Assisted reproduction treatment and epigenetic inheritance

Part I

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Introduction

- Some genes from babies conceived by IVF show a ***gene expression pattern*** different from naturally conceived children.

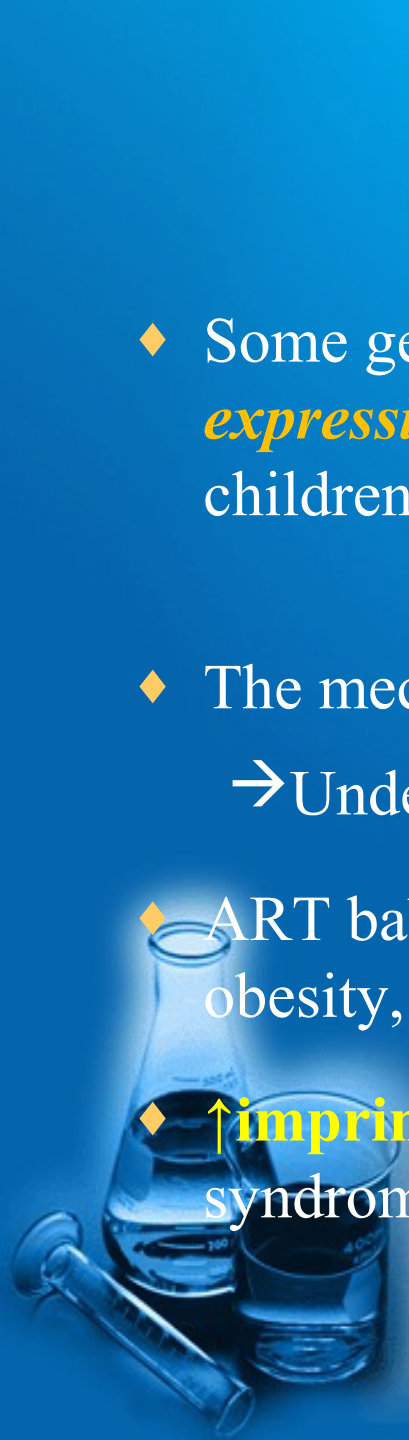
Katari et al., 2009

- The mechanism that switches genes on and off ??

  →Under ***epigenetic control***

- ART babies at a greater risk of diseases, such as diabetes and obesity, later in life

- **↑imprinting disorders**, especially Beckwith-Wiedemann syndrome (BWS)



Mechanism of inheritance

- **Classical Mendelian inheritance**
- **Transgenerational epigenetic inheritance**
  - Phenotypic alteration caused by transfer of chromosome / chromatin modifications through gametes
  - Proved in organisms ranging from bacteria and plants to the mouse and humans

Epigenetic mechanisms

- **DNA methylation**
- RNA-mediated chromatin modification
- Histone modifications and histone variants
- Other…organization of nuclear structure including chromosome replication behavior

Epigenetics

- Def.: the study of the process that underly developmental plasticity and canalization and that bring about persistent developmental effects in both prokaryotes and eukaryotes
- Important in **embryogenesis** and **cell differentiation**
  - Since all cells of an organism have the same genotype, epigenetic marks are deposited to alter transcription and achieve cell-type specific gene expression patterns in different tissues
- Sex-specific genomic imprinting and stable female X-inactivation – under epigenetic control

Epigenetically crucial phase

- Between generations, the germ line is subjected to two distinct **reprogramming events** …
  - Primordial germ cells (PGCs)
  - Preimplantation embryo ------- **ART**
- In order to prepare the cells for pluri- and toti-potency and down-regulate the inheritance of epigenetic information between generations

Epigenetic inheritance

- Imprinting disorders: some loci escaping reprogramming in the early embryo
- Epigenetic marks generally thought to be stable through rounds of somatic mitosis
- Careful balance between somatic maintenance of epigenetic marks and dynamic reprogramming in the germline → ***soft inheritance***
  - A more pliable system of inheritance, allowing organisms to quickly adapt to fluctuations in nutrition, predation or disease

The questions ??

1. If the conditions during gametogenesis and *in vitro* phases intrinsic to **ART** could elicit epigenetic effects ?

1. If the assumed epigenetic effects of ART can be **transmitted to the next generation** ?

Outlines

- **Epigenetic inheritance and germline reprogramming**
  - Mitotic inheritance of epigenetic marks
  - Reprogramming the Genome towards Totipotency
  - Transgenerational epigenetic inheritance
  - Stress, Hormone, and Nutrition Induced Transgenerational Epigenetic Variation
- **Epigenetic effects of ART**
  - Studies on mice designed to evaluate epigenetic and physiological aspects of ART
  - Epigenetic aspects of ART
- **Conclusions**

Methods

- **Literature databases (Pubmed, Medline)**
  - Trans-generational epigenetic inheritance
  - Epigenetic effects
  - ART

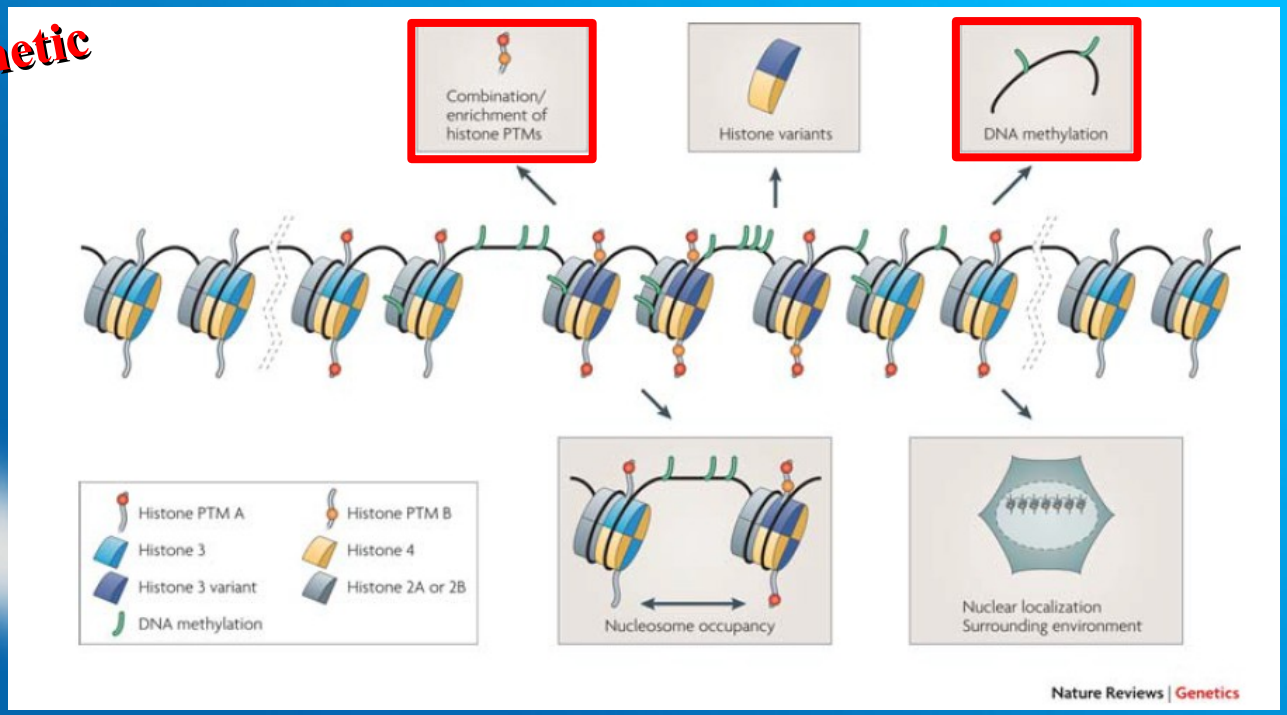
Epigenetic inheritance and germline reprogramming

- Mitotic inheritance of epigenetic marks
- Reprogramming the Genome towards Totipotency
- Trans-generational epigenetic inheritance
- Stress, Hormone, and Nutrition Induced Transgenerational Epigenetic Variation

Mitotic inheritance of epigenetic marks

- fundamental unit of Chromatin: nucleosome → 147 bp of DNA wrapped around a histone octamer containing two duplicates of H3, H4, H2A or H2B
- The degree of chromatin packing is dynamically regulated,
  - Heterochromatic: densely packed chromatin, transcriptionally repressed
  - Euchromatic: accessible chromatin, transcriptionally active

CONCEPT 1: Chromatin and epigenetic mechanisms



DNA methylation

- Occurs at CpG sites → 5methyl CpG
  - Gene inactivation, formation of heterochromatin
  - in mammals, by two families of DNA methyltransferases (DNMTs)
    - ***De novo* activity** (DNMT3)
    - Predominantly **maintenance** (DNMT1) **activity**
  - No demethylases described
- Tel 1,2,3 family of enzymes present in PGCs convert 5methyl CpG into 5hydroxymethyl CpG
  - Active in male pronucleus of the zygote
  - Epigenetic effect → ***under investigation***

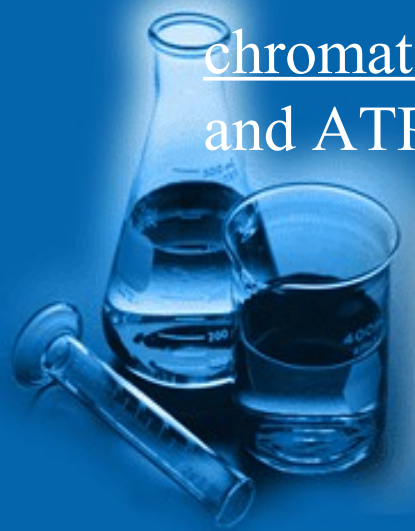
Histone tail modifications

- Methylation **- Acetylation**

- Phophorylation **- Ubiquitination**

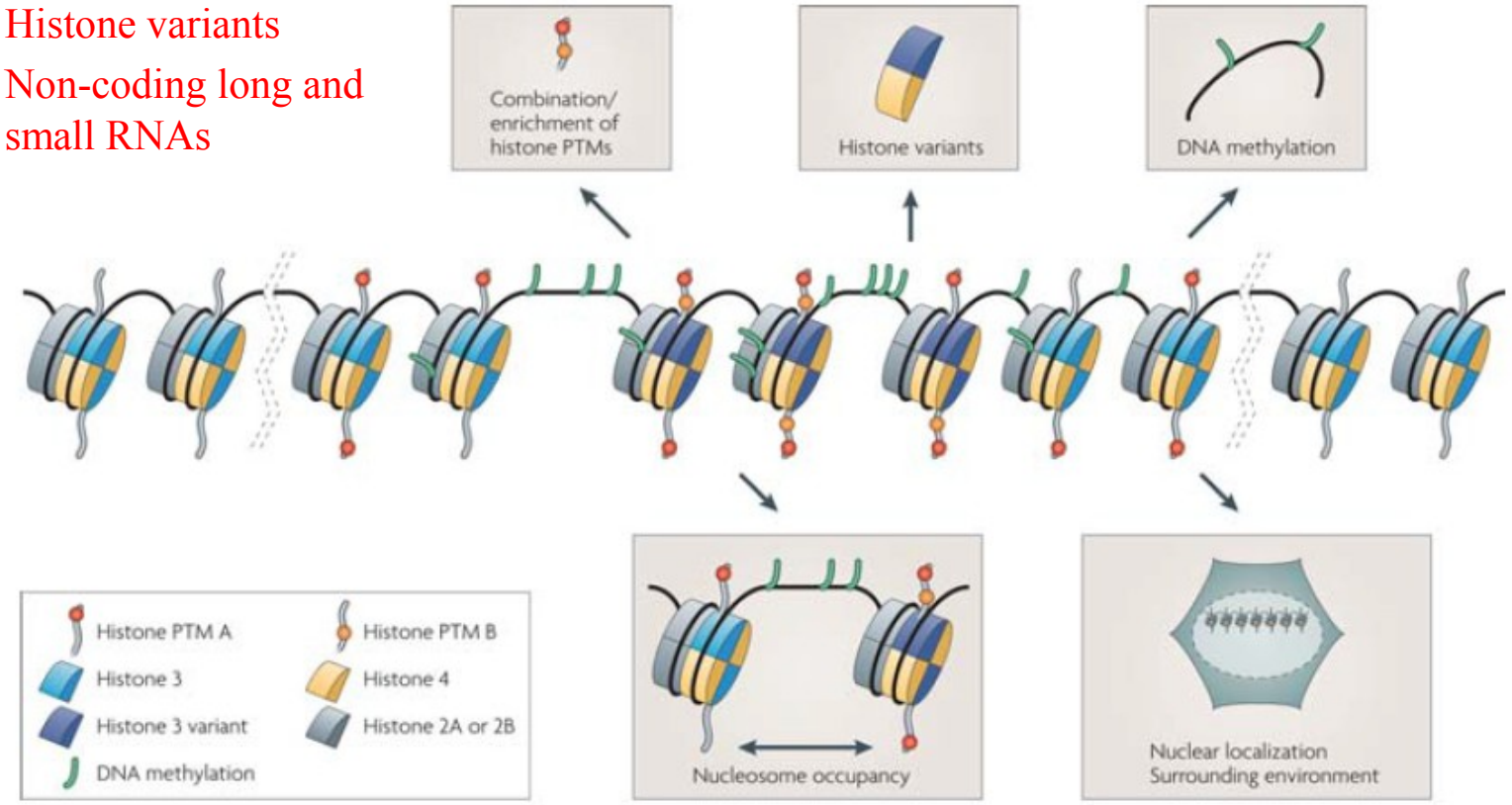
- Sumoylation: SUMO protein (small ubiquitin-like modifier)

- more dynamic than DNA methylation
  - Deposited and removed by a variety of enzymes
  - Both repressive and activating
- Exert influence either by directly changing structure of chromatin or via recruitment of chromatin-binding factors and ATP dependent chromatin-remodeling complexes



Chromatin and epigenetic mechanisms

- Histone variants
- Non-coding long and small RNAs



- Structure aspects of chromatin domains
  - Chromatin compaction
  - Nucleosome occupancy
  - Localization inside the nucleus

- The epigenetic chromatin state tightly linked to transcription.
- As cell differentiation, they acquired tissue-specific patterns of DNA methylation, histone modifications and other epigenetic chromatin marks.
- Mitotic transmission of epigenetic marks is observed throughout somatic cellular development.

- To enable replication of DNA during S-phase of the cell cycle → chromatin structure severely disrupted → partial loss of epigenetic marks
  - *Special mechanisms needed to ensure mitotic propagation of epigenetic information*

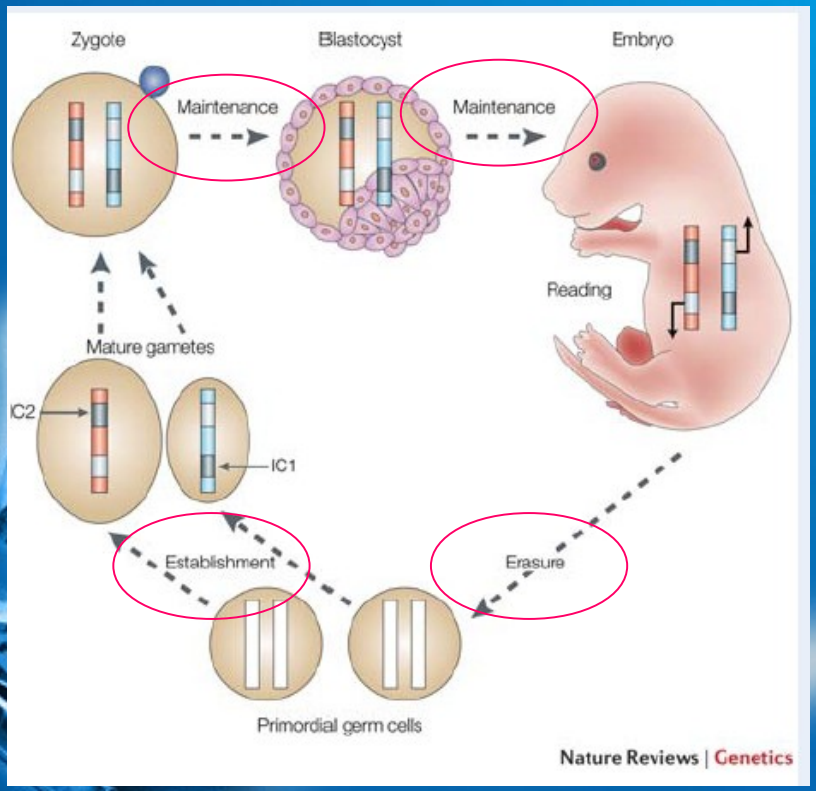
1. Mitotic inheritance of **DNA methylation**
   - transmitted semi-conservatively through DNA replication
   - DNMT1 interacts with PCNA (proliferating cell nuclear antigen) → recognize hemimethylated DNA → addition of a methyl group
     - error rate in base pairing : 1 in 10^9
     - error in adding -CH3: 1 in 40 in somatic cell divisions
   - more faithful inherited over mitotic divisions than histone modification

1. Propagation of **histone modifications**?? more complex
   - Current leading model: recruit histone-modifying enzymes directly after replication → further deposition of the mark in a positive-feedback loop

1. Relation between the **timing of replication** of a certain locus during S-phase and **gene activity**
   - transcriptionally active genes or alleles are replicated earlier than inactive genes or alleles
   - timing pattern linked to chromatin state
   - ex.: imprinting genes

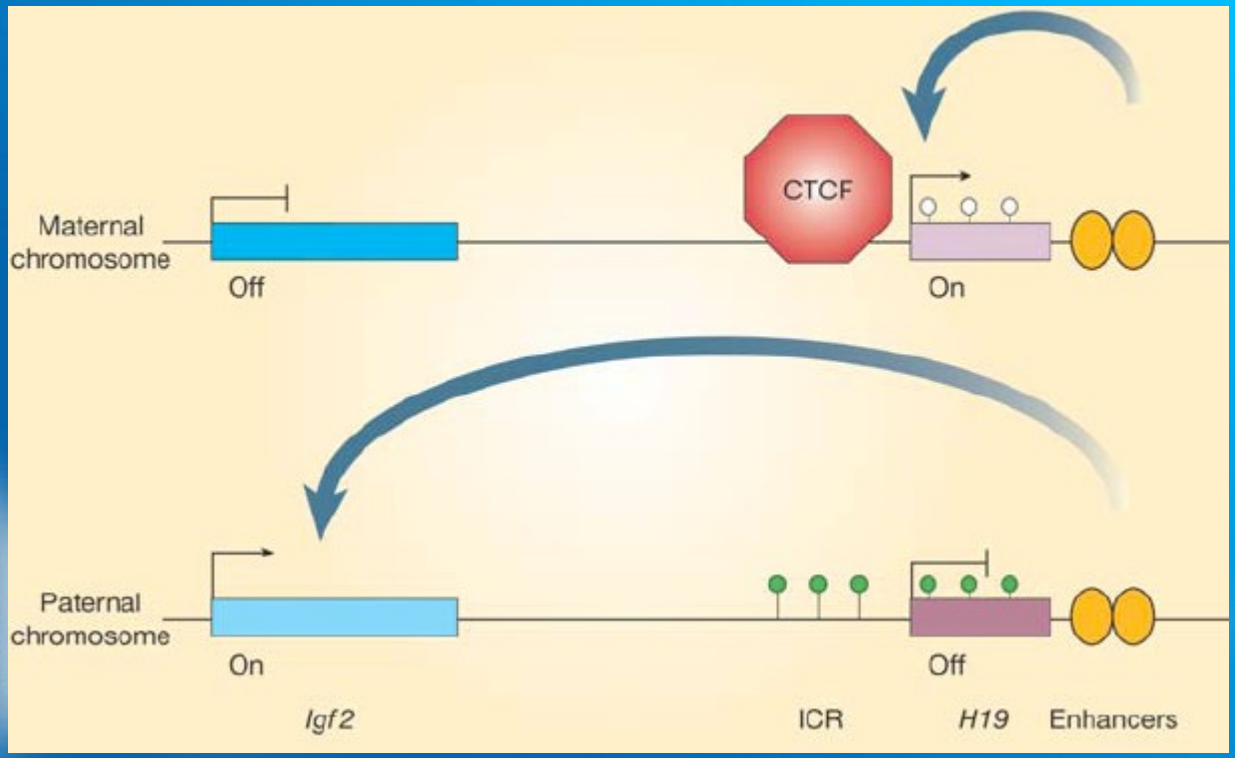
Concept 2: genomic imprinting

- **Operates to silence the maternal or paternal alleles of genes**
- Regulated by **imprinting control region** (ICR, also called a germline **differentially methylated region** or germline DMR)

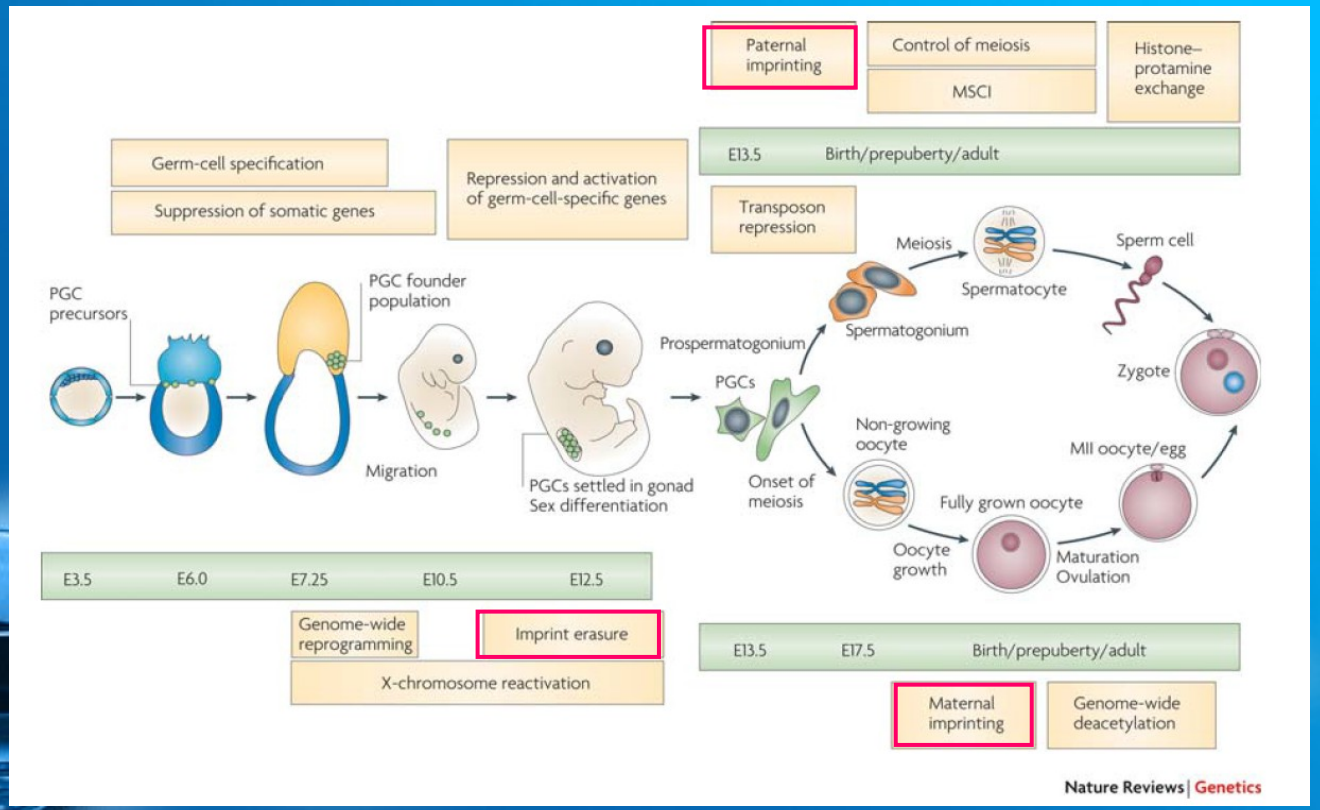


- DMR is marked by CpG methylation in one of two germlines
- **Methylation** occurs in sex-specific manner, maintained throughout fertilization, embryonic and subsequent development

- 15 germline DMRs listed in human →
  - 2 are methylated in male germline
  - in female, DMR always in promoter region
  - if more than one gene regulated by this DMR → long non-coding RNA

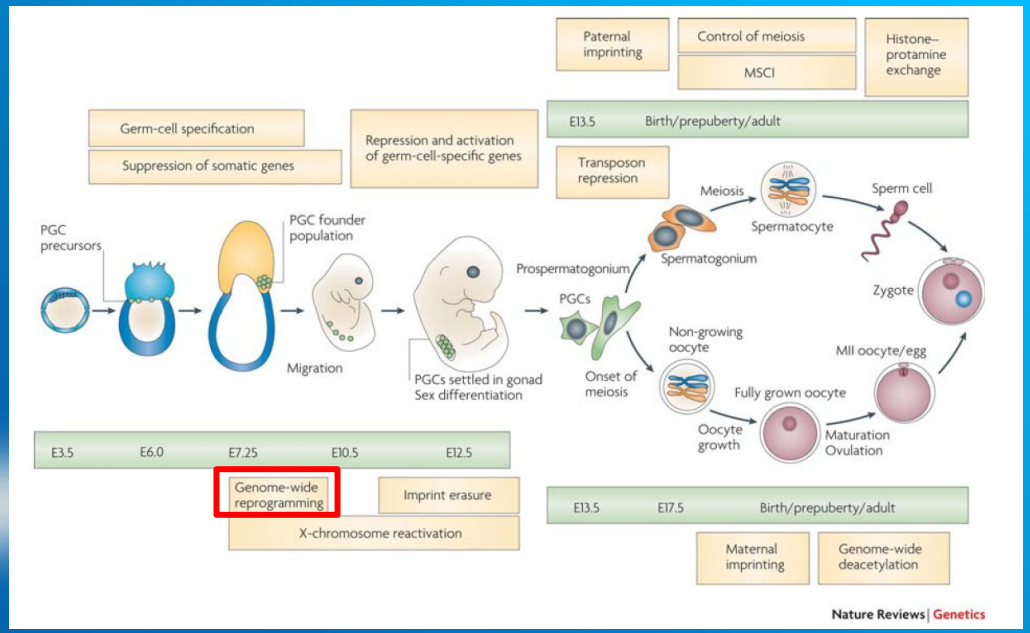


- When PGCs enter the developing gonad → imprinting erased then re-established later according to **sex**
  - ♂: remethylation → DNMT3a, from E15
  - ♀: remethylation → during postnatal follicle development
- in human: total 70 imprinted genes → type II DM, high-density lipoprotein cholesterol metabolism, social behavior

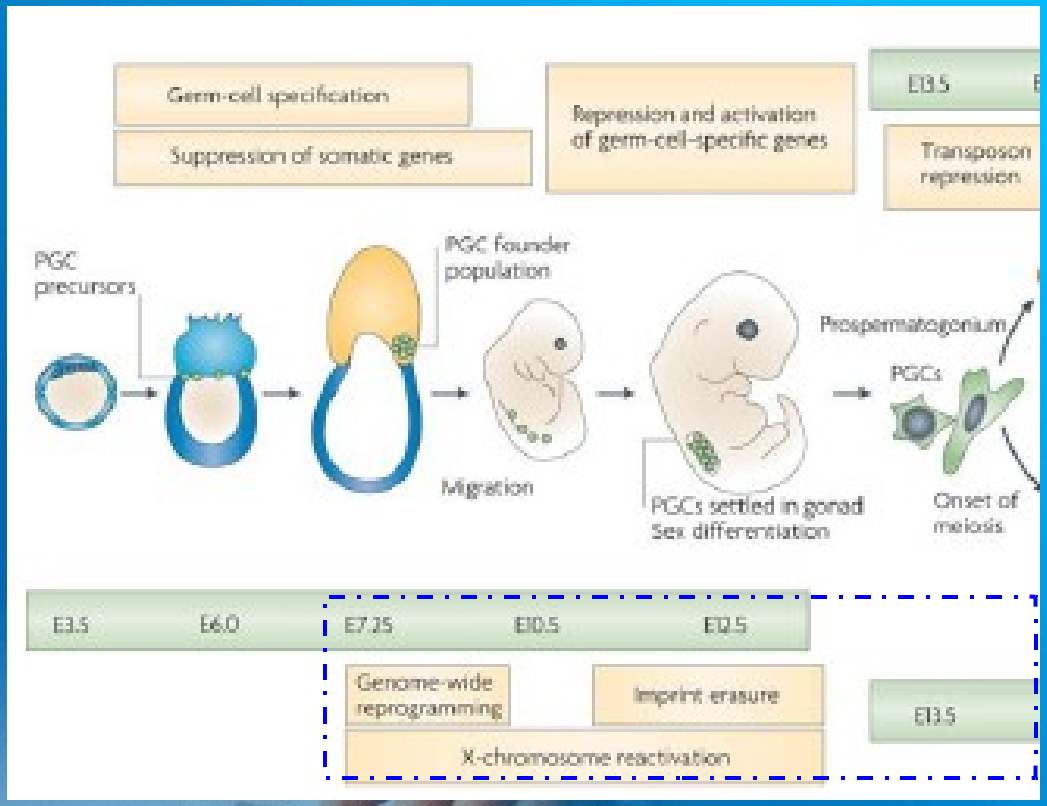


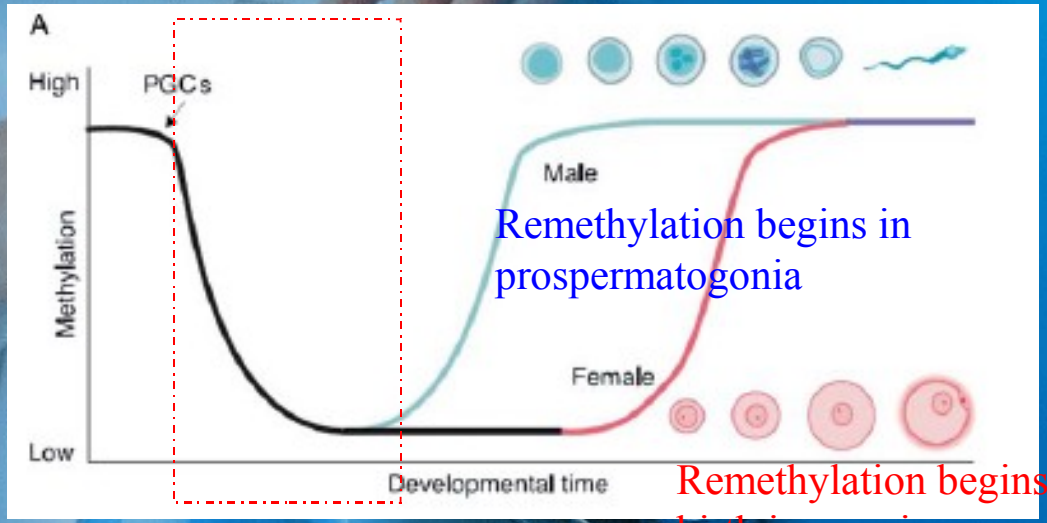
Reprogramming in the genome towards totipotency

- inheritance of epigenetic information between generations → generally actively prevented
- **Restore the germline to totipotent state**:
  - At PGC stage until after their entry in the incipient gonad
  - After fusion of sperm and oocyte in zygote and during the first cleavage divisions



1st phase of epigenetic reprogramming (demethylation)





- < 10% of CpGs retain methylation mark → **reprogramming**
- **demethylation** observed at nearly all sequence elements including promoters and genic, intergenic and transposon sequence
- allele-specific methylated DMRs of **imprinted regions** → demethylated between E10.5 and 12.5 (precise timing individually)
  - *de novo* methylase Dnmt3a, Dnmt3L
  - spermatogenesis (*H19*), oogenesis (*snrpn*)

Concept 3: Retrotransposons

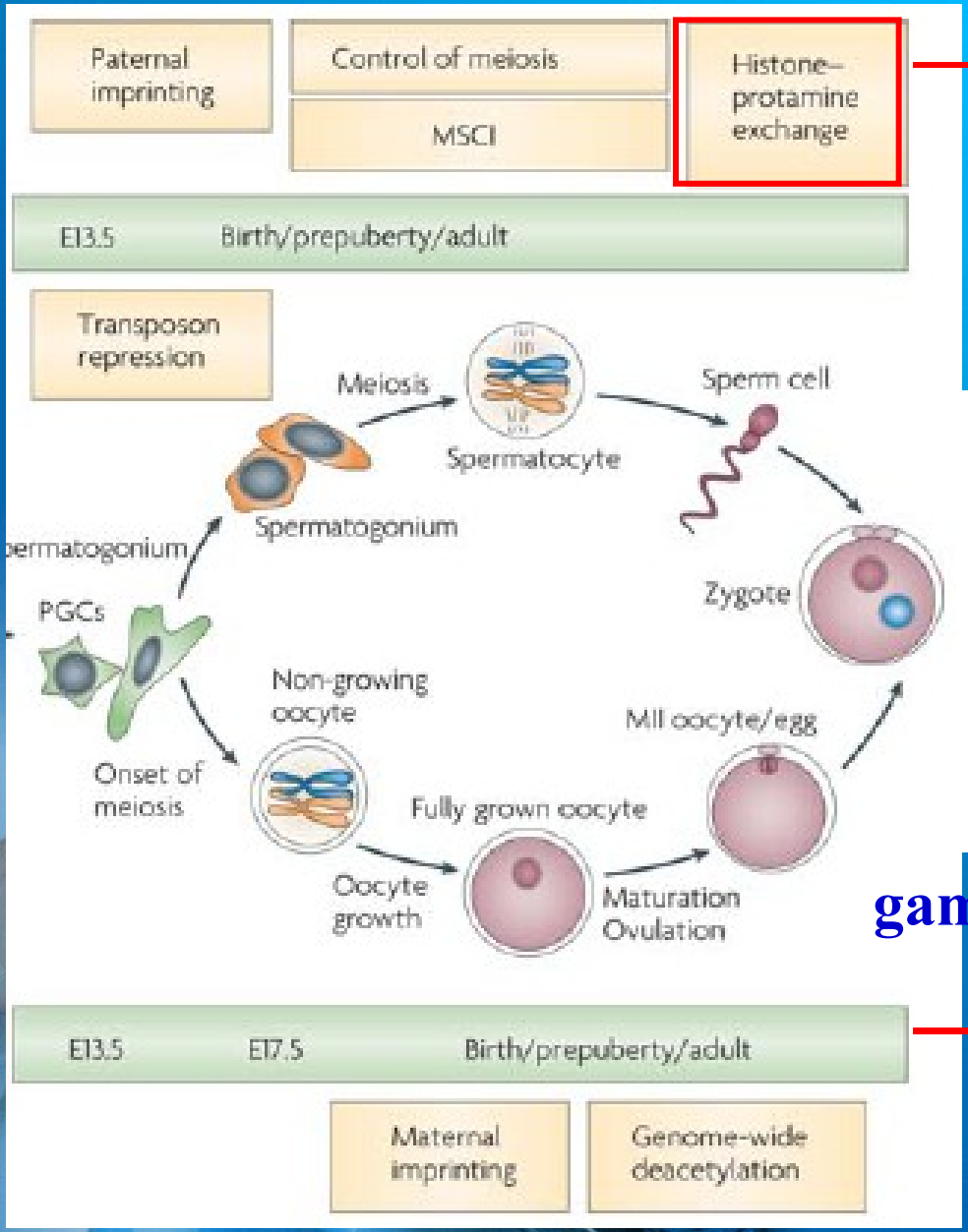
- exception to genome-wide demethylation → IAPs
- **transposable elements (TEs)**
  - ~ 50% in mouse genome and ~ 40% in human genome
  - **mobile DNA elements** with the ability to allocate transcription machinery for replication
  - **retrotransposon (majority)**
    - Require **RNA intermediate** to duplicate themselves
    - Long terminal repeats (LTR) & non-LTR retrotransposons

Retrotransposons

- LTR-retrotransposons
  - make up 8-10% of the genome
  - known as endogenous retroviruses → remnants of infectious retroviruses (lost "envelope" gene for caspid)
- Non-LTR retrotransposons
  - most abundant in mammalian genome
  - Short interspersed nucleotide elements (SINEs): no autonomous duplication potential
  - Long interspersed nucleotide elements (LINEs):
    - LINE-1 represents 17~20% of human and mouse genome mass
    - encode reverse transcriptase

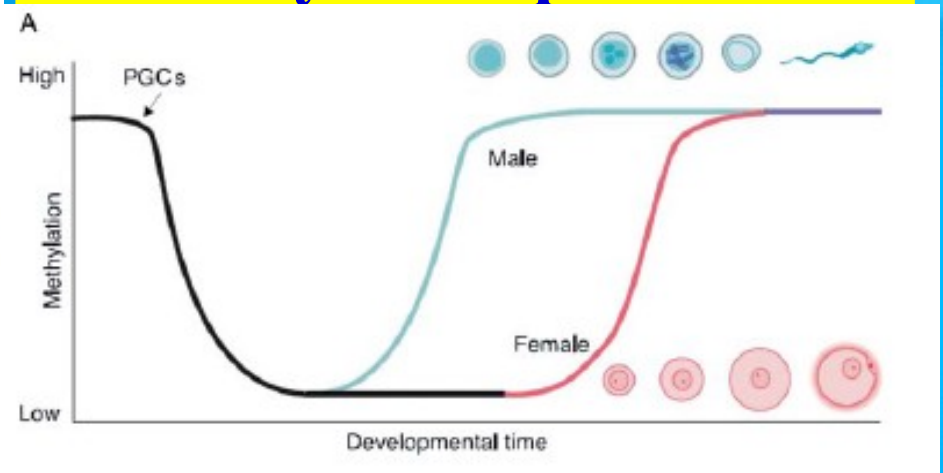
Retrotransposons

- various structural effects ranging from disruption of genes by insertional mutagenesis to chromosome rearrangements caused by homologous recombination
- mostly negative effects in short-term, **BUT...**involved in the expansion and structural ***evolution*** of genome → crucial in evolution of new proteins and regulatory sequences
  - Disturb expression of nearby genes (directly or via spread of repressive epigenetic marks)
- most are inactive, but some are still potentially able to duplicate and reintergrate in the genome
  - **LTR-containing intracisternal A particles (IAPs):** only **partial** demethylation



as stress resistant packing tool

Specific male and female methylation patterns



gamatogenesis

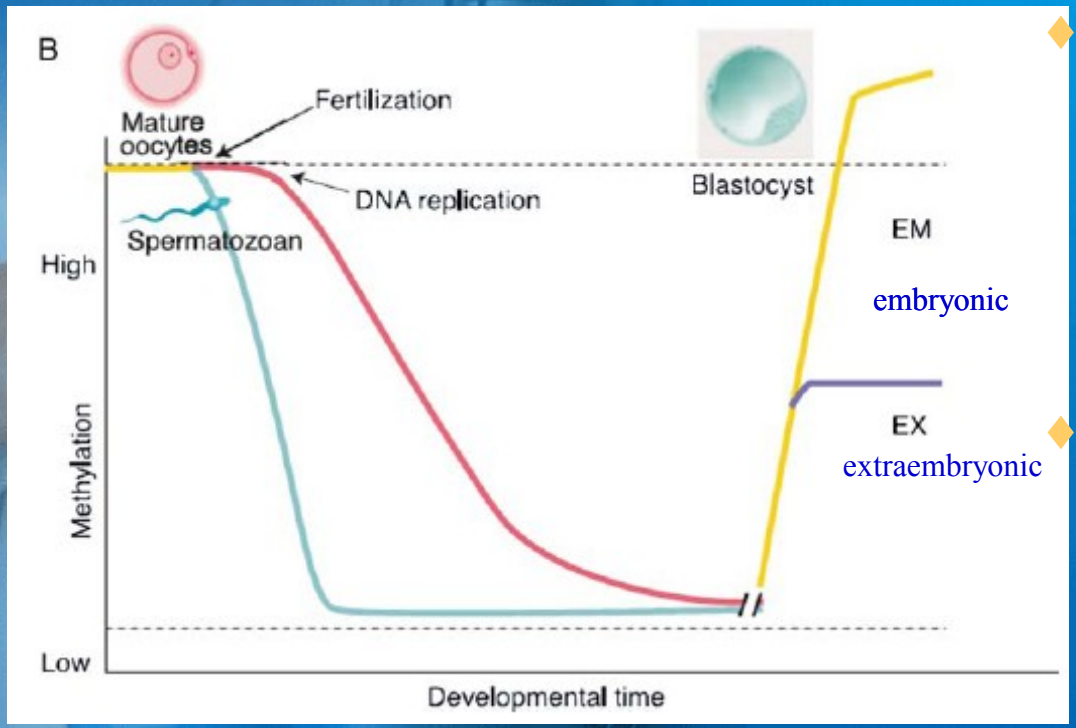
limited information

2nd phase of epigenetic reprogramming

- Starts after gamete fusion

  → rapid, active conversion of 5 methyl CpG dimers into hydroxymethyl group on mainly paternal DNA (active)

  → protamines exchanged for hyperacetylated histones from maternal pool



- in zygote, female genome passively loses methylation due to ↓ **DNMT1** activity → until morula stage → ICM differentially methylated
- Both paternally & maternally imprinted DMRs remain methylation

- At implantation, DNMT3b catalyses *de novo* methylation → mediate transition to terminal differentiation program
- Non-imprinted genes inherited promoter DNA methylation from paternal gametes → escaping early embryonic reprogramming

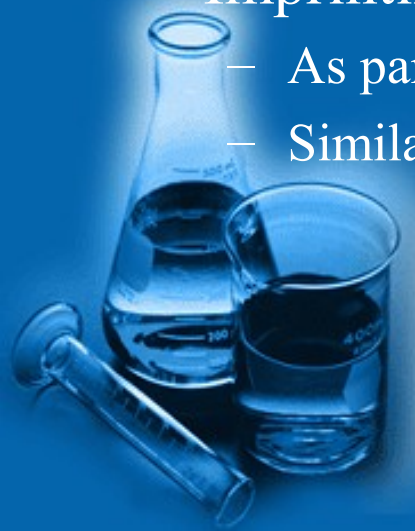
Borgel et al., 2010

- Chromatin reprogramming

- epigenetic asymmetry between male and female chromatin in the early embryonic phase of reprogramming originates from the grossly different chromatin states at the onset of fertilization
  - **Protamine-dominated** chromatin in sperm vs. **exclusively nucleosomal organization** of female meiotic chromosomes
  - Histone modification and variants (H3) in pronuclei
  - During the 1st cleavage divisions, become more similar as to the gross histone modification pattern

Transgenerational epigenetic inheritance: escaping reprogramming in the mouse occurs in both male and female germline

- IAPs: escape demethylation in mouse preimplantion embryo
  - Among the most active retrotransposons in the mouse genome
  - **Purpose of silencing**: original evolutionary function of repressive CpG methylation and **crucial for the prevention of retrotransposon induced mutation**
- Imprinting mechanisms: only in mammals, plants and insects
  - As part of sex-specific developmental program
  - Similar to retrotransposon silencing mechanism



- Both DMRs of imprinted loci and LTRs of IAP retrotransposons behave similarly in the targeting of ***de novo*** **methylation** in the developing oocyte after PGC reprogramming and in the protection from active demethylation in the zygote.
- Repeat-like nature of both sequence → acquisition of CpG methylation
- In the mouse, IAPs are still the best candidate for building up epigenetic inheritance as their methylation levels are relatively high at the time of active demethylation in early PGCs

Striking evidence for transgenerational epigenetic inheritance

- *Agouti (A)* locus: determinant of ***mouse coat color***
  - Carrying an upstream IAP element → the promoter causes aberrant expression of *Agouti* locus dependent on its level of CpG methylation
  - Phenotype:
    - Undermethylated LTR promoter → gene expression, yellow
    - Fully methylated LTR promoter → restricted expression, pseudo agouti

- Offspring coat color correlated to the mother
  - Epigenetic transmission by gamete
  - Paternal epigenetic transmission → less common

Another example

- *Axin-fused (Axin$_{Fu}$)* allele: ***mouse kinked tail***
  - Contains an inserted IAP retrotransposable element
  - Phenotype dependent on the methylation status of LTR promoter
- evolutionary conserved ability of IAPs to resist epigenetic reprogramming between generations → epigenetic inheritance ***stable***
- interindividual variation shown in each generation

- LTRs of IAPs and DMRs of imprinted regions able to conserve their methylation status throughout reprogramming event → mechanism not fully clear
- in the early mouse embryo:
  - **Dnmt 1 o** (stable maternal form, inherited from oocyte)
  - **Dnmt 1 s** (somatic form)
    - present in preimplantation embryo from 2-cell stage on
    - providing necessary tools for selective preservation of DNA methylation in the germline

Dnmt 1 o

- not present in the nucleus during the first 3 cleavage divisions
- migration to the nucleus at 8-cell stage → necessary for maintenance of imprints
- involved in both IAPs and DMRs
- loss of maternal Dnmt 1 o

→loss of imprinting at many loci, including maternally imprinted *Snrpn* and paternally imprinted *H19*

→profound phenotypic variation in the offsrping as retarded development at mid gestation in 60% of the embryos owing to deficiency in the maintenance of imprinting marks

- in addition to Dnmt1, various other proteins suggested to play a role in prevention of demethylation
- How to recognize these target sequence elements (rescue from demethylation) ?????
- CpG binding protein Mbd3, maternal effect proteins Zfp57, RNA elongator factor Elp3
  - Oocyte is involved in maintenance of transgenerational epigenetic marks → "maternal effect"

other epigenetic mechanisms

- A^{VY} and $Axin^{Fu}$ alleles: LTR of IAP shown to be demethylated at blastocyst stage
  - →Other mechanisms might contribute to conferring memory of the repressed epigenetic state
  - →Evidence: mouse mutations for Dnmt1
    - ✓No IAP transcripts detected despite absence of DNA methylation at IAP loci
  - →active / inactive $Axin^{Fu}$ alleles differentially modified at blastocyst stage with activating / inactivating histone marks

Regulatory small RNAs

- directly involved in the transfer of epigenetic variation via sperm in the mouse
- play a role in epigenetic inheritance in humans → **fertility**
- different species of small RNAs involved in the suppresion of retrotransposons
  - In the male mouse: piRNA (PIWI interacting RNAs) assist in degradation of retrotransposon RNA transcript → guide de novo methylation of partially demethylated TEs during epigenetic reprogramming in the embryo and during spermatogenesis
  - In the female germline: miRNAs and siRNAs assist in retrotransposon silencing
- necessary for early embryo developmental gene regulation

- "paternal-effect" genes
  - "chromatin metabolism" during spermatogenesis influences paternal gene expression in the next generation
- Both maternal and paternal germline possess the tools necessary for the transmission of epigenetic marks.
  - sex-specific manner → difference in maternal and paternal genotype, expressed before, at and after fertilization

Stress, hormone and nutrition-induced transgenerational epigenetic variation

- In animal models (mice and rats): external influences, including irradiation stress, exposure to hormones and nutrition → shown to induce variation in epigenome
  - possibility of this variation transmitted to subsequent generations ?

F0 pregnant female carrying F1 embryos

(containing germ cells to produce F2)

↓ treated with inducing agent

↓

↓

F3 generation

Genotoxic stress-induced transgenerational epigenetic variation

- **irradiation:**
  - direct genotoxic effects on cells (notably the nucleus)
  - induce an increased rate of DNA breaks and mutations in descendent cells and even across generations → **radiation-induced genomic instability**
    - Mutation frequency at expanded simple tandem repeat (ESTR) loci to detect transmission of delayed effects of irradiation

    ** mutation is read as a change in repeat number*
    - Epigenetic disturbance as a causative factor
    - results in a quickly induced **global hypomethylation of DNA**
      - After testis irradiation, hypomethylation of retrotransposed interspersed repeat elements (LINEs and SINEs) was found in the offspring. → genotoxic stress in the male germline can induce genetic and epigenetic variation in the offspring

Nutrition, hormones and epigenetic variation

- epigenetic effects caused by nutrition and hormones mainly described after induction in late embryos and early fetuses when PGCs are arising and migrate to the early gonad
- Adult male is also capable of acquiring nutrition-induced epigenetic variation in the germline.
  - fasting → altered serum glucose level in offspring (F1)
  - chronic exposure to a high-fat diet → pancreatic beta cell dysfunction in the offspring
  - low-protein diet → affect hepatic expression of genes involved in proliferation and cholesterol biosynthesis

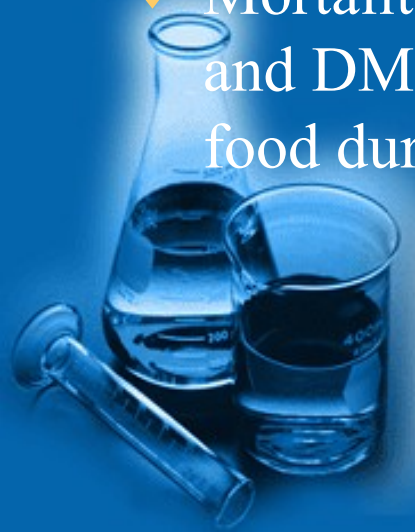
Nutrition, hormones and epigenetic variation

- in the mouse, **diet** found to be correlated with differential methylation of several lipid metabolism-related genes in the liver of both male and female offspring
- Sperm is sensitive to nutrition-induced epigenetic variation next to oocyte and developing embryo.

➔ *further research*: to verify if these effects transmitted to subsequent generations (F2) and elicit true long-lasting heritable epigenetic variation

Environmental influences

- DNA methylation level at the imprinted *IGF2* gene in individuals prenatally exposed to famine during Dutch Hunger Winter (1944-1945)
  - conceived during famine and exposed in earliest stages of development → **DMR of *IGF2* significantly hypomethylated** compared with non-exposed siblings
  - not seen if exposed at later gestational stage
- Mortality rates of tested individuals related to CV disease and DM reduced if their grandfather experienced scarcity of food during prepuberty



- exposure of midgestation rats to the anti-androgenic compound **vinclozolin**
  - → ↓spermatogenic capacity
  - → ↑incidence of male infertility up to F4
  - → persistent CpG methylation changes in selected gene promoters of F3 sperm
  - → change methylation levels in maternally and paternally imprinted gene

- Estrogen and androgen vs. epigenetic variations: mediated by **nuclear receptor**
- **Diethylstilbestrol (DES):**
  - estrogen receptor agonist
  - If exposed during pregnancy,

→ uterine anomaly
→ carcinogenesis

in mice: defects observed up to F3

in human: modest effect in F2

→ abnormal methylation of the lactoferrin promotor

- epigenetic variation → stress-induced chaperone Hsp90

Epigenetic inheritance and germline reprogramming

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To be continued …