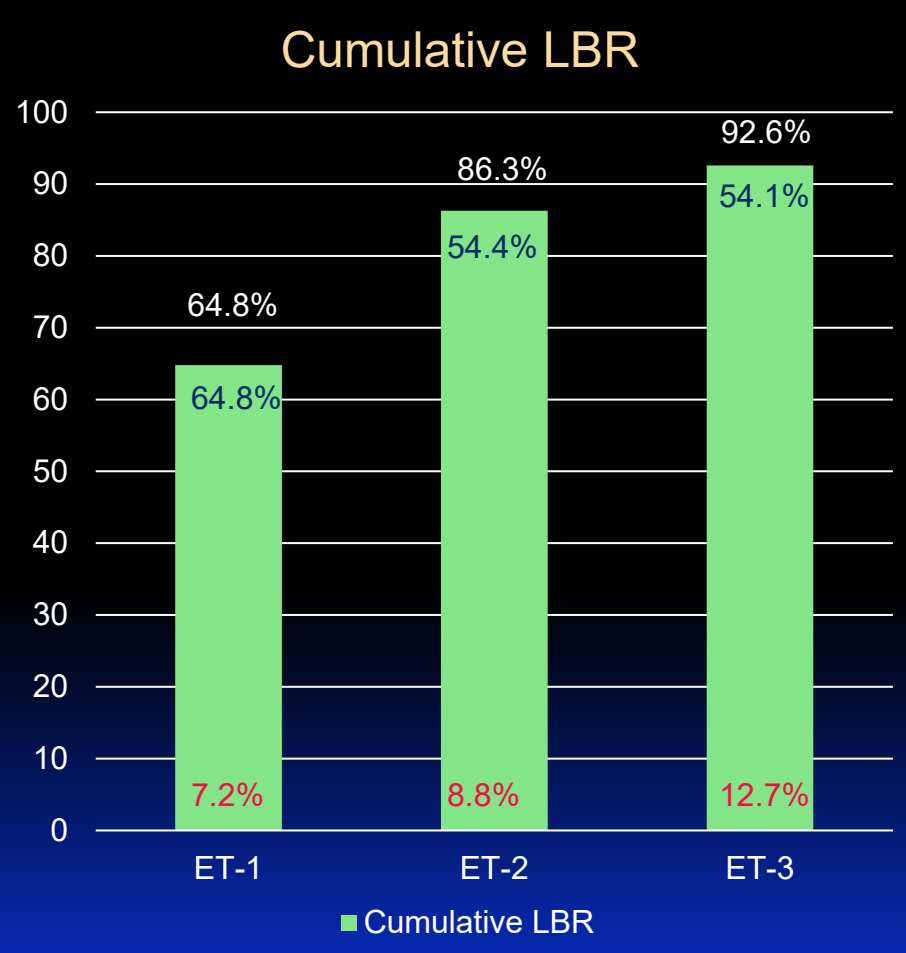
- Blastocysts could be from one or more cycles
  - 4111 had 1 VOR
  - 297 had 2 VOR
  - 21 had 3 VOR

Patients	mean
Age (y)	35
BMI (kg/m^2)	25
AMH (ng/ml)	3.01
M2's	12.5
2pn	10.5
Bx	5.45
Euploid	3.5
Non-euploid	1.8

The True Rate of RIF is Low

Pirtea P et al Fertil Steril 115:45-52, 2020





59.8%- per cycle IR or LBR

8.8%- per cycle SAB

The True Rate of RIF is Low

Pirtea P et al Fertil Steril 115:45-52, 2020

- Conclusions
  - RIF with euploid embryos is uncommon
  - Suggests that much of RIF with untested embryos is embryonic
  - Uterine causes after S/S or H/S is uncommon
  - Also explains why odd treatments appear to work…as they would have worked anyway
- Weaknesses
  - Retrospective
  - Some FETs after multiple stims
  - Confounding factors (e.g. smoking, obesity etc) not evaluated
  - Good Prognosis patients
  - Significant dropout rate
    - 43% after 1
    - 57% after 2
    - All ran out of embryos

The True Rate of RIF is Low

Gill P et al Fertil Steril 120, 4,Suppl, Oct 2023, P173

- Objective: CPR/LBR beyond 3 euploid ET
- Design:
  - Retrospective
  - International multi-center (n= 26 clinics)
  - 2012-2022
- Exclusions:
  - OD, GC, PGT-M, uterine anomalies, hydros,adenomyosis, non-obstructive-azoospermia, endometrium < 6 mm
- Patients
  - 123,987 VOR, 94,401 ET (64,572 euploid ET)
  - 34.6 yo, AMH 2.74, BMI 24.7
  - Mean endometrial thickness: 8.6 mm

# Euploid ET	n=	CPR/ET (%)	LBR/ET (%)
4th	105	51.4	40.0
5th	45	62.2	53.3
RR (CI)		1.21 (0.9-1.6)	1.33 (0.93-1.9)

Conclusion:

- CPR/LBR did not significantly decrease by the fifth euploid ET
- RIF is “incredibly rare” (1.5%) after 5 euploid ETs

Definition of Recurrent Implantation Failure

Cedars MI et al. Lugano Workshop on Recurrent Implantation Failure. Fertil Steril 120: 45-59, 2023

- Failure to have sustained implantation in at least 3 euploid embryo transfers
  - Or the equivalent number of unscreened embryos, adjusted to her age

Age (y)	Observed aneuploidy Rate	# untested blasts to achieve 95% chance of sustained implantation
<35	**20%**	**4**
35-37	**30%**	**5**
38-40	**50%**	**7**
41-42	**70%**	**13**
≥43	**85%**	**27**

Components

- Embryo
  - Egg
    - Age
    - Metabolic environment / lifestyle /exposures
    - Medications / supplements
    - Systemic pathologies
    - COH
  - Sperm
    - Age
    - Metabolic /lifestyle
    - Medications/Supplements
    - Systemic pathologies
  - Aneuploidy, metabolism, gene function
- Endometrium
  - Structural/Environmental
  - Biochemical/Synchrony
- Environment
  - Metabolic
  - Peritoneal / endometriosis
  - Hydrosalpinx
  - Systemic pathologies
  - Lifestyle / Exposures
- Technical
  - Lab quality
  - ET technique

Before we look at the patients, we should look in the mirror.....

SART Clinic Summary Reports 2021
Live Births/Intended Egg Retrieval

Clinic	< 35 yo	35-37 yo	38-40 yo	41-42 yo	>42 yo	OD
National	44.5%	32.4%	20.2%	9.6%	2.9%	41.4%
Clinic 1	72.7%	52.3%	44.4%	22.2%	0%	78.6%
Clinic 2	43.4%	38.2%	24.0%	6.5%	0%	58.3%
Clinic 3	43.9%	18.8%	20%	-	-	-
Clinic 4	38.0%	29.8%	19.1%	8.9%	3.5%	38.8%
Clinic 5	25%	6.7%	8.9%	5.8%	0.6%	33.3

How good are we at embryo transfers?

- Not everyone can do an ET well
- Not helpful
  - Antibiotics
  - Acupuncture
  - Analgesics
  - Massage
  - Delayed ambulation
- To Optimize:
  - Ultrasound guided
  - Removal of cervical Mucus plugs
  - Soft Catheter
  - Embryo location: (>1 cm from fundus)
  - Immediate ambulation
  - Monitor provider quality

Hearnes-Stokes Fertil Steril 74:80- 6, 2000
Angelini et al JARG 23:329-32, 2006
Uyara et al Fertil Steril 95:1860-2, 2011
Practice Committee, ASRM Fertil Steril 107:882-91,2017

The Embryo

Oocyte

- Age
- Metabolic environment / lifestyle /exposures
- Medications / supplements
- Systemic pathologies
- COH

Sperm: Role of the Male

- Contributes ½ genetic material
- Paternal Age is increasing
  - 1972: 27.4y
  - 2015: 30.9y
    - 8.9% . 40 y
- Typical Semen Analysis provides no information on Sperm DNA quality or epigenetics
  - Impacts all stages of early embryo development after genome activation

- A newborn has ~ 60 de novo point mutations (DNM's)
  - 80% are from paternal allele
    - Half are neurodevelopmental in nature
  - # of mutations increases with paternal age
    - Exponential
    - 70 yo: 8x mutations vs 20 yo
  - Epigenetic alterations also increase with age
  - Spermatognial stem cells (SSC)are continuously dividing, thus more chances for error
    - Age 25: ~ 350 SSC divisions
    - Age 45: ~ 750 SSC Divisions
  - DNM's also increase with oxidative stress

Khandwala YS et al Hum Reprod 32:2110-6, 2017
Wood KA, Goriely A. Fertil Steril 1001-12, 2022
Kong A, et al Nature 488:471-5, 2012

Role of the Male- Sperm Aneuploidy

- Can occur in presence of normal parental karyotype
- Influenced by:
  - Age
  - varicocoele
  - Radiation
  - Toxins (smoking/ETOH)
  - Medications
  - Obesity
  - Heat
- TEST: FISH
  - Use limited number of chromosomes (13/18/21/ X/Y)
  - Renders sperm unusable
  - Actual prognostic value in question
    - No information on specimen-to-specimen variability
  - Limited information on aCGH, qPCR, NGS for sperm

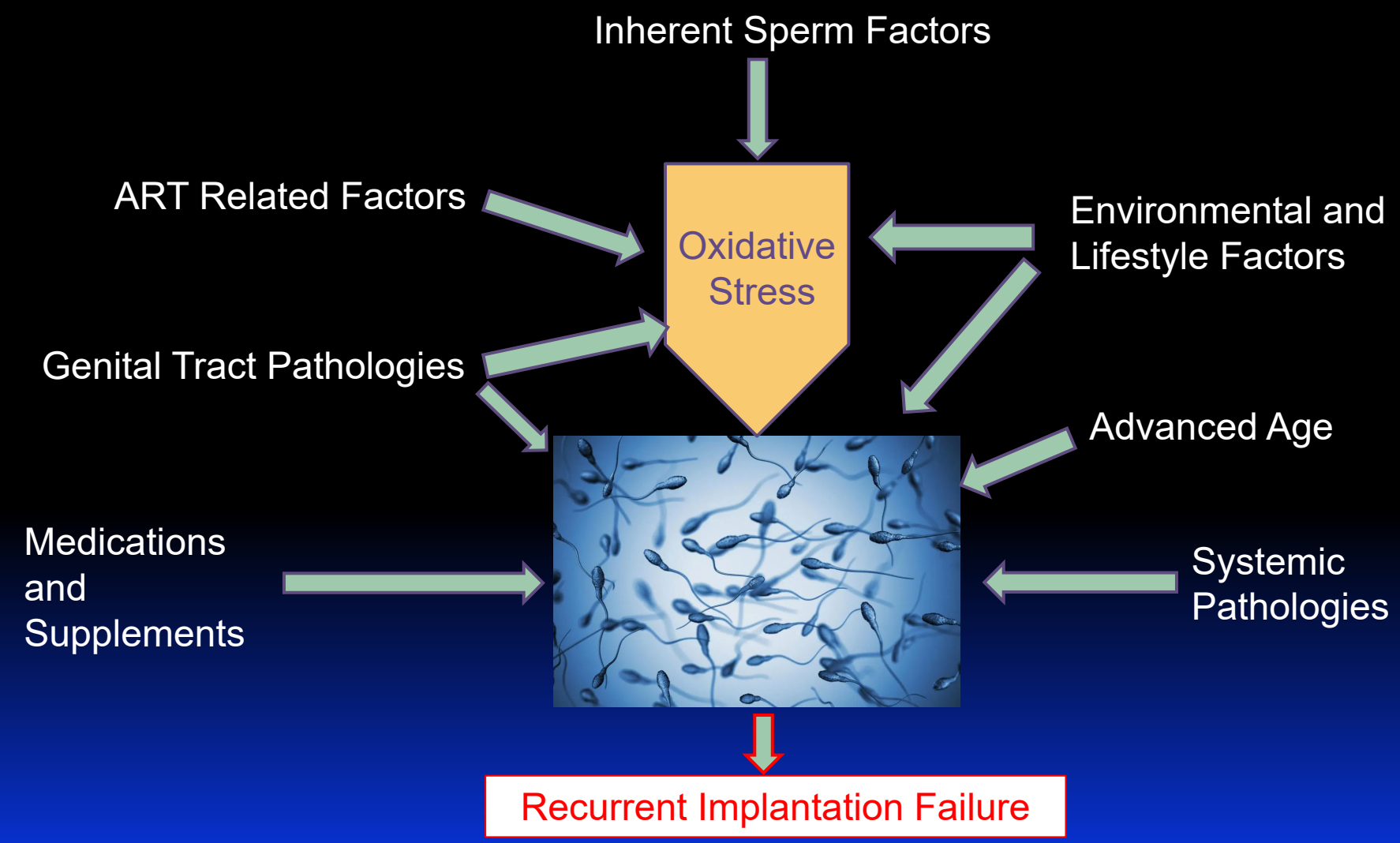
Keihani S et al RIF, p 222-58, 2018
Kohn et al JARG 33:571-6, 2016

Role of the Male: DNA Fragmentation

- Rationale:
  - Sperm have limited DNA repair mechanisms
  - Can be present with normal karotype, normal sperm FISH and normal semen analysis
  - Multiple Factors can impact nuclear and mitochondrial DNA

DNA Fragmentation: Potential Contributors

Modified from Keihani S et al RIF, p 222-58, 2018



Sperm Image from Medical News Today, 2017

DNA Fragmentation Tests

Test	How	Result	Strengths	Limitations
TUNEL	Incorporates modified nucleotides in Damaged area	% of sperm with DNA Damage	Detects both SS/DS breaks High sensitivity	Requires expensive technologies Variable protocols and thresholds
SCSA	Differential susceptible to denaturation according to level of DNA damage	% of sperm with DNA Damage	Measures large # cells rapidly Highly Std	Only SS breaks Not readily available
SCD (Halo Assa)	Differential susceptible to denaturation according to level of DNA damage	% of sperm with DNA Damage	Simple Inexpensive Convenient Low # sperm needed	Only SS breaks Interobserver variability
Comet Assay	Electrophoretic technique	% of damage in a single sperm	Inexpensive High sensitivity Small specimens	Nono-standardized Intralaboratory variation
AO Assay	Differential susceptible to de naturation according to level of DNA damage	% of sperm with fragmented DNA	inexpensive	Ss breaks Interobserver subjectivity

TUNEL- teerminal deoxy-nucleotide transferase-mediated dUTP nick end labeling
SCSA-Sperm chromatin Structure Assay
SCD-Sperm Chromatin Dispersion
AO- acridine orange assay

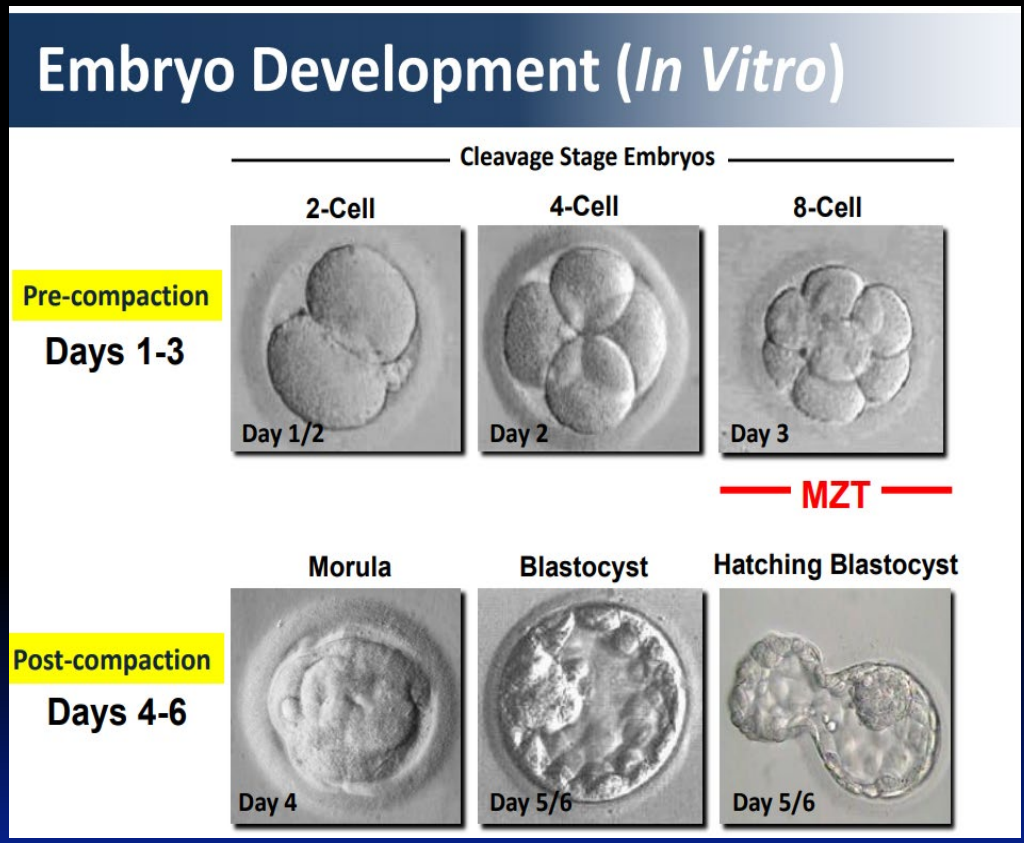
DNA Fragmentation Results

- TUNEL ~ correlates with RIF in meta-analyses
- Each test provides different information
  - Would prefer assays looking at DS breaks
    - TUNEL / Comet Alkaline
  - None can measure sperm actually used
  - Does not necessarily correlate with other assays
  - Most lack standard cut-off value
  - Poor standardization of test lab to lab and within many labs (CV's ~30%)
  - Do not address *specimen to specimen* variability
- Results are inconsistent and hard to generalize
  - Clinical data results and implantation are either missing or inconsistent

Cimadoma D et al Hum Reprod 36:305-17, 2021
Keihani S et al RIF, p 222-58, 2018

Embryonic Factors in RIF

- Embryos develop along a predictable timeline (after insem)
  - Fertilization: 18 h
  - 2c: 26-28h
  - 8c: 64-68h
  - D5: 116-120h



Jason Swain, PhD, University of Michigan 2013

Embryonic Factors: Things to judge

Cleavage Stage Embryos

- Rate of development
- Appearance
  - Cell #
  - Fragmentation
  - Symmetry
  - Granularity of cytoplasm
  - Cell sizes
  - Multinucleation
  - Zona thickness
- Early Cleavage and total cell # most predictive

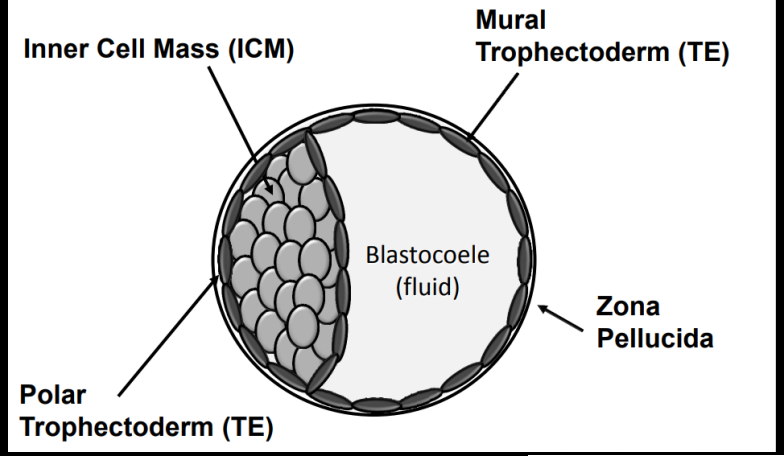
Blastocyst Stage

- Improves embryo selection
- More to judge:
  - Degree of Expansion
  - ICM
  - Trophectoderm
  - Gardner's criteria
    - Exp/ICM/TE
    - eg:4AB

Blastocyst Grading

Gardner DK et al Hum Reprod 13:3434-40, 1998
Jason Swain, PhD, University of Michigan 2013

Anatomy of a Blastocyst



Blastocyst Grading

Expansion – size of the blastocoel cavity

Stage 1 Stage 2 Stage 3 Stage 4

Stage 5 Stage 5 Stage 6

Blastocyst Grading

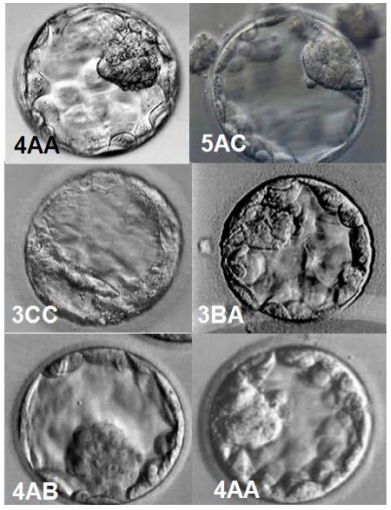
ICM

A – many cells, tight
B – many cells
C – few cells

TE

A – many cells, tight
B – many cells
C – few cells

Various systems exist



Embryonic Factors: Interventions

Time Lapse Imaging

- Advantages
  - Abnormal kinetic factors
  - Abnormal morphologic features
- Results
  - Multiple RCTs show no benefit
  - One RCT with benefit with significant biases
  - Large meta-analysis (9 RCTs)
    - Quality of evidence low
    - No difference in LBR/CPR/SAB

Other Interventions

- Blast Culture
  - Can you grow every baby to blast in the lab?
  - % blasts
  - Timing of blastulation
  - D2 or D3 ETs?
- Vitrification of slower blasts
  - Embryo/Endometrial synchrony
- Freeze-All
  - Embryo/Endometrial synchrony
  - Results mixed
- Biochemical Embryo Screening
  - PGT-A
  - Metabolic Screening
  - Mitochondrial DNA

Armstrong S et al Cochrane Database of System Rev #CD011320, 2019
Kahraman S et al J reprod Stem Cell Biotechnol 3:55-61, 2013
Goodman LR et al Fertil Steril 105: 275-85
Kasser DJ et al Fertil Steril 106(3)Suppl. E312, 2016
Rubio et al Fertil Steril 102:1287-94, 2014
George LC, et al Fertil Steril 120, 4, Suppl, P-248, 2023

Genetics of RIF

- Whole chromosome aneuploidy
- Segmental Imbalances
- Mosaicism
- Epigenetic
- Single Gene disorders

Genetics of RIF

- Whole chromosome Aneuploidy
  - PGT- ~ 30% in Early 30%; ~ 75% at 42 yo
  - SAB: > 60% aneuploid
- Age related
  - Increase in meiotic errors
  - Some studies report age related decline with euploid embryos
- Segmental Imbalances
  - Most de novo mitotic origin
  - Detection related to resolution of test used
    - SNP 13.8 mb
    - aCGH 5 mb
    - NGS 5 mb or less
  - Frequency
    - 6% SAB
    - 0.05% newborn
    - Embryos: 8.4%; 4.5% segmental only
  - Role in RIF unclear

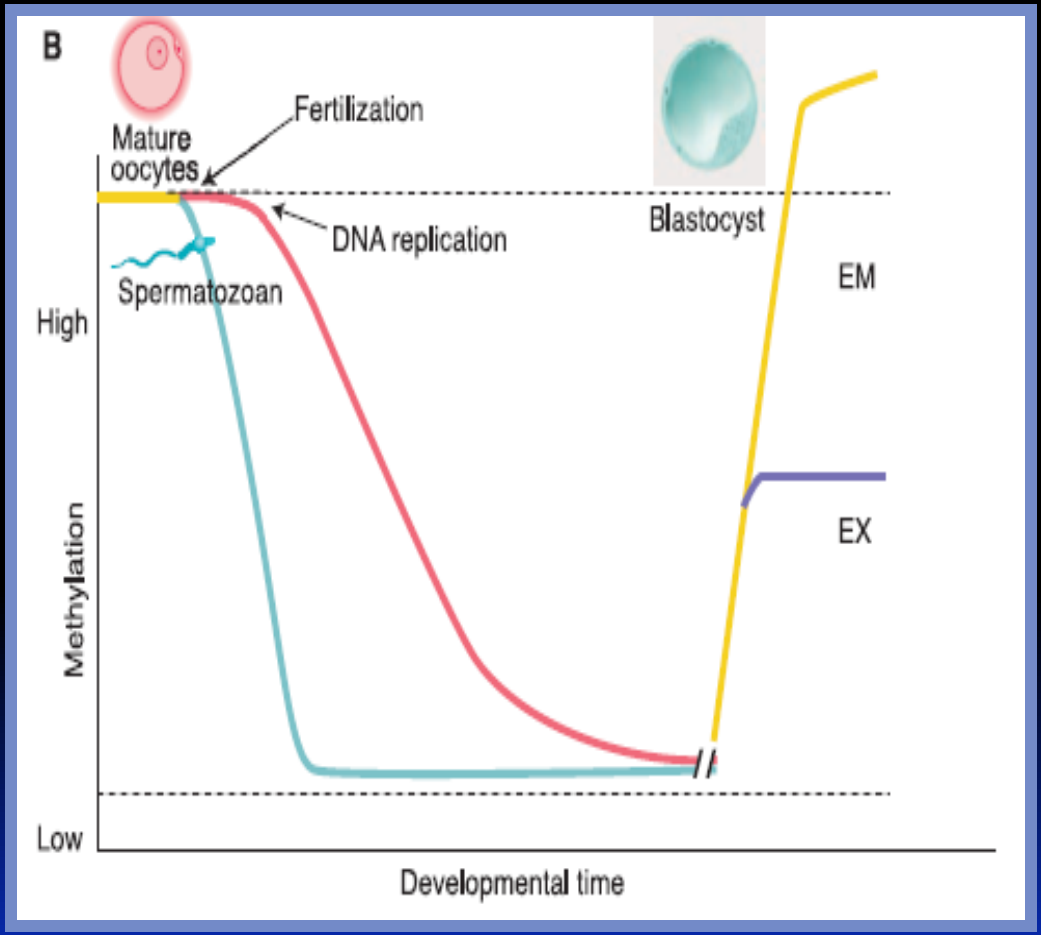
Franasiak J et al JARG 1501-9, 2014
Escriba et al Reprod Biol and Endocrinol 17: 2019
Viotti M. Genes 11:602-35, 2020
Horton GL etal Fertil Steril 100:1695-703-2013
Riely A et al JARG 37:595-602, 2020

Genetics in RIF

- Mosaicism
  - Presence of 2 or more cell lines
  - Frequency remains in question
  - Diagnosis remains in question
  - Some can clearly make babies
- Single Gene Disorders
  - Smith-Lemli-Opitz and Congenital Disorder of Glycosylation-1α implicated in miscarriage
  - None so far in RIF

Epigenetic Changes During Fertilization

- Dramatic changes in DNA methylation
- Restores toti-potency
  - Sperm imprint removed initially
  - Maternal genome slowly demethylated
  - Paternal transcription bursts ahead
  - Gradual re-methylation accompanies differentiation



Epigenetic Changes

- Environmental Alterations impact epigenetics
  - Starvation in pregnancy: altered lipid profiles, HTN, CAD
  - Maternal obesity in pregnancy: obesity, skeletal issues
  - Smoking in pregnancy
- Stimulations and lab conditions known to alter epigenetics of oocyte/embryo
- Role in RIF not established

Anatomic Factors in RIF

- The chances of finding uterine pathology in asymptomatic women with implantation failure can be as high as 50%

- Potential Findings
  - Suboptimal endometrial thickness
  - Fibroids
  - Polyps
  - Adhesions
  - Congenital Anomalies

Pundir J et al Reprod Biomed Online 28:151-61, 2014

Uterus: Endometrial Thickness

Liao Z et al Frontiers in Endocrinol 12: Article 814648, 2022

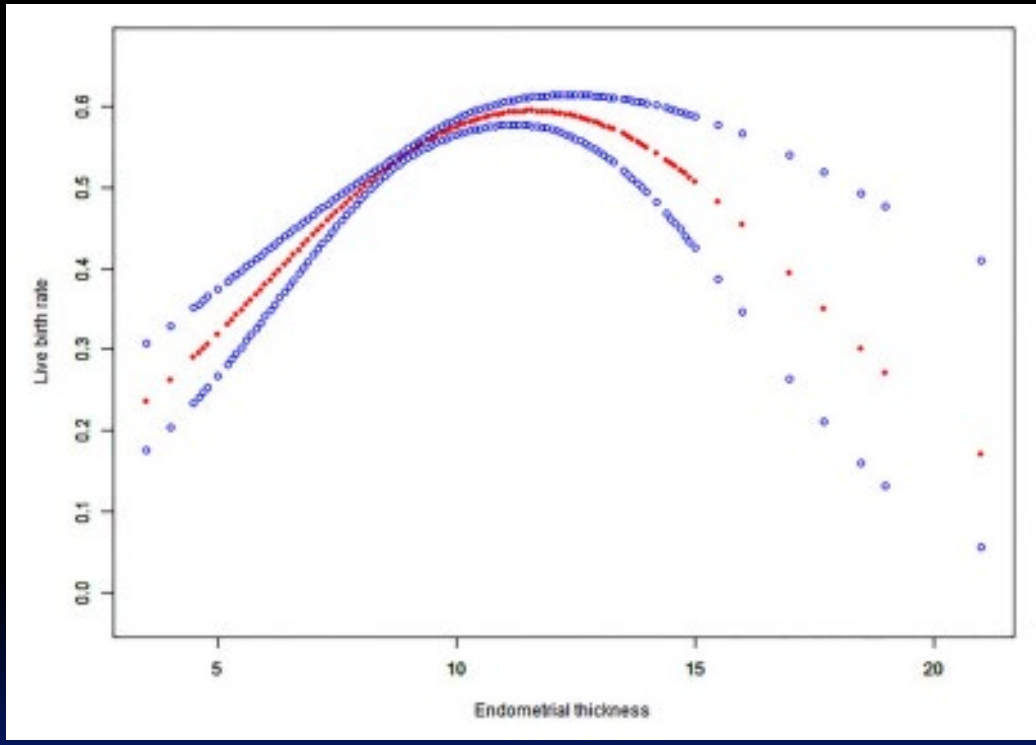
- Meta-analysis
  - N=22 studies
  - Fresh IVF cycles
  - Endometrial Thickness
    - <7mm,
    - 7-14mm
    - > 14mm

Vs normal (7-14mm)	Thin Endometrium (<7mm) OR (CI)	Thick Endometrium (>14mm) OR (CI)
LBR	0.47* (0.37-0.61)	1.08 (0.68-1.72)
CPR	0.48* (0.31-50)	1.22 (1.00-1.49)
IR	0.27 * (0.19-0.39)	1.14 (0.88-1.47)
SAB	1.43* (0.32-6.41)	0.90 (0.69-1.19)
Hypertensive Disorders	1.72* (1.01-2.04)	n/a
SGA	1.81* (1.16-2.83)	n/a
LBW	-0.12kg* (-0.19—0.04)	n/a

Uterus: Endometrial Thickness

Shaodi Z et al Plos One 15(9)e0239120, 2020

- Retrospective
- 10, 165 HRT-FET
  - 2013-2017
  - ~75%cleavage stage, ~ 25% blast ETs
  - Endometrial thickness at ET
- Compared to thickness at P4 start
  - 19% no change
  - 38% thicker
  - 48% thinner
    - CPR lower aOR 1.09 (CI 1.06-1.12)
- Lowest threshold for optimal LBR 8.7mm



Endometrial thickness vs LBR

Same seen for IR and CPR

Endometrium

- Synchronization of embryo and endometrium essential
- Specific histologic changes were noted during cycle
  - Not sufficient to determine cause of implantation failure
- Endometrial Markers
  - Suggest uncoupling of glandular and stromal development
  - Stroma seems unperturbable
  - Glands sensitive to delay

Noyes FW, Hertig At, Rock J Fertil Steril 1L2-25, 1950
Kliman HG Frankfurter D. Fertil Steril 111:618-28, 2019

Uterus: Endometrial Preparation

- Estrogen
  - Oral
  - Patch
  - Vaginal
  - IM
- Progesterone
  - IM
  - Vaginal
  - Combination
  - Oral- NO!

- Natural cycle
- Modified natural cycle
  - hCG
  - Progesterone
- Synthetic
  - Ocp's, LDR, antagonist, natural start
  - Estrogen
  - Progesterone

Endometrial Markers

- Endometrial Receptivity Assay (ERA)
  - 238 mRNAs
  - Bx done on day of ET
  - Standardized to 2 failed ETs with euploid embryos
    - 25% abnormal ERA
- Possibly helpful in subsequent ETs
  (Tan J et al JARG 35:683-92, 2018)
- Clearly not helpful in primary transfers
- Overall utility not clear

Endometrial Markers

- Endometrial Function Test
  - Combines histologic assessment with marker of endometrial development
    - Biopsies on CD15 (Cyclin) and 24 (p24)
    - If abnormal 10 x reduction in chance of pregnancy
  - Problems:
    - Requires 2 biopsies
    - Expert reproductive Pathologist needed
    - No blinded studies ever done
    - Only one person reads the results

Endometrial Markers

- Receptiva
  - Uses marker of inflammation (BL-6) as surrogate for presence of endometriosis
    - Can also be found with hydrosalpinges, PAD, ovarian cysts
    - CD-138 can be added to dx endometritis
    - Is not looking at WOI
  - EB done 7-10d after ovulation
  - If + rx with GnRHa recommended for 90d
  - Small studies suggest improved PR

Micro-RNAs

- miRNAs- small, single-strand, non-coding
  - Regulate gene expression via degradation or suppression of mRNAs
- May be involved in implantation
  - via endometrial extracellular vesicles
    - Altered Mucin 1 levels
    - Induce decidual rxn
    - Increase LIF
  - Via embryonic secretion to enhance receptivity
    - miRNAs can be found in culture medium
    - Some may be markers of aneuploidy and pregnancy failure
    - Also found in serum and endometrial fluid
  - Altering inflammatory environment of endometrium

Khosravizadeh Z et al. Fertil Steril 1:26-37, 2023

Endometrium: Inflammatory Lesions

- Infection- glandular pmn's- bacterial
- Lymphocytic infiltration- possibly viral
- Plasma cells
  - Chronic Endometritis
- TB
- Retained POC
- Malignancy

Kliman HG Frankfurter D. Fertil Steril 111:618-28, 2019

Anatomic Factors in RIF: Fibroids

Mechanisms

- Impaired implantation
- Blocked tubes
- Altered uterine contractions
- Altered endometrial perfusion
- Altered HOXA10, HOXA 11, glycodelin

Impact by location

- Submucosal:
  - Decrease IVF outcomes by 70%
- Subserosal:
  - Usually not an issue
- Intramural:
  - Decreases LBR by ~ 30%

Pritt EA et al Fertil Steril 91:1215-23, 2009
Goldberg et al. RIF p153-174, 2018

Anatomic Factors in RIF: Intramural Fibroids

- Question: Do intramural, non-cavity deforming fibroids < 6 cm alter IVF outcome
- Meta-analysis
  - 5 cohort studies
  - N=520 patients

Fibroid Size	LBR	Control LBR	OR (CI)
< 6 cm	23.8% (87/365)	36.12% (276/764)	0.48 (0.36-0.65)
< 4 cm	32% (58/181)	42.4% (131/309)	0.57 (0.36-0.90)
< 2 cm	30% (19/64)	36% (70-192)	0.74 (0.4-1.36)

Erden M et al. Fertil Steril 119:996-1007, 2023

Anatomic Factors in RIF: Treatment of Fibroids

Does Myomectomy Help?

- Submucosal
  - Improves CPR 2x
  - No impact on SAB
- Subserosal
  - Not evaluated
- Intramural
  - 2009 MA-Improves CPR 3.7X and decreases SAB 25% but NS
  - 2012 MA-Improves CPR 2x and decreases SAB 17% but NS

Other Interventions

- UAE- contraindicated
  - Decreased PR
  - Increased: SAB, PTD, breech, PPH
  - ? Difference in one done for PPH vs for fibroids
- MR guided focused U/S
  - Thermal ablation
  - Preliminary studies encouraging
- L/S radiofrequency thermal ablation
  - ? Fertility outcomes

Pritts EA Fertil Steril 91:1215-23, 2009
Metwally M et al Cochrane Database Syst Rev 11:CD003857, 2012

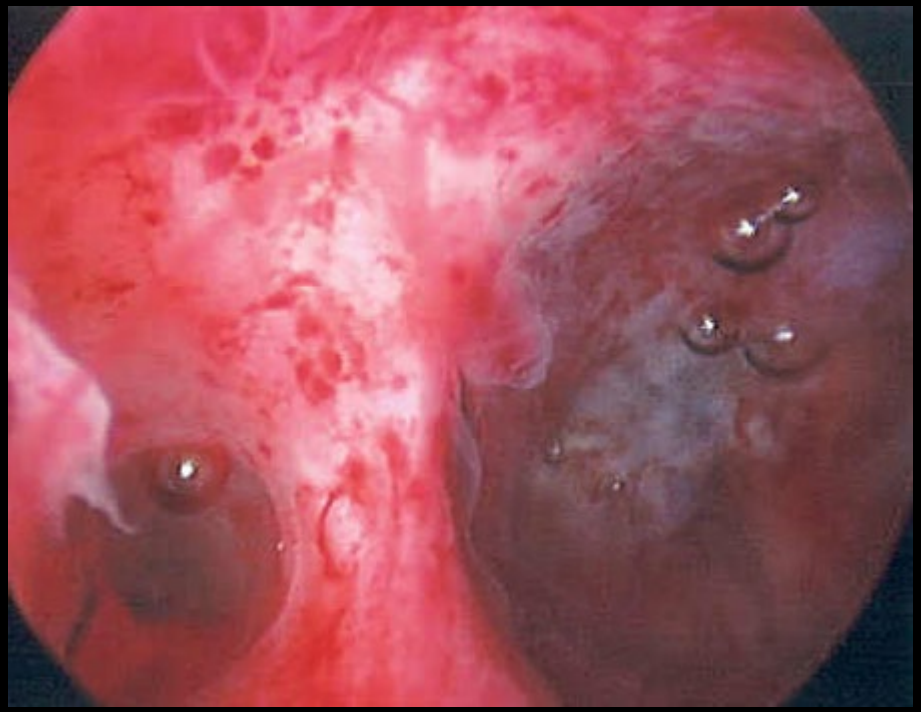
Anatomic Factors in RIF-Polyps

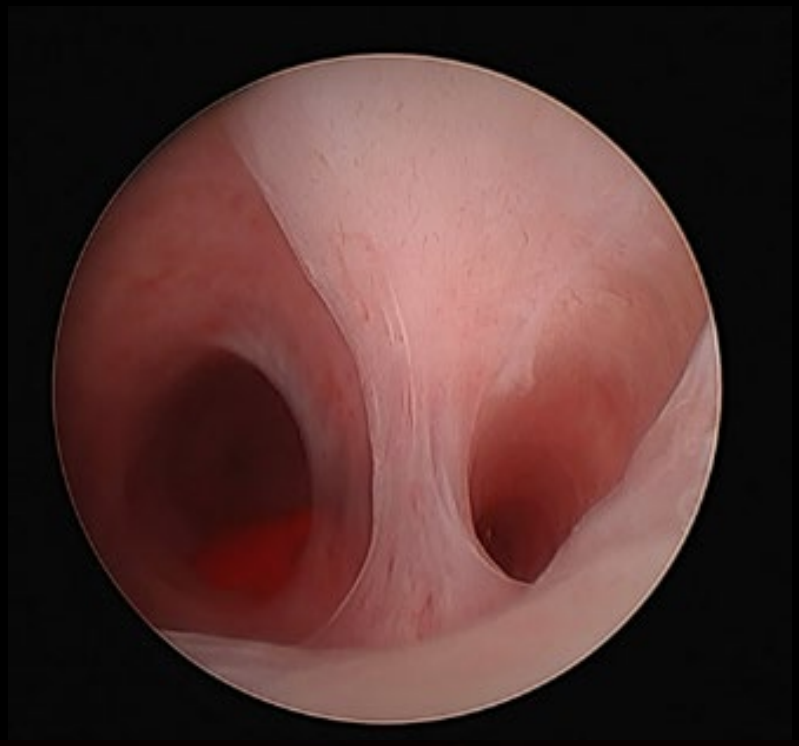
- Definition- focal overgrowths of endometrium supplied by single blood vessel
- Frequency
  - 3.2 % in BTL pts
  - 15.6% in unexplained infertility
  - 6-32% in ART patients
- Mechanism
  - Often asynchronous
  - Mechanical distortion
  - Decreased LIF, glycodelin, HOXA 10, IL 10, osteopontin
  - Increased IGFBP1, TNFα, NF-κB, PR, Cox-2, BCL-2
- Treatment
  - Hysteroscopy
- Results
  - IUI- RCT- 2x improvement in CPR after removal
  - IVF-2014 meta-analysis (n=3179)
    - H/S prior to first IVF
    - 44% improvement in CPR vs no H/S
    - No difference in CPR NL H/S vs Abnl H/S (33% vs 32.6%)

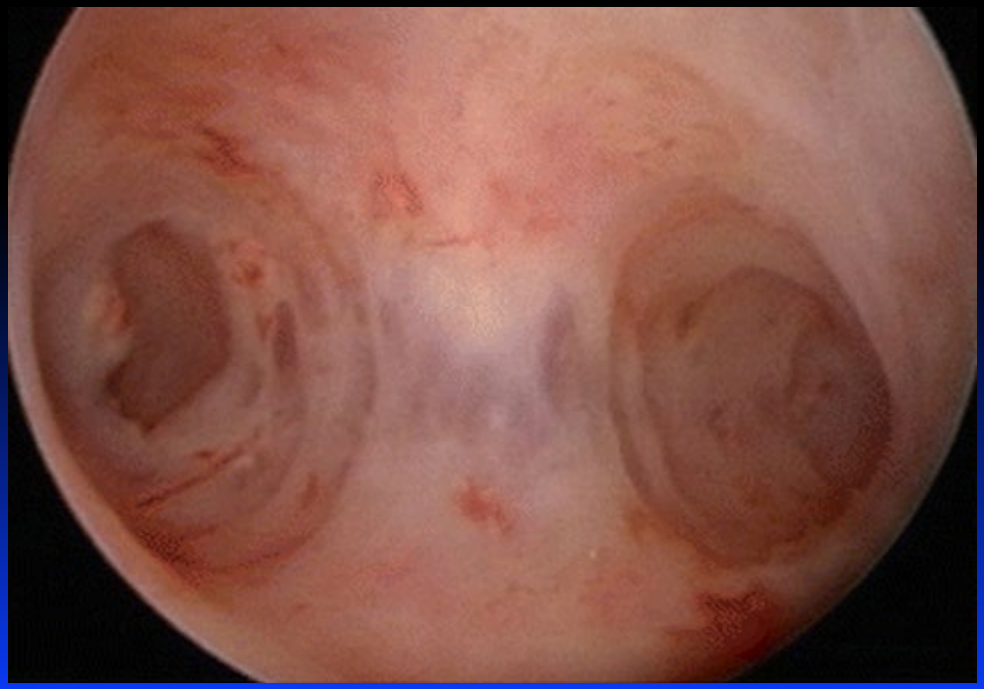
Ben-Nagi et al Reprod Biomed Online 19:737-44, 2009
Pinheiro et al Mol Med Rep 9:2335-41, 2014
Bozkuurt M et al Eur J Obstet Gynecol Reprod Biol 189:96-100, 2015
Pewez-Medina T et al Hum Reprod 20:1632-5, 2005
Goldberg JM et al in RIF, p153-74, 2018

Anatomic Factors in RIF- Adhesions

- Scar tissue in uterine cavity.
  - Need to distinguish between amount and symptoms
  - Asherman's Syndrome Endometrial damage, low Estrogen, Inflammation
- Mechanism:
  - Replacement of epithelium with fibrous tissue
  - Alterations in blood flow
  - Altered sperm transport
- Prevalence:
  - 8% in infertile women
  - >90% associated with prior pregnancy
- Evaluation:
  - Saline sono
  - HSG
  - H/S







Anatomic Factors in RIF- Adhesions

- Treatment:
  - H/S LOA
  - ? Benefits of post-op adjuvants
    - IUD
    - Balloon
    - Estrogen
    - Hyaluronic acid gel
- GCSF-May help endometrial thickness after LOA
  - 5.5 mm→7.9mm
  - But not on adhesion recurrence (42.5% v. 38.5%)
- Prognosis: How much damage?
- Outcomes:
  - 33-80% CPR
  - Pregnancy complications (SAB, PTD, Abnl placentation, IUGR, Uterine rupture)

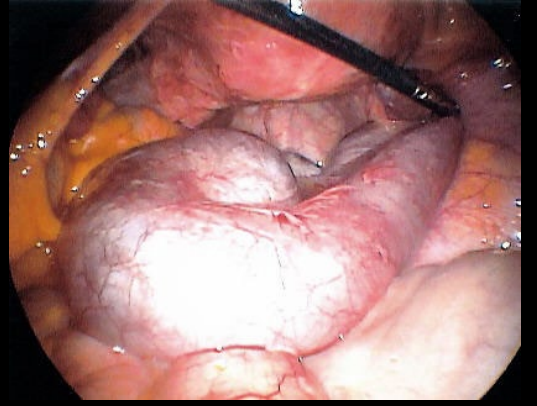
Deans R, Abbott J. J Min Invasive Gynecol. 17:555-69, 2010
Zhang Y et al Hum Reprod 37:725-33, 2022

Anatomic Factors in RIF: Congenital Anomalies

- Incidence:
  - 6.7% general population
  - 7.3% infertile population
- Impact:
  - ? On achieving pregnancy
    - Unicornuate may have reduced IR at IVF
  - Increased SAB, PTD, malpresentation
- Mechanism
  - Altered vascularity
  - Altered endometrial response to steroids
  - Altered VGEF expression
- Treatment: Surgery
  - H/S metroplasty for septum- decreases miscarriage rates
  - Removal of accessory horns for unicornuate
  - Unification procedures for bicornuate and didelphys rarely needed

Goldberg JM et al in RIF, 2018 p 153-74
Chan YY et al Ultrasound Obstet Gynecol 38:371-82, 2011
Probst AM, Hilll JA Semin Reprod MED 18:341-50, 2000

Anatomic Factors in RIF: Hydrosalpinges



- Definition: distal tubal blockage with fluid accumulation
  - Caused by inflammation (STD, Endometriosis, TB)
  - Beware of partial salpingectomies
- Possible Mechanisms
  - Mechanical
  - Altered endometrial receptivity
  - Embryo toxicity
- How problematic
  - 1998 meta-analysis (n=5569 patients): IR and CPR decreased by about 50% with hydro
  - 1999 meta-analysis (n=5592 patients): IR 38% lower and CPR 37% lower with hydro.
- Treatment
  - Salpingectomy/TL- improves CPR 2.3X
  - Aspiration alone not helpful
  - Aspiration + sclerotherapy improved CPR 78%

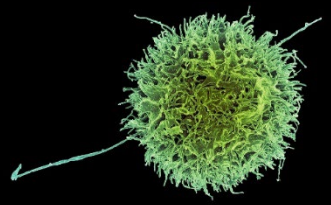
Zeyneloglu HB et al Fertil Steril 70:492-9, 1998
Camus E et al Hum Reprod 14:1243-9, 1999
Hammadieh N et al Hum Reprod 23:1113-7, 2008

Immune Factors: The Basics

Immune Factors

- How does a mother tolerate a genetically foreign embryo?
- Studying the immune impact on reproduction
  - Is really important: maternal tolerance starts at the uterine level
  - Is really hard to do
  - Is really complex due to great variety of participating
    - Cell types
    - Molecules
    - Processes
    - Locations
    - Genetic combinations

Immune system: Immune Cells



- Most are “tissue resident”
  - #, type, activated state depend on hormonal environment
  - Actively respond to fetal antigens *promoting immune tolerance*
- Immune hyperactivity (eg autoimmne disease) can damage trophoblasts

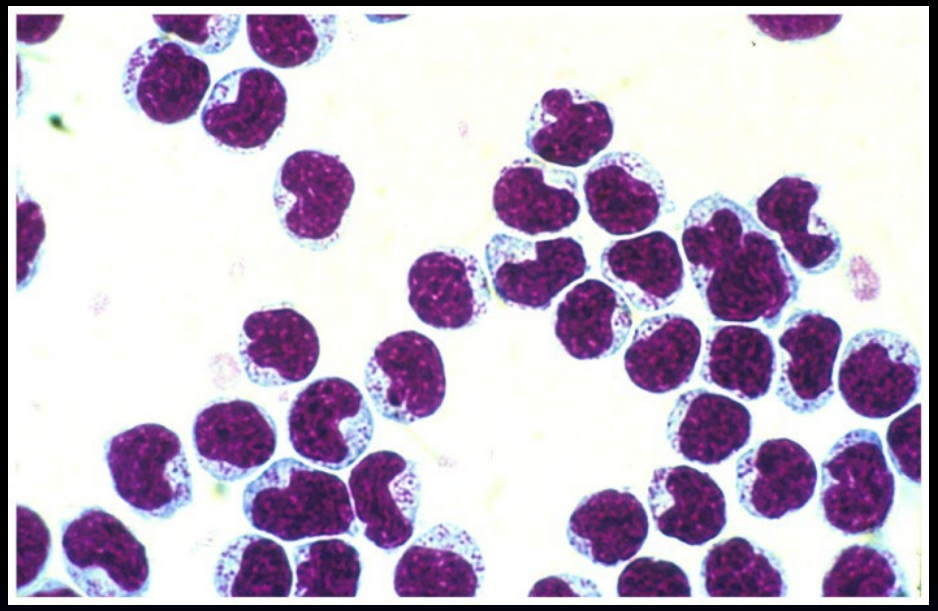
Franasiak JM et al Fertil Steril 116:1436-48, 2021
Photo: NIAID 29120480442.jpg, 2016

Immune System: Trophoblast invasion

- Maternal response to foreign antigens
  - Balance
  - Dysregulation *could* contribute to inefficiency
- Old model was that immune system was inactivated in some way
- Currently it is clear that specific immune *activation* is required at maternal fetal interface
- Cast of characters
  - Stromal cells, glands, arteries
  - uNK, macrophages
- Most important immune cells are
  - Tissue resident
  - Hormonal dependent
  - Change after fetal contact
  - Cytotoxic ability depends on balance of activity and inhibiting signals from surface receptors

Immune System: Natural Killer Cells

- Natural Killer cells
- 2 types:
  - Peripherial (pbNK)
  - Uterine (uNK)
- Are very different types of immune cells
- pbNK have been used in women with RIF based on mistaken notion that they are killing embryos



Rolstad B. Frontiers in Immunol 5:1-8, 2014

Immune System: Natural Killer Cells

Peripherial Blood NK

- 2 types
  - CD_{56dim} CD_{16+} (~ 90%)
  - $CD_{56Bright}$ CD_{162} (~ 10%)
- Cytotoxic-First line of defense
  - Viruses, tumors, damaged cells
  - Not trained to reject a healthy embryo

Franasiak JM, Scott RT Fertil Steril 107:1279-83, 2017
Alecsandru, Garcia-Velasco JA. Immune Factors in RIF, in RIF p93-102, 2018
Franasiak JM et al Fertil Steril 116:1436-48,2021

Immune System: Natural Killer Cells

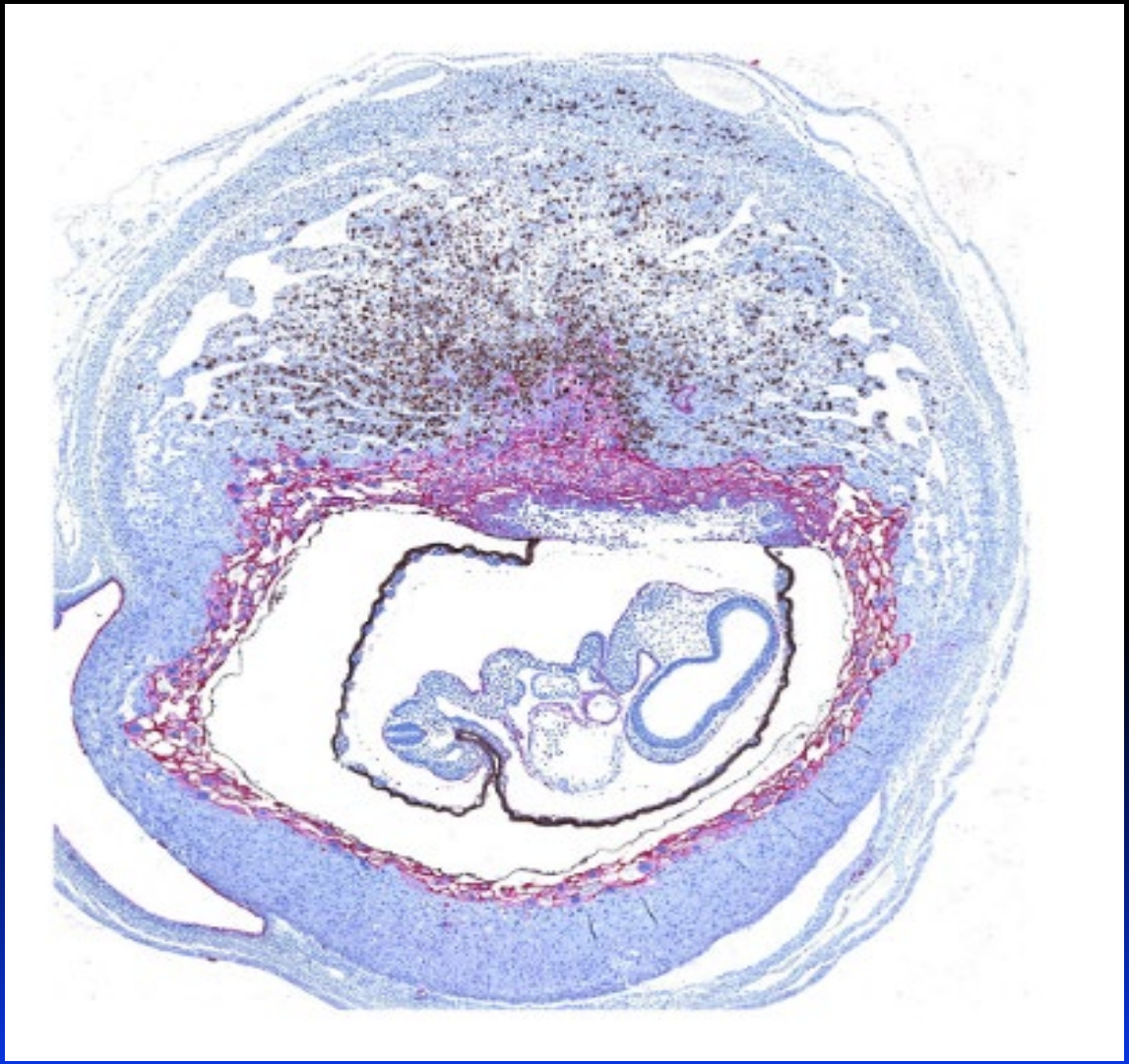
Uterine NK

- Primary type
  - CD $_{56\ superbright}$ CD $_{162}$
  - 70% of immune cells
  - 30% of cells overall in endometrium
- Weakly cytotoxic
- Source
  - Periphery→migrate?
  - Derived from precursor stem cells in uterus?
- Progesterone responsive
  - Very few prior to ovulation
  - Peak presence at time of implantation
- "Pro-implantation": via cytokines
  - control trophoblast invasion.
  - Remodel vasculature
  - Provide immune tolerance

Franasiak JM et al Fertil Steril 116:1436-48,2021
Franasiak JM, Scott RT Fertil Steril 107:1279-83, 2017

Localization of uNK

Gaynor LM Colucci F Front Immunol 8:2017



Locations

- Glands
- Spiral Arteries
- Site of invasion

Immune System: T-Helper Cells (T_H)

Franasiak JM, Scott RT Fertil Steril 107:1279-83, 2017
Alecsandru, Garcia-Velasco JA. Immune Factors in RIF, in RIF p93-102, 2018
Franasiak JM et al Fertil Steril 116:1436-48,2021

- CD4+
- Are characterized by type of cytokines

- T_{H1}
  - Proinflammatory cytokines
  - Interferon γ, TNF, IL1,2,12,15,18
  - In animal models increase pregnancy wastage
    - ? Cause or effect

- T_{H2}
  - Cytokines that limit activity of the TH1 cytokines
  - "anti-inflammatory"
- There is a variation in the balance of these based on type of immune challenge
- It is believed that in imbalance of the T_H cells can prevent successful implantation

Immune System: T-Helper Cells (T_H)

- Early Pregnancy is T_{H2} dominant
  - Induced by Progesterone (↑IL4, 6, ↓IL12,infγ)
  - Embryo contributes (↑IL10 TGF-B)
  - Strong T_{H2} response may be necessary for maintenance of pregnancy

- Before conception:
  - Peri-implantation period is T_{H1} dominant
  - No detectable differences in immune system of women noted to later have “abnormal” T_{H1} dominance
    - So no screening tests can be done in advance.

Presence of auto-antibodies

- Proposed Mechanism:
  - Ab bind to trophoblasts
    - Alterations in VEGF
    - Abnormal vascular invasion resulting in altered blood flow
  - Evidence:
    - Case control studies with mixed results
- Meta-analyses
  - 2 large studies show no impact
    - Hornstein MD et al Fertil Steril 73:330-3, 2000
    - ASRM Fertil Steril 90 (suppl5) 172-3, 2008
  - 1 Study (di Nisio Blood 118: 267-8, 2011)
    - Modest increased risk of failure with 1 or more Ab present
    - OR 3.33 (1.77-6.26)
    - Under powered

Immune Treatments

Immune Treatments

- Theory: balance between pro-inflammatory states and anti-inflammatory states determine the fate of the implanting embryo
- Empirical treatments have come to fill the gap between science and clinical need

Hviid MM, Macklon N Fertil Steril 107:6:1284-93, 2017

Corticosteroids

- Rationale: generalized immune suppression
  - Easy to take
  - Short regimens
  - cheap
  - ~ safe
- Often used with other immunmodulating agent

Results

- In general IVF population, “good evidence to recommend against” use of steroids to improve live birth
- In RIF with No Ab present
  - 2012 Meta-analysis
    - n 1759 patients
    - Other Rx included
    - OR 1.,21 (0.67-2.19)
- In RIF with Ab present
  - Retrospective studies suggest benefit in RIF
  - RCT: suggest improvement.

ASRM Fertil Steril 110 387-400-2018
Boomsma et al Cochrane Database Syst Rev 2012:CD005996

Corticosteroids: Meta-analysis

Study

- N= 681 patients, 7 studies
- ART used 6 IVF /1 COH/IUI
- Outcome: LBR, PR, IR/Couple
- Protocols: 7
  - 9 different Ab
  - 4 different corticosteroids
  - 7 different regimens (time/dose etc)
  - ASA also used for everyone
- Studies with RIF pts: 0

Results

- CPR: 43.6% v. 20.5%
  - OR 4.57 (1.19-1760)
- LBR: 42.7% v. 27%
  - OR 1.92 (1.17-3.16)

Conclusion

- Glucocorticoids improve CPR and LBR in women with unexplained auto Ab

Problems

Li T et al Front Med 9:819406, 2022

Aspirin

Proposed Benefit

- Improved uterine and ovarian blood flow
- Prevent thrombosis
- Improve endometrial thickness

Fresh-Meta-Analyses

- Sivislatidis CS et al 2011
  - 13 studies, 2653 pts, Fresh IVF
  - No Difference in:
    - CPR: 1.03 (0.91-1.17)
    - LBR : 0.91 (0.72-1.15),
    - SAB, Bleeding

FET- Retrospective

- He H et al et al 2023
  - Retrospective, n=4454 FETs
  - No Difference in:
    - CPR: 1.024 (0.89-1.17)
    - LBR : 1.003 (0.88-1.14)
    - SAB, PTB, PPH, Previa

Sivislatidis CS et al Cochrane Database Syst Rev 2011: CD 004832
He H et al. J Clin Med 13: 10.3390/jcm12031064, 2023

Heparin

- Mechanism:

  HB- EGF improves trophoblast invasion, decrease apoptosis
  Increase IGF-I, IGF-II- improves trophoblast invasion

RCT	# pts	IVF Failures	Timing	CPR (%) (rx v. Control)	P value	IR (%) (rx v. Control)	P value
Fawzy 2014	295	1-2	VOR- 8 wk	40. v. 27.5	ns	23.9 v. 14.7	0.01
Noci 2011	172	0	VOR- 8 wk	25 v. 20	ns	15 v. 12	ns
Berker, 2011	219	2+	VOR-12-wks	34.6 v. 33.9	ns	22.6 v. 21.1	ns
Uman, 2009	150	2+	VOR-12 wks	45.3 v. 38.7	ns	27.5 v. 19.8	ns
Qublan, 2008	83	3+	ET-Delivery	31.9 v. 9.6	<0.05	19.8 v, 6.1	<0.05

- Conclusion: support for heparin use in RIF weak at best.

Hviid MM, Macklon N Fertil Steril 107: 1284-93, 2017

IVIG

- From plasma of several thousand healthy donors
- Established uses:
  - ITP, Polyneuropathy, Guillen Barre, Kawasaki
- T ½ 21-25d
- Proposed Mechanism:
  - Decrease pNK, Increase T_{reg}, Decrease B cells
  - This "decreases cytotoxicity and "improves T_{H2} mileu"
- Cost: $ 7-14,000

- RCT (Stephenson MD, Flukor MR, Fettil Steril 74:1108-12, 2000)
  - No difference in IR, CPR, LBR

Meta-analysis	# studies	IR OR (CI)	CPR OR (CI)	LBR OR (CI)
Li, 2013	10	2.70 1.3-5.6	1.45 1.19-1.85	1.66 1.2-2.1
Abdolmohammadi-Vahid 2019	5		1.82 1.14-2.89	2.17 1.3-3.64
Rimmer, 2020	5 RCTS		1.55 1.16-2.07	1.83 1.42-2.35

- **Conclusions**
  - Some subpopulations may benefit
  - Overall quality of evidence poor
  - Expensive, inconvenient
  - ASRM: Insufficient evidence to recommend IVIG

ASRM Fertil Steril 110 387-400-2018
Rimmer Mpet al J Obstet Guyn Res 47:6:2149-56, 2021
Saab W Am J Reprod immunmol 2021:85:e13395

Adalimuab (anti-TNF-α)

- Uses: RA , UC, Crohn's
- Rationale: TNFα released by T_{H1}, blocked by adalimuab

- Results:
  - Observational studies all by one group
  - One non-randomized controlled trial (not RIF but "T_{H1}/T_{H2} elevation")
  - Multiple treatments used
    - ASA, IVIG, heparin, dex
  - Generally report improved IR, CPR, LBR

- Problems
  - Testing to Dx/stratify patients poorly defined and not routinely used
  - Very small sample sizes
  - Extensive heterogeneity in treatments and patients
  - Long term risks of adalimuab include infection and malignancy

- Needs:
  - Well-designed studies

- Should only be used in IRB approved studies

Winger EE et al Am J Reprod Immunol 61:113-120,2009
Hviid MM, Macklon N Fertil Steril 107: 1284-93, 2017
ASRM Fertil Steril 110 387-400-2018

Intralipids

- Fat emulsion made from soybean oil, glycerine and egg phospholipid
- TPN
- Proposed mechanism: decrease pNK activity

- RCT 2016
  - N 296
    - Unexplained infertility and RPL
    - Improved LBR (37,5% v. 22.4%)
  - Not in RIF population
- Prospective study (RIF and RPL patients) stopped because of no pregnancies in treatment group

Dakhly DM et al Int J Obstet Gynecol 1235L324-7, 2016
Check JH Clin Exp Obstet Gynecol 43:14-5, 2016

Granulocyte Colony Stimulating Factor (GCSF)

- Glycoprotein stimulating mobilization and migration of stem cells
- Implantation: suppression of immune response (lymphocytes, macrophages, T_{H2})
- Appears to improve idiopathic thin endometrium
  - (OR 0.47, -1.36—2.31)
- May help endometrial thickness after LOA
  - 5.5 mm→7.9mm
  - But not on adhesion recurrence (42.5% v. 38.5%)
- May Improve Pregnancy Outcome
- Natural conception after LOA (n=82 each)
  - 62.3% v. 50%
  - OR 0.609 (0.33-1.13)- NS
- RIF-IVF
  - CPR:40% v. 16%
  - OR 2.51 (1.36-4.63)
  - Problems:
    - Abstracts
    - Small numbers
    - No PGT
- RIF-OD- RCT
  - N=105 (52 cont, 53 rx)
  - SQ 0.3 mg/kg/d (day prior to ET to hCG)
  - LBR 67.9% v. 28.8% (p <0.00001)
  - Higher levels of periph T_{reg}

Kamath MS Et al Europ J Obstet Gynecol Reprod Biol 214:16-24, 2017
Sbracia M, Scarpellini F. Fertil Steril 120, 4, Suppl, P328, 2023
Zhang Y et al Hum Reprod 37:725-33, 2022

Peripherial Blood Leukocyte Injections

Pourmagahdan et al J Reprod Immunol 137:103077, 2020

- PBMC- consists of monocytes, T & B lymphocytes
- Rationale: provide the “initial inflammation” to enhance implantation
- Injected into uterus prior to ET

- Meta-analysis of RIF
  - 5 studies (1RCT)
- Improved outcomes
  - IR 14.3% v. 6.8%
    - OR 2.47 (1.31-4.67)
  - LBR 48.5% v. 21.3%
    - OR 3.57 (1.99-6.40)
  - SAB 19.4% v. 37.7%
    - OR 0.42 (0.23-0.77)

Problems:

Small numbers
High heterogeneity- patients, preps, protocols
No Mock rx
No PGT

Immune Factors: Conclusions

- Original focus was on markers in blood and quick solutions
- The immune therapies have failed because the tests have shown weak or no predictive value due to poor study design and great patient heterogeneity
- Studies on immunomodulation therapies are numerous but heterogeneous in
  - Design
  - Method of intervention
  - Study population
  - This makes them difficult to interpret and design an evidence based rational therapy strategy
- None of the studies are using euploid embryos
- While peripheral changes in pNK and TH1/TH2 can be noted, it is unclear if uNK are actually altered

Alecsandru A, Garcia Velasco, in Recurrent Implantation Failure, p 93-102, 2018
Hviid MM and Macklon N Fertil Steril 107:1284-93, 2017

Network Meta-analysis of 36 Therapies for RIF

He Y, et al. JARG 40:2343-2356,2023

- Network meta-analysis - comparing three or more interventions simultaneously by combining both direct and indirect evidence across a "network" of studies
- Objective: investigate effectiveness and safety of 36 different therapies for RIF
- 154 studies included
  - 74 RCTs + 80 others
  - 29,906 RIF patients
  - Only 10% high quality, with majority low quality

Results	Most Effective treatment on…	OR (CI)
IR	GH	3.32 (1.95-5.67)
CPR	IVIG + PBMC	5.84 (2.44-14.1)
LBR	Hyaluronic Acid	12.9 (2.37-112)
SAB	ASA + GC	0.208 (0.049-0.78)

GH- Growth Hormone
IVIG-intravenous immunoglobulins
PBMC-peripheral blood mononuclear cells
ASA-aspirin
GC-glucocorticoids

140 pages of supplemental information

Lifestyle Changes

ASRM Fertil Steril 117:53-62, 2022

Women	Mechanism	Recommendation
Alcohol	Studies mixed Decreased interval to conception with wine	Avoid ETOH or decrease use (< 2 drinks/d)
Smoking	Decreases E2 Increases oocyte depletion More prone to aneuploidy	Smoking Cessation Avoid Second-hand Smoke
Diet	Decreases risk of infertility (OR 0.34, 0.23-0.48)	Increase: monounsaturated fats, vegetable proteins, low gylcemic CHO
Marijuana	Alter oocyte development and gene expression Possible impact on fetal brain development	avoid
Caffeine	➢ 5 cups decreases fertility ➢ 3 cups increases SAB	Avoid or 2 cups or < / d
Environmental Toxins	DNA Damage Endocrine Disruptors	avoid
Weight	Extremes of either problematic	Maintain normal weight

Men	Mechanism	Recommendation
Alcohol	ROS Testicular pathology ED	Avoid ETOH or decrease use
Smoking	ROS Altered SA Sperm DNA damage	Smoking Cessation Avoid Second-hand Smoke
Testicular Heat Stress	Impairs spermatogenesis Sperm DNA damage	Avoid Wet Heat to groin area
Varicocoele	ROS Sperm DNA Damage Heat stress	Consider varicocoelectomy
Abstinence Time	Prolonged leads to oxidative sperm DNA damage	Consider shorter Abstinence times (1-2d)
Environmental Toxins	Sperm DNA Damage	Avoid
Weight	Altered metabolism Increased Scrotal Heat CVD	Maintain normal weight

Checklist

- Check the lab (how good are we really?)
- Check the physicians
  - Appropriate treatment choices?
  - ET technique
- Check the patient
  - Are meds being taken appropriately?
  - Clean up lifestyle
  - Optimize hormonal environment
- Check the embryos (PGT-A)
- Check the uterus
  - Endometrium
    - Thickness
    - Freeze-all?
  - Structural
    - H/S, S/S, HSG
  - Biochemical
    - EB: Endometritis, specific markers?

Conclusions

- RIF is frustrating for patients and physicians
- There is significant pressure to “do something”
- Human reproduction is inefficient
- Most of the problems are embryonic and true RIF is infrequent
- We must be thorough
  - In our evaluation
  - In our techniques
  - In our labs

Conclusions

- Immune / Reproductive interactions are very important and clearly there can be problems that lie at this level
- The immune interactions at implantation are complex and involve more than one or two cell types
- There really is not sufficient data to suggest that “immune balance” is a primary etiology for RIF
- Immune testing is complex, poorly standardized
- Impressive lack of good quality evidence to either support or refute the efficacy of these treatments

Conclusions

- With reproductive immunology there is a tremendous need
  - For better understanding
  - Develop testing that is reliable and predictive
  - Treatments that address the abnormalities and provide actual benefit
  - All of this comes through well designed studies
- Intervening *without* robust medical evidence requires discussion of :
  - There is no consensus diagnosis
  - Lack of consensus of the evidence
  - Risks
  - Costs
  - While most MD's are genuine, we have all seen the predatory nature of some

References- some places to start…

- Cedars MI et al. Recurrent implantation failure: reality or a statistical mirage: Consensus statement from the July 1, 2022 Lugano Workshop on recurrent implantation failure. Fertil Steril 120: 45-59, 2023
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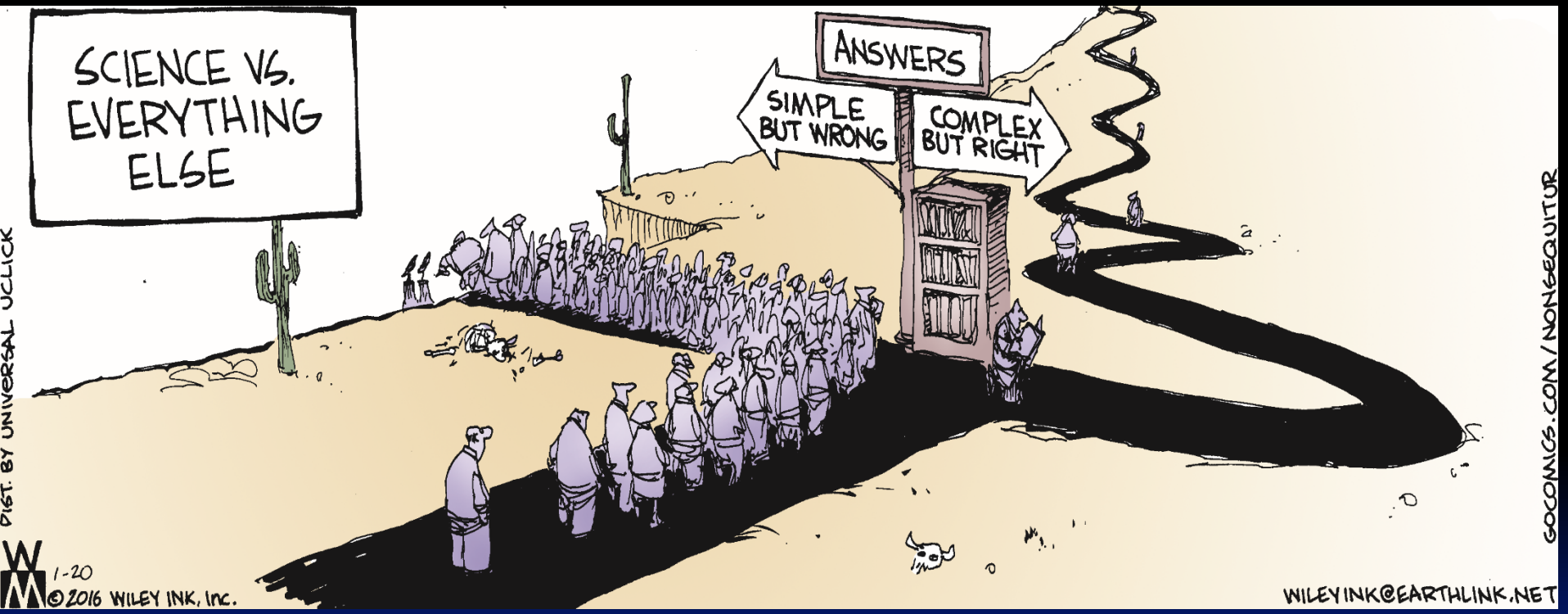
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