

10 April 2012 Infertility Journal Reading

Epigenetics and its Role in Male Infertility

Epigenetics and its role in male
infertility

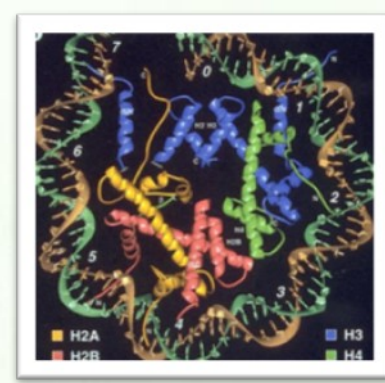
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Male infertility

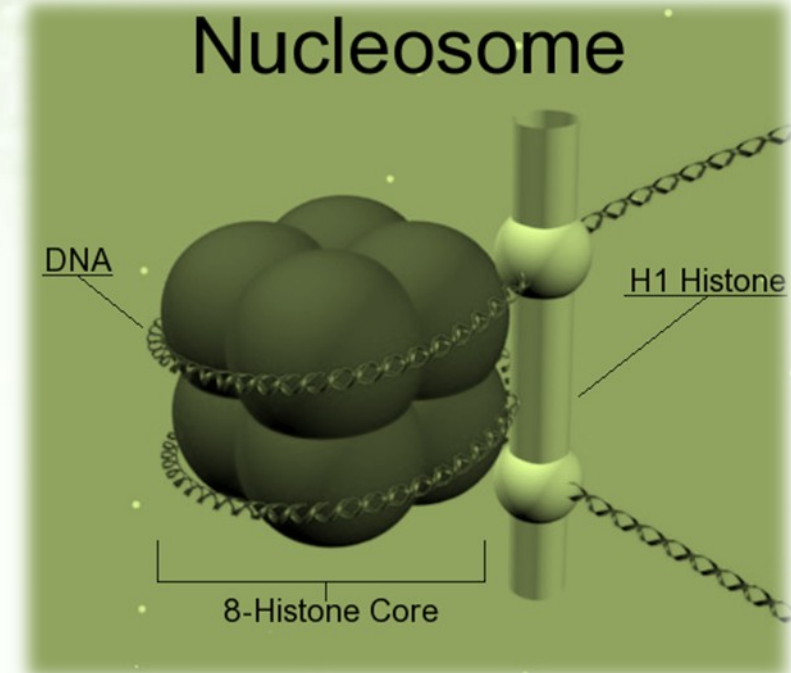
- A complex problem: genes, **epigenetic** factors
- Aberrant **epigenetic** reprogramming in male germ cells → Can lead to Sperm abnormalities
- **Epigenetic** changes contribute to male infertility
- Epi- (above or over)-genetics change:
 - Changes in the **phenotype** caused by mechanisms (*ex. gene action & expression*) other than changes in DNA sequences

Epigenetic changes



- DNA wraps around histones → nucleosomes
- Nucleosomes → packaged into chromatin
- Modifications in chromatin → control gene-expression in a spatiotemporal manner

- **Epigenomics:**
Genome-wide approach to studying epigenetics



Epigenetic mechanism of gene regulation

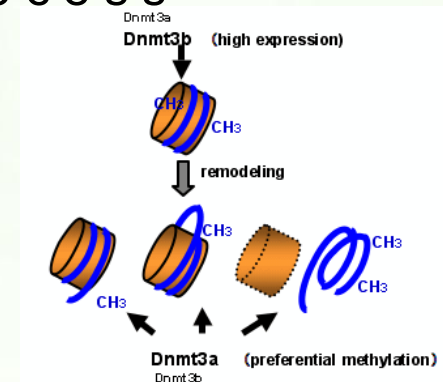
- 2 major modifications that occur in chromatin
 1. DNA methylation
 1. Post-translational histone modifications

DNA methylation

- A biochemical process
- Add **methyl group** → **5'-cytosine pyrimidine ring**
- A result of DNA methyltransferase (**DNMT**) activity
- Typically occurring in a **CpG** dinucleotide

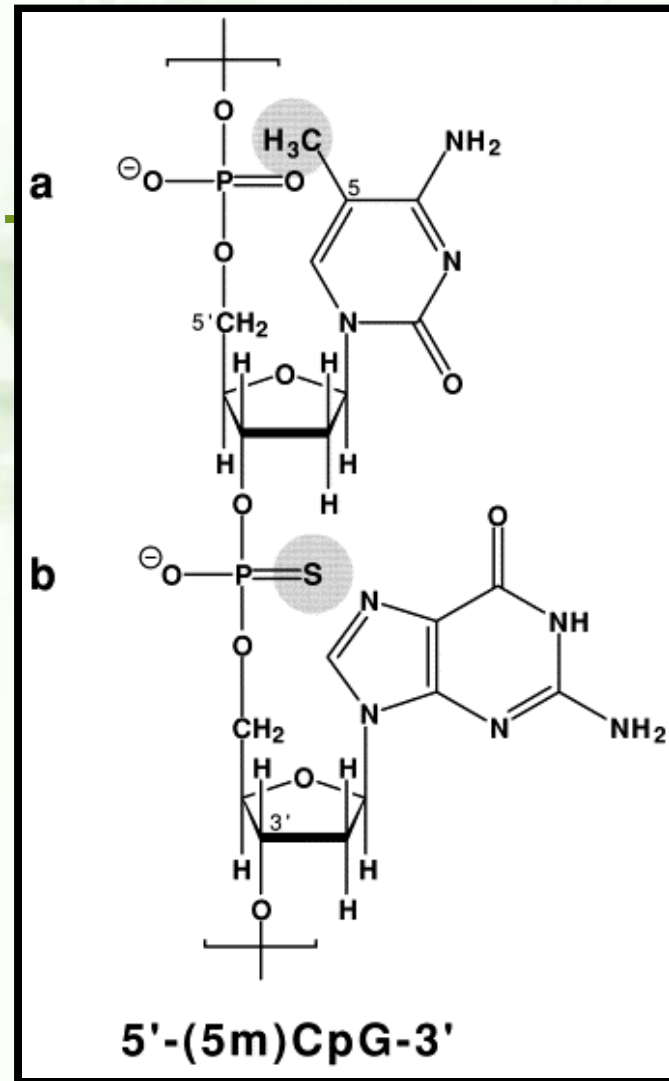
DNA methyltransferase (DNMT)

- 3 main DNMTs:
 - i) DNMT1: key role in maintenance of methylation
 - ii) DNMT3a/3b:
 - De novo methyltransferases
 - Methylate the genomic DNA during early embryonic development
 - A gradual rather than an abrupt process



CpG dinucleotide

- Cytosine
- phosphodiester
Guanine



C p G islands

- Genomic regions, approximately 500 base pairs long (200 at least)
- High frequency of CpG sites (CG : GC ratio >55%)
- Located within the about 40% of **promoter region** of mammalian genes (70% of human)
- Methylated → **transcriptional silencing**
- Hypomethylation & hypermethylation can occur simultaneously at different regions in the genome

Post-translational histone modifications

- Acetylation, methylation, ubiquitylation, phosphorylation
- Main players in epigenetic regulatory mechanisms
- H2A, H2B, H3 and H4: integral part of nucleosomes
- H3 ↔ H4 (intricate interplay) → antagonistically to regulate (active chromatin → inactive histone code)

A c e t y l a t i o n

- A marker of **a c t i v e**, transcriptionally competent regions
- Hypoacetylated histones:
In transcriptionally **i n a c t i v e** euchromatic or heterochromatic regions

M e t h y l a t i o n

- M a r k e r f o r **a c t i v e & i n a c t i v e** r e g i o n s o f c h r o m a t i n
- H i s t o n e H 3 - N t e r m i n u s - l y s i n e 9 o n t h e (H 3 - K 9) :
 - A f e a t u r e o f **s i l e n t** D N A
- H i s t o n e H 3 - l y s i n e 4 (H 3 - K 4)
 - D e n o t e s **a c t i v i t y**
 - P r e d o m i n a n t l y a t t h e p r o m o t e r s o f a c t i v e g e n e s

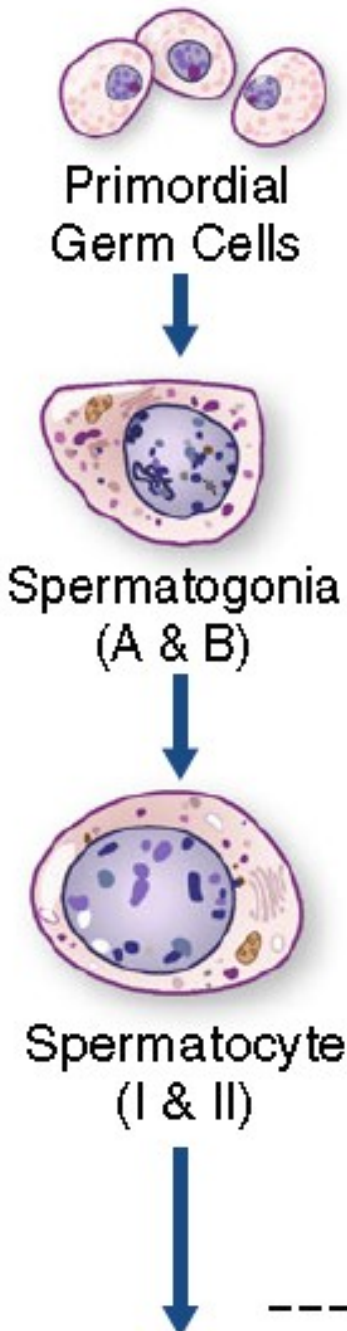
Epigenetic gene regulation during germ-cell development

- Epigenetic mechanisms - specific sets of genes
 - Regulate **DNA accessibility** throughout an organism's lifetime
 - Active at any stage of development
- Each cell type has its own **epigenetic signature**:
 - Developmental history, environmental influences
 - Ultimately reflected in the phenotype of the cell and organism

- Before fertilization:
 - Paternal genome delivered by the mature sperm, has a haploid genome, packaged densely with protamines
 - Maternal genome arrested at metaphase II is packaged with histones
- At the time of fertilization:
 - ➔ Protamines are rapidly replaced by histones
 - ➔ Oocyte: complete 2nd metaphase → polar body
 - ➔ The H3 and H4 histones that associate with the paternal chromatin are more acetylated than those present in the maternal chromatin

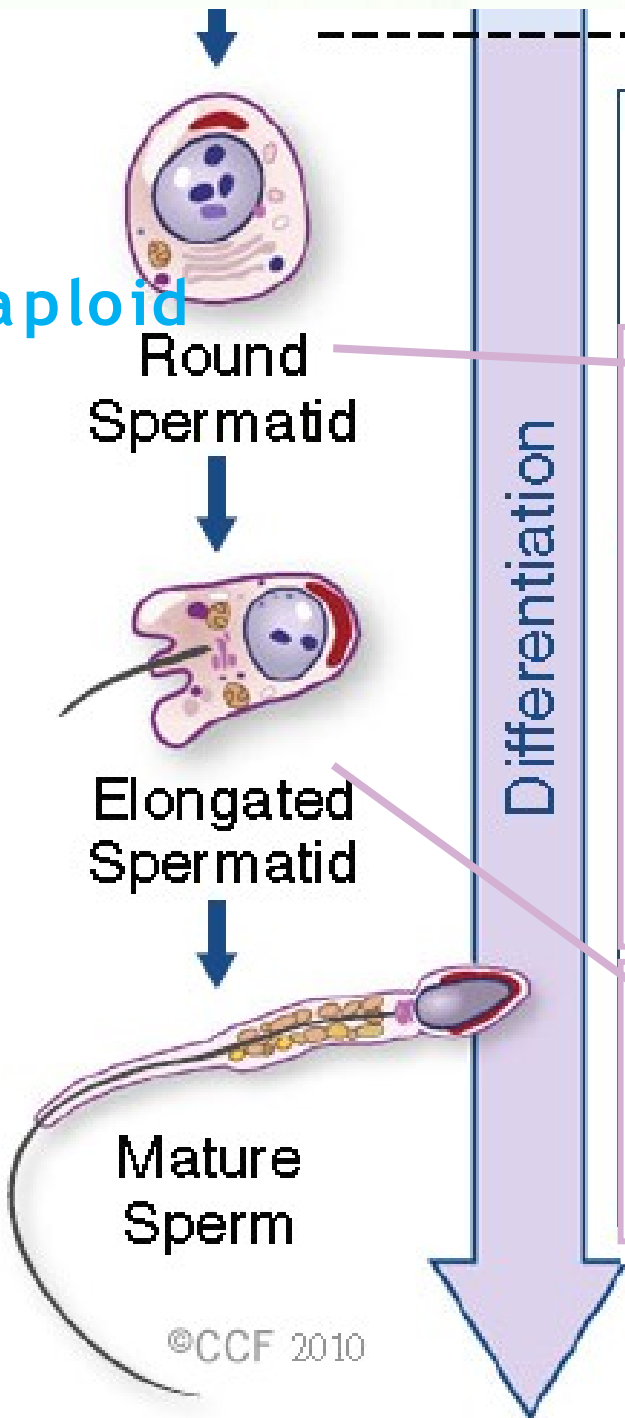
Spermatogenesis

Post-implantation



- Pluripotent cells in the epiblast → PGCs
 - ♀ Arrested in the prophase of meiosis
 - ♂ Enter mitotic arrest
- **Premeiotic** PGCs and spermatogonia: Unique patterns of histone modifications such as low H3K9me2 levels
 - change dynamically upon initiation of **meiosis**
- Changes in the composition and modification of **histones** could contribute to **chromatin modifications**

haploid



Spermiogenesis

- Final developmental change after meiosis

- Global nuclear remodeling occurs
 - ✓ Some histone marks such as H3K9me2 on the inactive X chromosome are retained

- ✓ **Testis-specific linker histone** variant H1T2 →

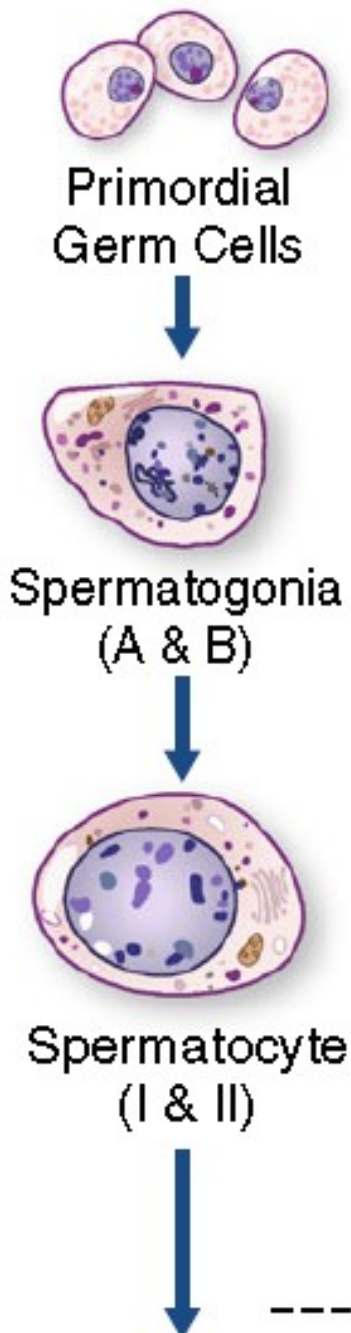
crucial chromatin condensation

- A linker histone variant H1Is1 (histone-1-like protein in spermatids 1) is expressed

histone-protamine exchange process

- Hyperacetylated nuclear histones
 - Replaced by transition proteins (TP1 and TP2)
 - Removed and replaced by protamines
 - Incorporated into sperm chromatin
 - Induces DNA compaction (important for the formation of spermatozoa and providing a safe environment for the genome)
- The presence of somatic-like chromatin in the sperm nucleus could transmit different epigenetic information to the offspring

Spermatogenesis



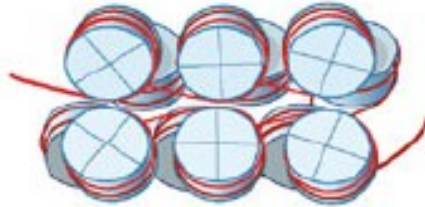
Mitosis

Meiosis

Spermatocytogenesis

Epigenetic Events

- Methylation of DNA



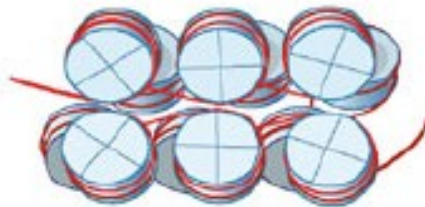
Modification occurs while structure is condensed

Possible Errors

- Abnormal DNA methylation
- Altered expression of mRNAs and other non-coding RNAs

Epigenetic Events

- Phosphorylation of DNA (2 events)



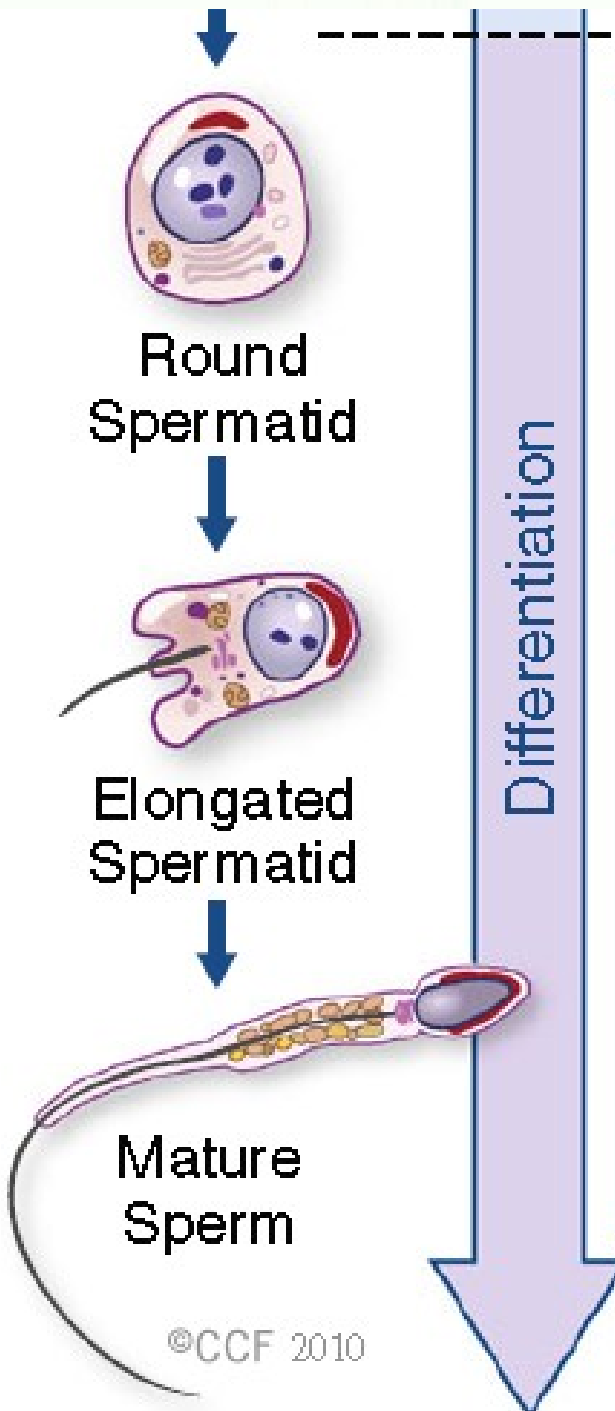
- Ubiquitylation
- Sumoylation
- H2AZ and H3.3 incorporation

Possible Errors

- Double strand breaks
- Chromosomal nondisjunction
- Abnormal histone modification

Set up the paternal specific imprints

Assist in both recombination and XY body formation and XY body formation involved in XY Body formation

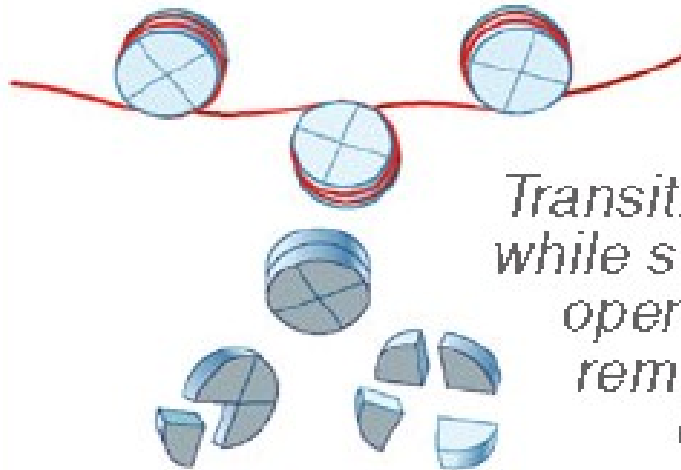


Spermiogenesis Assist in the

Epigenetic Events

- Hyperacetylation
- Histone to protamine transition
- Histone removal and degradation

Histone-
Protamine
exchange



Transition occurs while structure is open; histones removed and degraded

Possible Errors

- Protamine replacement errors
- Abnormal centrosome formation
- Apoptotic DNA fragmentation

Consequence of apoptosis following double strand breaks or abnormal

B R D T (b r o m o d o m a i n t e s t i s s p e c i f i c)

- D o u b l e b r o m o d o m a i n - c o n t a i n i n g p r o t e i n
- B i n d s h y p e r a c e t y l a t e d h i s t o n e H 4 b e f o r e a c c u m u l a t i n g i n c o n d e n s e d c h r o m a t i n
- H e l p o r g a n i z i n g t h e s p e r m a t o z o o n ' s g e n o m e b y
 - M e d i a t i n g a g e n e r a l h i s t o n e a c e t y l a t i o n - i n d u c e d c h r o m a t i n c o m p a c t i o n
 - M a i n t a i n i n g a d i f f e r e n t i a l h i s t o n e a c e t y l a t i o n o f s p e c i f i c r e g i o n s

B O R I S

(brother of regulator of imprinted sites)

- Specifically expressed in male gonads
- Could be directly involved in the resetting of methylation marks during male germ cell differentiation
- Linked with methyases mediating de novo methylation & demethyases mediating erasure of imprinting marks
- Domains: the same 11 Zinc Finger as **CTCF**

CTCF

- C C C T C binding factor
- A somatic regulator for expression of imprinted genes
- Binds to specific target DNA sequences
- Plays an important role in the maintenance of differential methylation patterns in somatic cells
- Present in both somatic and germ cells
(\Leftrightarrow *BORIS is expressed specifically in the male germ line*)

Paternal impact on early embryogenesis

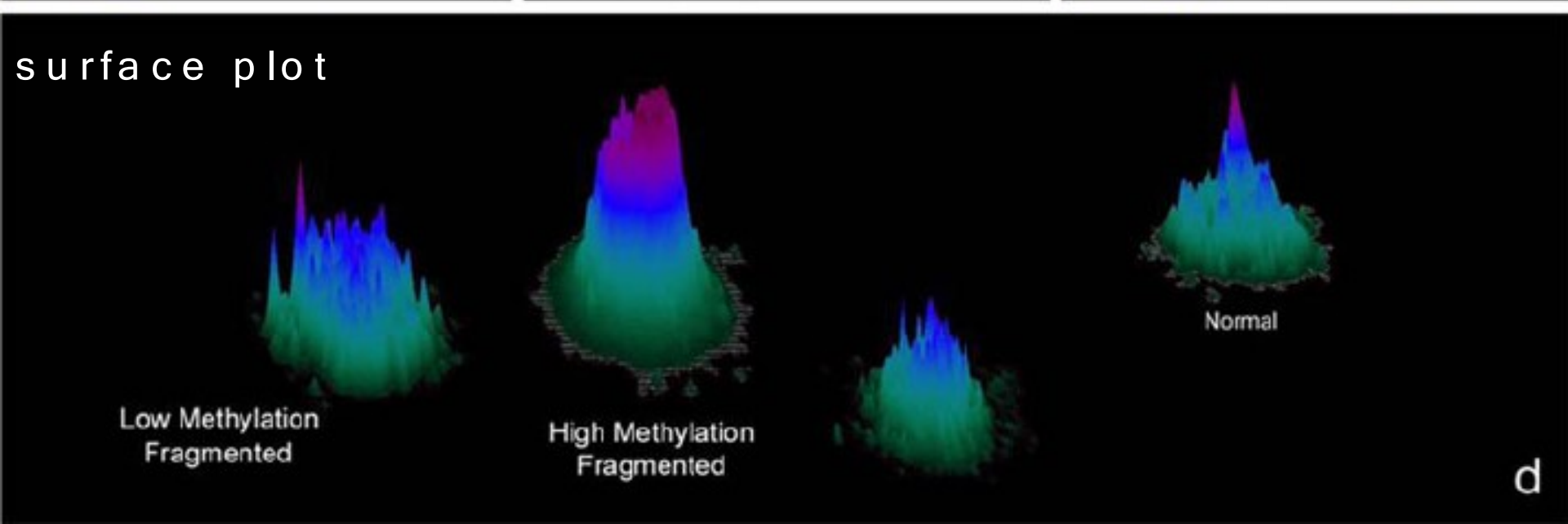
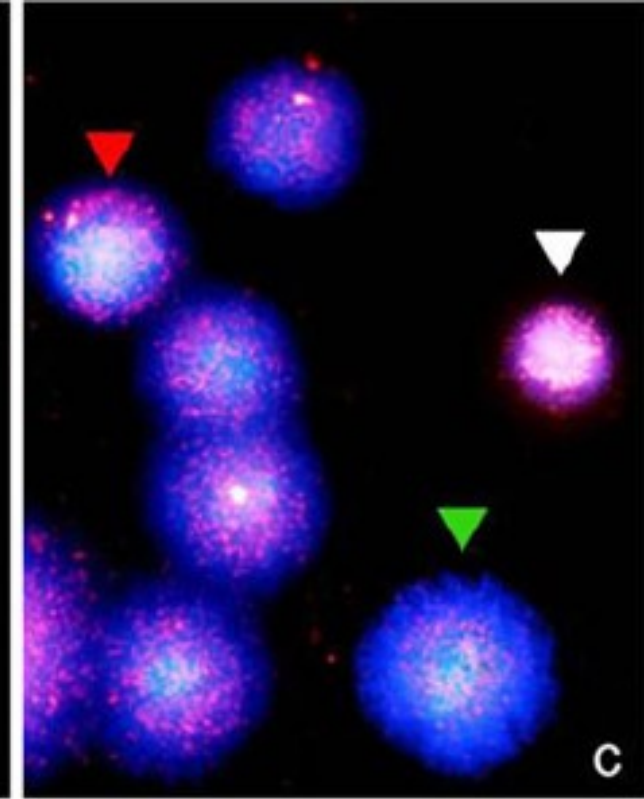
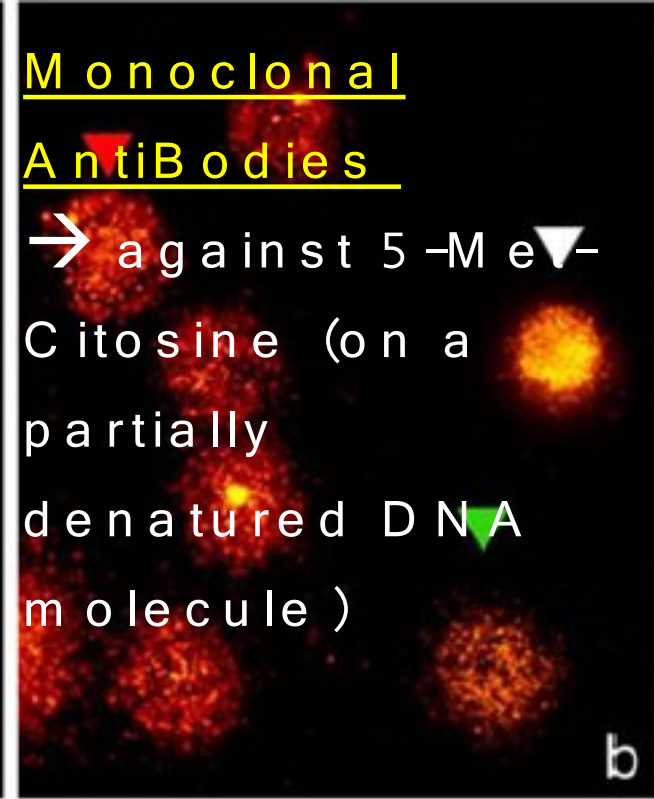
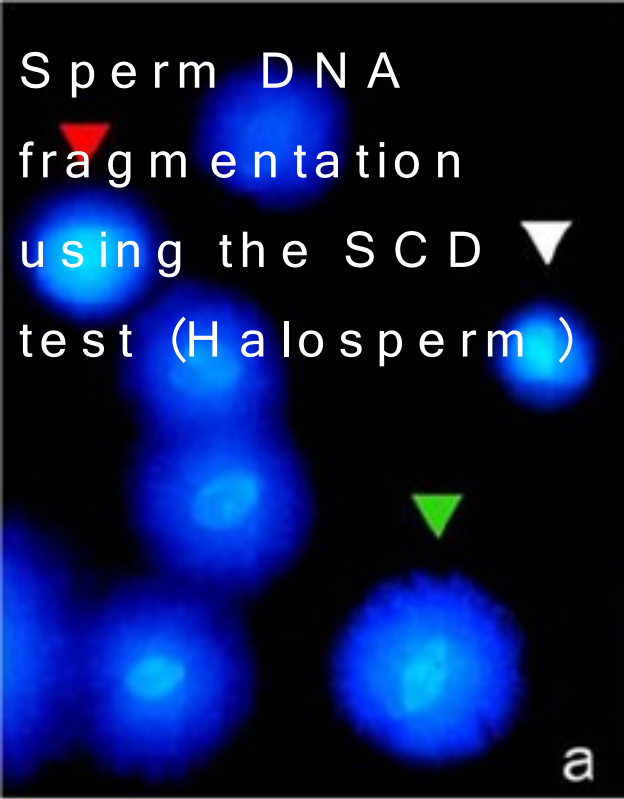
- **Advanced paternal age:**
 - ↓ semen volume, sperm morphology / motility
 - No significant reduction in sperm concentration
- **High DNA fragmentation:**
 - Diminished sperm count, motility, morphology
 - Decreases fertilization and implantation rates
- **Methylation depletion:** 5-aza-deoxycytidine (base analog) → incorporated into DNA → ↓ level of DNA methylation → varying the gene expression

Methodology

- ? Actual role of DNA modifications on fertilization?
- Absence of reliable techniques, easy, reproducible, for the level of DNA modification in each gamete

Sperm Chromatin Dispersion (SCD) based technology

- ✓ Detection of DNA breaks in lysed sperm nuclei by specific DNA Breakage Detection - Fluorescence In Situ Hybridization (DBD-FISH) assay



Histones

- Best for **transmission of epigenetic information** (∴ influence on the modification of chromatin structure & access of transcriptional machinery to genes)
- Methyltransferases → mono-, di- or trimethylation of lysine or arginine → Facilitate gene silencing
- ? Whether modified histones play a crucial role in gene expression during **early embryogenesis**?
- ? If abnormal histone modifications in the sperm → ↓ **Embryo development**?

- Alterations in methylation patterns → can effect biallelic expression or repression of imprinted genes → various pathologies
- Impaired spermatogenesis ⇔ aberrant H4 acetylation
- H4 hyperacetylation: observed in infertile men exhibiting Sertoli cell only (SCO) syndrome
- Sperm of patients with asthenozoospermia and teratozoospermia: ↓ levels of DNA methylation

Epigenetic changes important for male gametes

- Gonadal sex determination and testis development
 - In the rat: Occur between embryonic days 12 ~ 15 (E12 to E15)
 - In the human: after midgestation
 - Initiated by differentiation of precursor Sertoli cells that result from Testis-determining factor SRY

- Aggregation of the precursor Sertoli cells, PGCs, migrating mesonephros cells (precursor peritubular myoid cells)
→ Promotes testis morphogenesis & cord formation
- Fetal testis: Steroid receptors (+), Target for endocrine hormones
 - Androgen receptor (AR) & estrogen receptor- β (ER β) → present at the time of cord formation (E14), in Sertoli cells, precursor peritubular myoid cells, germ cells

1. Testis does not produce steroids at this stage of development
 2. Estrogens and androgens have the ability to influence early testis cellular functions
- Endocrine disruptors (*vinclozolin*, *methoxychlor*), → at a critical time during gonadal sex determination (E 8 ~ E 15 in the rat)
→ adult testis phenotype: ↓ spermatogenic capacity + infertility

- External factors → can induce an epigenetic transgenerational phenotype through an apparent reprogramming of the male germ line
- ? Steroidal factors acting inappropriately at the time of gonadal sex determination → reprogram the germ line epigenetically (altered DNA methylation) → transgenerational transmission of an altered phenotype or genetic trait ?

- Many epigenetic modifiers
 - DNA methyltransferases, histone modification enzymes & their regulatory proteins
 - play essential roles in germ-cell development
- Some are germ-cell-specific genes (such as Dnmt3L and Prdm9)
- Numerous intra- and inter-individual differences in DNA methylation in human sperm samples → phenotypic differences in the next generation
- Sperm samples from oligospermic patients: often contain DNA-methylation defects at imprinted loci

Epigenetics and protamine abnormalities

- During differentiation of the male gamete → the genome undergoes major changes
 - **Homologous recombination** → Affect the DNA sequence and genetic information → Alter its nuclear structure and epigenetic information
- How the specific **nucleoprotamine / nucleohistone** structure of the sperm nucleus conveys epigenetic information?

Protamines 1 & 2 (P1 / P2)

- Essential for sperm function
 - **haploinsufficiency** of either P1 or P2 → ↓ amount of the respective protein
 - Mutation of the **calmodulin-dependent protein kinase Camk4** (phosphorylates P2) → defective spermiogenesis & male sterility
- P1 / P2 in fertile men lies close to 1.0 (0.8 ~ 1.2)
 - Perturbation of this ratio → poor semen quality,
↑ DNA damage, ↓ fertility

Spermatogonia

Core Histone Acetylation

Primary Spermatocyte

Core Histone Deacetylation

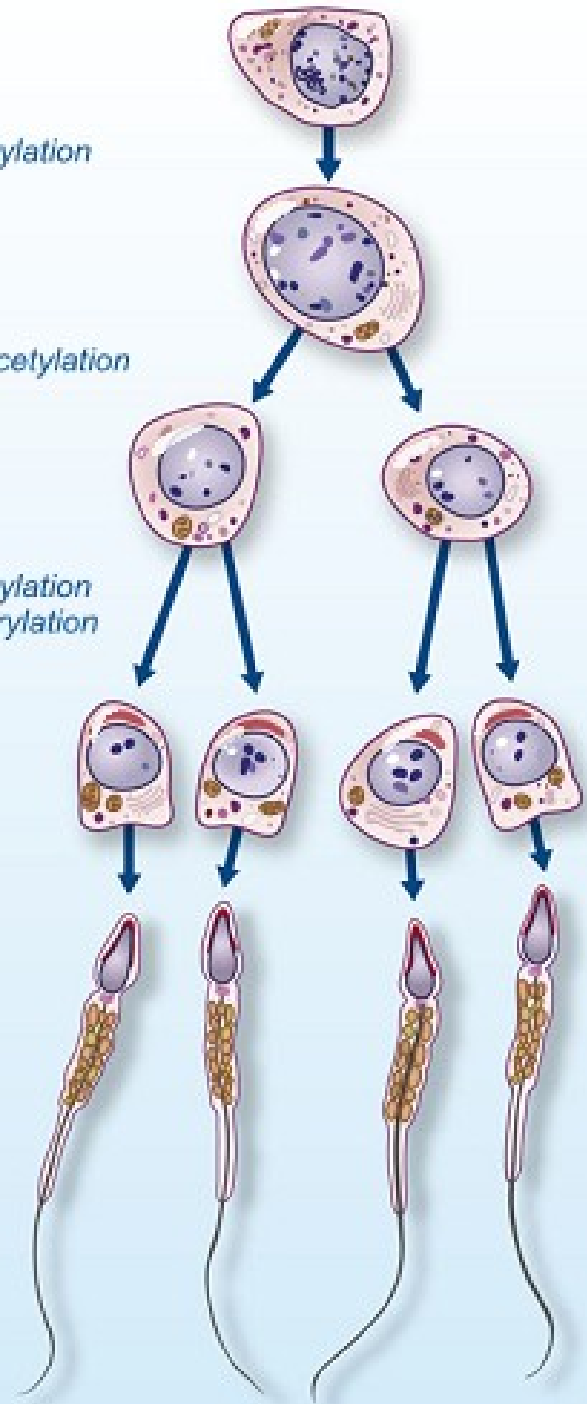
Secondary Spermatocytes

Core Histone Acetylation
Protein Phosphorylation

Round Spermatids

Protamine Disulfide Bond

Elongated Sperm



- Elongating spermatid
 1. Long after the completion of meiosis
 → Protamine replacement
 2. During the period of protamine replacement
 → Undergoes other maturation events that affect its motility and fertilization ability (not been linked to sperm count)

- In mature mammalian spermatozoa, DNA is actually not homogeneously packed with protamines
- Some histones are retained in humans
- ➔ The **persistent histones** could be an **important epigenetic code** in the sperm and may not be the result of inefficient protamine replacement

- “Abnormal protamine replacement ⇔ generally diminished semen quality”
→ may be due to a defect in the unique system of :
during spermatogenesis → temporal uncoupling
of transcription and translation

→ Abnormal protamine incorporation into chromatin → may affect transcription of other genes

Ex.: Deregulation of protamines (in mice)

→ Precocious chromatin condensation

→ Transcription arrest

→ Spermatogenic failure

- Human sperm nucleus:
 - Retains 10–15% of its original histone content
→ distributes it in a heterogeneous manner within the genome
 - Histones (retained bind specific regions) → convey epigenetic information to the early embryo
- Sperm with abnormal protamine replacement for IC SI → obvious and profound implications

Future research:

- (Fertile men \leftrightarrow patients with known chromatin abnormalities)

→ mature sperm

→ Role of retained histones throughout the sperm genome

Epigenetics and ART

- ART → Induce epigenetic alterations
 - Affect fetal growth and development
- ✓ May depend on genomic imprinting (*such as the Beckwith-Wiedemann or Angelman syndrome*)
- ✓ Loss of gene imprinting may occur during **preimplantation** under certain conditions of gamete handling
- Possible epigenetic risks linked to ART:
 - ✓ Use sperm with incomplete reprogramming
 - ✓ IVF procedures at a time of epigenetic reprogramming

Loss of epigenetic control (epimutation)

1. May 1~2 orders of magnitude > somatic DNA mutation
2. Inappropriate expression of the affected gene
3. May also expose hidden genetic variation
4. Some sub-fertile couples may have a genetic predisposition to **epigenetic instability**
→ offspring **more susceptible to epigenetic changes** (independently of whether or not conceived by ART)
5. Affecting imprints can arise during imprint erasure, establishment or maintenance

Some studies...

- Loss-of-function mutations of DNA methyltransferases
 - Affect all imprinted domains
 - As well as other chromosomal regions
- Mutations of Dnmt3a (de-novo methylase) and maintenance methylase (Dnmt)
 - Lead to loss of imprinting & embryonic lethality
- Targeted disruption of Dnmt3L in mice
 - Homozygous mutant males: Azoospermia
 - Homozygous females: heterozygous progeny died before midgestation

Imprinting defects and subfertility

- Can have a common and possibly genetic cause
- Super-ovulation instead of ICSI
 - ↑ risk factor of child with an imprinting defect
 - Maturation of epigenetically imperfect oocytes
 - Disturb process of DNA methylation in the oocyte
- Can lead to spontaneous abortions
- The level of DNA methylation in human sperm
 - ↔ could be linked to their ability to initiate pregnancy by ART

Epigenetics and testicular cancer

- Testicular germ cells tumor (TGCT)
 - ~98% of all testicular neoplasms and is
 - The most common malignancy among young males
- Epigenetic changes that **deregulate gene expression** are frequently observed during the development of cancer
- The epigenetic equilibrium of the normal cell is disrupted during tumorigenesis

In human neoplasms

- DNA methylation defects:
 - ✓ Hypomethylation, global loss of 5-methylcytosine (less)
 - ✓ Hypermethylation of regulatory regions of promoters (more)
 - silencing of tumor suppressor genes
 - The first epigenetic abnormality to be identified in cancer cells
- TGCs are believed to arise from primordial germ cells (PGCs) — (DNA methylation → erase parental imprints → totipotency is restored)

- The genome of seminomas
 - Extensively hypomethylated
 - Virtually completely devoid of CpG island hypermethylation
- Nonseminoma group
 - Less extensively hypomethylated
 - Variable CpG island hypermethylation levels (comparable with tumors of other tissues)

- ↑ methyltransferases (DNMT3A & DNMT3B), their homologue DNMT3L ⇔ **embryonal carcinoma subtype** significantly
- The presumptive testis-specific chromatin regulator CTCFL (BORIS) and the pluripotency marker POU5F1 (OCT3/4) ⇔ cancer/testis associated genes
 - hypermethylated in somatic tissue
 - hypomethylated in normal testis tissue

The effect of **epigenetic sperm abnormalities** on early embryogenesis

- Imprinting errors in developing fetus → severe pathologies
- ART → ↑ the risk of imprinting diseases
- In oligospermic men, ↑ Abnormal methylation of the H19 gene ↔ Beckwith-Wiedemann syndrome
- ↓ Genome-wide methylation pattern in sperm (ex.: 5-methyl-cytosine immunostaining)
 - Poor embryo quality in rats
 - In normospermic men
 - Poor pregnancy outcome during IVF
 - Independently affects embryogenesis

- Men undergoing testicular sperm extraction (TESE) for ICSI: Frequency of P1 transcripts ↔ pregnancy rates
- Epigenetic regulation of DNA (via nuclear packaging) in the sperm ↔ function of the mature sperm
- *Complex path of sperm production & delicate balance of epigenetic and genetic factors during sperm maturation*
- Formation of a mature sperm with the ability to fertilize an oocyte
- Developing embryo

Epigenetic regulation and nutrition

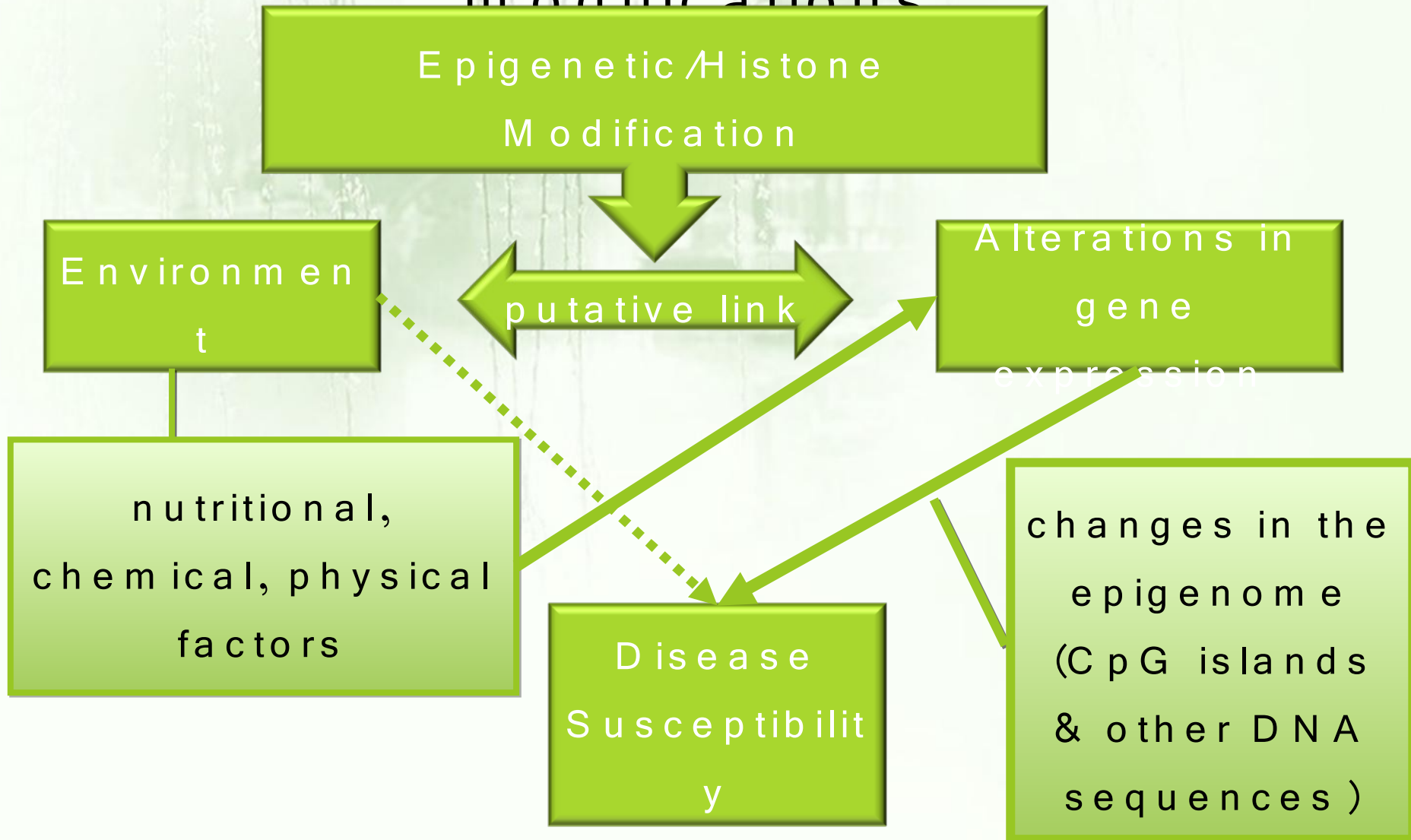
1. Epigenetic programming:
 - Tightly during fetal development and lactation
2. Dietary supplement with a **methyl donor** during pregnancy → ↑ methylated IAP (Intracisternal-A particles) sequence
3. There are many environmental and metabolic factors that can influence patterns of **histone acetylation** and **DNA methylation**

- Nutrition during early development
 - Can influence DNA methylation
 - 1-carbon metabolism is dependent on dietary methyl donors & co-factors (*methionine, choline, folic acid, vitamin B-12*) → critical during development
- Folate supplementation → Can prevent epigenetic change caused by a decrease in DNMT1 activity
- Maternal behavior → epigenetic regulation of the glucocorticoid receptor gene in the hippocampus and determines the stress responses of the offspring

- Methyl-donor-deficient diet in postnatal life
 - Can permanently affect the expression of IGF 2
 - Resulting in **growth retardation**
- Patient with Hyperhomocysteinaemia
 - Accumulation of Sadenosylhomocysteine (an inhibitor of DNA methyltransferases)
 - Diet: affect the DNA methylation status

★ The impact of diet, nutrients or drugs on early epigenetic programming → seriously in spermatogenesis

The role of Environmental Factors in epigenetic modifications



M o n o z y g o t i c T w i n s

- *An interesting model for studying the role of environmental factors in epigenetic modifications*
 - Epigenetically concordant at birth in most cases
 - Differences (DNA methylation and histone modifications) accumulate with age
 - Greatest in those who had lived together for the smallest amount of time
- This finding underlines the relative importance of environmental factors in addition to intrinsic factors

Future perspectives

- Common diseases ↔ epigenetic basis ↔ medicine
- Knowledge of genetic & epigenetic modifications of **germ cells** → necessary for the production of functional gametes & overcoming infertility
- Analysis of **DNA methylation** patterns → Categorization of infertile men → A new level of ↓ fertilization, implantation or pregnancy rates
- In causing disease: Environmental interaction ↔ genome → modulating to improve human health
- Clinical spectrum → Molecular Dx & targeted Tx



**THANK YOU FOR
LISTENING**