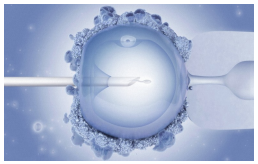
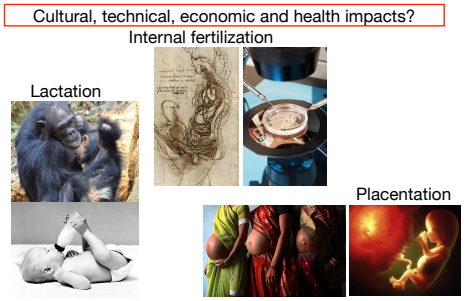
17. Assisted Reproduction Technologies ART



ANBI 116 Evolution of Sex –
Pascal Gagneux

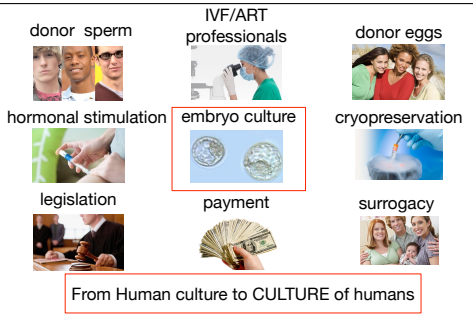
March 3, 2022

Hallmarks of mammalian reproduction



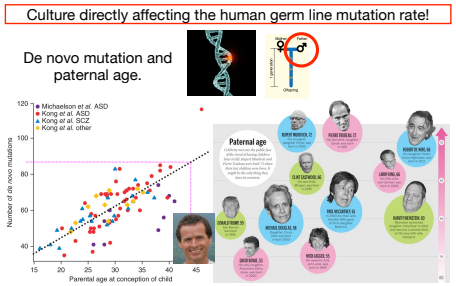
Mammalian reproduction: internal fertilisation, gestation, lactation
Human culture and technology: artificial insemination, IVF, egg donation, surrogacy, wet nursing, baby formula
Practice question: which aspect of human reproduction was first replaced by human technology?
Lactation, infants could be wet-nursed or fed with milk from other mammals.

The reproduction technology niche



Most recent third, fourth and fifth party involvement: sperm, donors, reproductive medical teams, egg donors, surrogate mothers, lawyers.
Practice question: How can gametes and even embryos be frozen without damaging the fragile cell membranes? They are frozen in special freezing media that prevent the formation of crystals, that would damage the lipid membranes.

Paternal age and germline mutation rate



Sequencing the whole genome (all 2 times 3 billion letters of the entire diploid genome) of parent child trios allows the measurement of total number of de novo mutations that a child inherits from both parents.

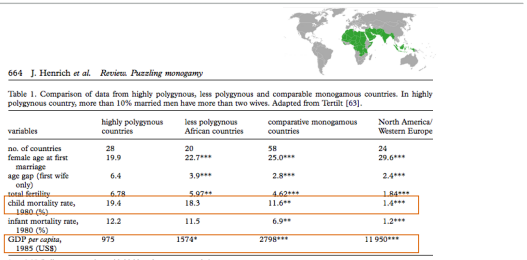
Practice question: How is it possible to measure de novo mutations in a child?

By sequencing the genome of both parent and the child (trio).

The father, especially if he is older than 30 year old. Practice question: Who does a baby inherit more de novo mutations from, the father or the mother?

The father, especially if he is older than 30 year old.

The spread of monogamy - leveling reproductive opportunities?



664 J. Henrich *et al.* *Review. Puzzling monogamy*

Table 1. Comparison of data from highly polygynous, less polygynous and comparable monogamous countries. In highly polygynous country, more than 10% married men have more than two wives. Adapted from Tertilt [63].

variables	highly polygynous countries	less polygynous African countries	comparative monogamous countries	North America/ Western Europe
no. of countries	28	20	58	24
female age at first marriage	19.9	22.7***	25.0***	29.6***
age gap (first wife only)	6.4	3.9***	2.8***	2.4***
total fertility	6.78	5.97**	4.62***	1.84***
child mortality rate, 1980 (‰)	19.4	18.3	11.6**	1.4***
infant mortality rate, 1980 (‰)	12.2	11.5	6.9**	1.2***
GDP *per capita*, 1985 (US$)	975	1574*	2798***	11 950***

*$p < 0.05$ (indicates comparison with highly polygynous countries).
**$p < 0.01$.
***$p < 0.001$.

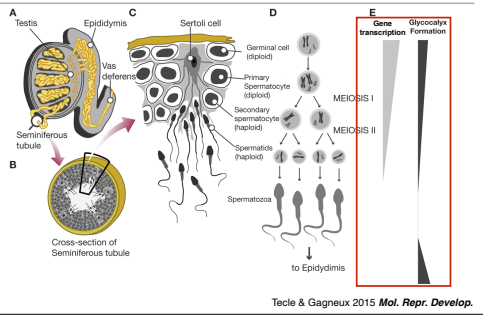
Henrich, Boyd and Peterson ***Phil. Tran R Soc. B*** *2012*

The richer a country and the lower its child mortality, the more likely that monogamy is the rule.

Practice question: What is the relationship between wealthy societies, child mortality and monogamy?

Wealthier societies with lower child mortality are more likely to have laws enforcing monogamy.

Spermatogenesis (74 days plus 6 in epididymis)



Tecle & Gagneux 2015 ***Mol. Repr. Develop.***

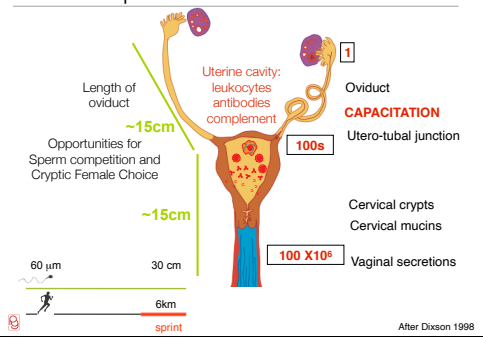
epididymis 20 ft or 7 m in humans

transit time 2 to 6 days in humans

Practice question: How can sperm maturing in the epididymis continue to modify their surfaces if they do not express any genes any more at this stage?

The cells lining the epididymis secrete glycoconjugates that can insert themselves in the sperm membrane “painting” the sperm surface.

Primate female reproductive tract



After Dixson 1998

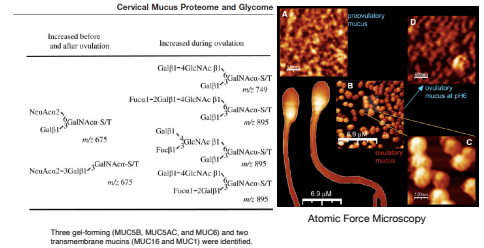
Practice question: What is cryptic female choice?

Cryptic female choice refers to selection of sperm by the female after she was inseminated and before sperm meet the egg.

Where does natural fertilization take place in the female reproductive tract?

The ampulla (upper most part of the fallopian tube). On the side where ovulation occurred.

Cervical Mucins during cycle



Atomic Force Microscopy

Andersch-Björkman et al., ***Molecular & Cellular Proteomics*** (2007)

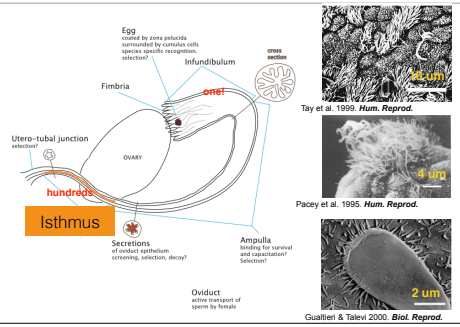
Brunelli et al., ***FASEB J.***(2007)

Different structures of human cervical mucus during the menstrual cycle. Tapping mode Atomic Force Microscopy images of: preovulatory mucus (A), ovulatory mucus (B) with a detail enlarged (C); ovulatory mucus at pH 6 (D). Two spermatozoa are also imaged (left side of the image) at the
same magnification as the ovulatory mucus (B) to facilitate comparison.
Hyaluronan plug during pregnancy blocks the cervix completely.

Practice question: how does cervical mucus (a glycoprotein) change during ovulatory phase?

The composition and length of sugar chains attached to the core protein of the mucus glycoproteins change, altering the stiffness of the mucus and creating pores for sperm to pass through.

Close Scrutiny: Isthmus Sperm Reservoir



Tay et al. 1999. ***Hum. Reprod.***

Pacey et al. 1995. ***Hum. Reprod.***

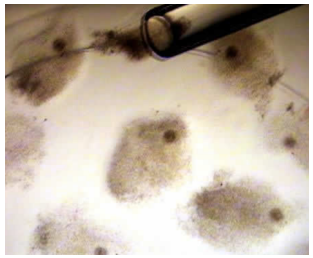
Gualtieri & Talevi 2000. ***Biol. Reprod.***

Sperm are closely inspected by the female in the lower oviduct.

Practice question: What happens to the tiny minority of inseminated sperm that reach the lower part of the fallopian tube?

They attach to the cell membranes of the female tract and undergo capacitation.

Cumulus: hyaluronan rich matrix



Advanced Fertility Center of Chicago

Practice question:

What is the vestment surrounding an ovulated human egg in vivo?

A thick matrix of hyaluronan (a polysaccharide) secreted by cumulus cells that are also present in the vestment.

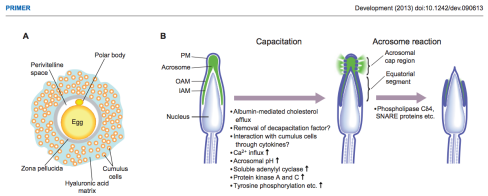
What happens to the vestment of human eggs used for ART?

It is digested away with enzymes in the laboratory prior to IVF or ICSI.

Gamete glycocalyces

Egg and Sperm have special glycocalyces.

Egg Zona Pelucida



Okabe, M ***Development*** (2013)

The glyccalyx is the “sugar coat” coating every living cell. Capacitation of sperm prepares sperm for subsequent acrosome reaction. This “blowing of a gasket” exposes important enzyme and receptors allowing the sperm to advance towards the egg and bind to its membrane.

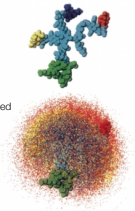
Practice question: What is the Zona Pellucida?

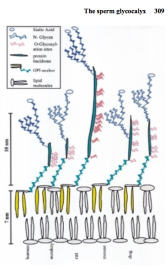
A complex layer of glycoproteins that forms a species-specific molecular pattern surrounding oocytes.

Sperm Glycocalyx

CD52 protein

CD52 protein with attached sugar chains (glycans)





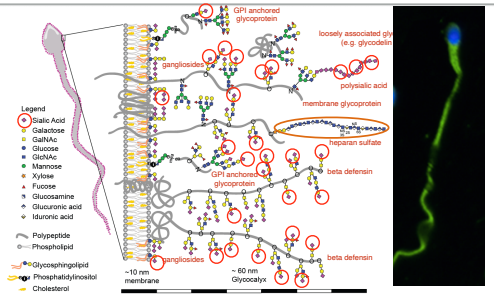
Schröter, S, *et al.* ***Human Repr. Update .*** (1999)

CD52 glycosylation. Dynamic molecular modeling a glycoprotein called CD52 without any sugar chains/glycans (above left) or with the glycans (below left). The sperm glycocalyx is more than three times as thick as the membrane itself.

Practice question: What is the sperm glycocalyx?

The thick glycan shield coating each sperm cell.

The mammalian sperm glycocalyx

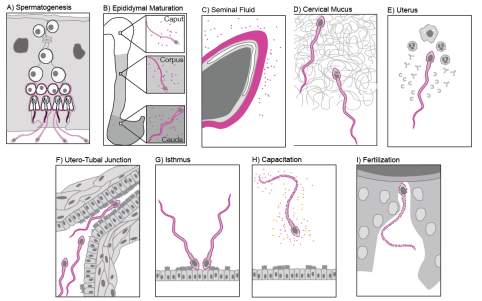


Tecle & Gagneux 2015 ***Mol. Reprod. Devel.***

Schematic diagram of a sperm cell surface. Many of the glycans on the surface of sperm have a sialic acid (magenta diamond symbol) as the last sugar of each chain.
The microphotograph shows a chimpanzee sperm stained for silica acids with a fluorescent antibody, the blue stain shows DNA in the head.

Practice question: which genes are expressed in mature sperm?
None, there is no active gene expression in mature sperm.

Functions of the Sperm Glycocalyx



Tecle & Gagneux 2015 ***Mol. Repr. Develop.***

the sugar coat of sperm has many important functions during fertilization.
Practice question: Can you list two or more functions of the sperm glycocalyx?
1.allowing sperm to penetrate cervical mucus;
2. protecting sperm from female immune cells;
3. allowing interaction between the sperm and the female oviduct

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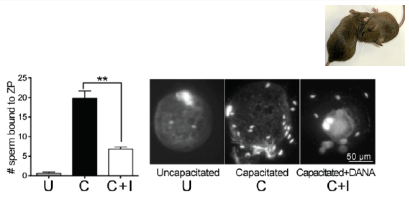
Effect of Sialidase* inhibition on
egg zona binding by mouse sperm



Ma *et al.* ***JBC 2012***

Sialidase*: enzyme that cleaves sialic acid, a sugar that coats most of the cell surface

Experimentally blocking the function of sialidase a sperm enzyme, that can cleave terminal sialic acids during capacitation (a process resulting from exposure of sperm to the female reproductive tract and necessary for sperm to fertilize the egg) interferes with sperm function and fertilization. The inhibitor used was the drug (DANA).
Practice question: What does sialidase enzyme do on sperm?
The enzyme cleaves terminal sugars (squalid acids) coating sperm when sperm get ready to meet the egg during capacitation.

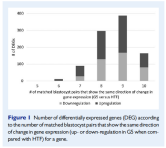
Assisted Reproductive Technologies | ART

Intrauterine Insemination (IUI): millions of sperm
Ovarian stimulation and egg harvesting
In vitro fertilization (IVF): millions of sperm
Gamete freezing
Intracytoplasmic sperm injection (ICSI): **one sperm**
Donor Sperm
Donor Egg (hormonally induced superovulation)
Embryo Culture
Embryo Freezing
Surrogate Mothers
Three parent child
Embryo editing

Practice question: How could an IVF child end up with three parents?
The nucleus of the egg of an elder woman can be implanted into the egg of a younger woman, from which the uncles was artificially removed. The mitochondria of that egg come from one woman, the nuclear DNA (genome) from another, and the sperm genome from a third parent.

Peanut butter

Lack of regulation for embryo culture media.
EU regulates peanut butter more strictly.
Effects of embryo culture medium on infant development and health?
HTF vs G5 media: effects on differential gene expression in day 6 blastocysts:

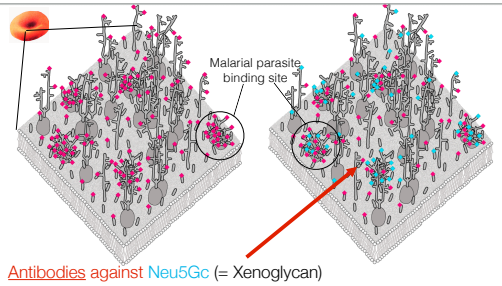


HTF vs G5 media: effects on birth weight!

Kleijkers *et al.* ***Hum. Reproduction*** (2015)
Evers ***Hum. Reproduction*** (2016)
Sunde *et al.* ***Hum. Reproduction*** (2016)
Kleijkers *et al.* ***Hum. Reproduction*** (2016)

Media in which human embryos are cultured for several days have demonstrable effects on gene expression.
Practice question: Which is more strictly regulated in the European Union: peanut butter or human embryo culture medium?
Peanut butter.

Human Glycocalyx underwent a watershed event:
Loss of **Neu5Gc** and excess of **Neu5Ac**



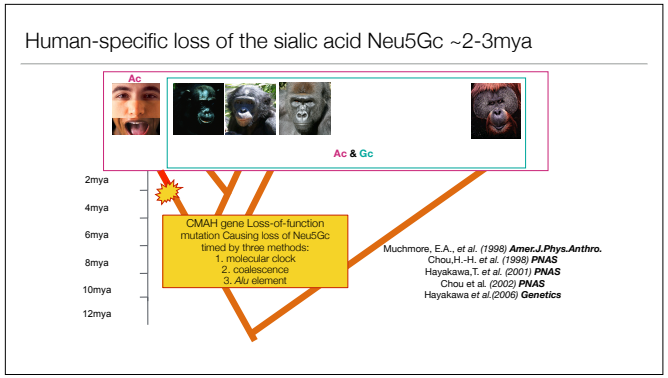
Altman & Gagneux, modified from Viitala & Järnefelt, 1985

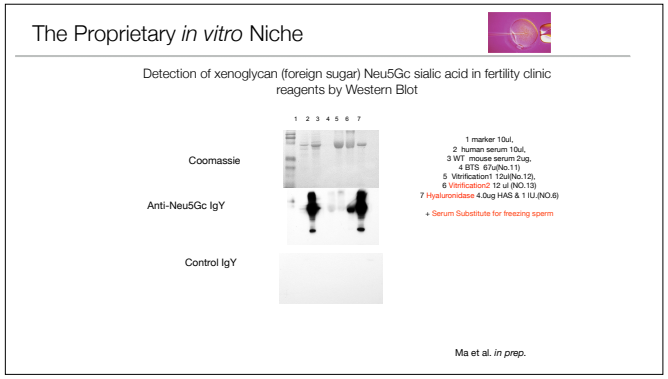
Two major differences between humans and most other mammal species:
More Neu5Ac, No Neu5Gc – shaped by what? Pathogens?

Humans thus have cells with a unique sialic acid profile among mammals (the glycocalyx of most cells differs!

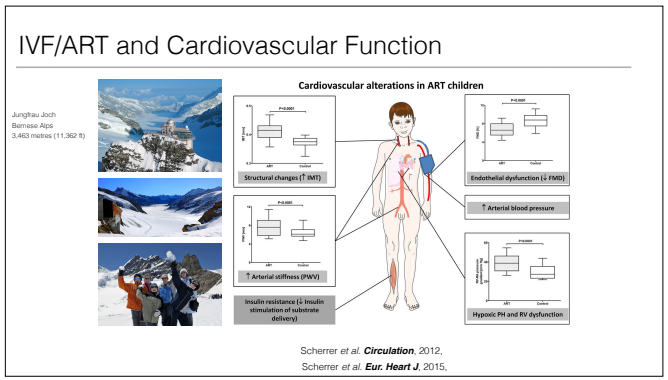
No Gc and more Ac a difference of hundreds of millions of molecules per cell.

Humans have lost the capacity to modulate Gc/Ac composition, which most other mammals may still do in response to specific pathogen pressure.





Reagents used to treat human eggs and freeze human embryos contain non-human sugars that can be immunogenic to the mother!

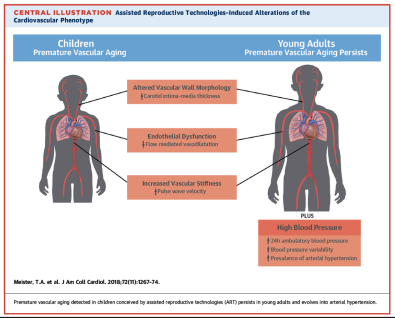


Assisted reproductive technology-induced cardiovascular alterations and consequences thereof in young apparently healthy children. Assisted reproductive technology alters systemic and pulmonary vascular function. There is evidence that systemic vascular dysfunction translates into increased arterial blood pressure and contributes to insulin resistance, whereas pulmonary vascular dysfunction predisposes to exaggerated hypoxic pulmonary hypertension and right ventricular dysfunction. IMT, carotid intima-media thickness; PWV, pulse wave velocity; FMD, flow-mediated dilation; PH, pulmonary hypertension; RV, right ventricle.

Practice question: What experiment was used to test for potential effects of IVF on vascular functions in a large group of children?

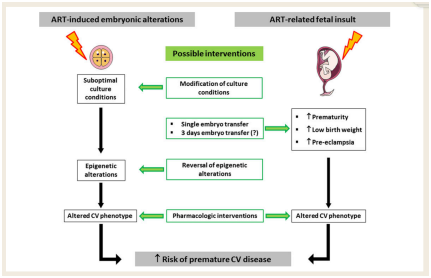
A trip to a high altitude location in the Swiss Alps that included a cardio vascular test panel on location.

IVF/ART and Cardiovascular Function



Assisted reproductive technology-induced cardiovascular alterations appear to lead to altered cardio-vascular aging in young adults.

IVF/ART and Cardiovascular Function

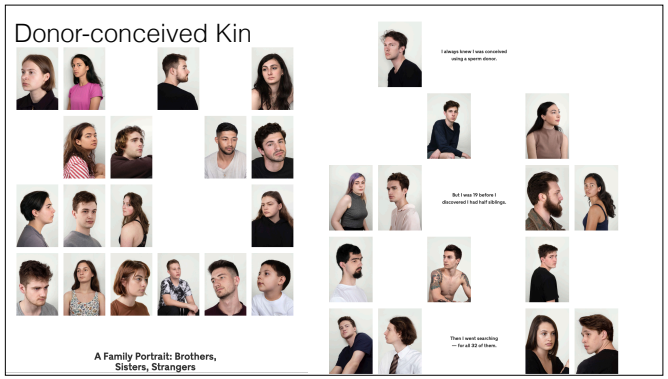


Scherrer *et al.* ***Eur. Heart J***, 2015.

Mechanisms underpinning assisted reproductive technology-induced alteration of the cardiovascular phenotype and possibilities for prevention and/or intervention. Assisted reproductive technology alters the cardiovascular phenotype and increases cardiovascular risk in two ways.
(i) Suboptimal culture conditions cause epigenetic changes in the embryo that result in an altered cardiovascular phenotype. Improve- ment/modification of culture conditions may attenuate assisted reproductive technology-induced epigenetic alterations and administration of drugs that restore epigenetic changes reinstate a normal cardiovascular phenotype.
(ii) Assisted reproductive technology increases the prevalence of pathological

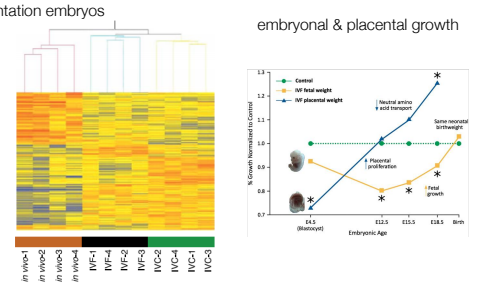
events during the foetal period which are known to increase cardiovascular morbidity and mortality in naturally conceived persons. Single embryo transfer is expected to eliminate the additional risk of foetal insults related to multiparity, implantation of 3-day embryos appears to be associated with a lower risk of foetal insults than blastocyst transfer. Finally, pharmacological interventions aimed at restoring a normal cardiovascular phenotype are expected to decrease ART-related cardiovascular risk in the offspring.

Practice question: How are assisted reproductive technologies a potential risk for “sneaky fucking”?
Men involved in the industry can use their own sperm for fertilizing eggs.



One Photographer made it his mission to take a picture of all his 31 donor conceived half-siblings.

Effect of IVF on gene expression and development in mice

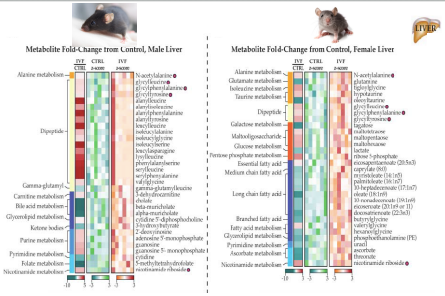


Giritharan *et al.* **Reproduction** (2007)

Feuer *et al.* ***Molec. Hum. Reproduction*** (2013)

Both IVF and *in vitro* embryo culture change the gene expression patterns of hundreds of genes in laboratory mice.
Despite resulting in comparable birth weight, both the placenta and the fetus differ in developmental trajectories of their weight!!

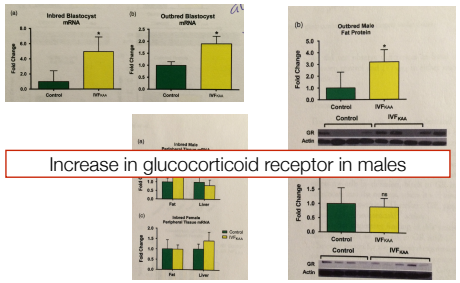
Sexually Dimorphic Effect of IVF on Adult Mouse Fat and Liver Metabolomes



Feuer *et al.* **Endocrinology**, November 2014.

Effects of IVF on the metabolic functions of the liver in lab mice.

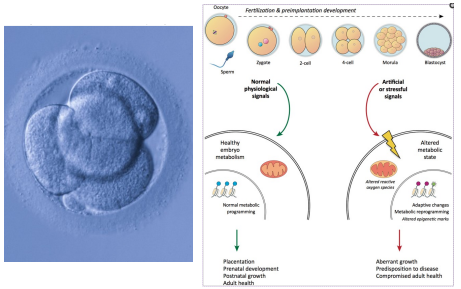
Glycocorticoid receptor in male fat tissue



Simbulan *et al.* ***J. Dev. Origin Health Disease*** (2016)

Prenatal development is highly plastic and readily influenced by the environment. Adverse conditions have been shown to alter organ development and predispose offspring to chronic diseases, including diabetes and hypertension. Notably, it appears that the changes in glucocorticoid hormones or glucocorticoid receptor (GR) levels in peripheral tissues could play a role in the development of chronic diseases. In vitro fertilization (IVF) and preimplantation embryo culture is associated with growth alterations and glucose intolerance in mice.

Effects of the *in vitro* niche on imprinting?



Feuer *et al.* ***Molec. Hum. Reproduction*** (2013)

Suboptimal preimplantation environments may irreversibly alter developmental competence. During fertilization and preimplantation development, embryos respond to the environment (growth factors, nutrient availability) by fine-tuning gene expression networks, metabolic pathways and epigenetic marks. Aberrant or suboptimal signals may alter the intracellular metabolic states, leading to permanent adaptations to gene expression and the establishment of altered metabolic pathways and epigenetic marks. This will affect prenatal development and post-natal growth, with deleterious consequences for adult fitness.

Practice question: why could exposure to artificial culture media profoundly affect embryo development?

Because it happens in the first few days after fertilization, when embryos strip and re-establish DNA imprinting.

Imprinting changes associated with ART

Human Molecular Genetics, 2009, Vol. 18, No. 20 3769–3778
doi:10.1093/hmg/ddp319
Advance Access published on July 15, 2009

DNA methylation and gene expression differences in children conceived *in vitro* or *in vivo*

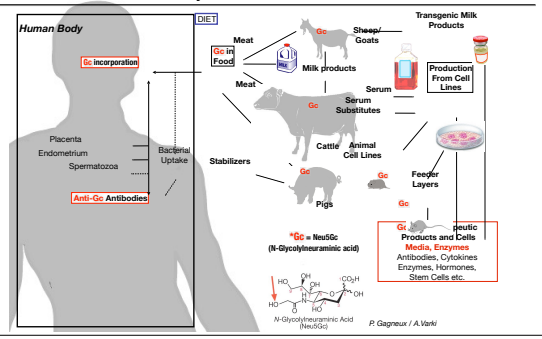
Sunita Katari[1†], Nahid Turan[1†], Marina Bibikova[2], Oluwatoyin Erinle[1], Raffi Chalian[3], Michael Foster[3], John P. Gaughan[4], Christos Coutifaris[3] and Carmen Sapienza[1,5,*]

[1]Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140, USA, [2]Illumina Inc., San Diego, CA 92121, USA, [3]Department of Obstetrics & Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Philadelphia, PA 19104, USA, [4]Biostatistics Consulting Center, Temple University School of Medicine, Philadelphia, PA, USA and [5]Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA, USA

Received May 7, 2009; Revised June 18, 2009; Accepted July 9, 2009

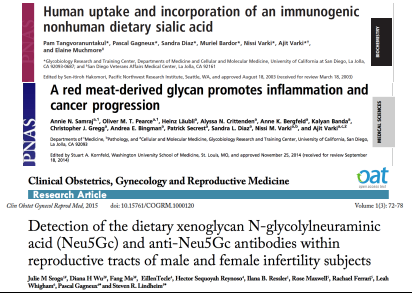
The current study provides support for the hypothesis that ART or associated subfertility may be
associated with genome-wide changes in DNA methylation, and these changes appear to be, at least in part, due to epigenetic instability in ART pregnancies. Further studies are required in order to determine the extent to which such ART-related epigenetic instability may have phenotypic consequences.

Contamination of Humans by Gc*:



The non-human sugar Neu5Gc, a silica acid not naturally made by humans but present at high levels in red meat (pork, beef and sheep/goat) can get into the human body via food and then gets incorporated into human cells. Once incorporated into glycolipids or glycoproteins of human cells and tissues, it can trigger autoimmune reactions.

Modern H. sapiens take up Neu5Gc in red meat and are immunized by this foreign sugar



Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid

Pam Tangvoranuntakul*, Pascal Gagneux*, Sandra Diaz*, Muriel Bardor*, Nissi Varki*, Ajit Varki*†, and Elaine Muchmore‡

A red meat-derived glycan promotes inflammation and cancer progression

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Clinical Obstetrics, Gynecology and Reproductive Medicine

Research Article

Detection of the dietary xenoglycan N-glycolylneuraminic acid (Neu5Gc) and anti-Neu5Gc antibodies within reproductive tracts of male and female infertility subjects

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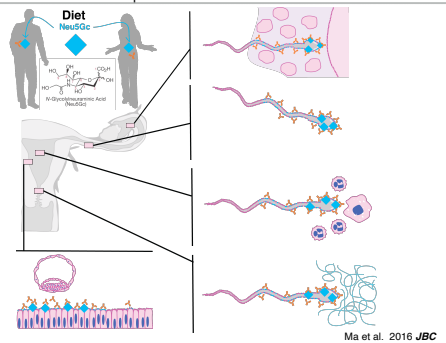
Xenosialitis in Human Reproduction



Ma et al. 2016 ***JBC***

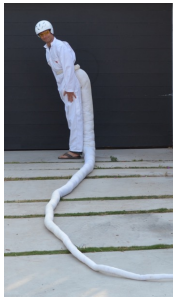
Xenosialitis: inflammation caused by the presence of a non-human silica acid from the diet incorporated into human tissues and secretions. On sperm, the presence of this non-self sugar can cause antibodies of the male or the female to target sperm and interfere with their survival and function, one the cells lining the users (endometrium) the presence of this foreign sugar can cause inflammation that negatively affects implantation and or pregnancy.

Summary

- ART is a logical extension of human niche construction and tendency for leveling of reproductive opportunities.
- Female QC exists, but is poorly understood.
- ART bypasses female screening of sperm and paternal immunological priming.
- ART exposes gametes and embryos to bio-contaminants.
- IVF and embryo culture affect mouse development and human embryonal gene expression.
- ART is associated with increased perinatal risk: twinning, preterm, C-section, pre-eclampsia, sex ratio…
- ART is associated with increased pediatric risk: imprinting disorders, cardiovascular dysfunction….

Large scale experiment is ongoing regarding longterm effects.

Thank You !



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