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Transgenerational Ageing (2)

Rossella E. Nappi
(UNIPV-ITALY)

GLADE – VIRTUAL INSTITUTE FOR GOOD HEALTH AND WELL-BEING,
18 – 25 September, 2022

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Ageing Across the Life-Course and Generations

Rossella E. Nappi
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The power of early intervention to prevent NCDs

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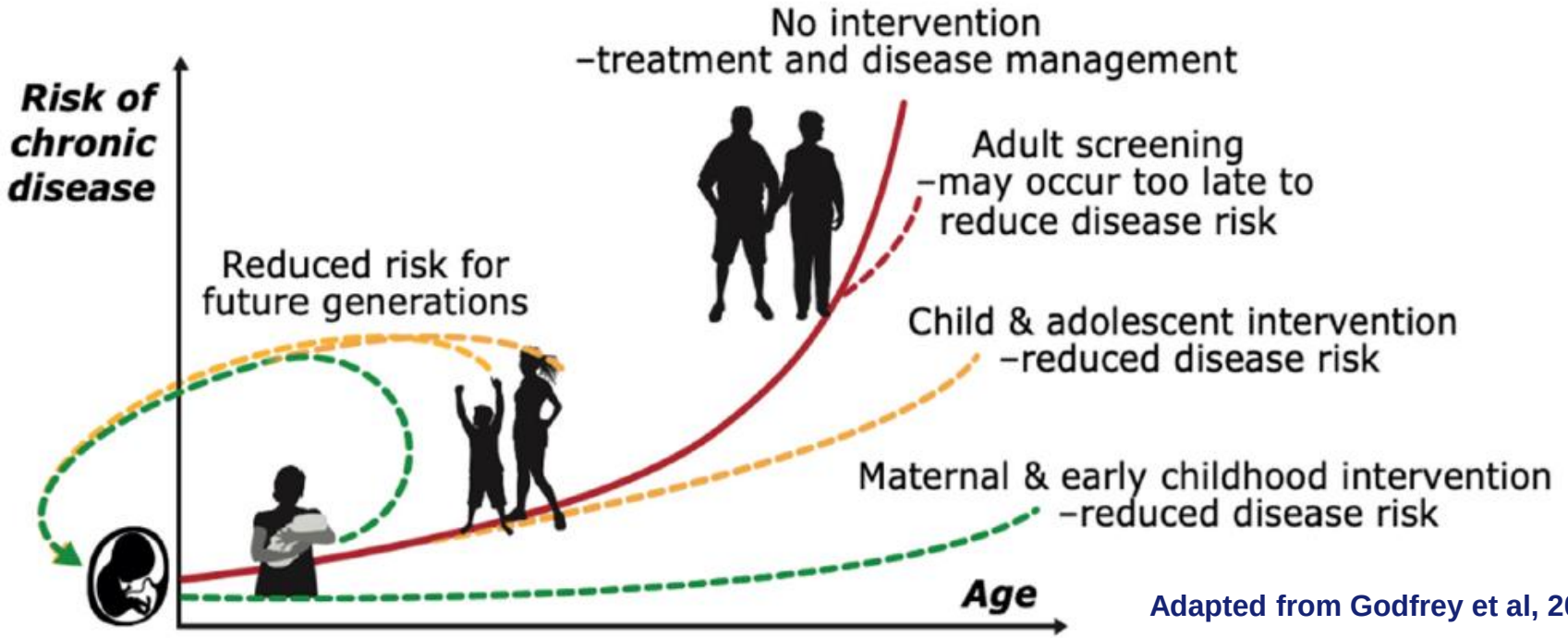

Adapted from Godfrey et al, 2010

- The life-course approach emphasizes the complex interactions between environmental exposures from pre-conception onwards that influence chronic disease risk.
- Such an approach also helps mitigate the potential for transgenerational transmission of disease traits.

Tohi et al, 2022

The First Thousand Days Perspective (1)

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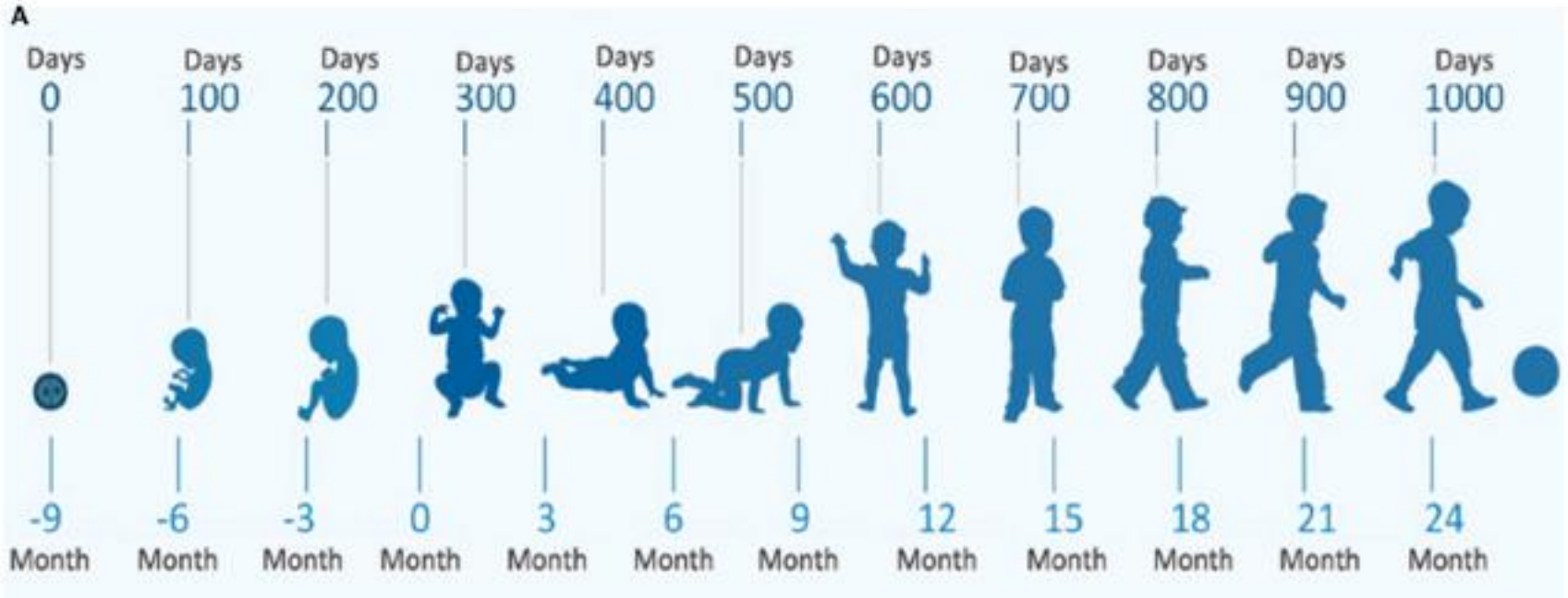
WHO program diagram depicts the horizontal perspective over the first 1,000 days from conception through 2 years of age.



- Gene–environment interactions begin at conception to influence maternal/placental/fetal triads, neonates, and children with short- and long-term effects on organs and systems.

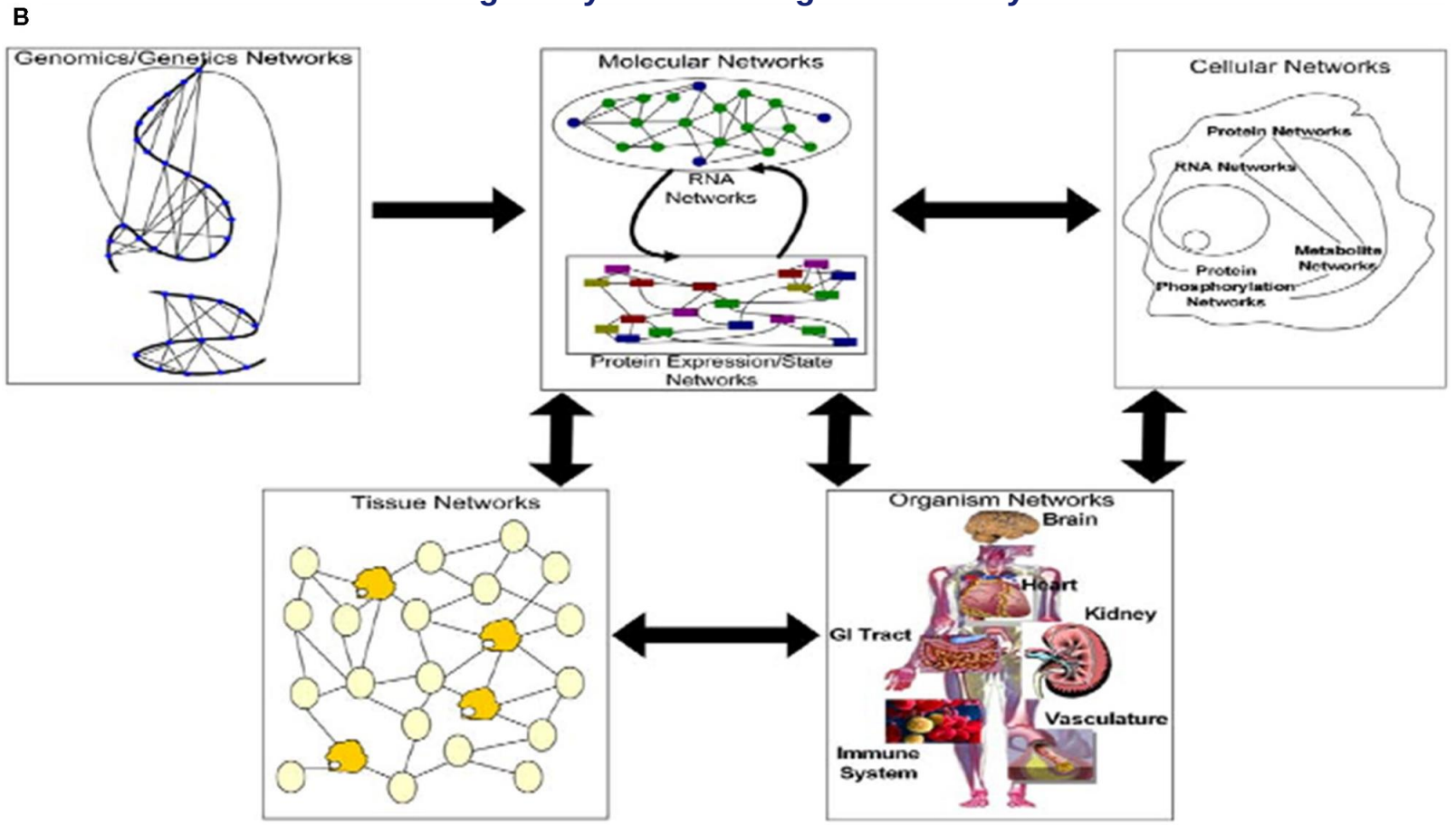
MS Scher, 2021

The First Thousand Days Perspective (2)

Co-funded by the Erasmus+ Programme of the European Union

The vertical diagnostic perspective depicts the flow of information across biological systems through a hierarchy of networks.

- Each panel highlights a different set of networks at play in a biological system.
- **Genomics networks** represent interactions among DNA sequences that may give rise to longer-range as well as more local chromosome structures that modulate gene activity, in addition to inducing synergistic effects on higher-order phenotypes. Genomics networks drive molecular networks composed of RNA, protein, metabolites, and other molecules in the system.
- **Molecular networks** are components of **cellular networks** in which the complex web of interactions among these networks gives rise to the complex phenotypes that define living systems.
- **Tissue networks** comprise cellular networks that are clearly influenced by the molecular and genomics networks, and organism networks comprise tissue networks that are clearly defined by the component cellular and molecular networks
- **Complex phenotypes like disease** emerge from this complex web of interacting networks, given genetic and environmental perturbations to the system

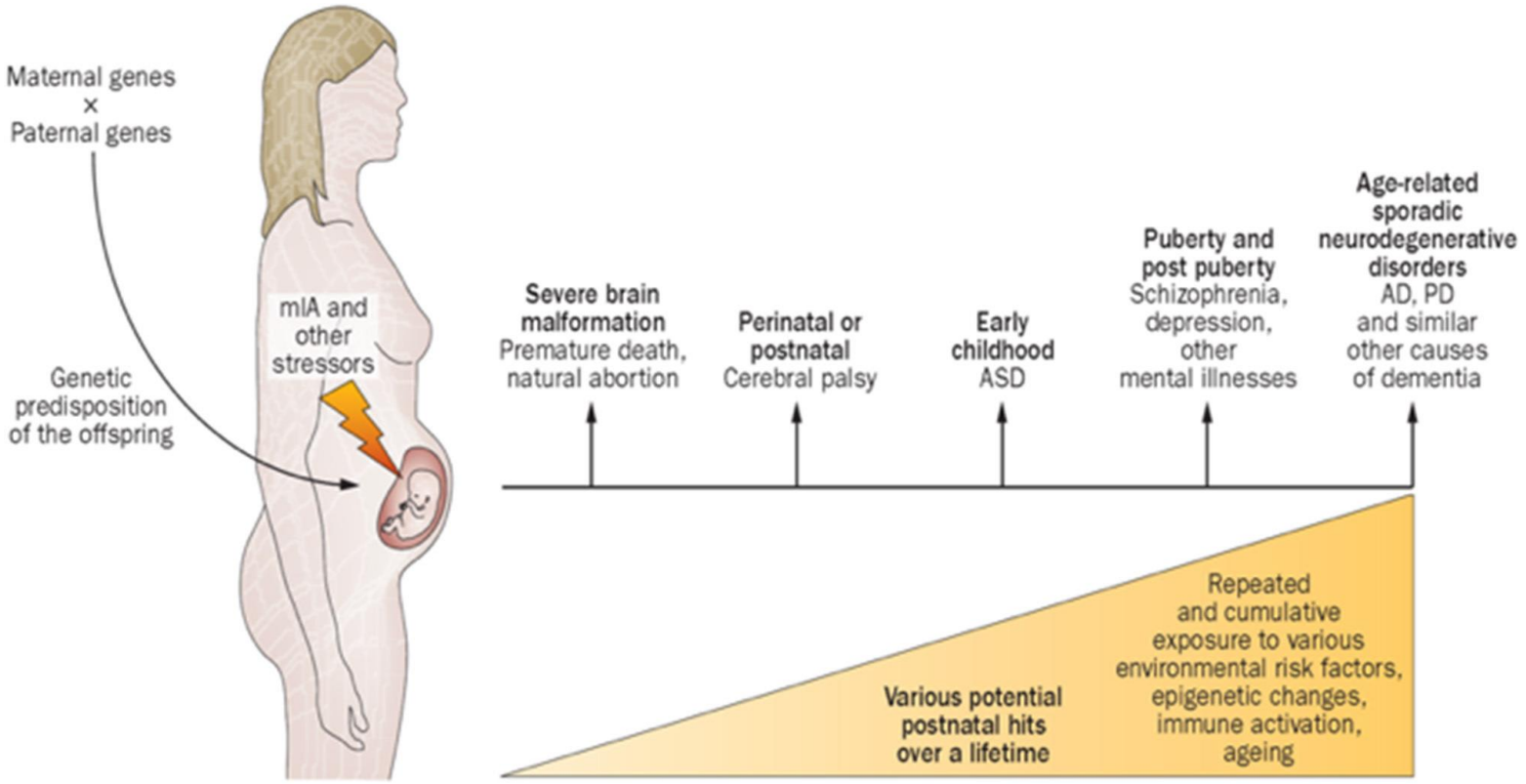


MS Scher, 2021

The Example of the Fetal/Neonatal Neurology Program

- Proposed causal chain of events with maternal Immune Activation (IA) in humans, leading to a wide spectrum of neuronal dysfunctions and behavioral phenotypes observable in the juvenile, adult, or aged progeny.

AD, Alzheimer disease; ASD, autism spectrum disorder; mIA, maternal immune activation; PD, Parkinson disease

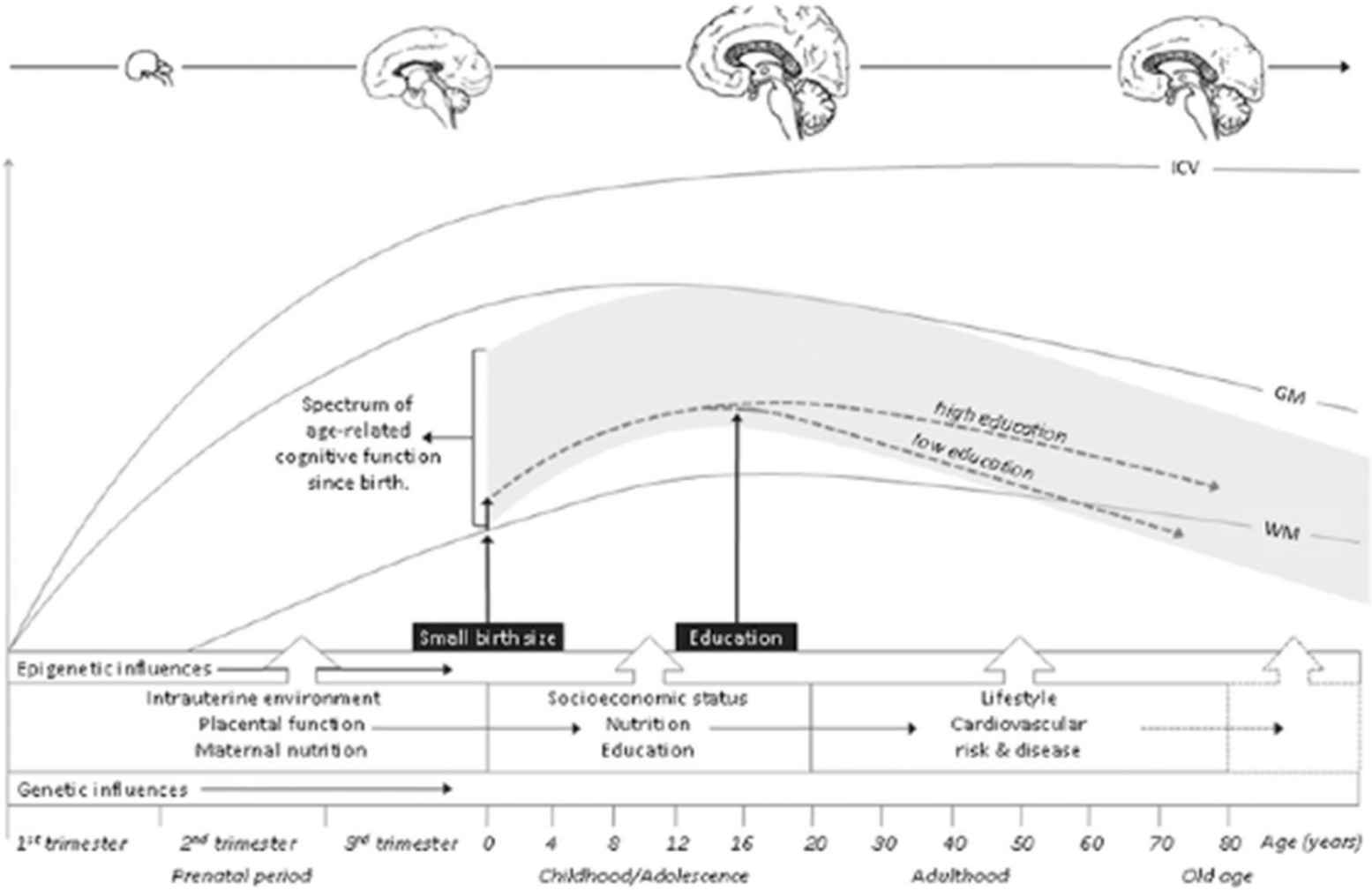


MS Scher, 2021

A hypothesized model of the origins and life course of brain aging

Co-funded by the Erasmus+ Programme of the European Union

- **Several "critical periods"** (prenatal period, childhood/adolescence, adulthood, and old age) are identified during which an individual is at greatest risk of damage if exposed to putative risk factors.
- Normal development of ICV and brain volumes (GM and WM) is presented for these critical periods, and the possible different risk factors influencing brain development throughout these periods are described.
- Allegedly, **genetics and epigenetic influences could alter brain structure and function throughout life, but their impact would probably fade with age**. In addition, the spectrum of age-related cognitive ability from birth to old age is presented in this figure, with a schematic view of our findings that small birth size is related to poor cognitive functioning only in those with lower educational levels.



MS Scher, 2021

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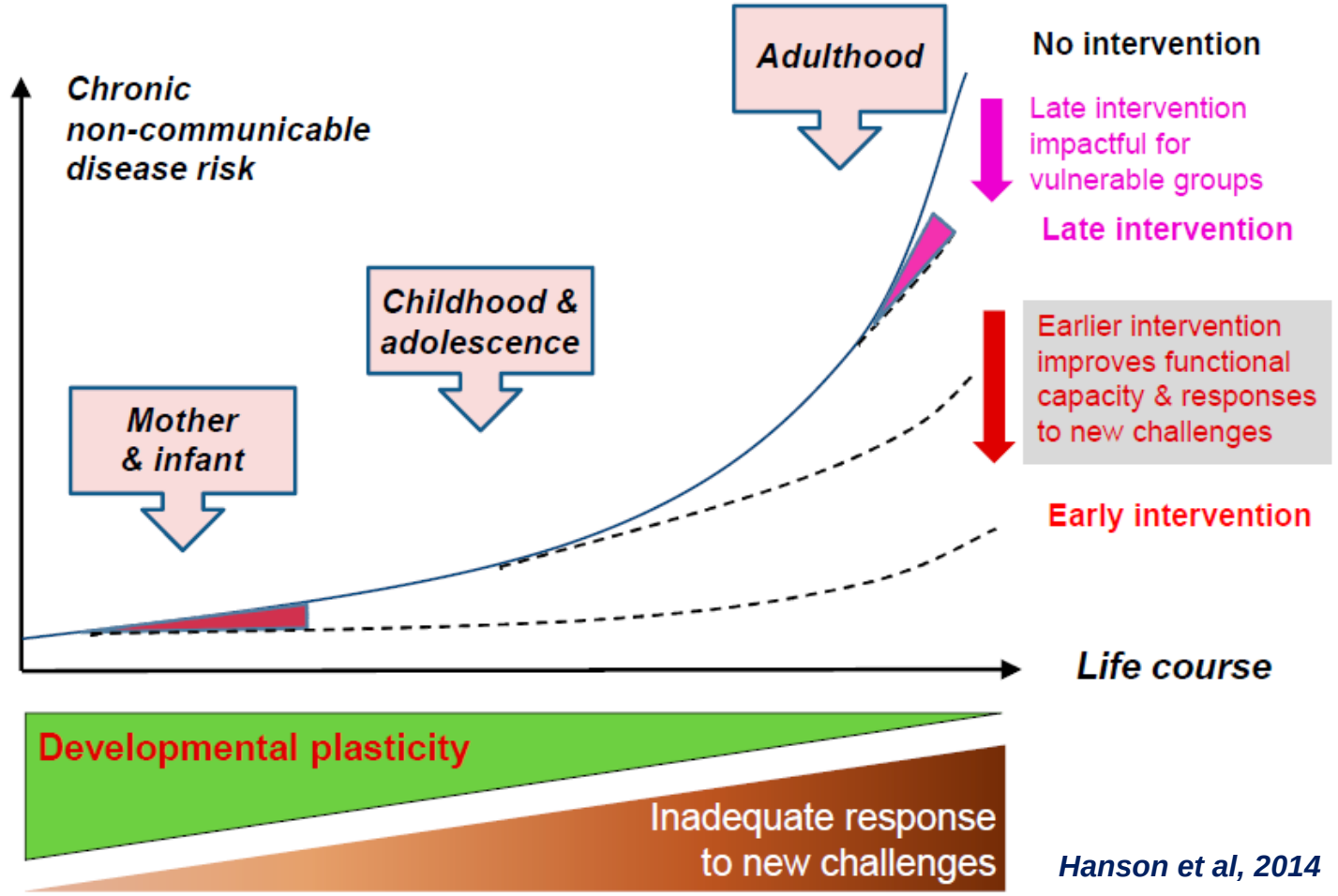
Timing of interventions and effect on disease risk

Co-funded by the Erasmus+ Programme of the European Union

- Non-communicable diseases (NCDs), such as cardiovascular disease and osteoporosis, affect individuals in all countries worldwide.
- Given the very high worldwide prevalence of NCDs across a range of human pathology, it is clear that traditional approaches targeting those at most risk in older adulthood will not efficiently ameliorate this growing burden.
- It will thus be essential to robustly identify determinants of NCDs across the entire life course and, subsequently, appropriate interventions at every stage to reduce an individual's risk of developing these conditions.
- A life course approach has the potential to prevent NCDs, from before conception through fetal life, infancy, childhood, adolescence, adulthood and into older age.



Hanson et al, 2014

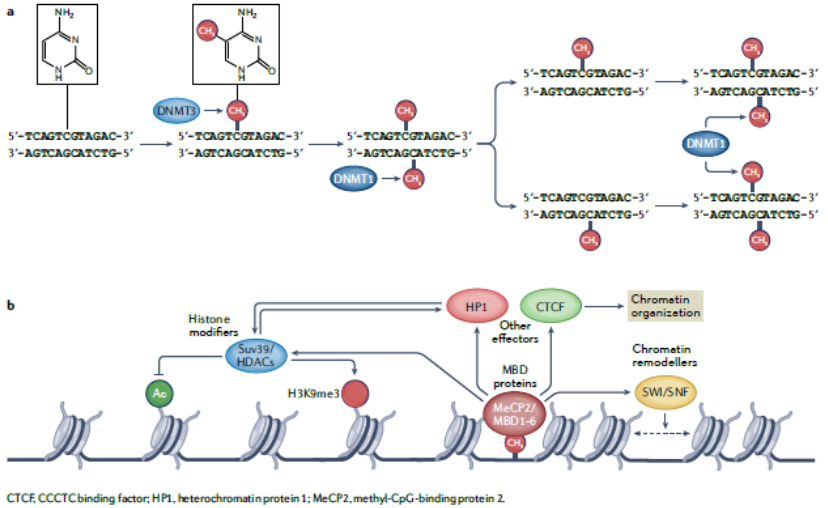
Baird et al, 2017

Molecular mechanisms of transgenerational epigenetic inheritance

Co-funded by the Erasmus+ Programme of the European Union

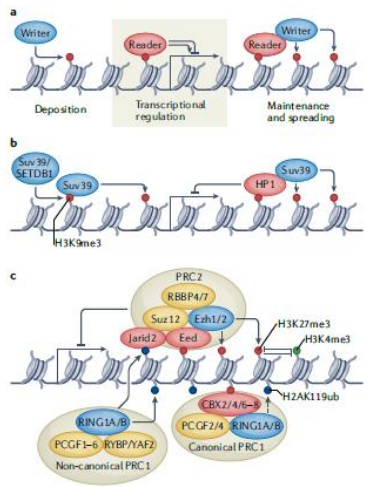
- As the basis of genetic information, DNA sequence is the foundation of life on Earth. However, its proper implementation requires another level of information, in the form of regulatory signals.
- The study of these signals is known as ‘epigenetics’, a term originally coined by Conrad Waddington in 1942 that has since undergone several redefinitions.
- In here, epigenetics denote “the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA sequence”.
- This definition encompasses those molecular signals peripheral to the DNA that are generally referred to as epigenetic, such **as DNA methylation or modification of histone proteins** around which DNA wraps to form the nucleosome (the basic structural unit of chromatin), as well as more recently discovered gene regulatory signals such as 3D genome organization. It also includes both mitotic inheritance of these signals and inheritance across generations.

DNA methylation deposition and inheritance



CTCF, CCCTC binding factor; HP1, heterochromatin protein 1; MeCP2, methyl-CpG-binding protein 2.

Histone modifications



Fitz-James & Cavalli, 2021

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Intergenerational and transgenerational epigenetic inheritance

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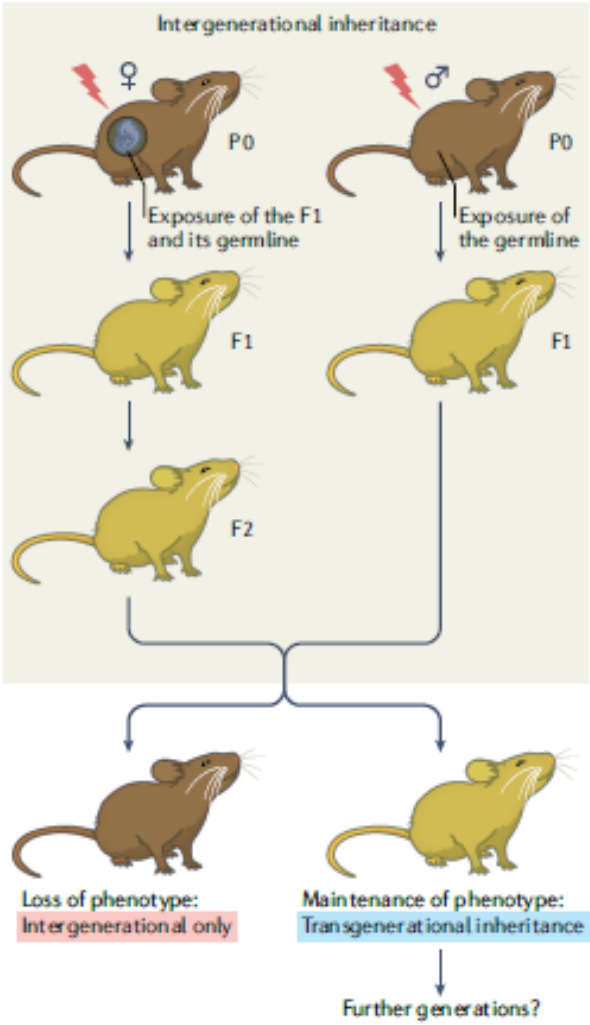
- Epigenetic change can arise in an individual sporadically or by exposure to some environmental stimulus.
- If this change is passed on to the next generation, it becomes a heritable epigenetic mark.
- Inheritance in the immediate offspring of the individual in which the change arose is termed ‘intergenerational’.
- In mice this corresponds to inheritance in the F1 generation for an exposed male parent or in the **F1 and F2 generations** for an exposed female.
- This is due to the exposure not only of the individual mouse but also its germline and potentially, in the case of the female, of its unborn offspring’s germline. Beyond these first generations many epigenetic signals are lost, and inheritance does not proceed past the intergenerational stage.
- In some cases, however, the signal is maintained in the **F2/F3 generations** and beyond. Past this point, it is termed ‘transgenerational’ epigenetic inheritance, because the epigenetic signal is maintained even in the absence of the initial stimulus or epigenetic trigger.

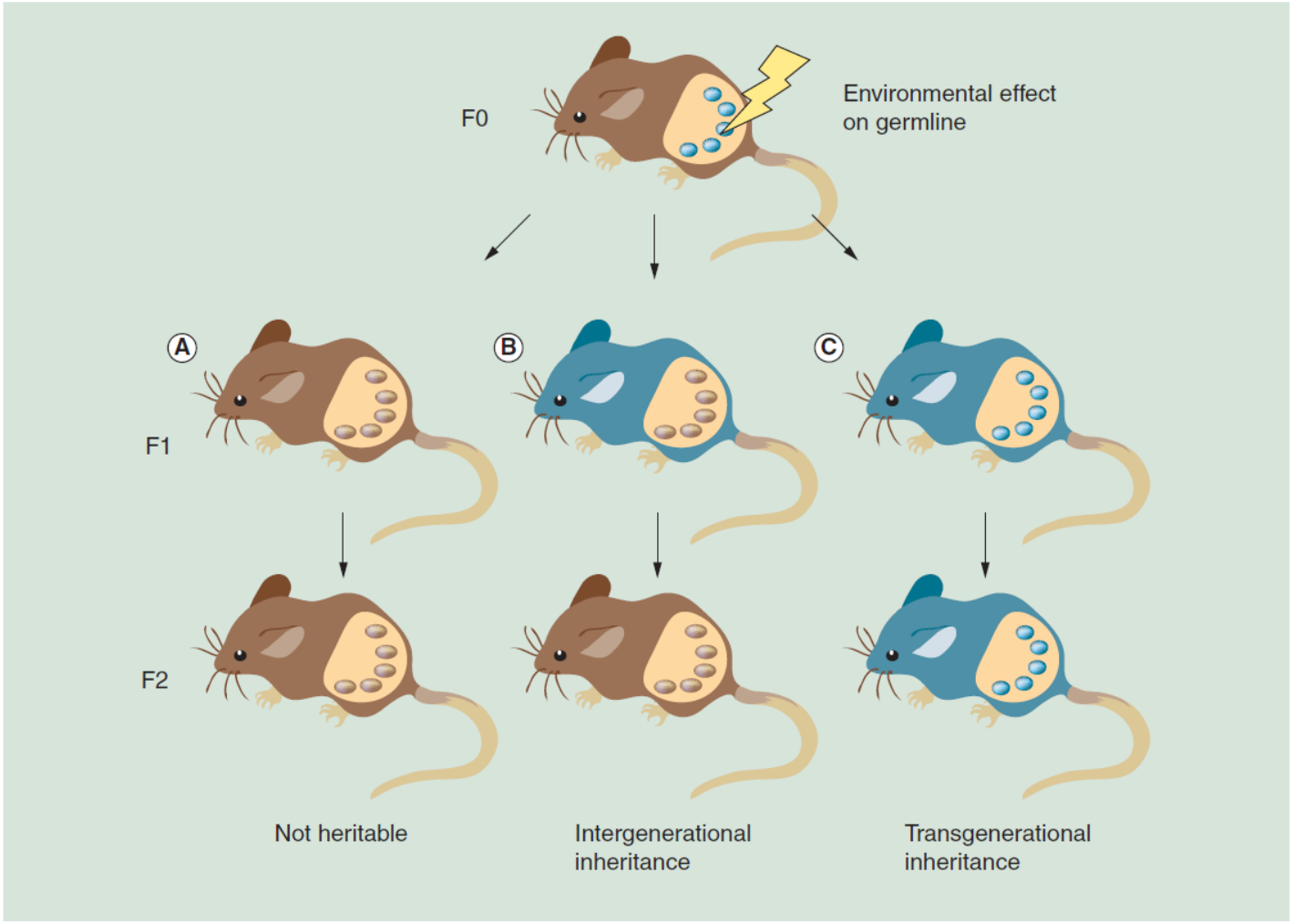


Fitz-James & Cavalli, 2021

Epigenetics: where environment, society and genetics meet

Co-funded by the Erasmus+ Programme of the European Union

(A) No inheritance: epimutations in the parental F0 germline do not affect the offspring (F1). Such epimutations are presumably corrected during F1 preimplantation reprogramming.
(B) Intergenerational inheritance: epimutations transmitted through the F0 germline escape preimplantation reprogramming and alter development in the F1 generation. However, these epimutations are corrected in the germline of F1 animals and are not transmitted to the F2 generation.
(C) Transgenerational inheritance: epimutations escape both preimplantation and germline reprogramming in F1 and subsequent generations and affect development over multiple generations.



Majnik & Lane, 2014

The crucial role of motherhood in transgenerational inheritance in both sexes

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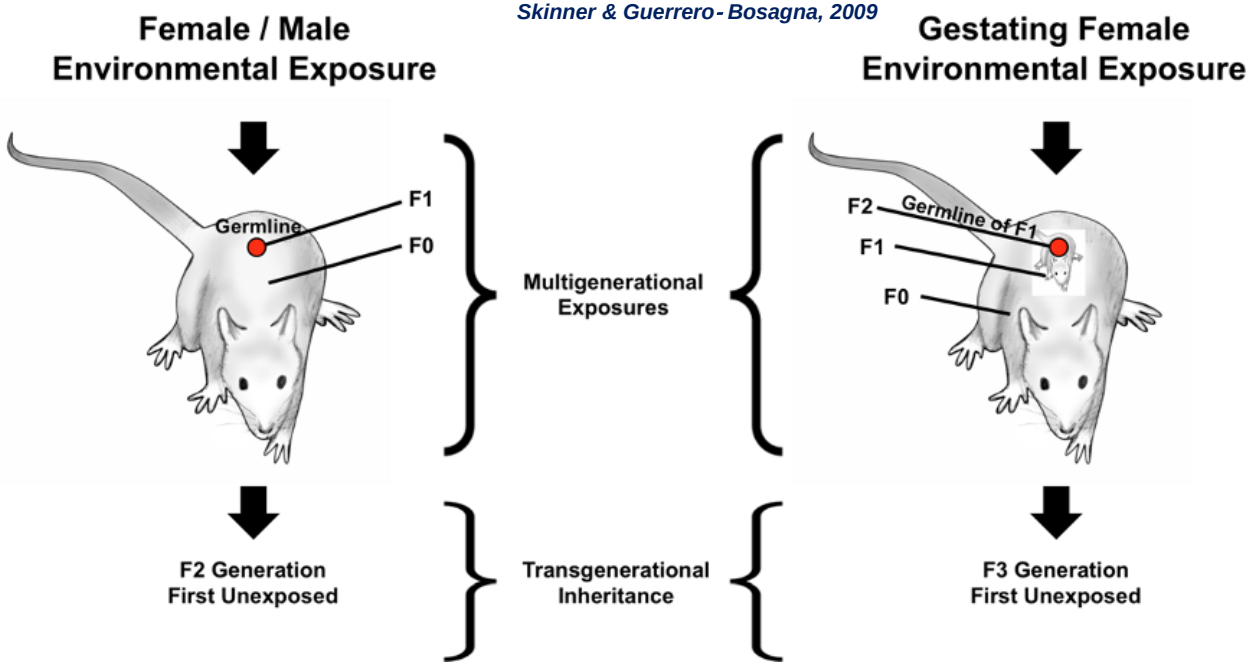
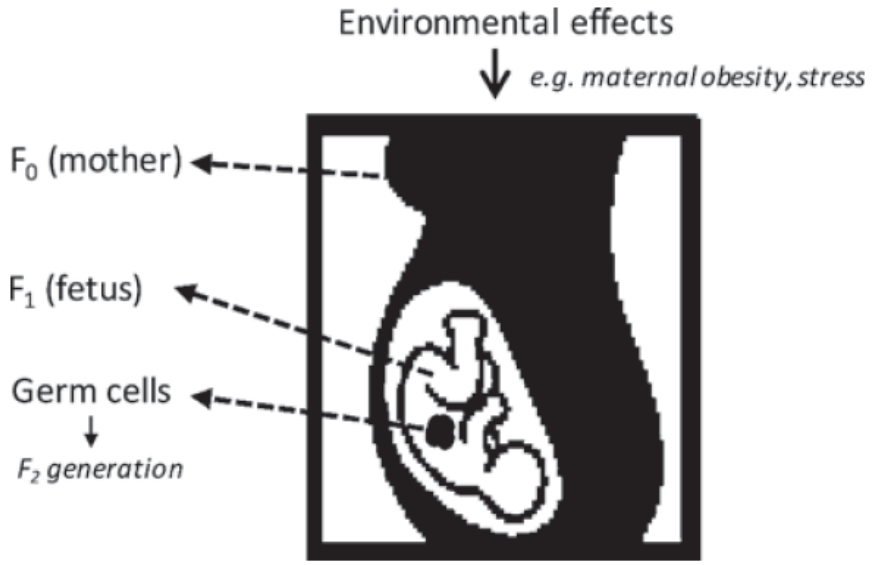
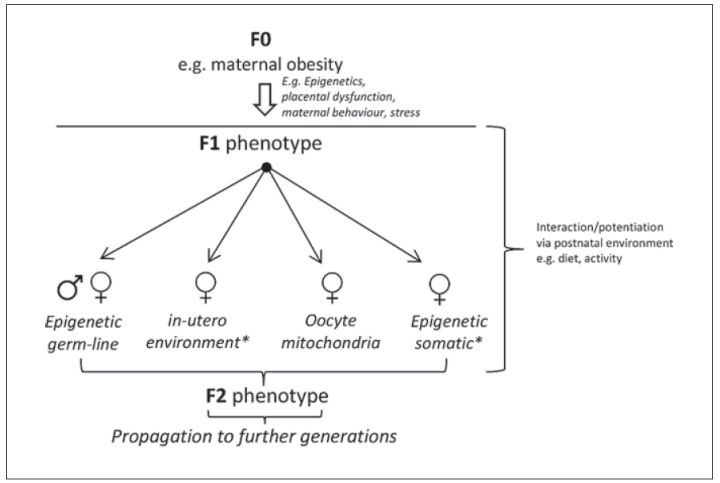
Table 3. Transgenerational versus multigenerational phenotypes and toxicology.

Phenotype	Exposure	Definition
Multigenerational	Direct	Coincident direct exposure of multiple generations to an environmental factor or toxicant promoting a toxicology in the multiple generations exposed
Transgenerational	None, except the initial (ancestral) generation	After the initial exposure, the transgenerational phenotype is only transmitted through the germline in the absence of direct exposure

Skinner & Guerrero-Bosagna, 2009







Multigenerational exposure and transgenerational inheritance

Skinner, 2014

Vickers, 2014

Transgenerational Effect of Grandmaternal Inheritance

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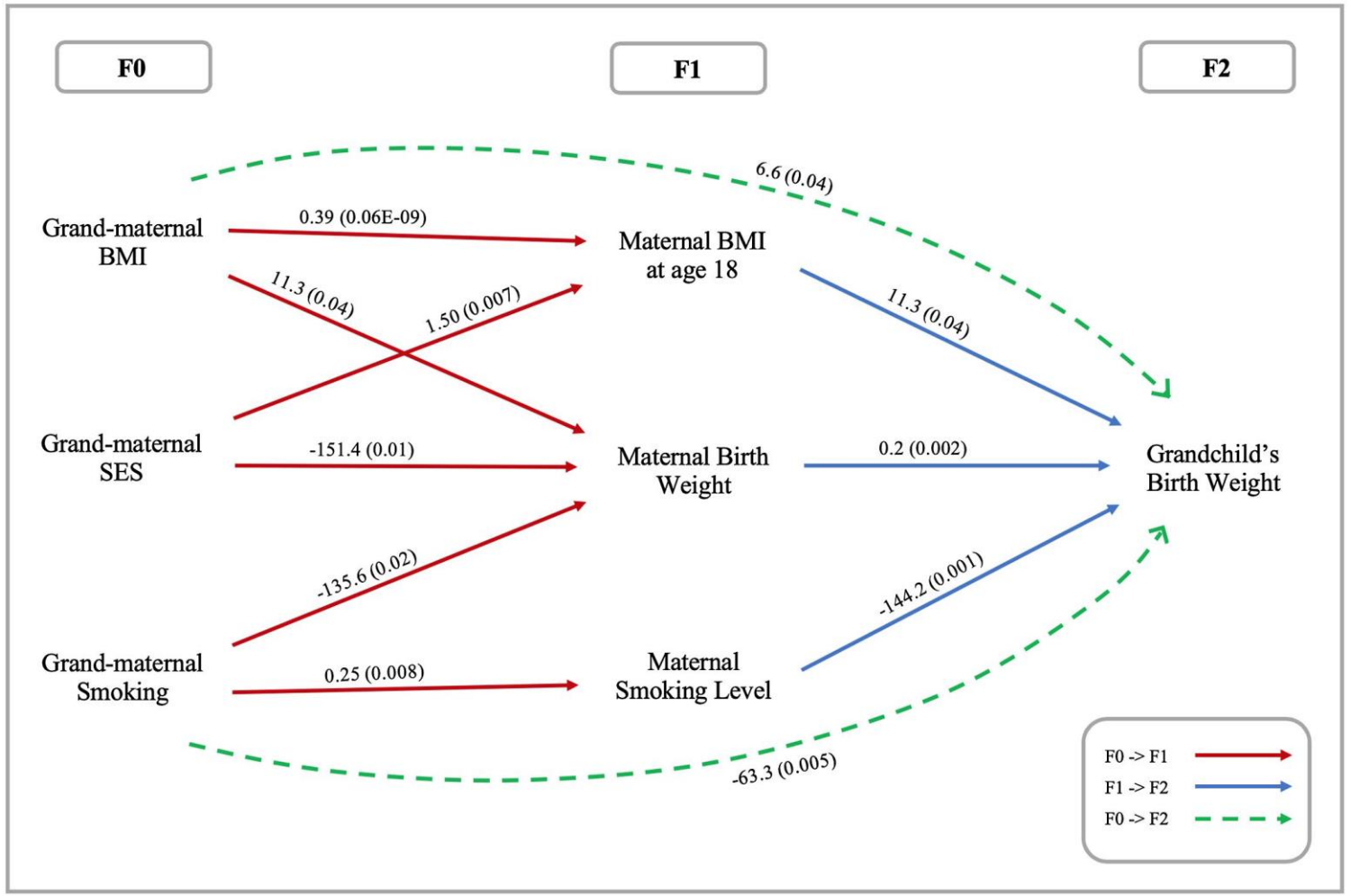
- **Larger grandmaternal BMI indirectly increased grandchild's BW via maternal BW and BMI.**
- **Grandmaternal smoking during pregnancy indirectly reduced grandchild's BW via maternal smoking during pregnancy and BW.**



Shen et al, 2020

Overview of potential maternal prenatal stressors during the current COVID-19 pandemic

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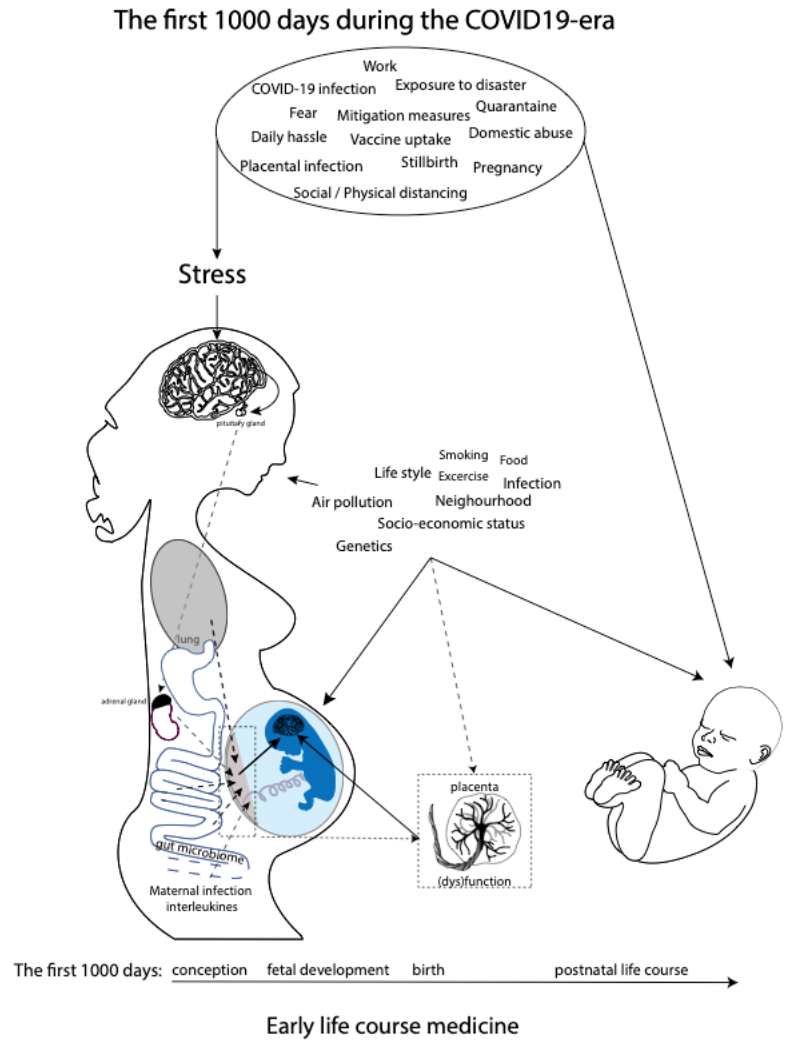
EARLY LIFE COURSE MEDICINE

Several pathways affecting fetal (neuro)development in utero, including maternal HPA axis, maternal inflammation, maternal gut microbiome and placental dysfunction, and postnatal neurodevelopment during the first 1000 days are evident.

The COVID-19 pandemic has a major impact on society, particularly affecting its vulnerable members, including pregnant women and their unborn children. Pregnant mothers reported fear of infection, fear of vertical transmission, fear of poor birth and child outcomes, social isolation, uncertainty about their partner's presence during medical appointments and delivery, increased domestic abuse, and other collateral damage, including vaccine hesitancy.
Accordingly, pregnant women's known vulnerability for mental health problems has become a concern during the COVID- 19 pandemic, also because of the known effects of prenatal stress for the unborn child.



Schoenmakers et al, 2022

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Examples of stressors during pregnancy

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Table 2. Stress during pregnancy and associated pregnancy complications in humans

Type of stress	Pregnancy complications	References
Exposure to Hurricane Katrina	Induction of labour	Oni *et al.* 2015
	Increased perceived stress	
Death of close relative	Preeclampsia	László *et al.* 2013
	Gestational diabetes	László *et al.* 2015
Obesity during pregnancy	Gestational diabetes	Chu *et al.* 2007
Overweight during pregnancy	Preeclampsia	Dempsey *et al.* 2003
Maternal low birth weight	Preeclampsia	Dempsey *et al.* 2003
	Gestational diabetes	Seghieri *et al.* 2002
Vitamin D deficiency	Preeclampsia	Bodnar *et al.* 2014

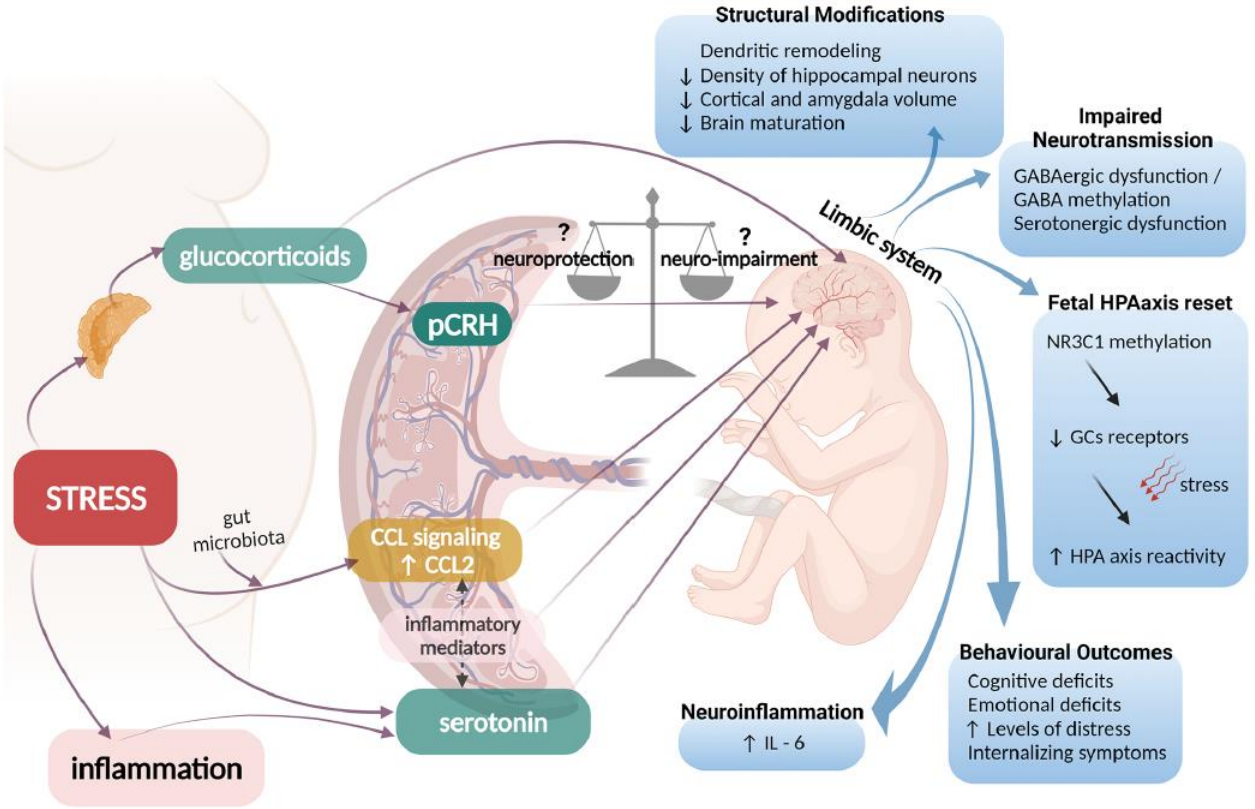
Stressful maternal events that can contribute to the development of various pregnancy complications as demonstrated in epidemiological studies.

Cheong et al, 2016

Possible pathophysiological mechanisms linking prenatal maternal adversity to disrupted fetal brain programming

Co-funded by the Erasmus+ Programme of the European Union

- Maternal stress could activate adrenal production of glucocorticoids (GCs) that can cross the placenta and regulate fetal brain neurogenesis. GCs also enhance production and release of placental CRH (pCRH) into the fetal compartments, a neuropeptide that can exert either neuroprotective or neuro-impairment effects.
- Excess levels of GCs and pCRH have been associated with structural fetal brain modifications, impaired neurotransmission and disrupted programming of the HPA axis of the fetus that involves epigenetic modifications of the glucocorticoid receptor (GR) gene and is linked with increased HPA axis reactivity of the neonate and adverse behavioral and emotional outcomes later in life. Additionally, maternal stress or inflammatory conditions can enhance placental output of serotonin (5-HT) to the fetal brain leading to serotonergic dysfunction.
- Excess maternal stress, can influence signals arising from gut microbiota to affect placental CCL signaling. The interplay between placental 5-HT, CCL-2 and other inflammatory mediators ultimately drives fetal neuroinflammation and IL-6 elevation in the fetal brain.



Kassotaki et al, 2021

The downstream effects of maternal care

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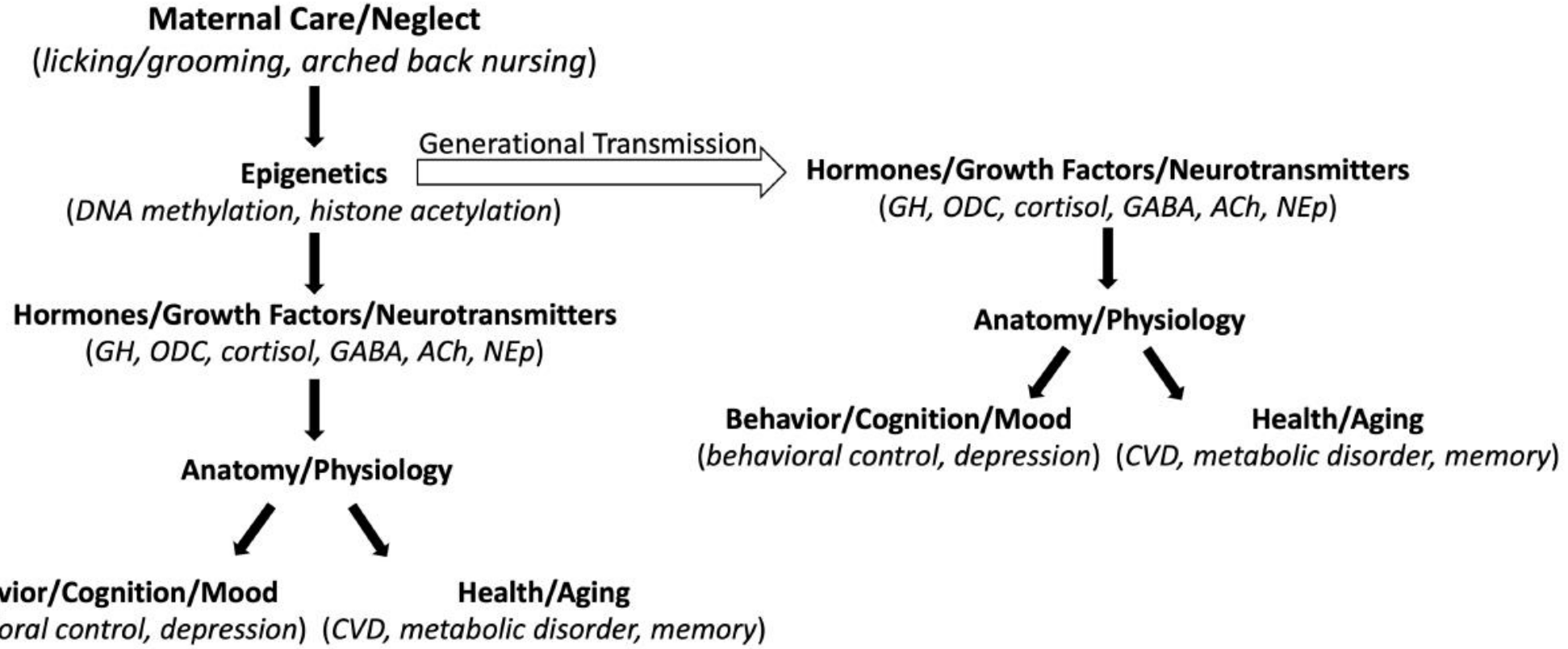


- **Maternal care alters gene expression (Epigenetics) in the offspring that then alters expression of various molecules (Hormones/Growth Factors/Neurotransmitters) that impact quality of life (Behavior/Cognition/Mood) and longevity (Health/Aging). The Epigenetic effects can be transmitted across generations to alter phenotypes in subsequent generations.**

GH, growth hormone; ODC, ornithine decarboxylase; GABA, gamma-aminobutyric acid; ACh, acetylcholine; NEp, norepinephrine; CVD, cardiovascular disease.

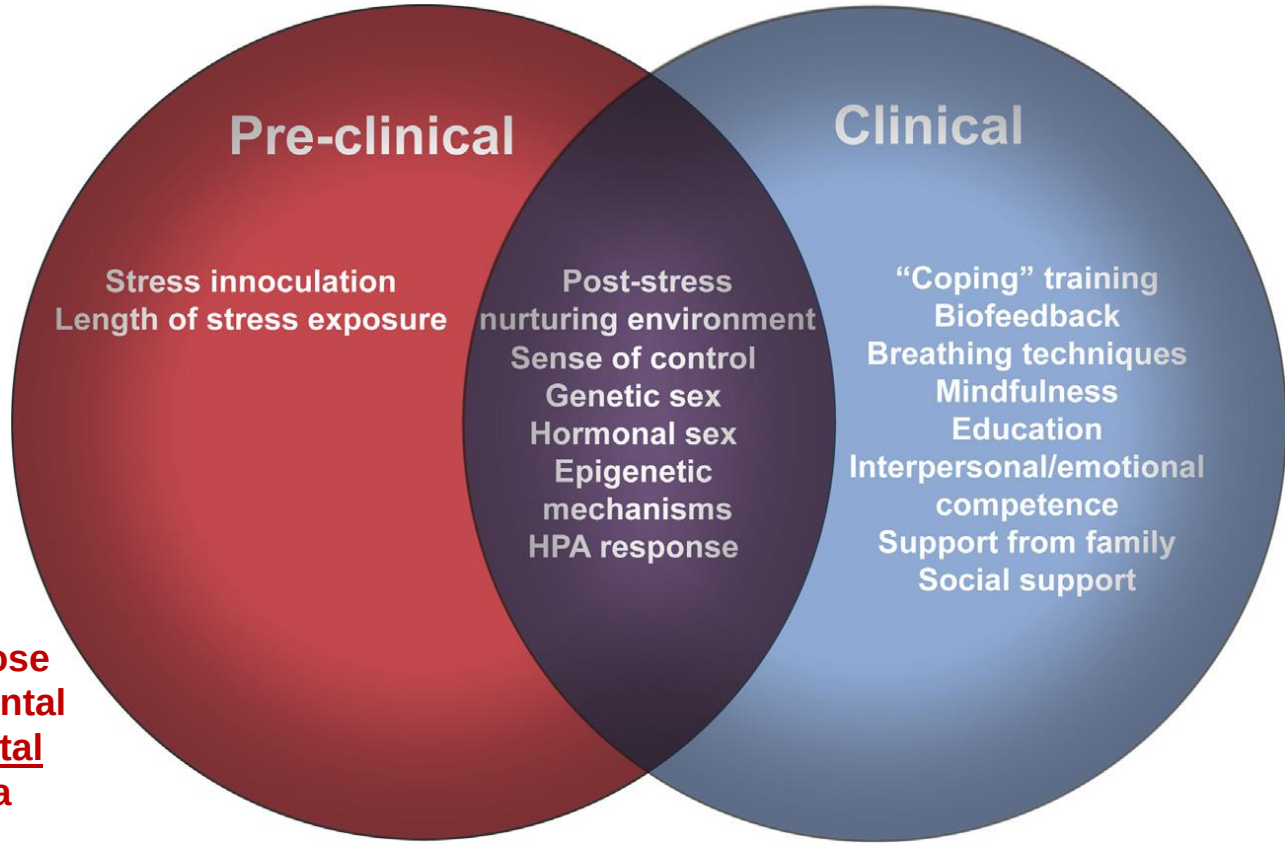
Dumas, 2022

Methods and mechanisms of promoting resilience

Co-funded by the Erasmus+ Programme of the European Union

Resilience is an active and dynamic process that is shaped in part by genetic sex, gonadal steroids, and epigenetic regulation of stress physiology and changes across epochs.

- **In preclinical models, resilience is ascribed to animals that experience a stressor, yet demonstrate biological or behavioral phenotypes similar to unstressed control animals.**
- **In clinical research, the ability to experience significant stress without subsequent psychopathology is considered a sign of resilience. However, studies of immune, hypothalamic-pituitary-adrenal (HPA) axis, and brain function suggest that such exposures have a physiologic impact even in asymptomatic individuals. Such alterations create risk for adverse health conditions later in life.**



<u>Proximal responses</u> are defined as those that occur within the same developmental epoch as the stress exposure, and <u>distal responses</u> as those that occur within a subsequent developmental epoch.

Hodes & Neill Epperson, 2019

Relationship between stress effects on behavior across the life span and psychiatric disease (1)

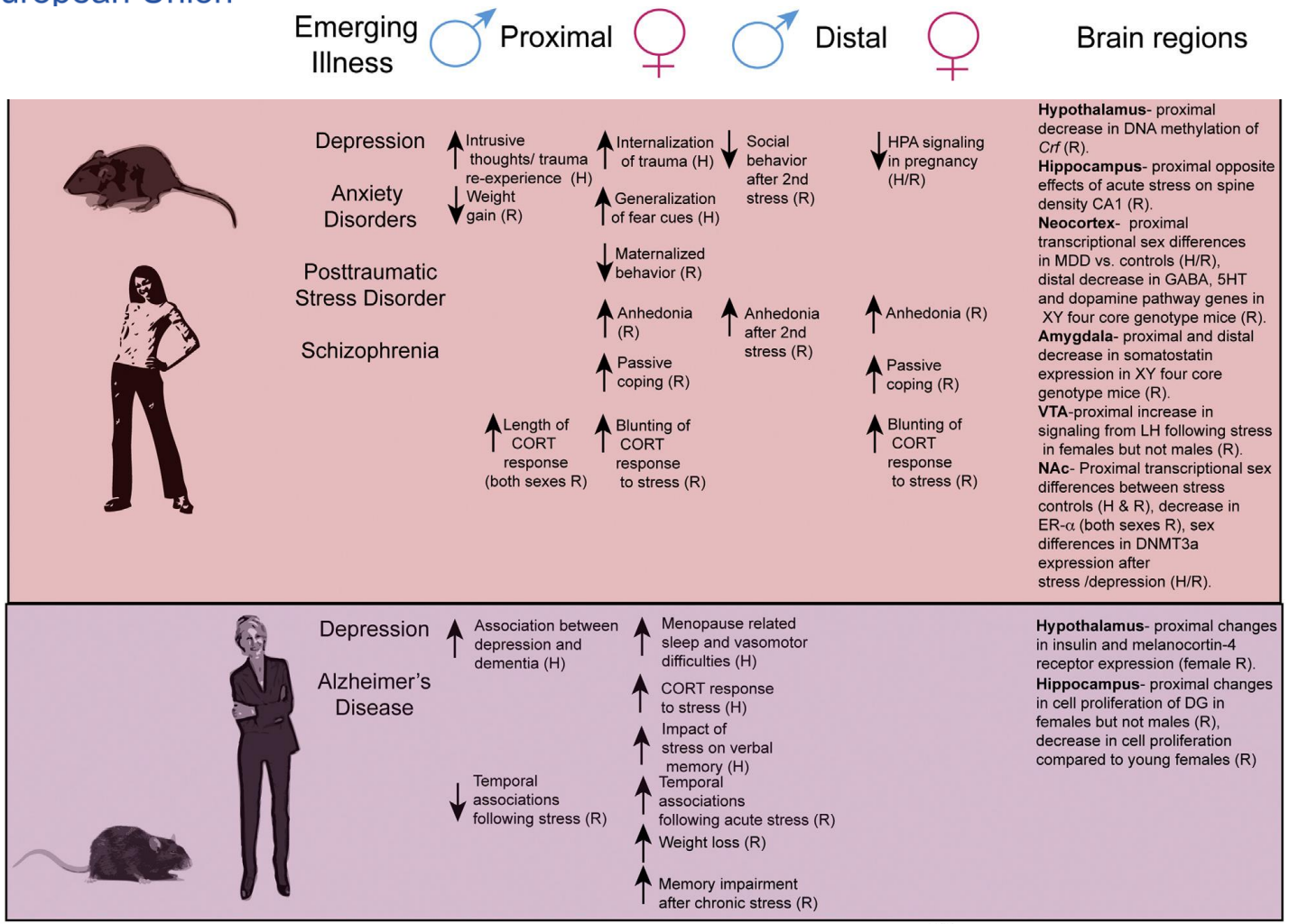
Co-funded by the Erasmus+ Programme of the European Union

Emerging Illness	Proximal ♂	Proximal ♀	Distal ♂	Distal ♀	Brain regions
 Autism Spectrum Disorder Attention Deficit Disorder	↑ Peripheral vasodilation (H) ↑ Risk of stillbirth (H)	↓ Fetal energy (H) ↑ Risk of premature birth(H) ↓ Fetal growth (H)	↓ Social behavior (H/R) ↑ Anhedonia (R) ↑ Passive coping (R) ↑ Conditioned place preference (R) ↓ Paired plus inhibition (H/R) ↑ CRF Immunoreactivity (R)	↑ Passive coping (R) ↓ Conditioned place preference (R) ↓ Spatial learning (R)	**Hypothalamus**- distal changes in methylation status of *Crf* and *Gr* (male R). **Hippocampus**- proximal changes NMDA/GABAA (female R), distal changes NMDA/GABAA (both sexes-R). Neuronal atrophy CA3/2 (male R), CA1/CA3 (female R). **Neocortex**-distal increase in GR binding (female R), distal decrease in binding (male R). **Amygdala**- distal effect of higher GR binding in adulthood F>M (R).
  Autism Spectrum Disorder Attention Deficit Disorder	↑ CORT (both sexes H&R)		↑ Impulsivity & conduct disorders (H) ↓ Social behavior (H/R) ↑ Anhedonia (H/R) ↑ Cognitive impairment (R)	↓ CORT levels in response to offspring separation or distress (H&R) ↓ Maternal care (R) ↑ Depression & anxiety (H) ↓ Exploratory anxiety (R) ↓ Weight gain (R) ↓ Conditioned freezing (R)	**Hypothalamus**- proximal and distal decrease in CRF mRNA (male R), proximal decrease of CRF binding (both sexes R). **Hippocampus**- proximal decrease in CRF mRNA CA1/DG (both sexes R), distal CA1 dendritic atrophy, CA3 mossy fiber expansion, reduced LTP, increase of CRF receptor expression (male R). **Neocortex**- distal increase in correlation between blood *OTX* methylation and increase in connectivity between vmPFC and cingulate (both sexes H), increase in activation of DLPFC by tryptophan depletion post menopause (female H), increase in connectivity between ACC and POA, PAG, thalamus, M1 during early lactation (female R). **Amygdala**- larger structure, stronger activation by threatening stimuli (both sexes H).

Hodes & Neill Epperson, 2019

Relationship between stress effects on behavior across the life span and psychiatric disease (2)

Co-funded by the Erasmus+ Programme of the European Union

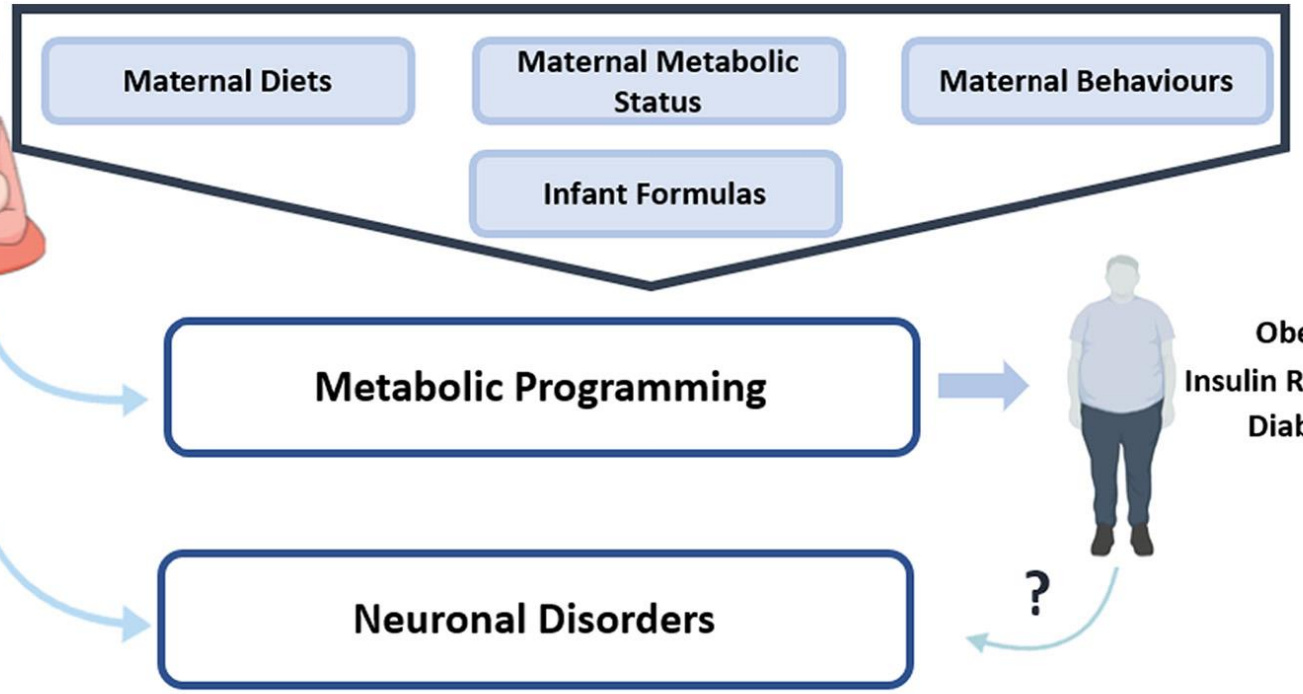


Hodes & Neill Epperson, 2019

Programming of future generations during breastfeeding

Co-funded by the Erasmus+ Programme of the European Union

- Maternal metabolic condition, diet and behaviours adopted during lactation period may predispose the offspring for metabolic syndrome such as obesity, insulin resistance and diabetes, at adulthood.
- These conditions can also have an impact in offspring neurodevelopment and increase their susceptibility for anxiety, depression, learning and memory impairments and higher risk for autism spectrum disorder and attention deficit hyperactivity disorder.

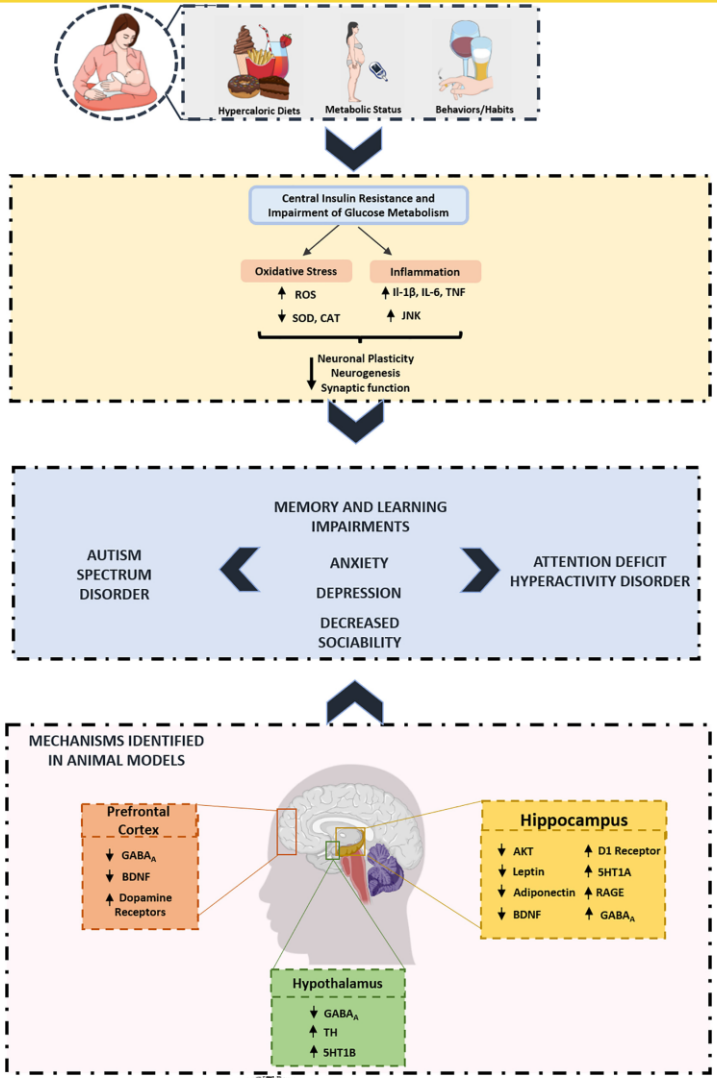


Amaro et al, 2022

The intricate relation between metabolic and neurodevelopment disorders during breastfeeding

Co-funded by the Erasmus+ Programme of the European Union

- The possible mechanisms underlying neurodevelopmental and behaviour alterations are still poorly understood. Nevertheless, the consumption of hypercaloric diets, the metabolic status and the behaviours adopted by the mother during breastfeeding period, may predispose the offspring for the development of central insulin resistance and impairment of glucose metabolism.
- As consequence, this can potentiate alterations in BNDF signalling, GABAergic, dopaminergic, and serotoninergic systems, in different central brain regions, affecting the proper neurodevelopment of the offspring.

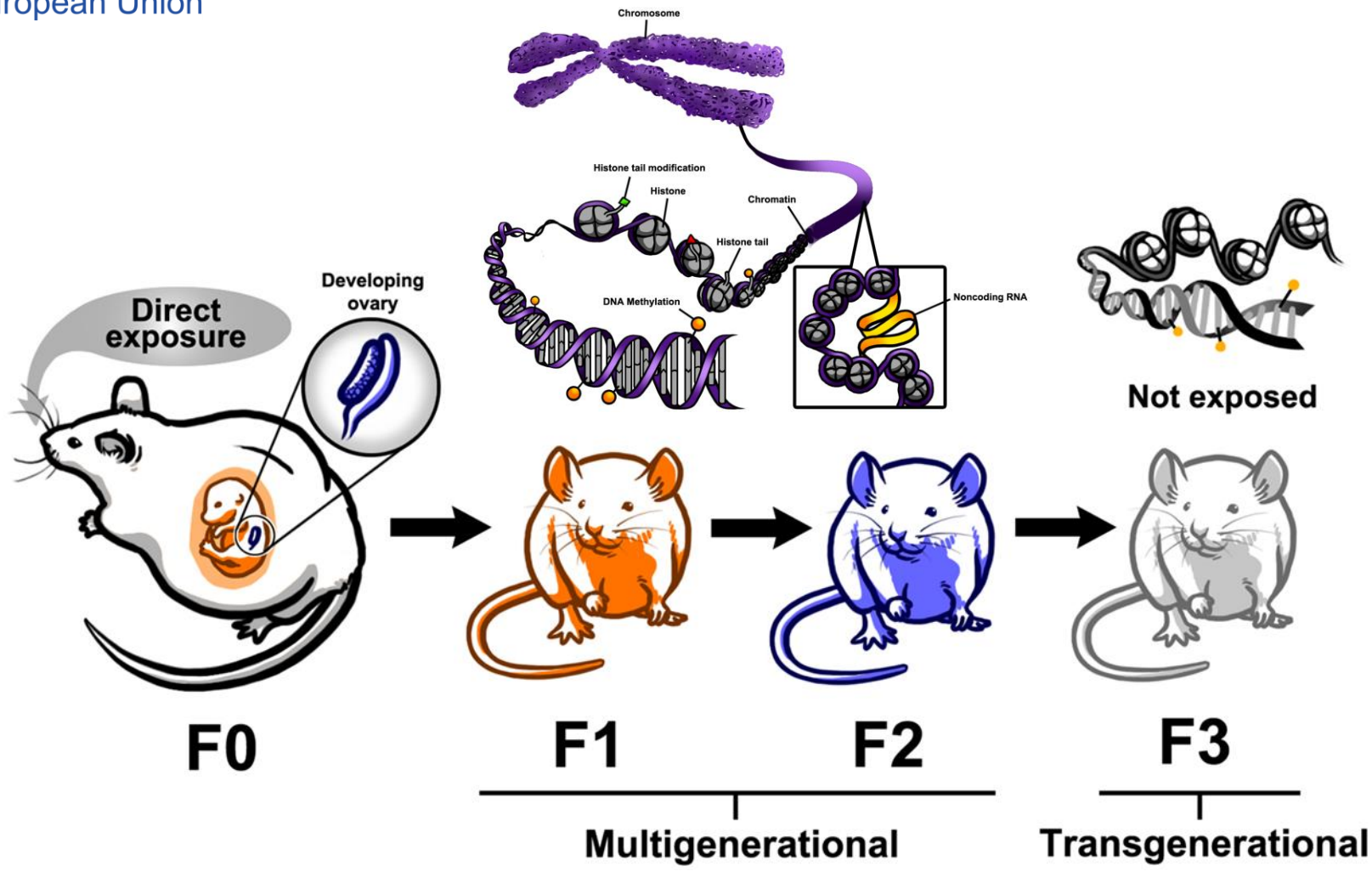


Amaro et al, 2022

The epigenetic impacts of endocrine disruptors on female reproduction across generations

Co-funded by the Erasmus+ Programme of the European Union

- Exposure to endocrine disruptors during prenatal development causes multigenerational effects in the F1 and F2 generations and transgenerational effects in the F3 generation.
- The F1 and F2 generations are directly exposed to the endocrine disruptor as a fetus and germ cell, respectfully.
- The F3 generation is not directly exposed and the mechanisms governing the effects in the F3 generation are thought to be epigenetic in nature.

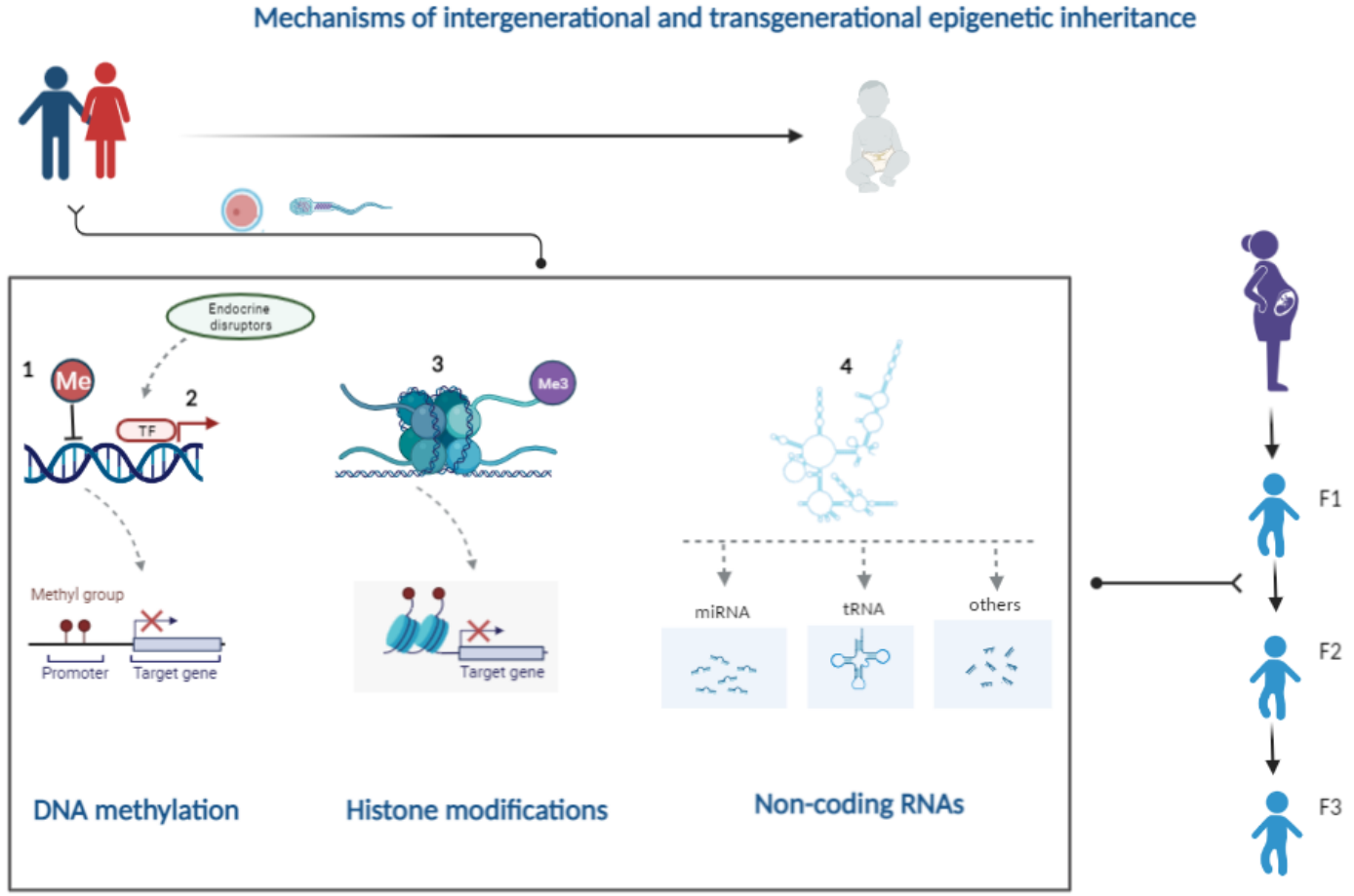


Rattan & Flaws, 2019

Epigenetic Inheritance: Intergenerational Effects of Pesticides and Other Endocrine Disruptors

Co-funded by the Erasmus+ Programme of the European Union

- Parental pre-conception and in utero exposure to chemical substances, such as pesticides, cause epigenetic alterations in the germline that can be transmitted between generations and affect disease (including cancer) susceptibility in the progeny.
- Several mechanisms play a potential role in intergenerational and transgenerational transmission of disease predisposition including
- (1) DNA methylation
- (2) transcription factor-associated DNA methylation patterns
- (3) histone modifications
- (4) non-coding RNAs.

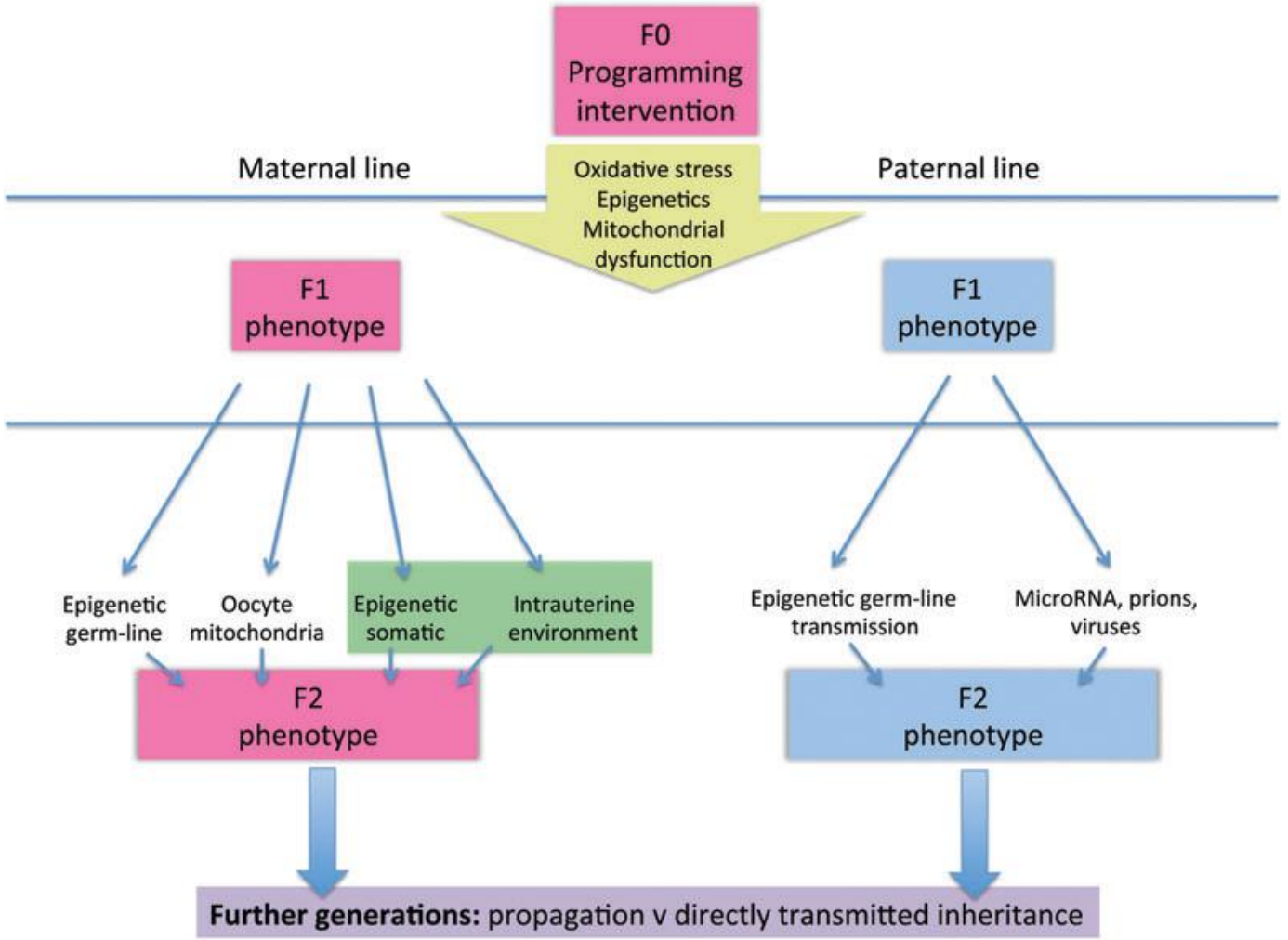


Nicolella & de Assis, 2022

Transgenerational developmental programming

Co-funded by the Erasmus+ Programme of the European Union

- Potential mechanisms of transgenerational developmental programming via maternal (orange) and paternal (blue) lines.
- Mechanisms that involve phenotype propagation de novo in each subsequent generation are highlighted in green.
- Proposed mechanisms of developmental programming to F1 generations are shown in yellow.

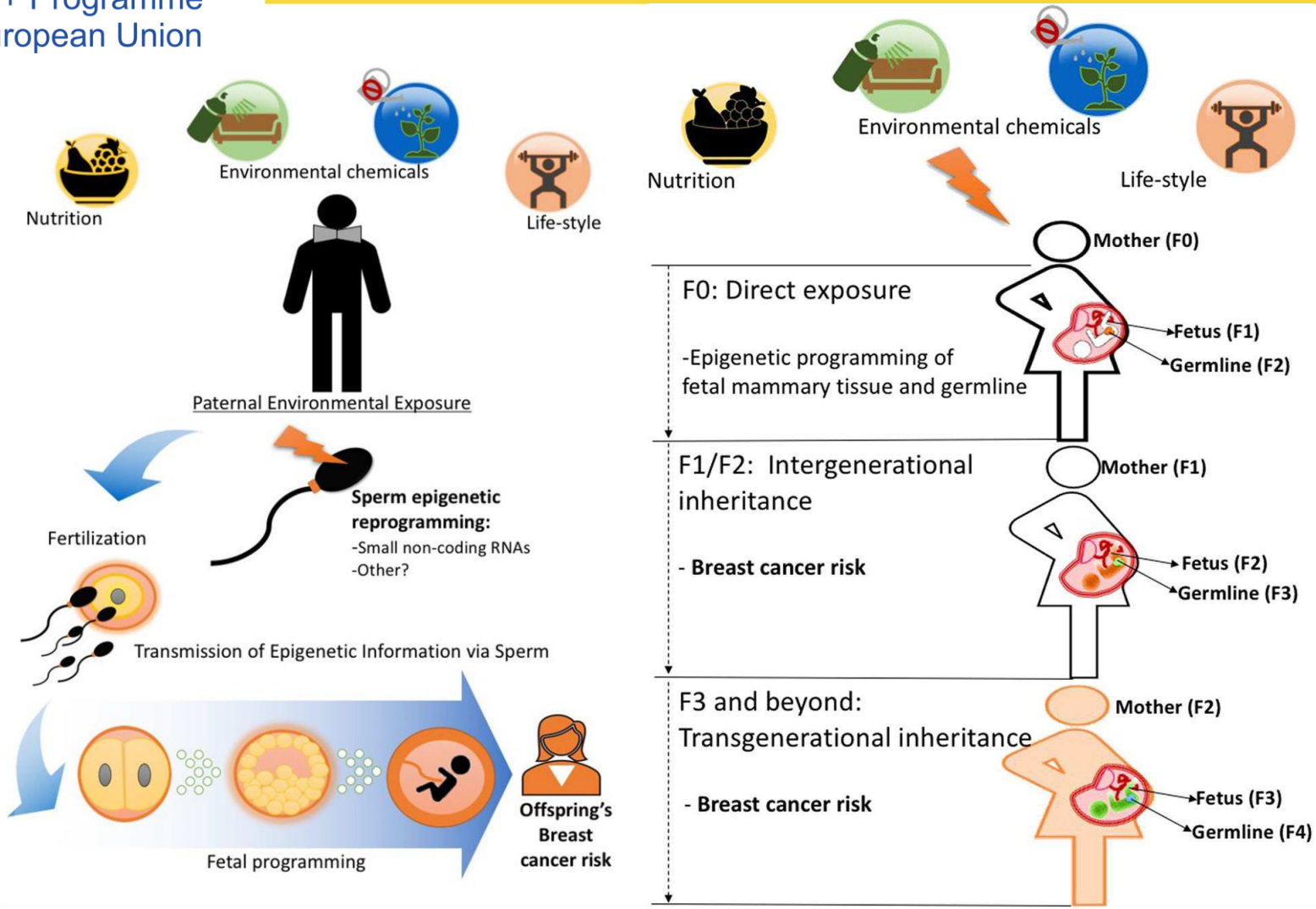


Aiken & Ozanne, 2014

Diet and Transgenerational Epigenetic Inheritance of Breast Cancer: The Role of both Parents

Co-funded by the Erasmus+ Programme of the European Union

- **Maternal environmental insults could directly affect the developing fetus' mammary gland.**
- **However, the past decade showed that history of exposure in the parental generation could lead to multigenerational and transgenerational predisposition for breast cancer.**

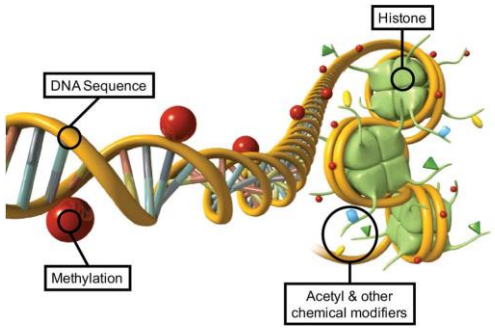


Cruz et al, 2020

Epigenetic transgenerational inheritance, gametogenesis and germline development

Co-funded by the Erasmus+ Programme of the European Union

Epigenetic mechanisms and processes (marks)

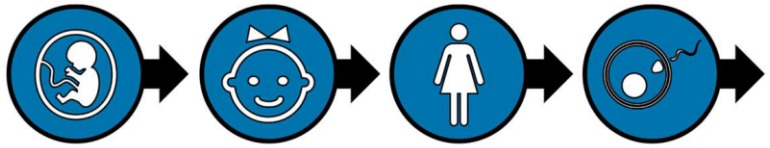


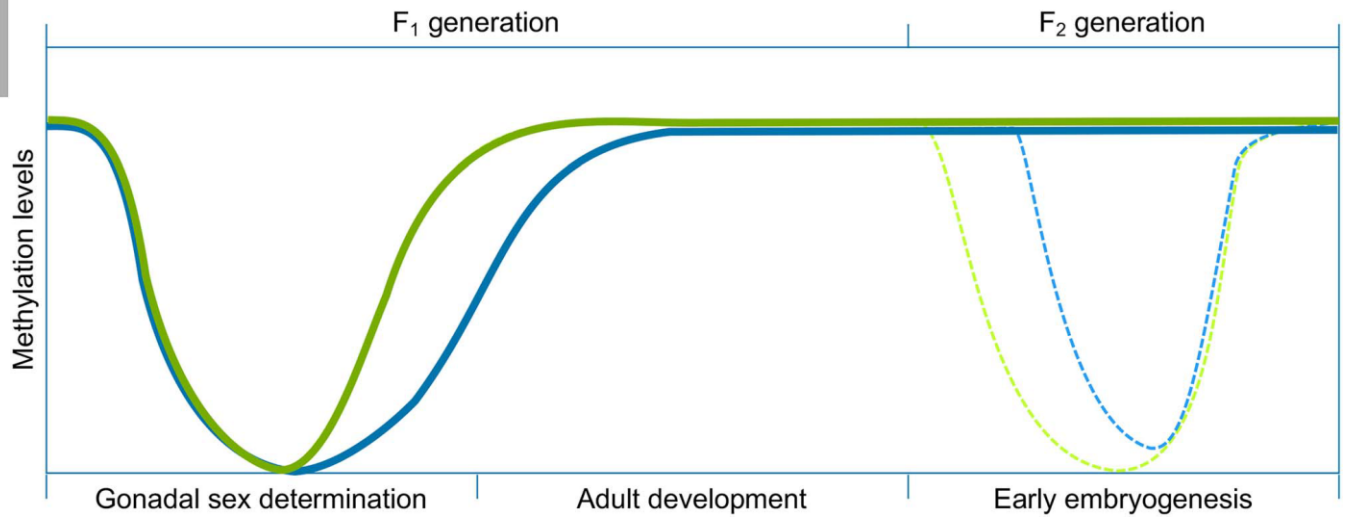
EPIGENETIC MECHANISMS AND MARKS

- DNA Methylation
- Histone Modifications
- Chromatin Structure
- Non-coding RNA
- RNA methylation

- One of the most important developing cell types in any biological system is the gamete (sperm and egg).
- The transmission of phenotypes and optimally adapted physiology to subsequent generations is in large part controlled by gametogenesis.
- In contrast to genetics, the environment actively regulates epigenetics to impact the physiology and phenotype of cellular and biological systems.
- The integration of epigenetics and genetics is critical for all developmental biology systems at the cellular and organism level.

Epigenetic reprogramming (DNA methylation erasure) during primordial germ cell development at gonadal sex determination and following fertilization in the early embryo.





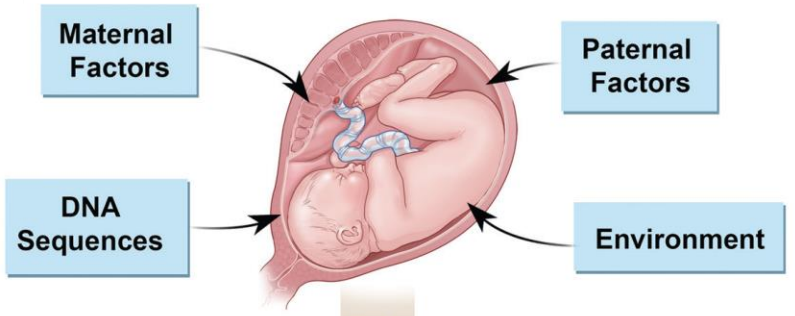
Maamar et al, 2021

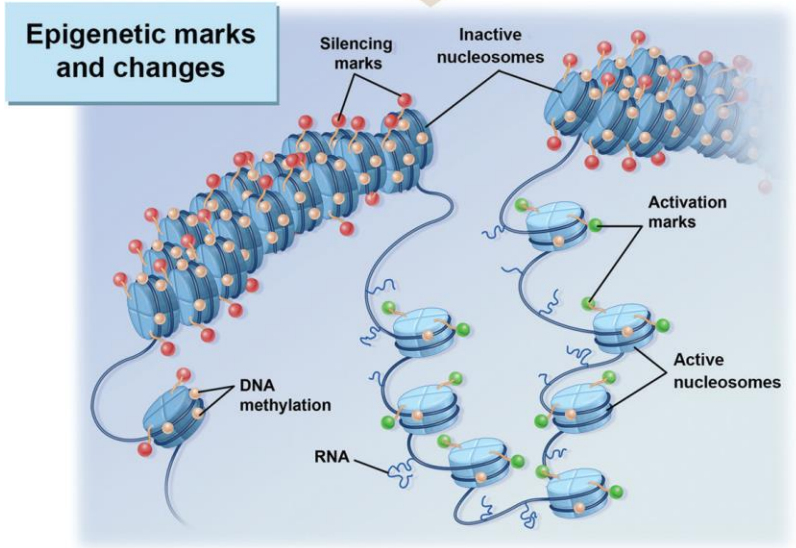
UNIVERSIDADE D COIMBRA · UNIVERSITATEA „ALEXANDRU IOAN CUZA" din IAȘI · FRIEDRICH-SCHILLER-UNIVERSITÄT JENA · UNIVERSITÀ DI PAVIA · Université de Poitiers · VNiVERSiDAD D SALAMANCA · TURUN YLIOPISTO

Epigenetic changes and developmental origins of health and disease (1)

Co-funded by the Erasmus+ Programme of the European Union

- Fetal DNA sequences are not altered by epigenetic changes, yet these profoundly influence DNA configuration.
- The processes of DNA methylation, post-translational histone modification, RNA modulation are involved and can affect gene availability and activation over the lifespan.





Epigenetics plus Mitosis → early life experiences resulting in long-term effects

some maternal effects

Epigenetics plus germ-cell reprogramming → transgenerational effects

some paternal effects

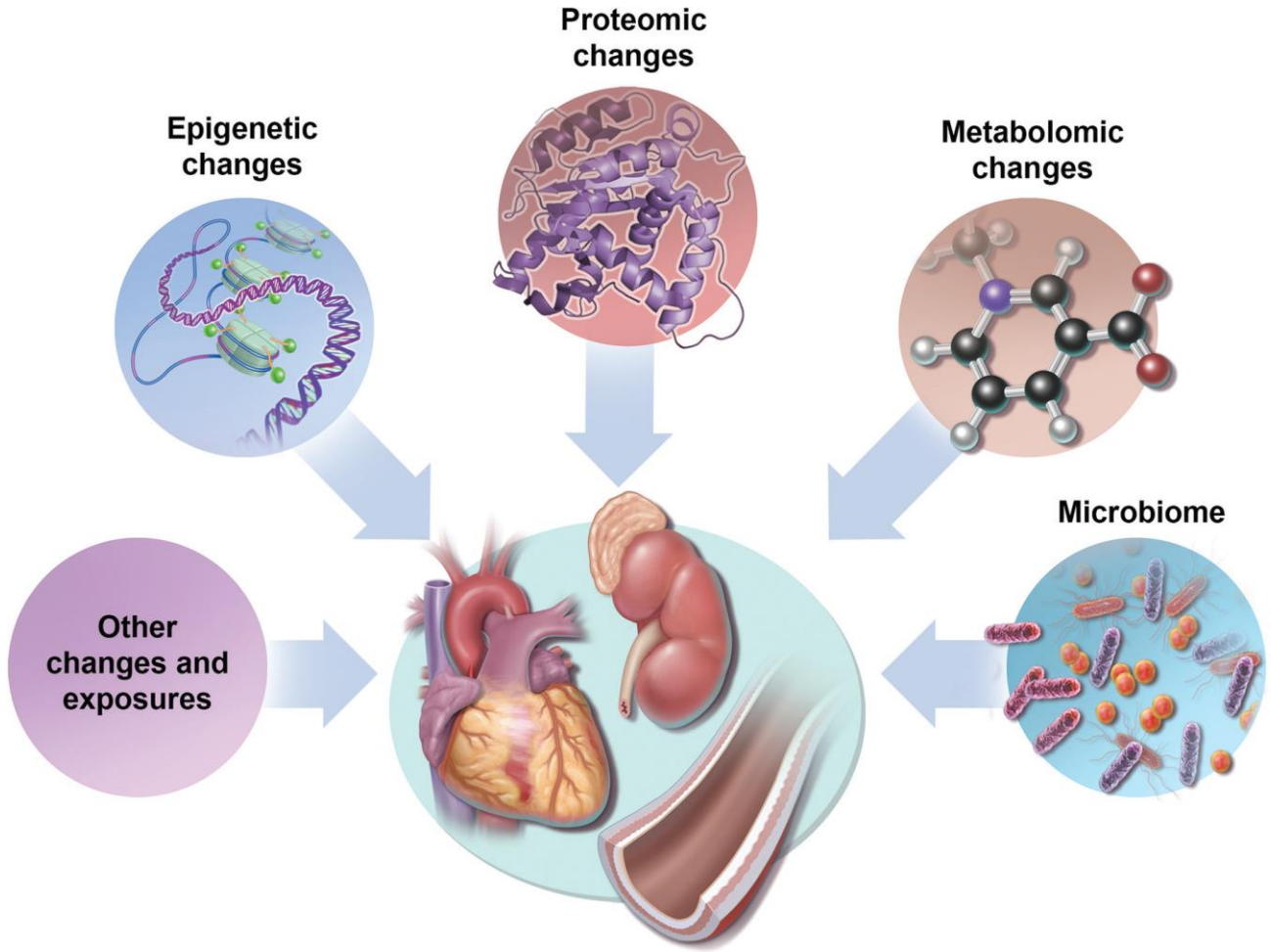
Lurbe & Ingelfinger, 2021

Epigenetic changes and developmental origins of health and disease (2)

Co-funded by the Erasmus+ Programme of the European Union

OMICS and programming

- A variety of noncoding events impact the future health of offspring.
- New insights associated with a life course perspective demand longitudinal rather than cross-sectional studies.
- It is essential to design new preconception and birth cohorts with precise phenotypic observations and long-term perspectives. After that, it will be necessary to pair them with molecular studies.
- The identification of molecular changes, together with later phenotypes, is critical to better understanding the underlying mechanisms and to delineating sensitive and specific biomarkers.
- This can facilitate the detection of risk and monitor the efficacy of preventive interventions to reduce the occurrence of future disease.



Lurbe & Ingelfinger, 2021

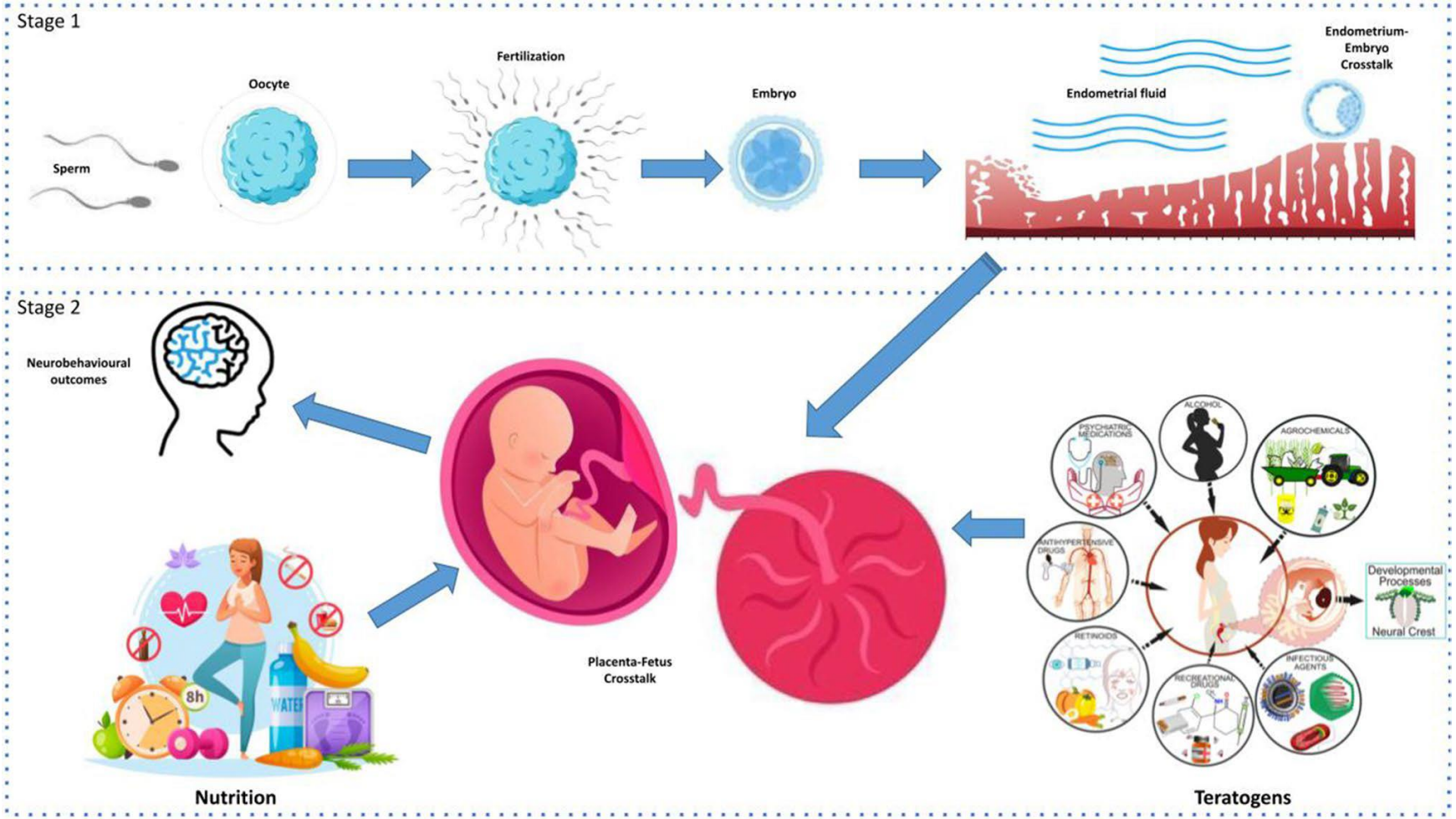
Hot spots of epigenetic action

Co-funded by the Erasmus+ Programme of the European Union

Epigenetics of pregnancy: looking beyond the DNA code

<u>Stage 1</u>: from gametes to embryo-endometrium cross-talk

<u>Stage 2</u>: from placenta-fetus crosstalk to brain development, with an overview on environmental factors as well (nutrition and teratogens).

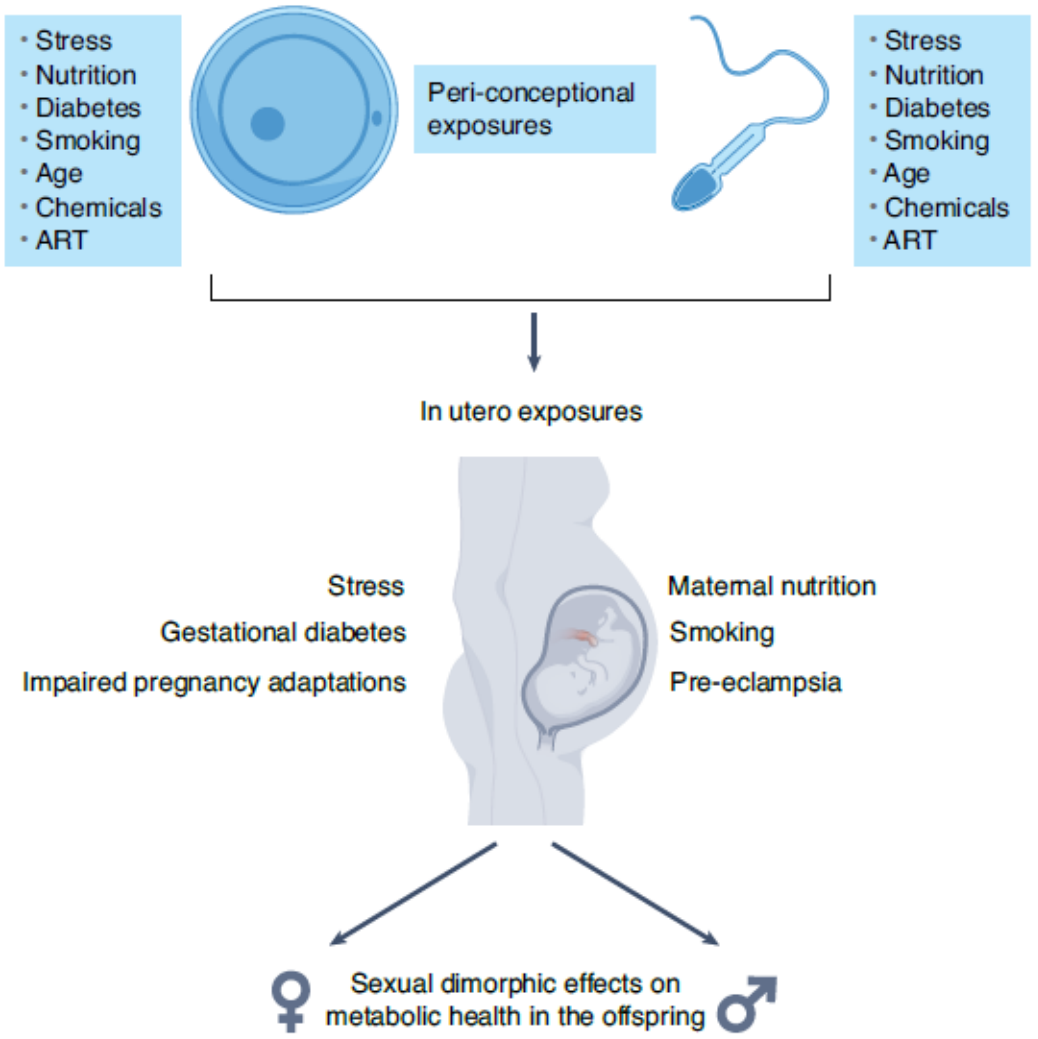


Zuccarello et al, 2022

The intergenerational inheritance of metabolic traits

Co-funded by the Erasmus+ Programme of the European Union

- **Parental germline exposures before pregnancy affect sperm and oocytes.**
- **In utero exposures experienced by the developing embryo during pregnancy due to adverse maternal environments may program sex-related dimorphic effects on the short-term and long-term health of the offspring.**

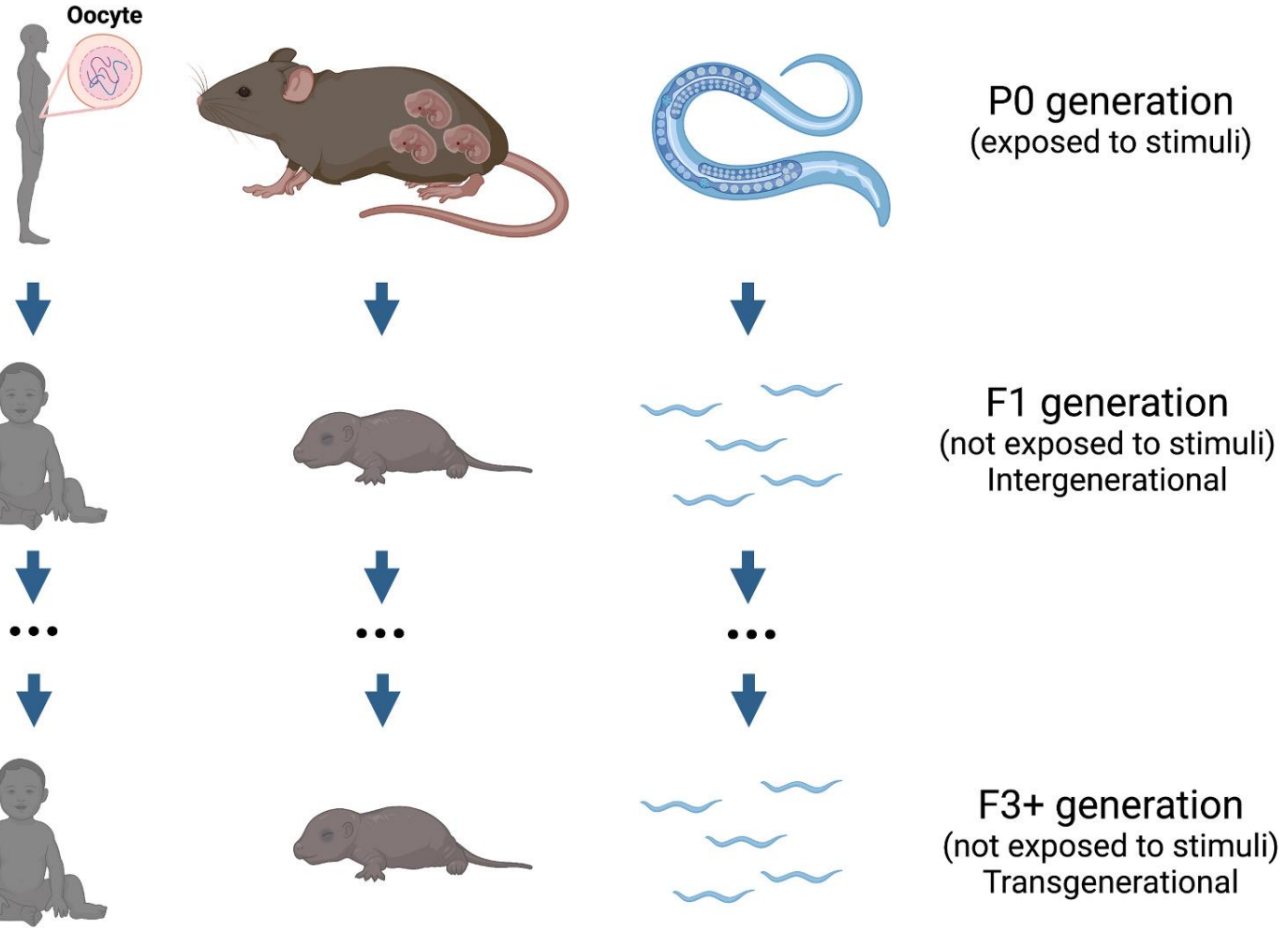


Sandovici et al, 2022

Multigenerational epigenetic inheritance: Transmitting information across generations

Co-funded by the Erasmus+ Programme of the European Union

- **Numerous different parental (P0) stresses can have multigenerational effects on offspring.**
- Intergenerational effects represent any effect of parental stress on F1 progeny that either directly acts on or is communicated through P0 germ cells or developing F1 embryos *in utero.* By comparison, all effects that are initiated in the P0 generation and persist into the F3 (or later) generations are transgenerational effects. Effects that are initiated in the P0 generation and persist to the F2 generation are intergenerational if any germ cells of F1 animals have formed *in utero* when the initiating event/stress was present and transgenerational if no F1 germ cells have formed.
- These original distinctions between intergenerational and transgenerational effects in F2 progeny are still used as definitions in the literature irrespective of the mechanisms that mediate multigenerational effects in progeny, including cases where such effects might not be transmitted via germ cells.

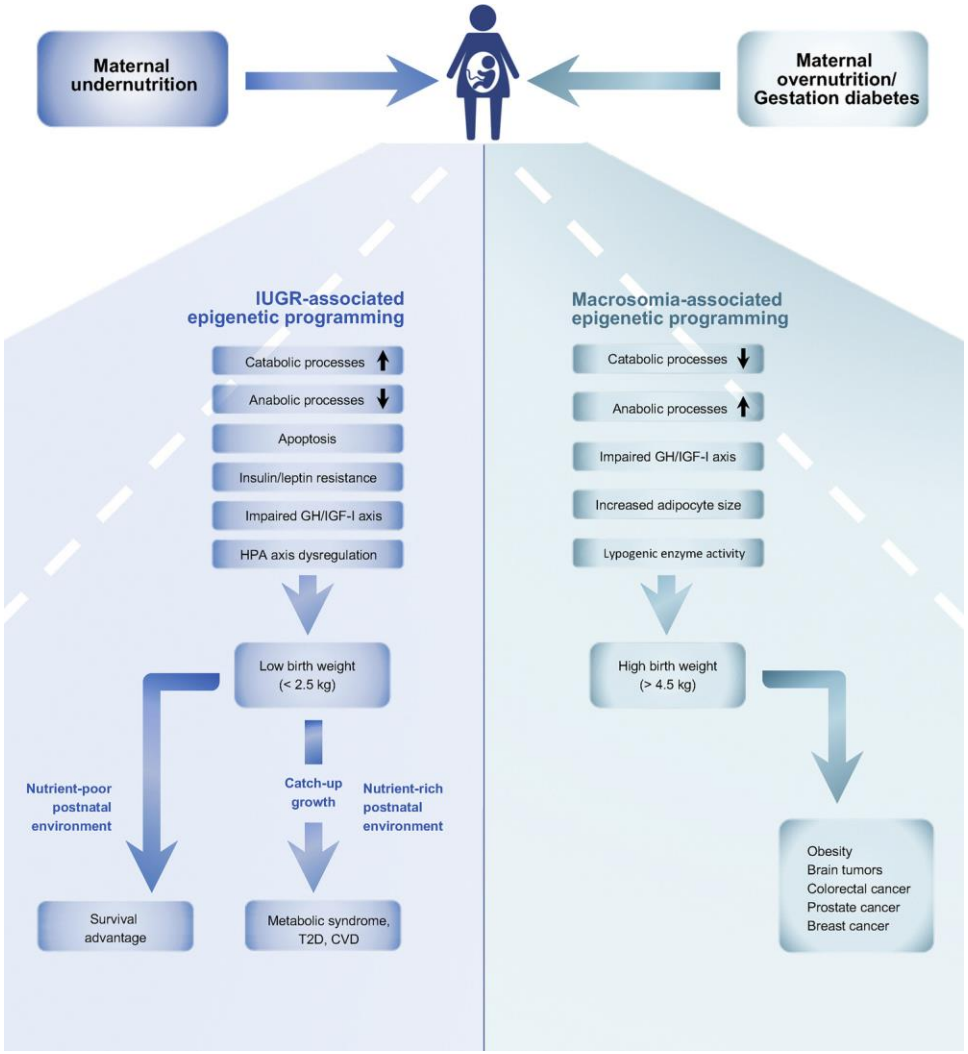


Burton & Greer, 2022

Developmental programming of aging trajectory

Co-funded by the Erasmus+ Programme of the European Union

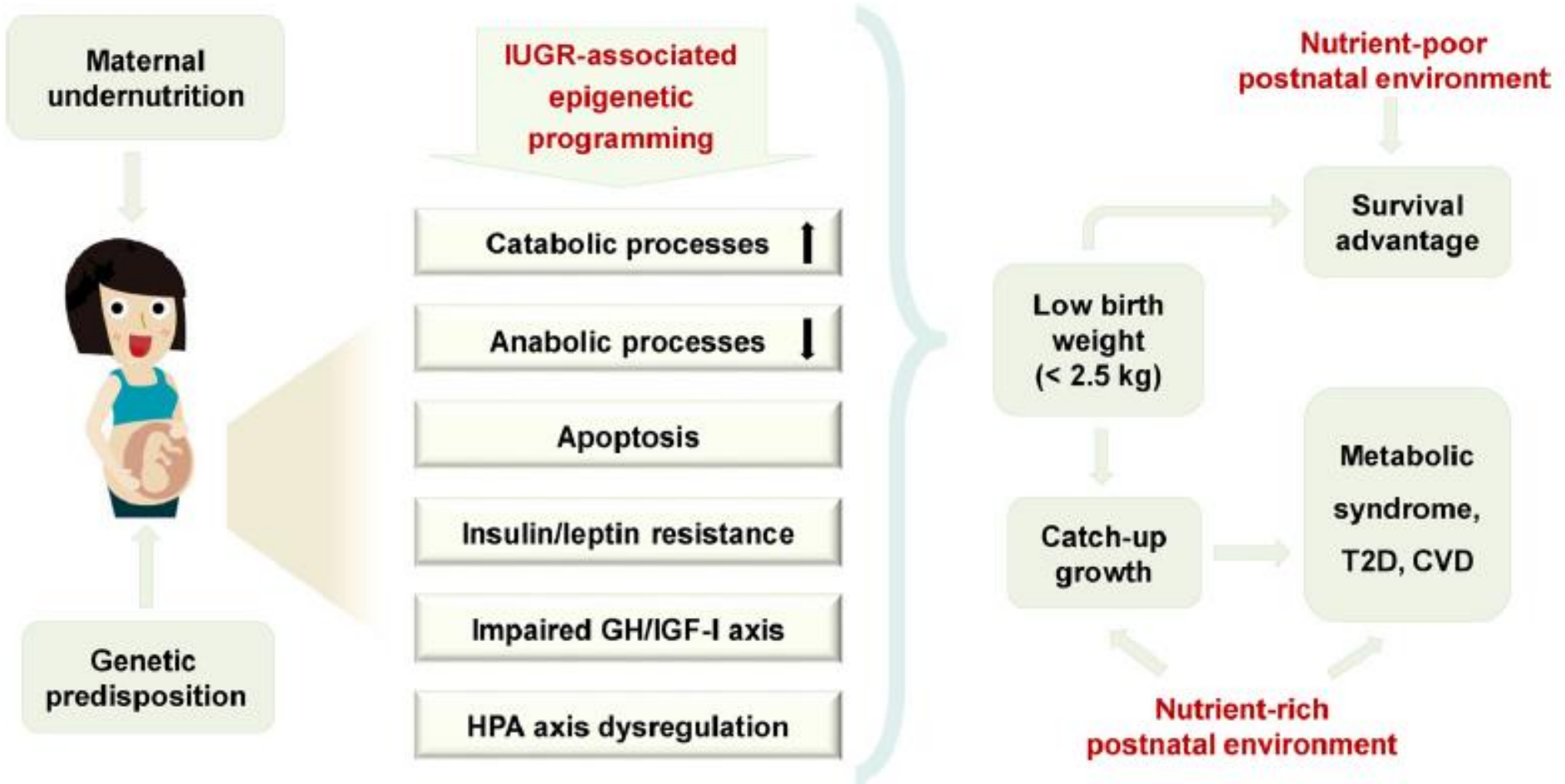
- There is accumulating evidence that aging phenotype and longevity may be developmentally programmed.
- Main mechanisms linking developmental conditions to later-life health outcomes include
  - persistent changes in **epigenetic regulation**
  - **(re)programming** of major endocrine axes such as growth hormone/insulin-like growth factor axis and hypothalamic-pituitary-adrenal axis and also early-life immune maturation.
- Recently, evidence has also been generated on the role of telomere biology in developmental programming of aging trajectory.
- In addition, persisting changes of intestinal microbiota appears to be crucially involved in these processes.



Vaiserman et al, 2018

Birth weight predicts aging trajectory: A hypothesis (1)

Co-funded by the Erasmus+ Programme of the European Union

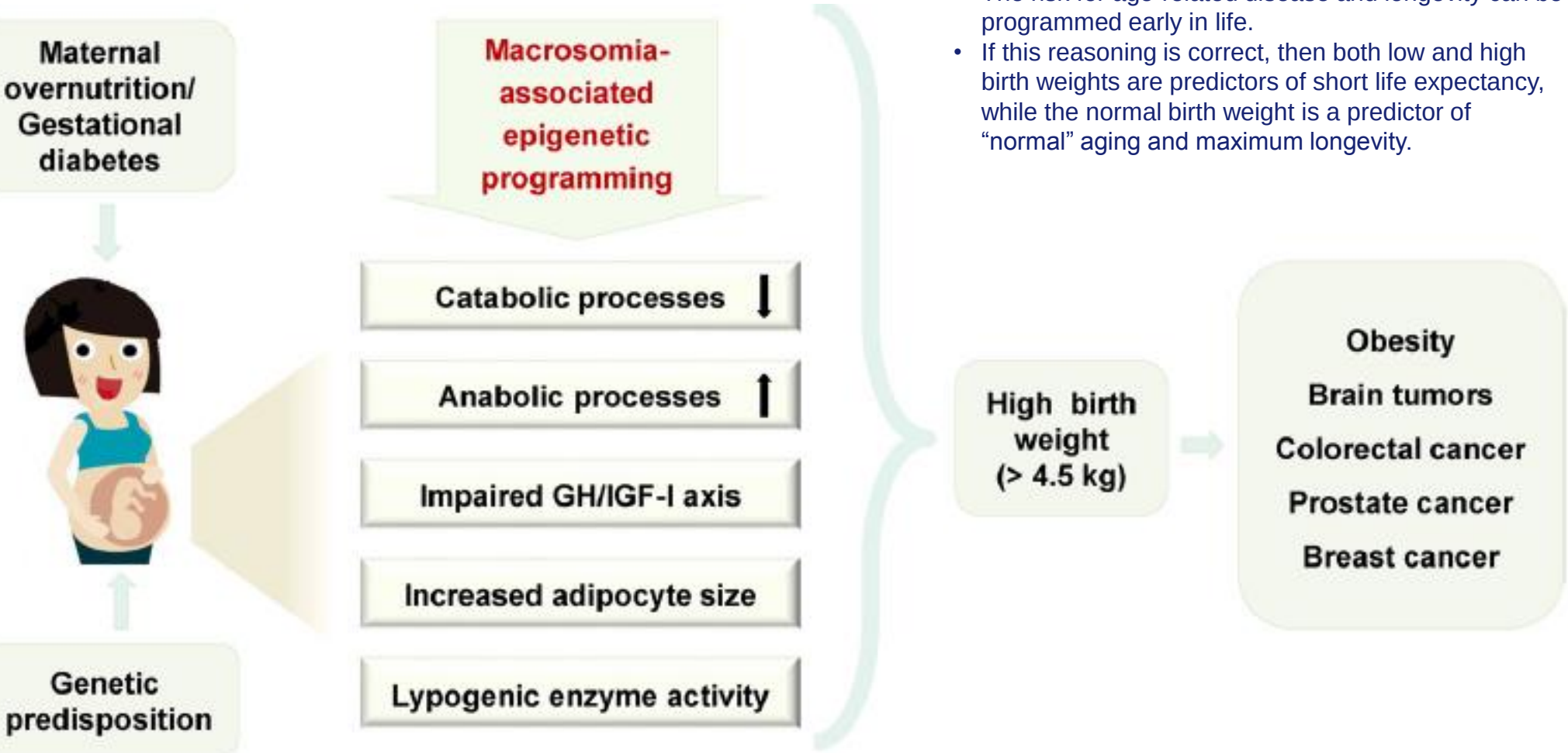


AM Vaiserman, 2018

Birth weight predicts aging trajectory: A hypothesis (2)

Co-funded by the Erasmus+ Programme of the European Union

- The risk for age-related disease and longevity can be programmed early in life.
- If this reasoning is correct, then both low and high birth weights are predictors of short life expectancy, while the normal birth weight is a predictor of "normal" aging and maximum longevity.



AM Vaiserman, 2018

Intrauterine growth restriction (IUGR) phenotypes are sex-specific– transgenerational inheritance

Co-funded by the Erasmus+ Programme of the European Union

F0 ♀

Intrauterine insults

Uteroplacental insufficiency
Maternal undernutrition
High-fat diet
Low-protein diet
Restraint stress

Offspring phenotypes

F1	F2	F3
↓ birth weight ↓ insulin secretion, ↑ insulin resistance,↑ glucose intolerance, sex-specific ↓ kidney weight, ↓ glomerular number, ↓ nephron, ↑ renal cell apoptosis, ↑ mean arterial pressure, ↑blood pressure; sex-specific	↓ β-cell mass, ↓ first-phase insulin response, ↑ insulin resistance; sex-specific ↑ liver weight, impaired hepatic *de novo* lipogenesis ↓ nephron, ↑blood pressure	↓ body weight; sex-specific altered glucose metabolism; sex-specific

Epigenetic changes:

Kidney	lncRNA: altered TCONS_0014139, TCONS_00014138, TCONS_00017119 ↓ *Dnmt3a* expression, ↓ *p53* DNA methylation
Pancreas	lncRNA: ↓ *Tug1;* ↑ *Gch1* DNA methylation, ↓ *Pdx1* promoter histone H3 and H4 acetylation
Liver	miRNA: altered *miR-709, miR-122, miR-192, miR-194,miR-26a, let-7a, let-7b, let-7c, miR-494, miR-483, miRNA19a-3p* ↓ genomic DNA methylation ↑ H3 acetylation, ↓ H3K4 dimethylation in *Igf1*
Brain	↓ *Dnmt1* expression, ↓ genomic DNA methylation, ↑ H3 acetylation
Lung	miRNA: altered *miR-29, miR-128-3p, miR-34c-5p, miR-19b-3p, miR-449a-5p, miR-30e-5p*

Liver	lncRNA: ↓ *H19* ↑ *H19* DNA methylation, ↓ *Lxra* DNA methylation
Sperm	↓ *Lxra* DNA methylation

?

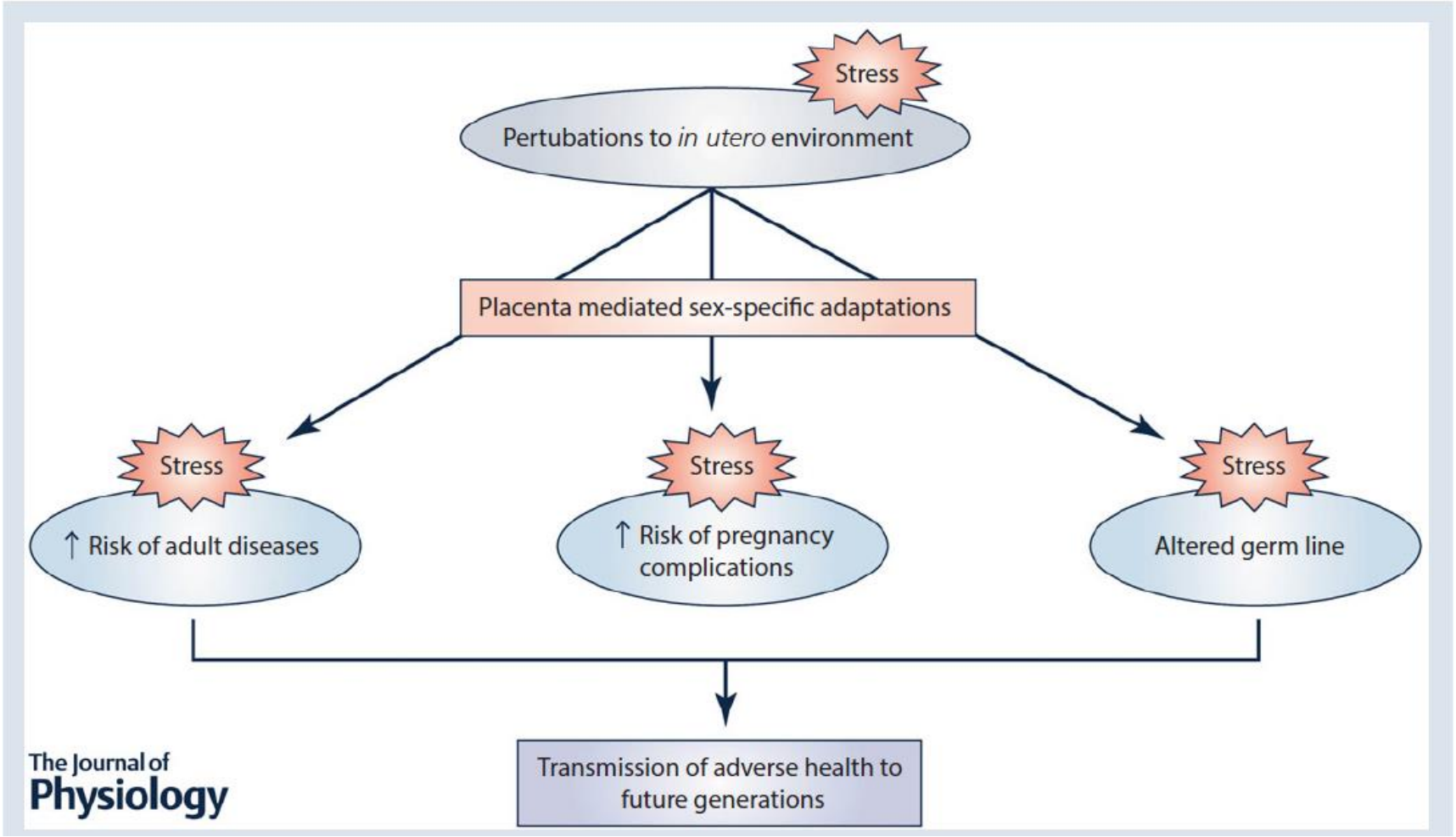
Created with BioRender.com

Similar to human studies, altered epigenetic mechanisms such as non-coding RNA modifications, DNA methylation, and histone modifications were also found in these offspring. Results obtained from different rat and mouse IUGR models.

Doan et al, 2022

Sex-specific fetal programming and the transgenerational transmission of diseases

Co-funded by the Erasmus+ Programme of the European Union

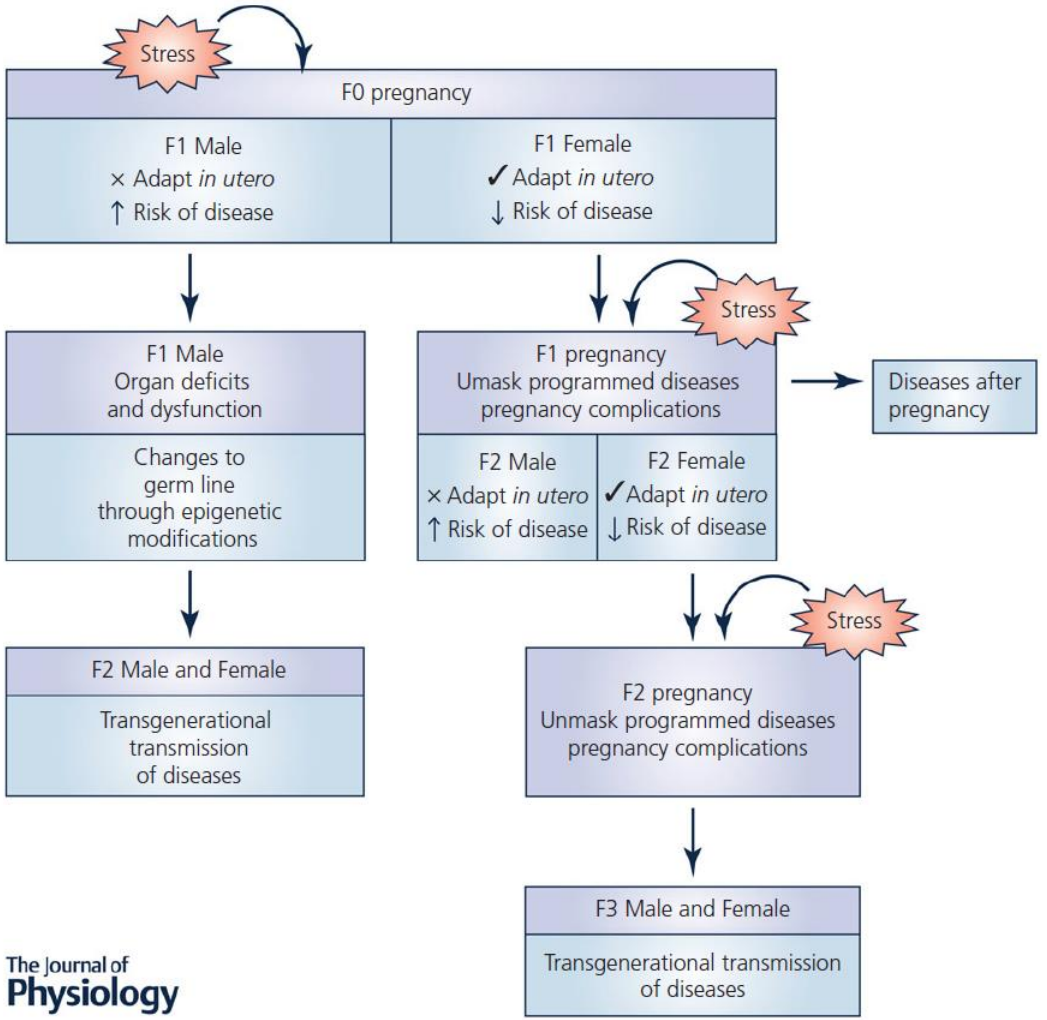


Cheong et al, 2016

Transgenerational transmission of programmed outcomes through the maternal lineage

Co-funded by the Erasmus+ Programme of the European Union

- **Female fetuses generally adapt well to perturbations *in utero* and alter their developmental strategy in accordance with their environment**. However, when these **F1 females** become pregnant, programmed diseases can be unmasked leading to the development of pregnancy complications. This in turn creates a suboptimal intrauterine environment for the developing F2 fetus. Similarly, **F2 female fetuses** are programmed for adult disease, and when they become pregnant, pregnancy complications may arise leading to programming consequences in the **subsequent F3 generation**.
- If the cycle continues, this would result in transgenerational programming of disease that may persist across multiple generations. Furthermore, **pregnancy complications impair the long-term health of the mother**, and she may experience long-term diseases even after the conclusion of pregnancy. Although the maternal lineage has been thought to be mainly responsible for the transgenerational transmission of disease in the past, recent studies have demonstrated **important roles of the paternal lineage**.
- When the developing **F1 male fetus** is exposed to a suboptimal environment *in utero*, this increases the risk of postnatal organ deficits and dysfunction. This increased susceptibility to adverse health may impact on the germ cells of these males during reproductive age and **when they mate with a normal female, the alterations in the germ line may be passed on to the offspring.** As the germ cells that give rise to the F2 generation were not present during the initial insult, phenotypes observed in this generation are sufficient to classify this as transgenerational transmission of programmed disease.



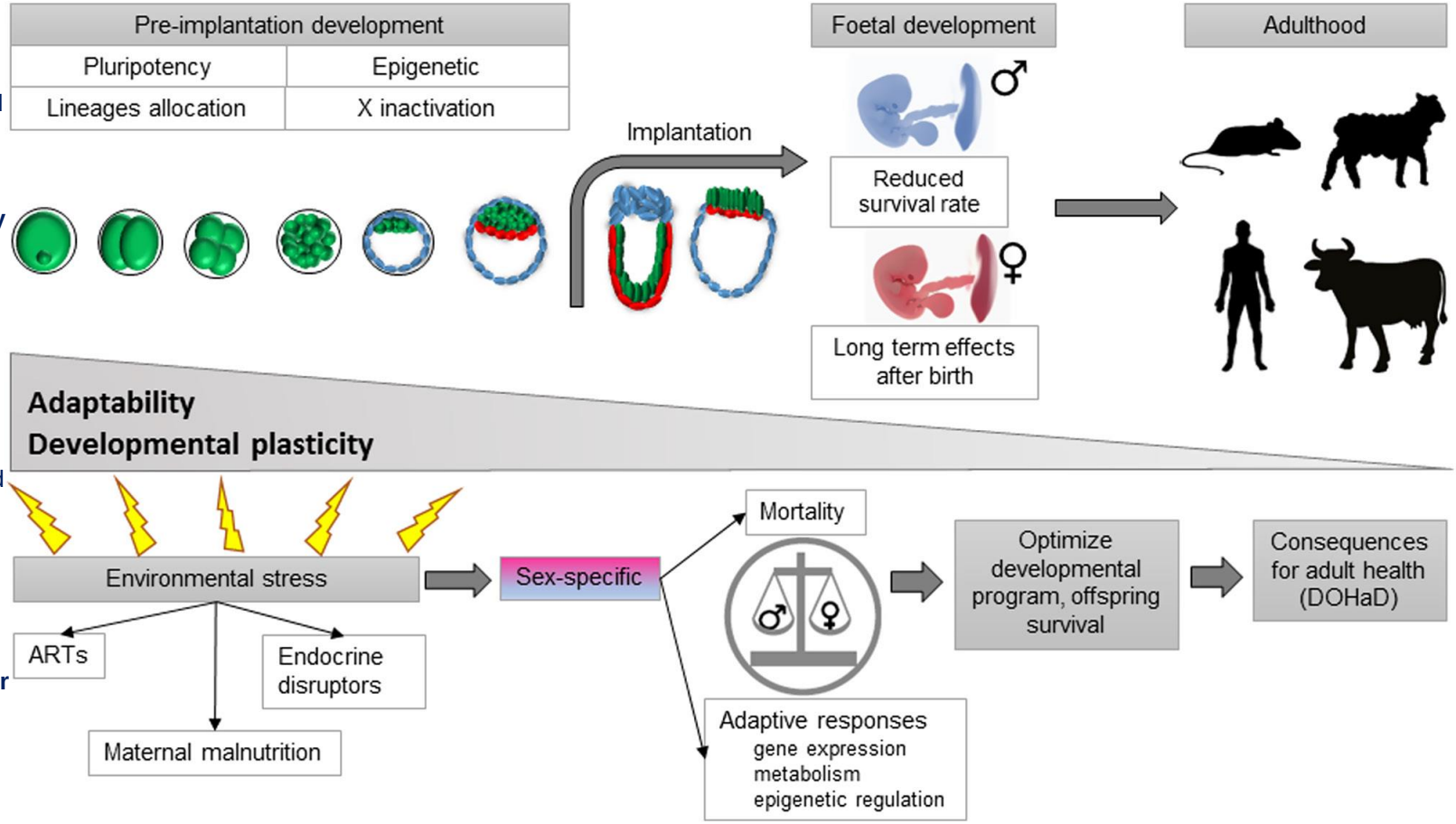
The Journal of Physiology

Cheong et al, 2016

Pre- and peri-implantation embryo adaptations to environmental stress

Co-funded by the Erasmus+ Programme of the European Union

- Critical events taking place during this developmental window (pluripotency emergence, epigenetic reprogramming, lineage allocation and X chromosome inactivation) render the embryo especially sensitive to environmental stress. **Such embryos and the resulting foetus respond to stressors by sex-specific mortality or by adaptive responses in order to optimize their developmental program and offspring survival.**
- The adaptability due to developmental plasticity (the ability of the genotype to produce different phenotypes in response to different environments) decrease throughout embryo and foetal development until early postnatal life.
- Later in adult life, this adaptability disappears and only the brain maintains a certain degree of plasticity.
- **The female placenta (in pink), due to its higher adaptability, buffers more efficiently the impact of endogenous and exogenous stressors on the foetus and it is less compromised than male foetus (in blue) under similar stress conditions.** However, compensatory mechanisms can compromise adult health according to the developmental origins of adult health and disease (DOHaD).

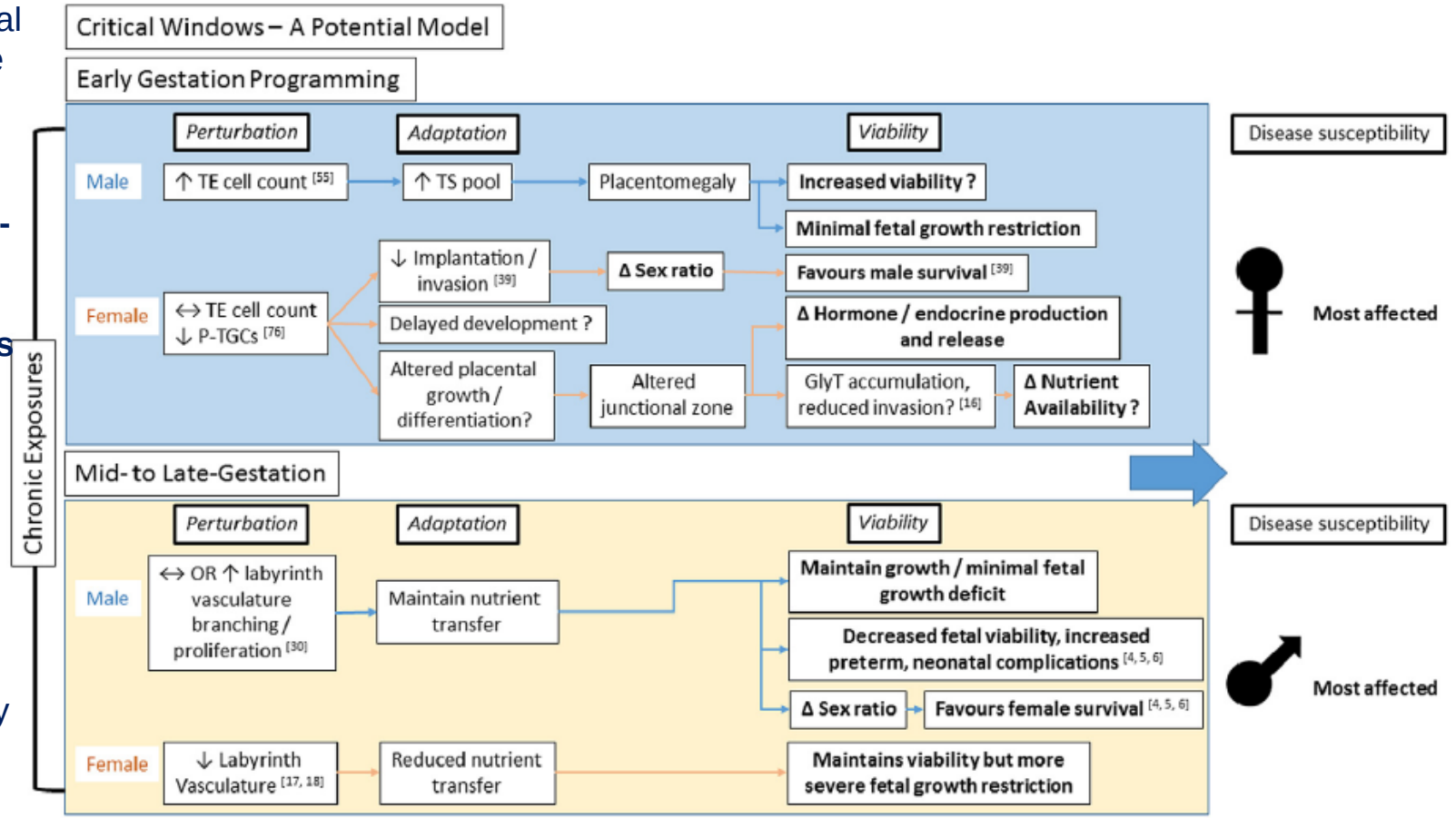


Pérez-Cerezales et al, 2018

Sexual dimorphism in the formation, function and adaptation of the placenta

Co-funded by the Erasmus+ Programme of the European Union

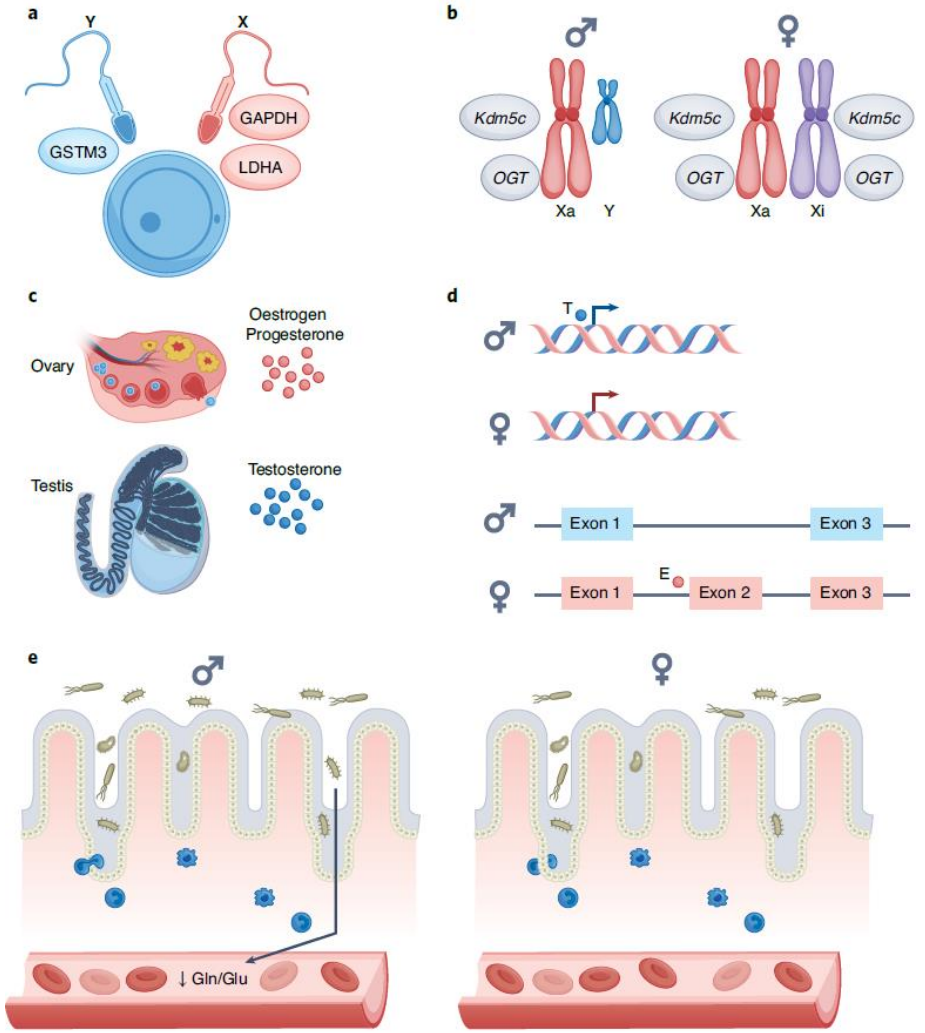
Sexual dimorphism during placental development encompasses a wide variety of pathways contributing to growth, differentiation, and metabolism of the placenta. **Early exposure models (IVF, ICSI, peri-conceptional alcohol exposure) show females to develop the most deleterious phenotypes** e.g. differentiation, which may be associated with female specific disease phenotypes in adulthood (e.g. metabolic disease. Conversely, males seem most at risk for exposures during mid-late gestation and show limited placental responsiveness, which may come at a cost of fetal viability later in gestation, as well as earlier and more severe disease phenotypes e.g. cardiovascular disease.



Kalisch-Smith et al, 2017

Sexual dimorphic effects related to sex chromosomes and sex hormones

Co-funded by the Erasmus+ Programme of the European Union



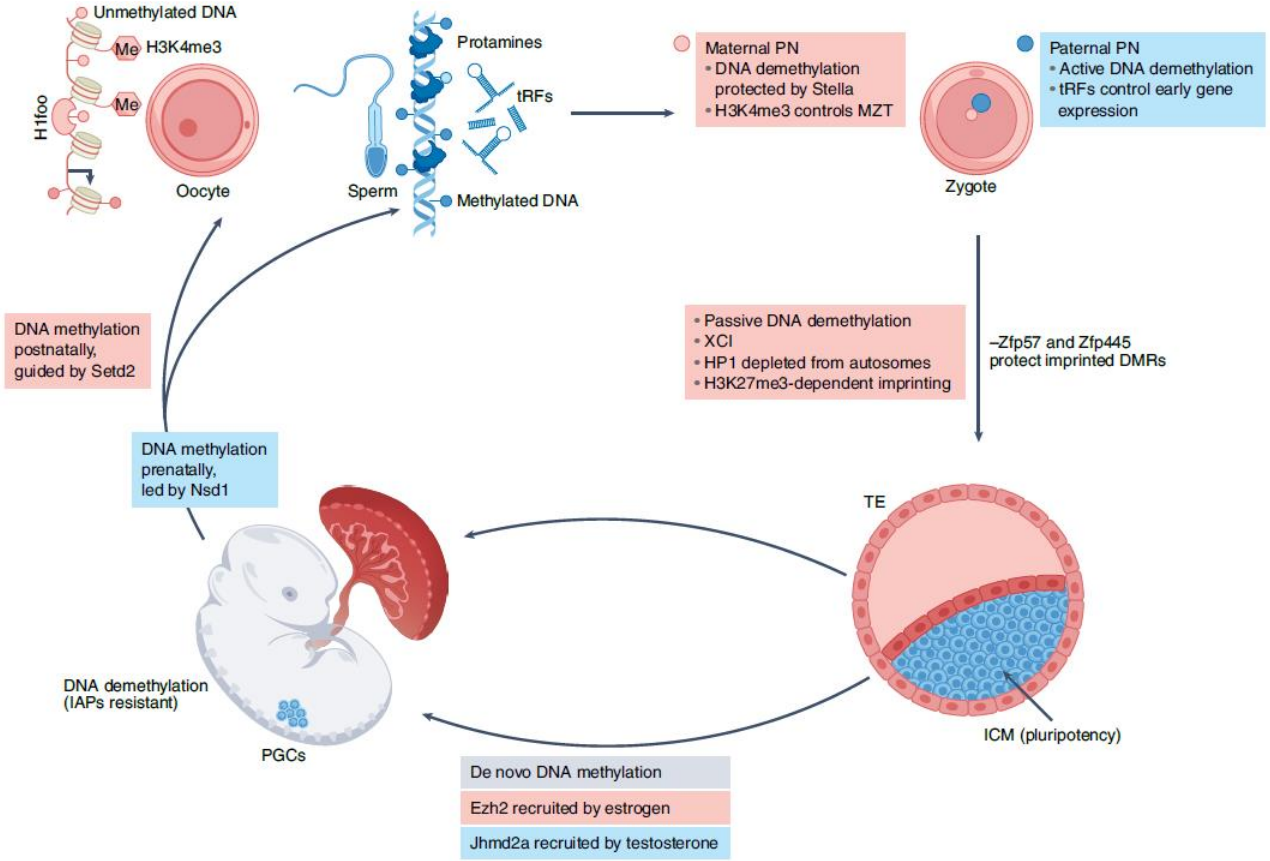
a, X- and Y-chromosome-bearing spermatozoa may have differences in their proteome content and some of these proteins are related to metabolic processes.
b, X-linked genes that escape XCI result in gene dosage differences between males and females, for example OGT, encoding O-linked-N-acetylglucosamine transferase, or Kdm5c, encoding a histone demethylase (Xa, active X chromosome; Xi, inactive X chromosome).
c, Gonadal steroid hormones have been suggested as the underlying mechanism responsible for the sexual dimorphism observed in metabolic diseases.
d, Upper, binding of testosterone (T) to the promoter regions of genes containing androgen-response elements leads to their transcriptional upregulation (thicker blue arrow), which in turn is responsible for sex-related differential expression (sex-DE). Lower, interaction between oestrogen (E) and the splicing machinery induces retention of exon 2 into the mature mRNA of a hypothetical gene and leads to expression of a female-specific alternative transcript.
e, Sex hormones influence the composition of the gut microbiota, with lower Gln/Glu being described in male mice.

Sandovici et al, 2022

Developmental epigenetic reprogramming leading to sex-related effects on gene expression patterns

Co-funded by the Erasmus+ Programme of the European Union

Key sex-related differences identified in mouse studies are colour coded (red, female specific; blue, male specific). Mature oocytes have large chromatin blocks enriched in H3K4me3, associated with low DNA methylation levels and contribute with histone variants, such as histone H1foo, acting as maternal factors in the zygote. Actively transcribed
gene bodies exhibit high DNA methylation levels. In sperm, most histones are replaced by protamines and DNA is overall highly methylated. Males also contribute to the zygote and early embryogenesis by small non-coding RNAs, such as tRFs. At fertilization, the paternal pro-nucleus (PN) is subject to active DNA demethylation. Oocyte-derived Stella protects maternal PN against active demethylation, and methylation of maternal DNA is gradually diluted through DNA replication during subsequent cell divisions, in the absence of nuclear Dnmt1. H3K4me3 inherited from the mother controls the maternal-to-zygotic transition (MZT). Imprinted DMRs are protected against DNA demethylation by binding of Zfp57 and Zfp445 in both sexes.
Before implantation, female embryos initiate XCI. Binding of HP1 to the inactive X chromosome depletes its levels on autosomes, inducing sex-related differences in gene expression for hundreds of genes. A few genomic loci are transiently imprinted because of the inheritance of maternal H3K27me3. The embryo reaches its lowest DNA methylation levels at the blastocyst stage; the first cell differentiation event corresponds to the formation of the inner cell mass (ICM, the future embryo proper) and trophectoderm (TE, the future placenta). After implantation, lineage establishment is accompanied by cell-type-specific de novo DNA methylation. After the formation of the gonads, sex hormones recruit specific epigenetic modifiers (for example, Ezh2, which contains an oestrogen response element, and Jhmd2a, which interacts with the androgen receptor). In the developing embryo, PGCs are first demethylated (with the exception of DNA demethylation-resistant sequences such as IAPs, followed by gain of DNA methylation in a sex-specific manner.
De novo DNA methylation is mediated by Nsd1 before birth in sperm, and guided by Setd2 in oocytes, in the postnatal life.

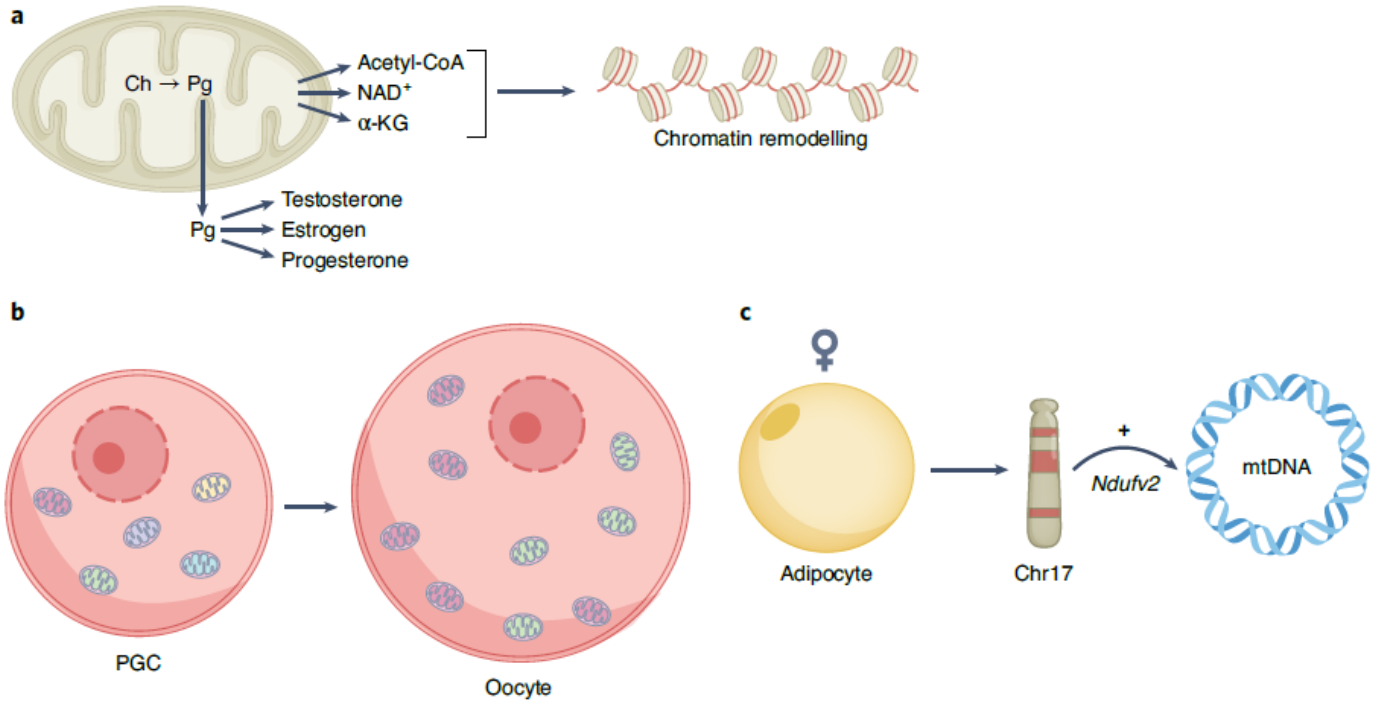


Sandovici et al, 2022

Sexual dimorphic effects related to mitochondria

Co-funded by the Erasmus+ Programme of the European Union

a, Mitochondria are implicated in the conversion of cholesterol (Ch) into pregnenolone (Pg), which is then converted into sex hormones in the endoplasmic reticulum, in a sex-dependent manner. Mitochondrial metabolism (through the Krebs cycle) is also the source of substrates and cofactors used for chromatin remodelling.
b, During female gametogenesis, differentiation of primordial germ cells (PGCs) into mature oocytes is accompanied by an increase in the total number of mitochondria. In addition, oocyte maturity is associated with a selective amplification of a fraction of mitochondria present in PGCs, which leads to a more homoplasmic mature oocyte in comparison with the heteroplasmic progenitor (depicted here by a reduction in the number of colours painting mitochondria in the oocyte), a process known as mitochondrial bottleneck.
c, In the white adipocytes of female mice, a locus on chromosome 17 containing Ndufv2, controls in trans the expression of at least 89 genes implicated in mitochondrial biogenesis and oxidative phosphorylation.

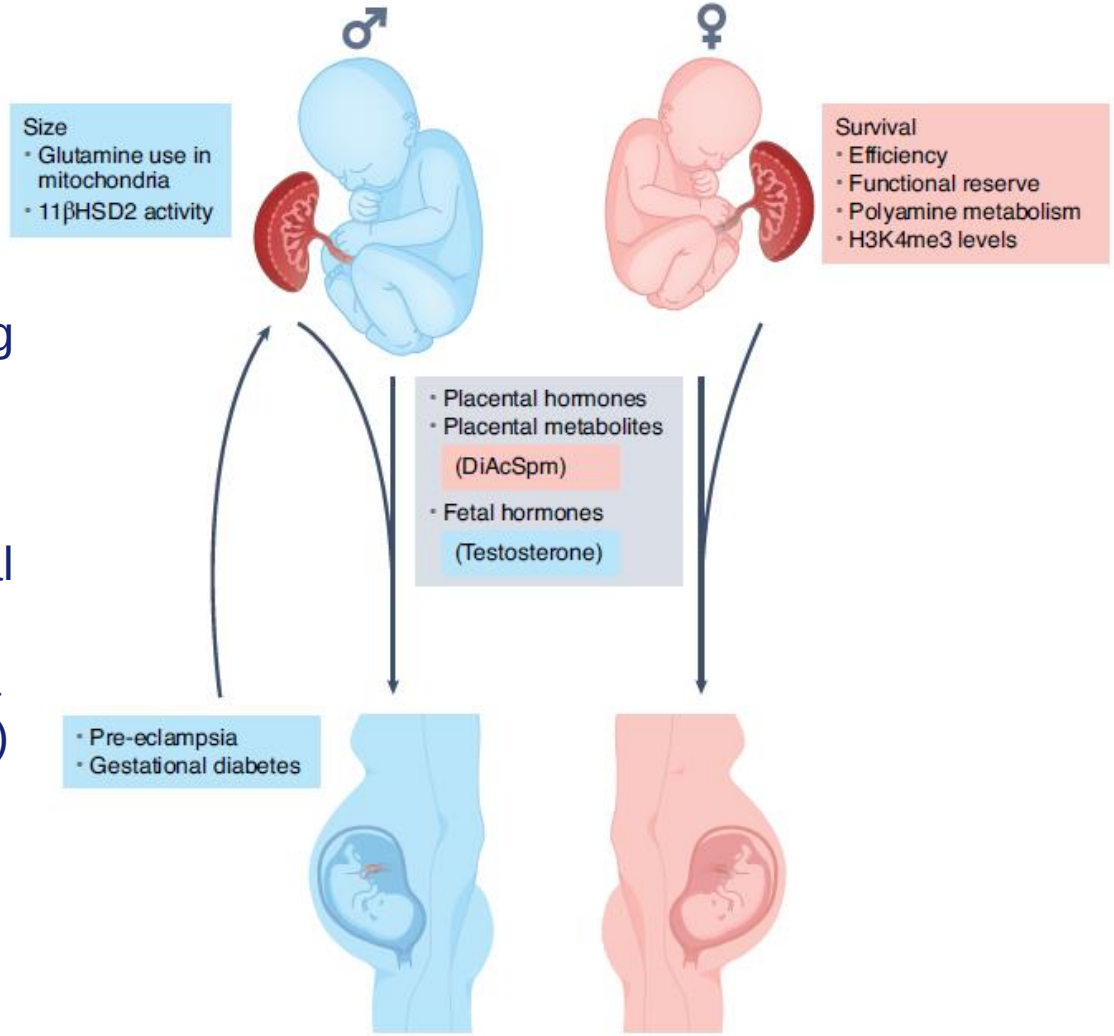


Sandovici et al, 2022

Sex differences in placental and maternal adaptations to pregnancy

Co-funded by the Erasmus+ Programme of the European Union

- Sex-related differences in feto-placental unit development and function favour growth in males and survival in females.
- The fetus and the placenta are also influencing maternal adaptations to pregnancy in a sex-dependent manner, leading to increased risk for pregnancy-related diseases in male-bearing pregnant women and intergenerational effects.
- Characteristics and parameters enhanced in a sex-specific manner are shown in blue (males) and red (females), respectively, while those that have not been explored so far for sex-related effects are shown in black.



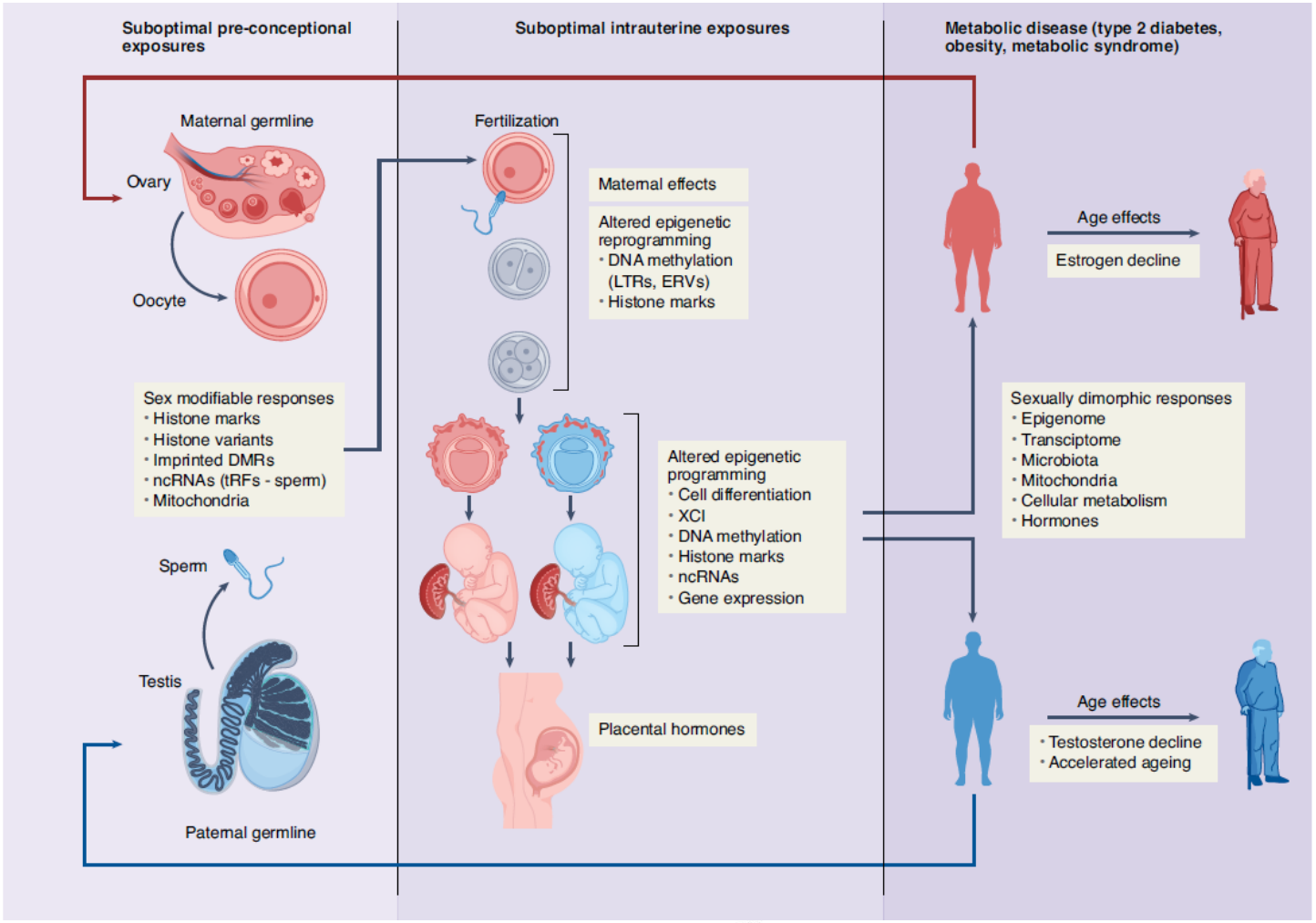
Sandovici et al, 2022

Pathways leading to sex differences in the intergenerational inheritance of metabolic disease

Co-funded by the Erasmus+ Programme of the European Union

- Suboptimal exposures during peri-conceptional or intrauterine development can lead to sexually dimorphic molecular changes that contribute to sex-related differences in the frequency, age of onset and severity of metabolic disease in adult life.
- The suboptimal metabolic milieu can exert detrimental effects on the germline, thus contributing to the intergenerational inheritance of metabolic diseases.

ERVs, endogenous retroviruses; LTRs, long terminal repeats; ncRNA,
non-coding RNA.

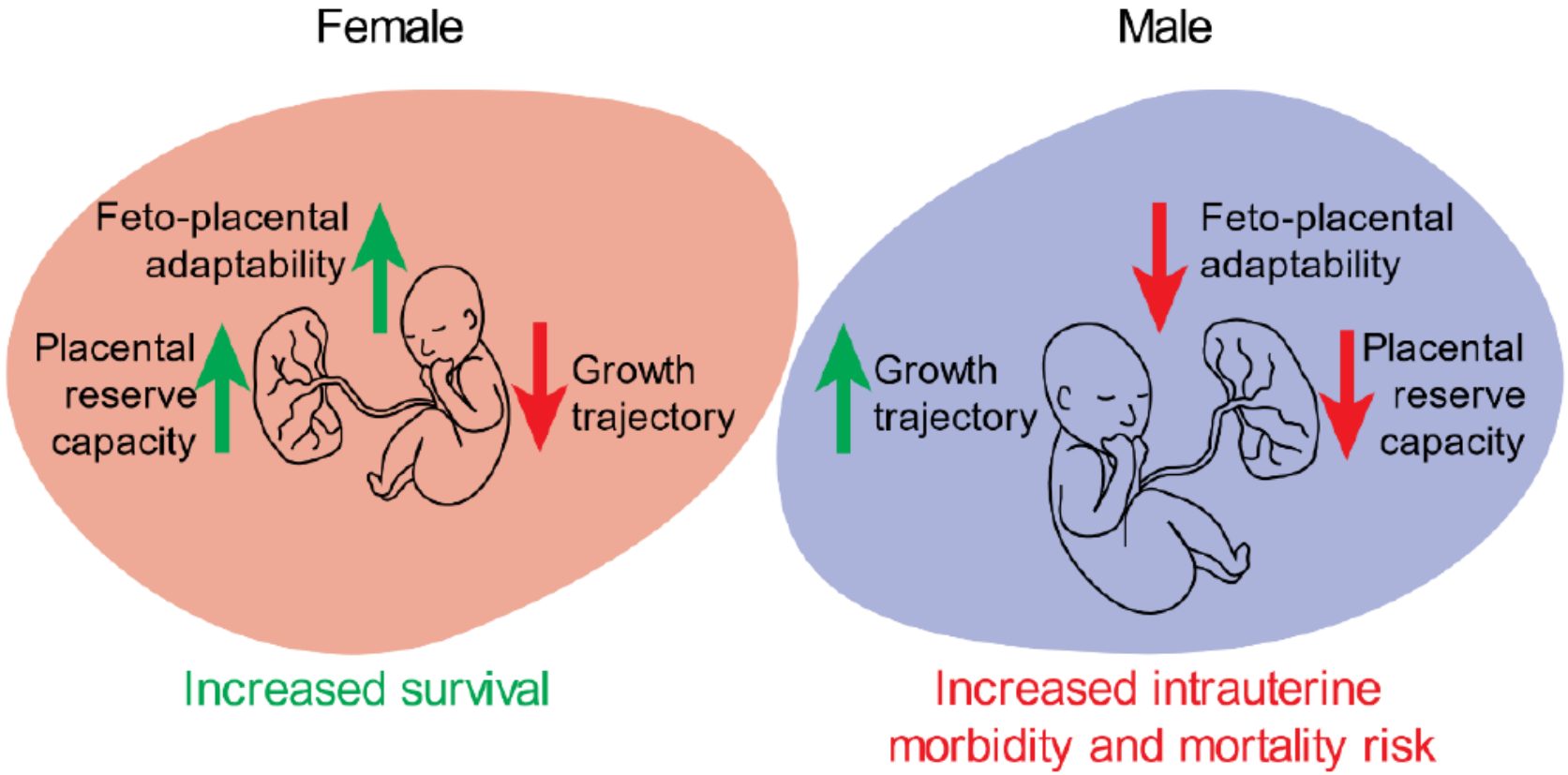


Sandovici et al, 2022

Let's Talk about Placental Sex, Baby (1)

Co-funded by the Erasmus+ Programme of the European Union

In females, increased feto-placental adaptability and placental reserve capacity result in increased survival rates at the expense of a reduced growth trajectory, whereas the opposite is observed in males.



- The occurrence of pregnancy complications differ according to fetal sex **with a higher cardiovascular and metabolic load for the mother in the presence of a male fetus**.

Meakin et al, 2021

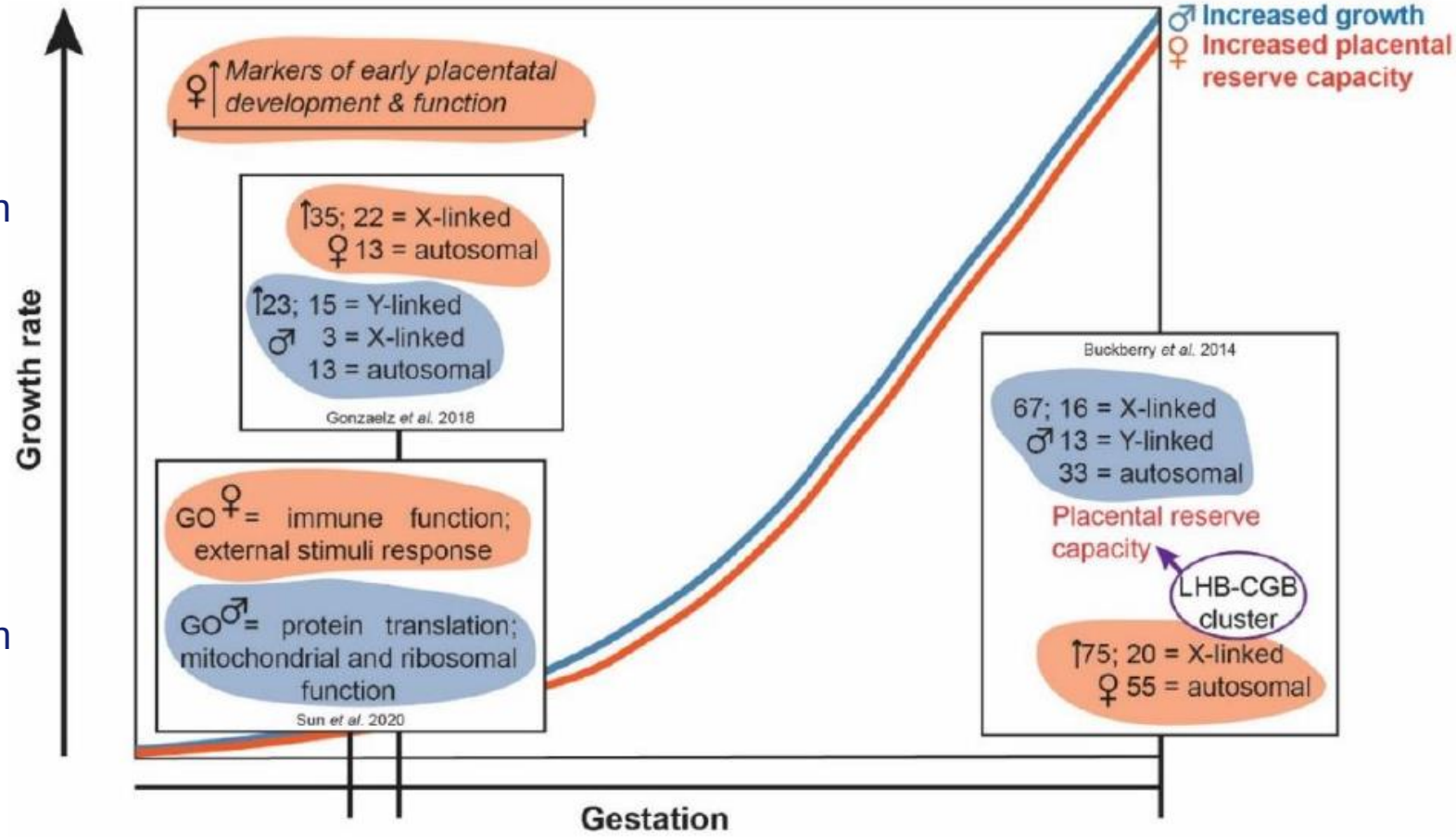
Let's Talk about Placental Sex, Baby (2)

Co-funded by the Erasmus+ Programme of the European Union

Understanding Mechanisms That Drive Female- and Male-Specific Fetal Growth and Developmental Outcomes

- Alterations in female and male placental transcript expression across gestation may contribute to sex-specific feto-placental growth and function outcomes.
- It is postulated that the differential expression of transcripts in females increases placental reserve capacity and feto-placental adaptability to an altered maternal environment at the expense of a reduced growth trajectory, relative to males.

GO = gene ontology; LHB-CGB = luteinising hormone beta subunit and chorionic gonadotropin beta subunit.

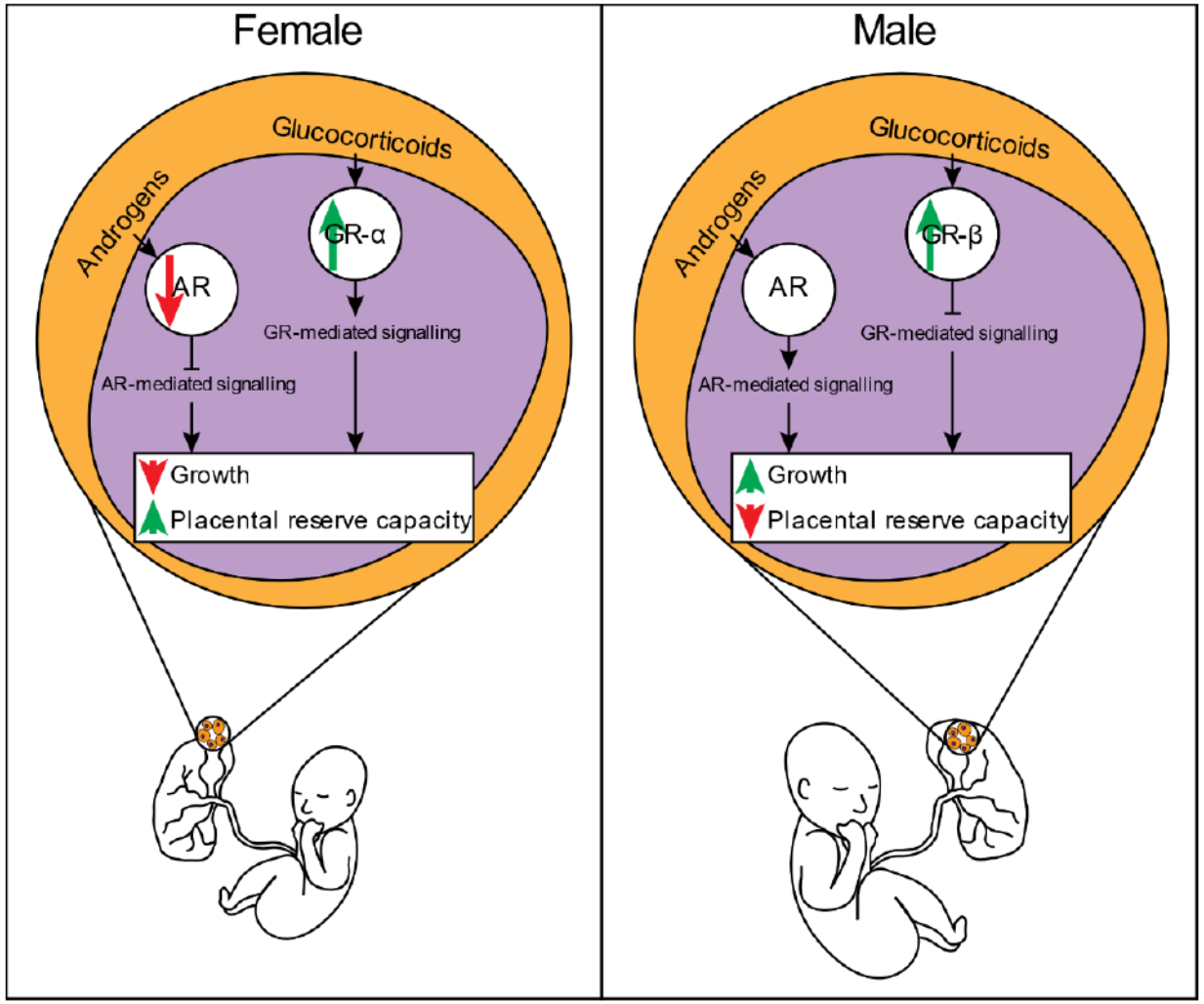


Meakin et al, 2021

Let's Talk about Placental Sex, Baby (3)

Co-funded by the Erasmus+ Programme of the European Union

Sex-specific differences in androgen and glucocorticoid-mediated signalling within the
feto-placental unit. Females prioritise pathways regulated by glucocorticoids to enhance placental
reserve capacity at the detriment of growth, whereas males prioritise androgen-mediated signalling pathways to enhance growth at the expense of placental reserve capacity

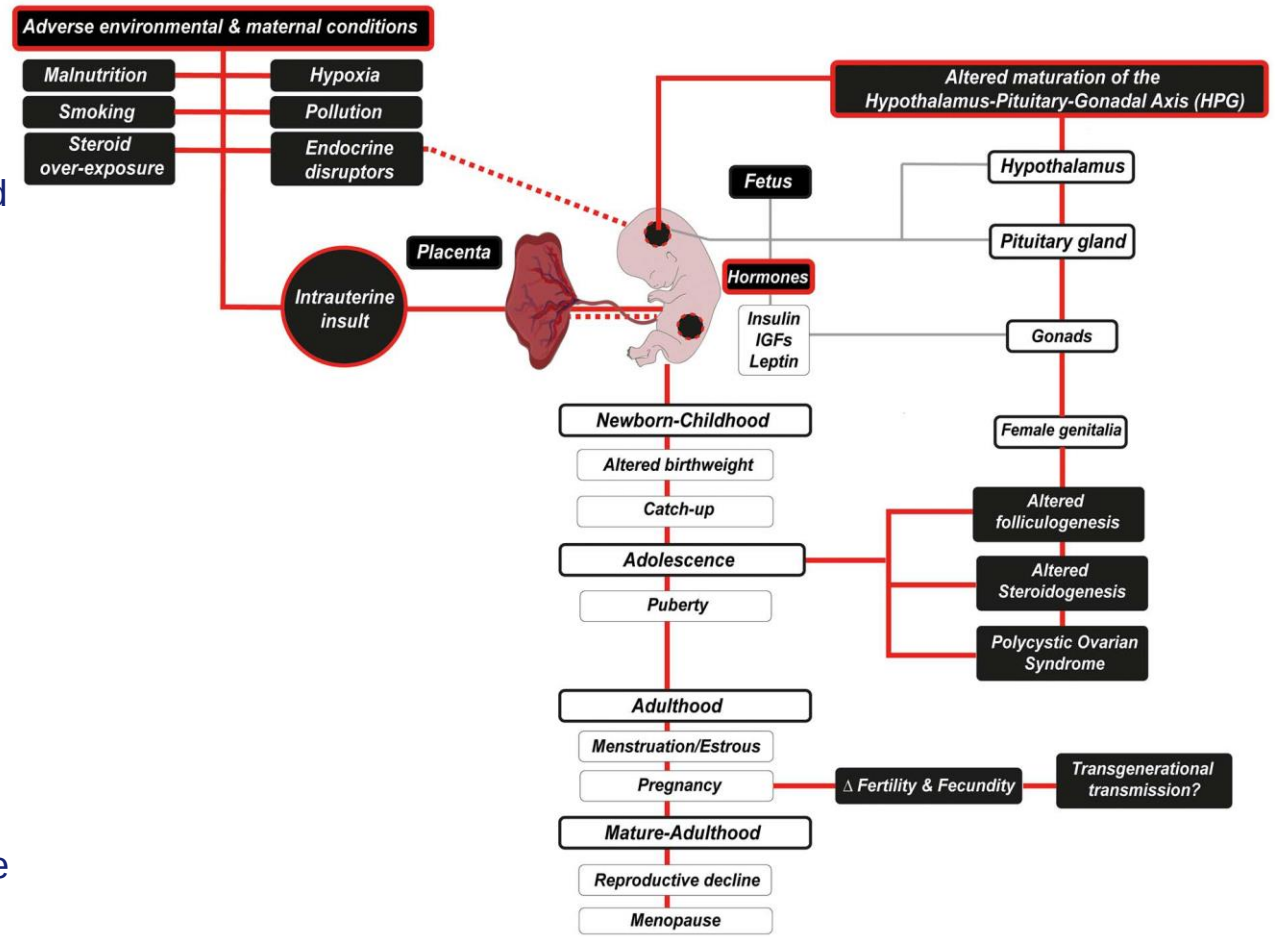


Meakin et al, 2021

Developmental programming of the female reproductive system

Co-funded by the Erasmus+ Programme of the European Union

- Environmental conditions during pregnancy can adversely impact the reproductive phenotype of female offspring with consequences for fertility and future reproductive capacity. These changes are mediated via programmed changes in the structure, maturation, and function of reproductive tissues and the HPG axis.
- Reproductive alterations may or may not be dependent on a change in birthweight and may be secondary to changes in the placenta in utero, as well as to programmed alterations in offspring growth rate and/or the production of HPA hormones, insulin, leptin, and IGFs postnatally.
- Reproductive programming may be observed as changes in the timing of puberty onset and menopause/reproductive decline, altered menstrual/estrous cycles, polycystic ovaries, and elevated risk of reproductive tissue cancers. These reproductive outcomes can affect the fertility and fecundity of the female offspring and may lead to negative impacts on the second generation.

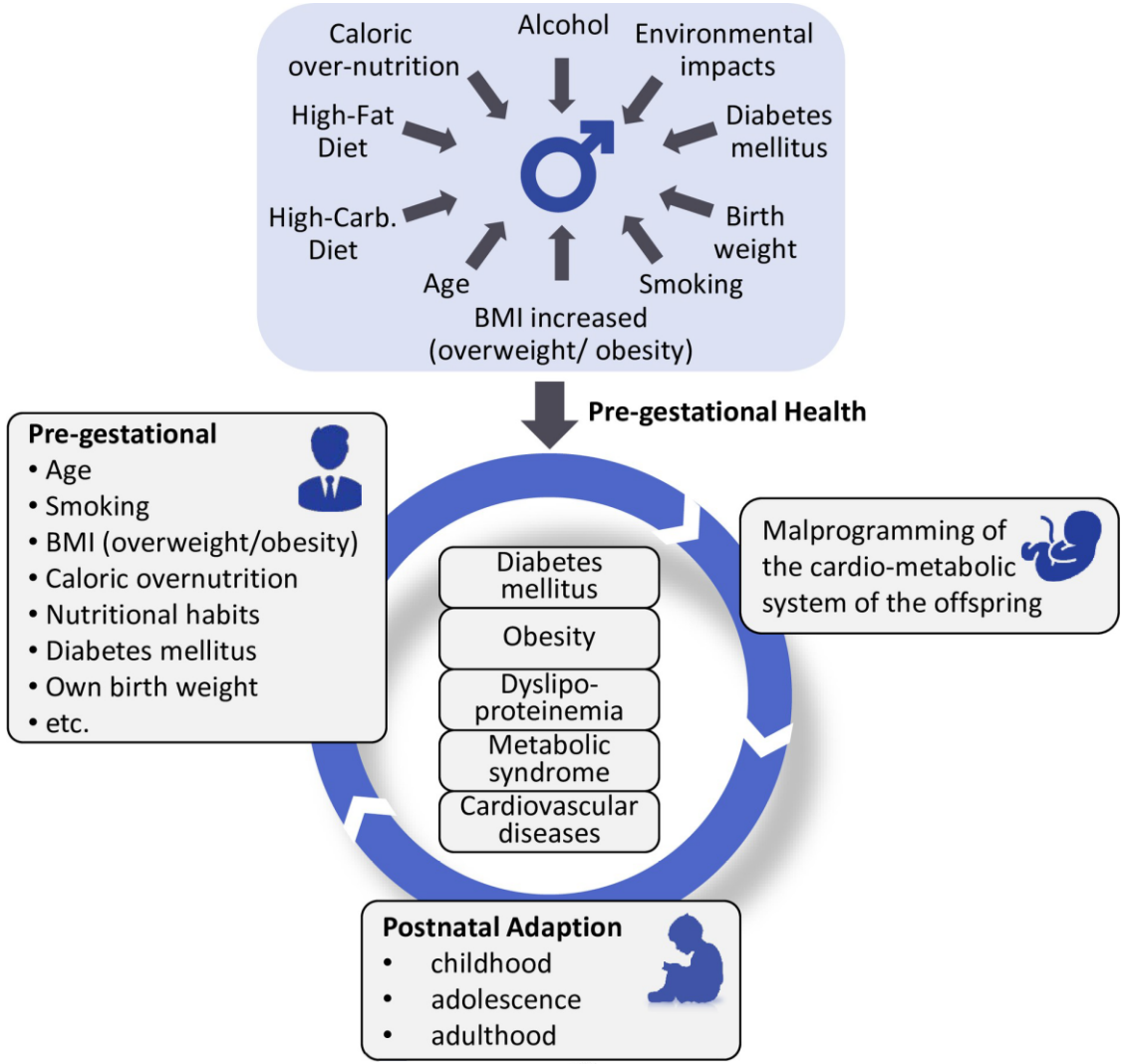


Yao et al, 2021

Life cycle of paternal programming metabolic and cardiovascular health of their offspring

Co-funded by the Erasmus+ Programme of the European Union

- There is emerging evidence that paternal risk factors, such as paternal obesity, diabetes mellitus, nutritional habits, advanced age and exposure to environmental chemicals or cigarette smoke, are clearly associated with adverse effects in metabolic and cardiovascular health in their offspring.
- Compared to maternal programming, pre-conceptional paternal factors might also have also a substantial effect in the sense of trans-generational programming of their offspring and need further research.

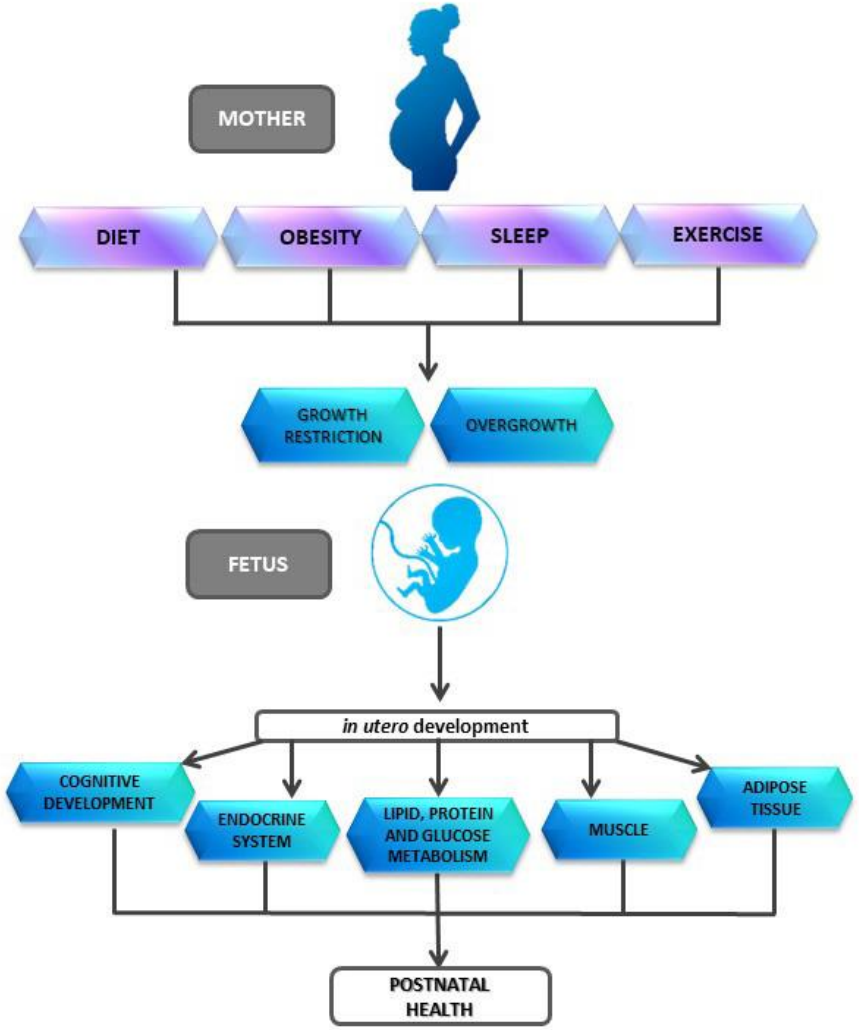


Eberle et al, 2020

Impact of Early Nutrition, Physical Activity and Sleep on the Fetal Programming of Disease in the Pregnancy

Co-funded by the Erasmus+ Programme of the European Union

- Adequate caloric intake, protein, mineral, vitamin, and long-chain fatty acids, have been noted for their relevance in the ospring brain functions and behavior.
- Fetus undernutrition/malnutrition causes a delay in growth and have detrimental eects on the development and subsequent functioning of the organs. Pregnancy is a particularly vulnerable period for the development of food preferences and for modifications in the emotional response.
- Maternal obesity increases the risk of developing perinatal complications and delivery by cesarean section and has long-term implications in the development of metabolic diseases.
- Physical exercise during pregnancy contributes to overall improved health post-partum. It is also interesting to highlight the relevance of sleep problems during pregnancy, which influence adequate growth and fetal development.



Moreno-Fernandez et al, 2020

Effects of maternal under-or overnutrition in male offspring reproductive phenotype

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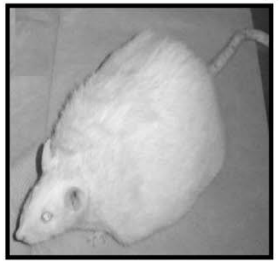
Programming (F0)

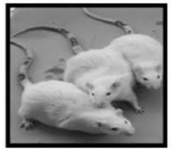
Maternal obesity



Maternal protein restriction

Male offspring (F1) reproductive phenotype:

- Delay in sexual development markers
- Low testosterone serum levels
- Increased oxidative stress
- Decreased sperm quality
- Reduced fertility
- Premature aging of reproductive capacity



Interventions in F1 from obese mothers

Exercise

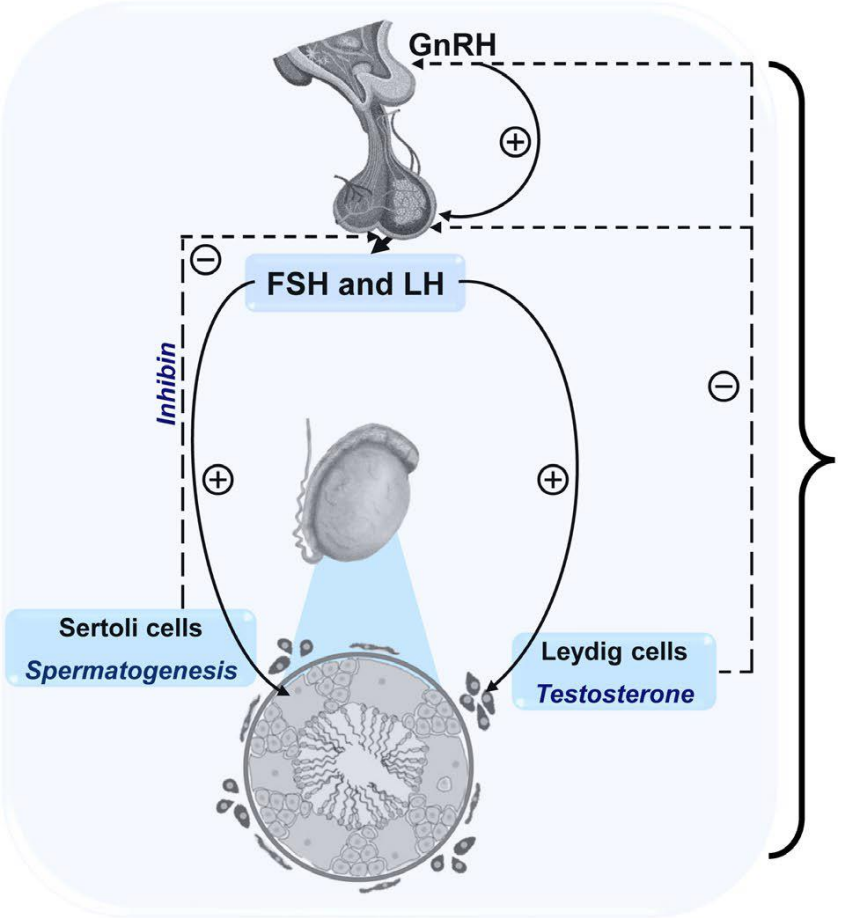
→ **Restores hormonal, oxidative stress and sperm quality parameters** → **Improves male offspring reproductive capacity**

Zambrano et al, 2021

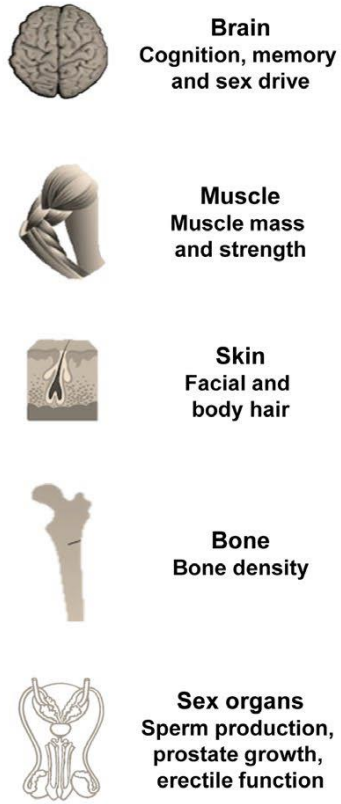
Male reproductive axis and the ageing-related changes

Co-funded by the Erasmus+ Programme of the European Union

Male reproductive axis



Influence of testosterone



Brain
Cognition, memory and sex drive

Muscle
Muscle mass and strength

Skin
Facial and body hair

Bone
Bone density

Sex organs
Sperm production, prostate growth, erectile function

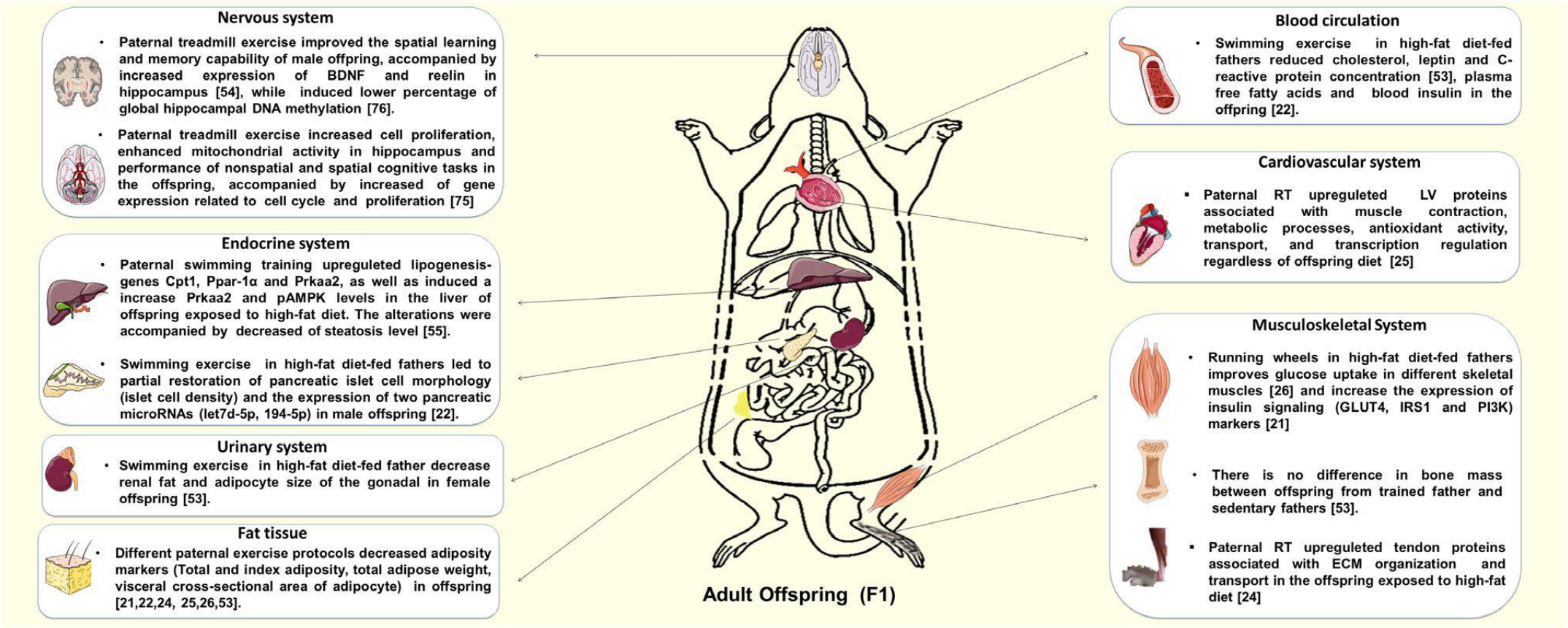
Age-related changes in male reproductive system

- Decrease in testicular size and weight
- Decay in testosterone secretion
- Decline in sperm production
- Prostate enlargement
- Diminished prostate secretion
- Reduced libido
- Sperm DNA fragmentation

Zambrano et al, 2021

Overview of the effects of paternal exercise on physiological systems in the offspring

Co-funded by the Erasmus+ Programme of the European Union



- Different exercise protocols might induce beneficial effects on distinct tissues and organs in the first generation (F1).

Vieira de Sousa Neto et al, 2021

Role of Inflammaging on the Reproductive Function and Pregnancy (1)

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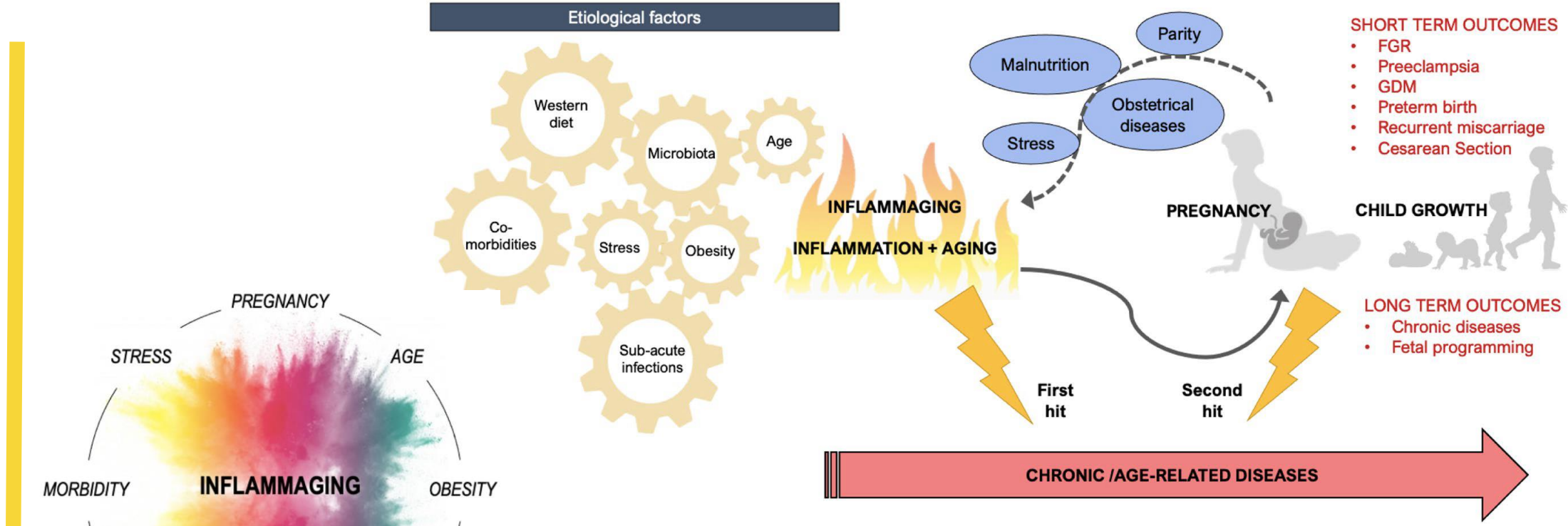
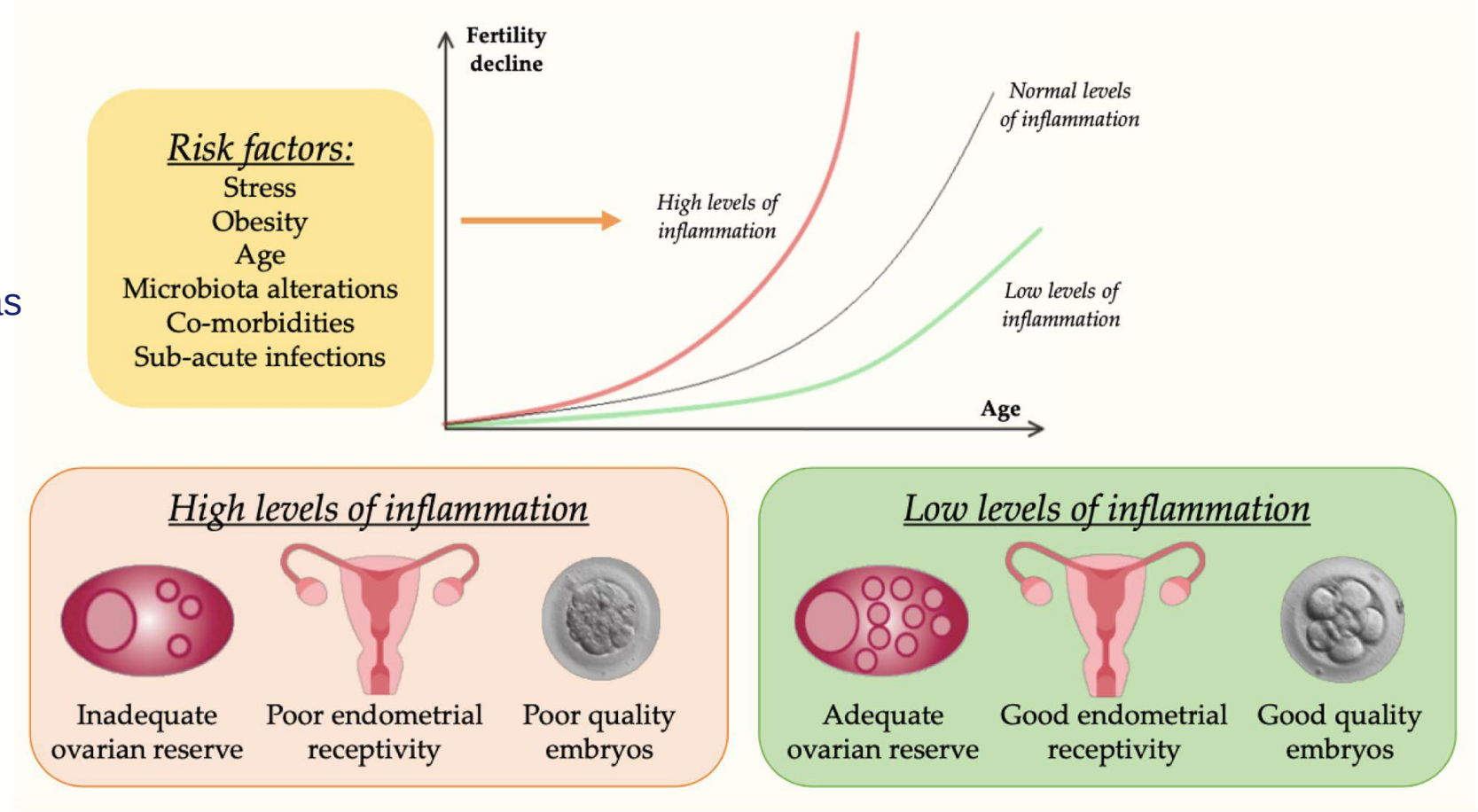


Zavatta et al, 2022

Role of Inflammaging on the Reproductive Function and Pregnancy (2)

Co-funded by the Erasmus+ Programme of the European Union

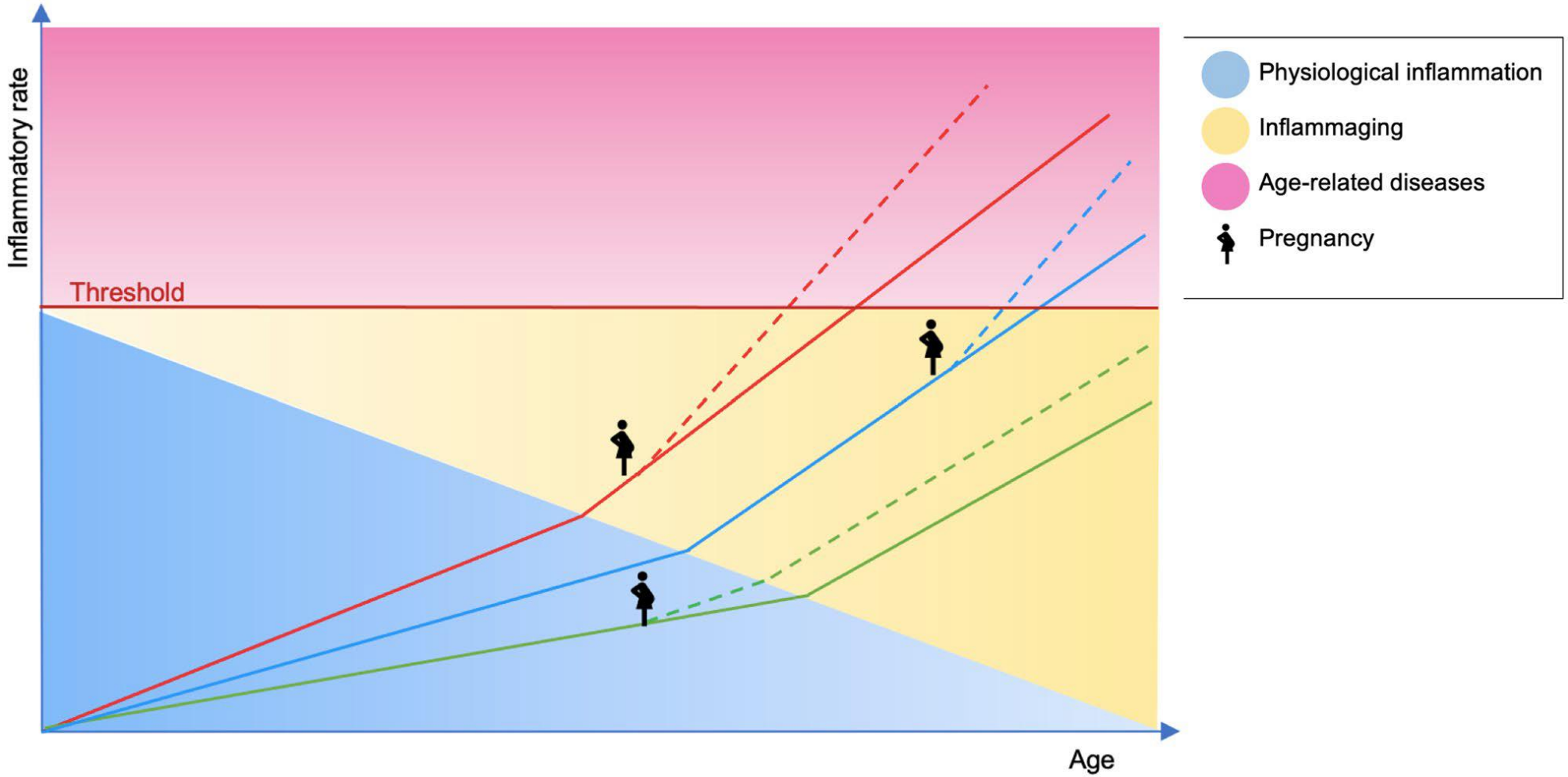
- Fertility spontaneously declines as women age.
- However, higher levels of inflammation lead to a faster reduction of the ovarian reserve, as well as are associated with poor quality embryos and inadequate endometrial receptivity in In-Vitro Fertilization (IVF) treatments.
- Reduction of risk factors has a positive impact on inflammation and, in turn, on female fertility.



Zavatta et al, 2022

Inflammaging and pregnancy effect on aging trajectories and age-related diseases outbreak

Co-funded by the Erasmus+ Programme of the European Union

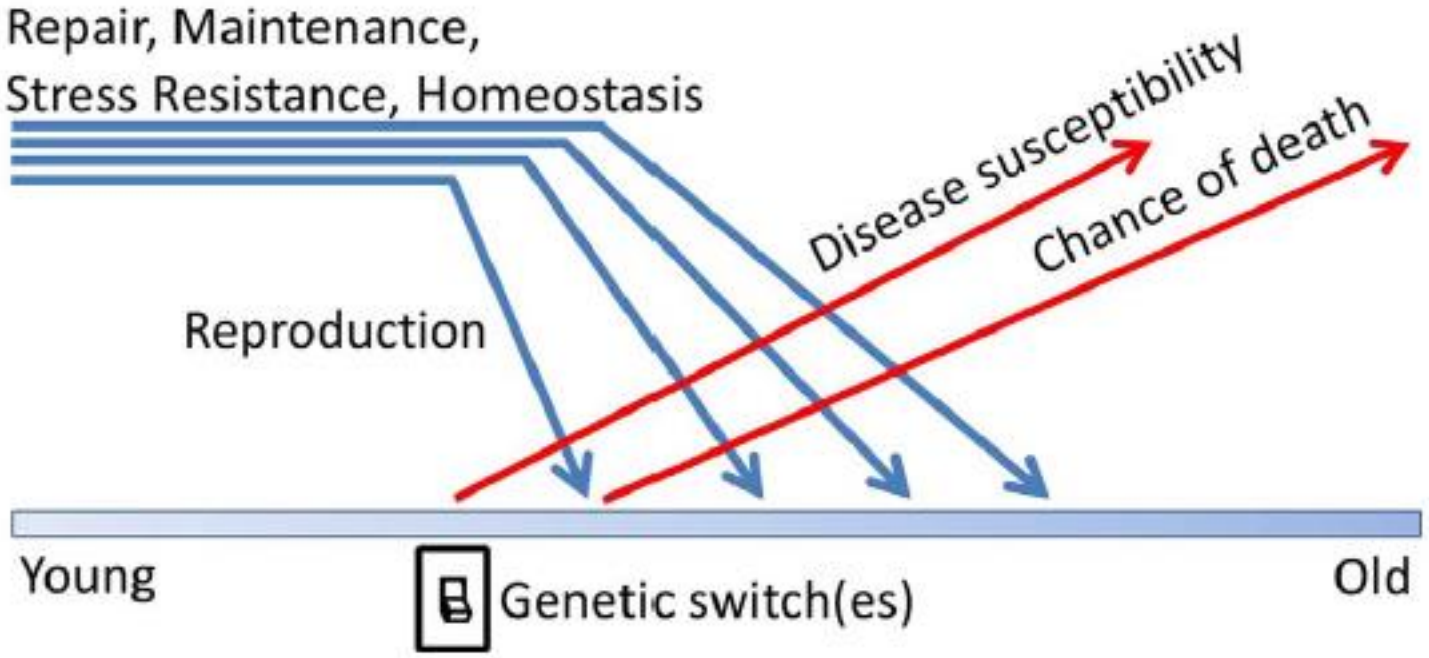


Zavatta et al, 2022

A genetic switch model of aging

Co-funded by the Erasmus+ Programme of the European Union

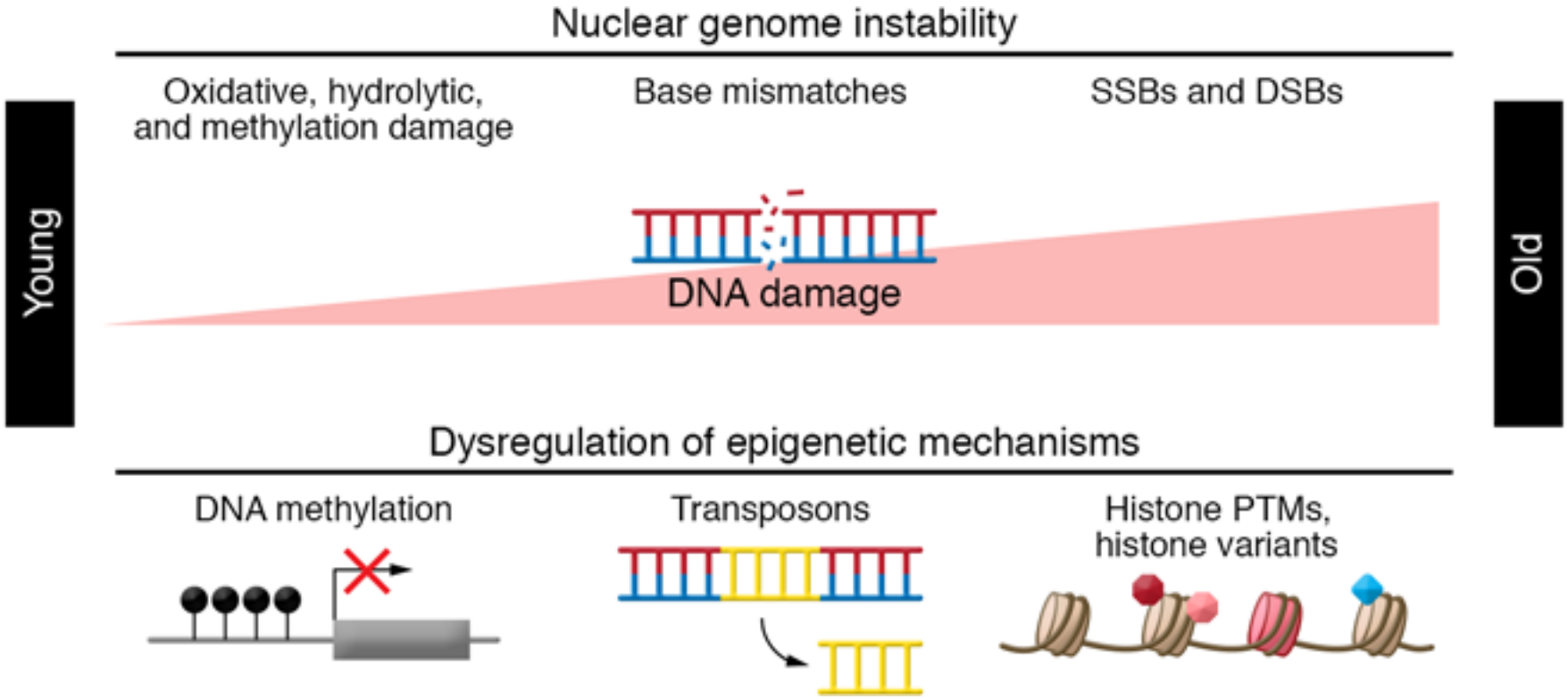
- In young organisms energy is invested into repair, maintenance, stress resistance and homeostasis pathways. This keeps the organism in a youthful state with low risk of disease and death by internal causes.
- At some point during or after reproduction a genetic switch, or switches, occurs in which the repair, maintenance, stress resistance and homeostasis pathways are turned off, or turned down, even though these pathways are still able to function optimally. The genetic switch may also trigger a redistribution of resources from somatic maintenance to reproduction.
- The decline resulting from the loss of these survival pathways increases susceptibility to disease and the chance of death, thereby causing aging.



JM Van Raamsdonk, 2018

Epigenetics, DNA damage, and aging

Co-funded by the Erasmus+ Programme of the European Union



- **Aging is determined, in part, by interrelated mechanisms that affect nuclear genome integrity: macromolecular damage to DNA and epigenetic alterations.**

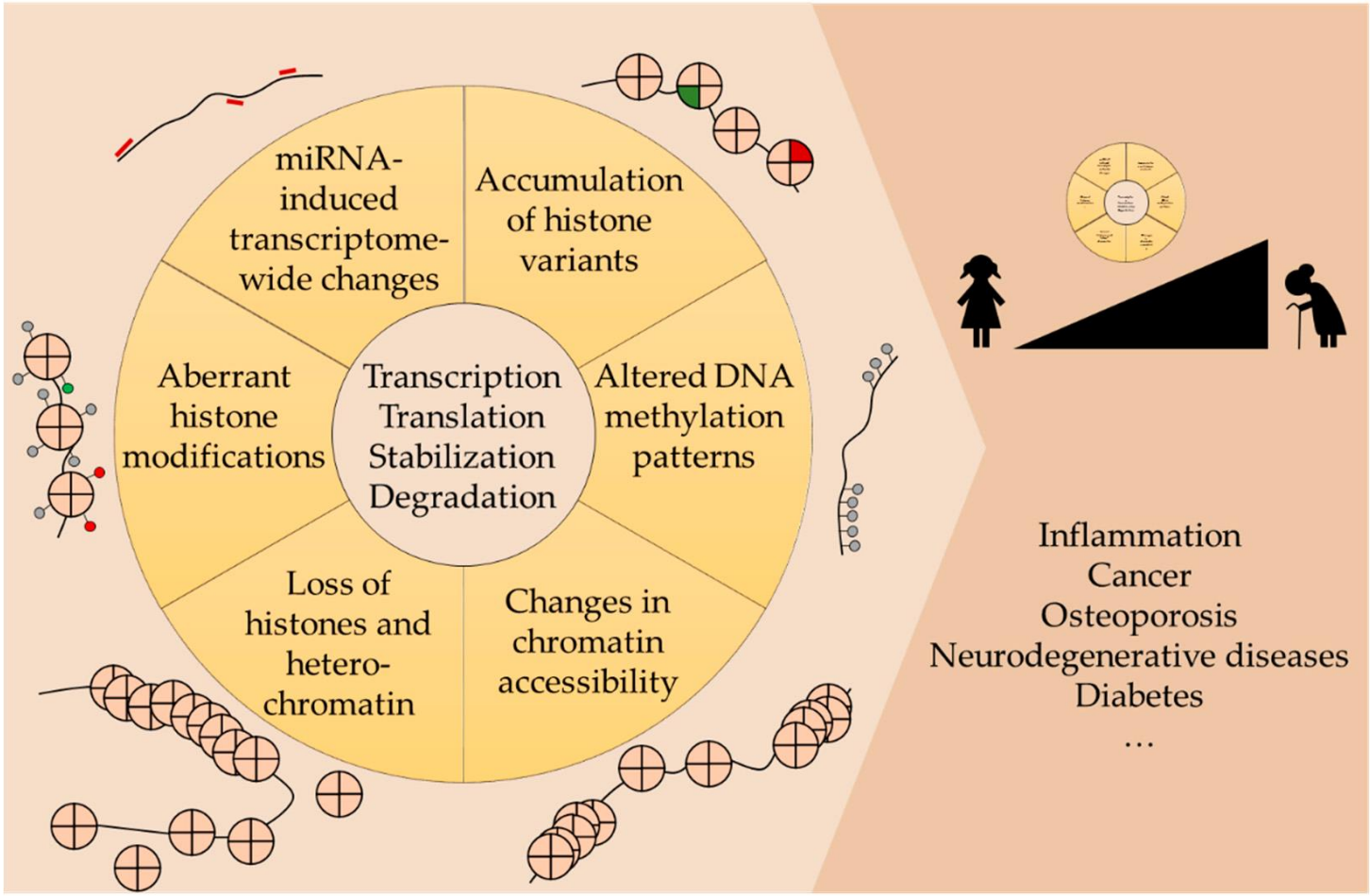
DSBs, double-strand breaks; PTMs, posttranslational modifications; SSBs, single-strand breaks.

Soto-Palma et al, 2022

Epigenetics of aging and aging-related diseases

Co-funded by the Erasmus+ Programme of the European Union

- During aging, various epigenetic alterations occur including accumulation of histone variants, changes in chromatin accessibility mediated by chromatin remodeling complexes, loss of histones and heterochromatin, imbalance of activating/repressing histone modifications and aberrant expression/activity of miRNAs.
- These deregulations can affect transcription and, subsequently, translation, as well as the stabilization or degradation of molecular components.
- Consequently, these aberrant epigenetic processes can promote morbidities, which are frequently observed in the elderly populations, including inflammation, cancer, osteoporosis, neurodegenerative diseases, and diabetes.

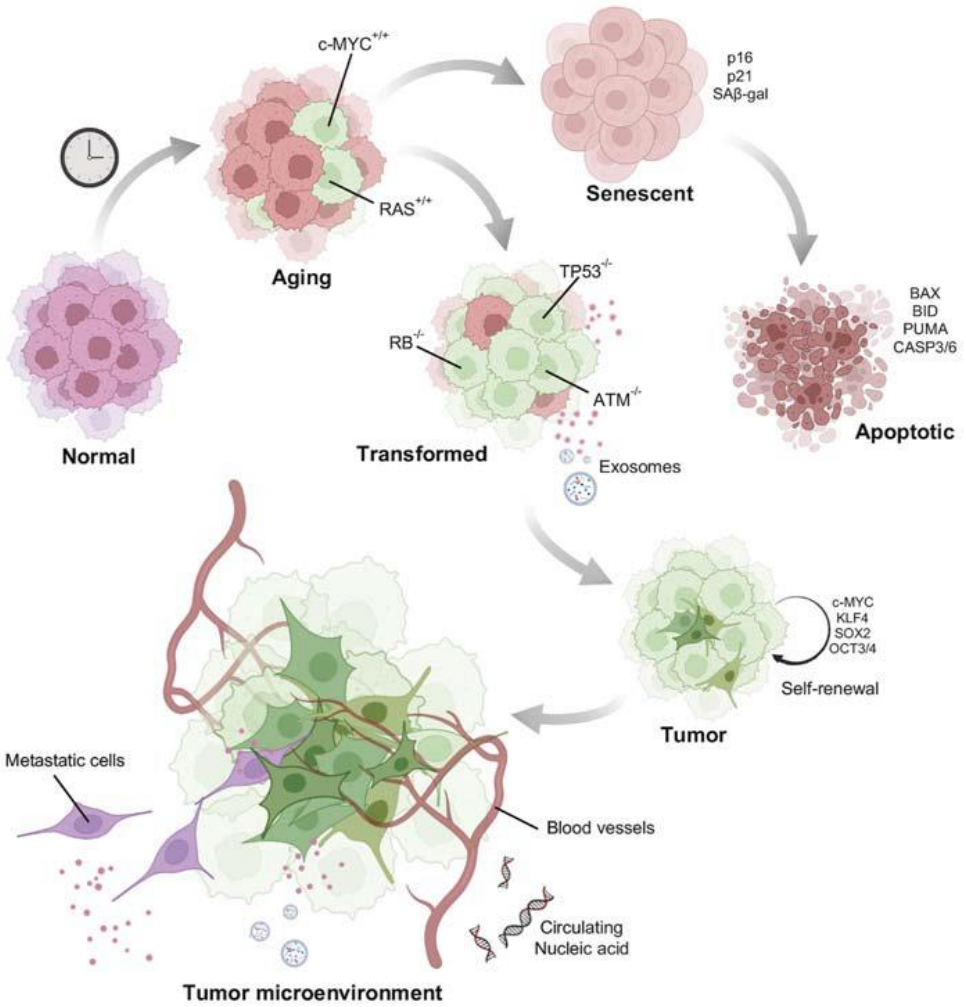


Saul & Kosinsky, 2021

Model of how aged cells potentiate tumor formation

Co-funded by the Erasmus+ Programme of the European Union

- **Normal young cells accumulate DNA damage and senesce. A few of the damaged old cells acquire mutations such as activation of oncogenes to induce oncogene induced senescence.**
- **Senescent cells after acquiring enormous DNA damage might be directed towards apoptotic pathway. Some cells however, escape death by acquiring other mutations and gain self-renewal property behaving as potential stem cells. Tumor cells once formed is also facilitated by the aging stroma for its growth and metastasis.**

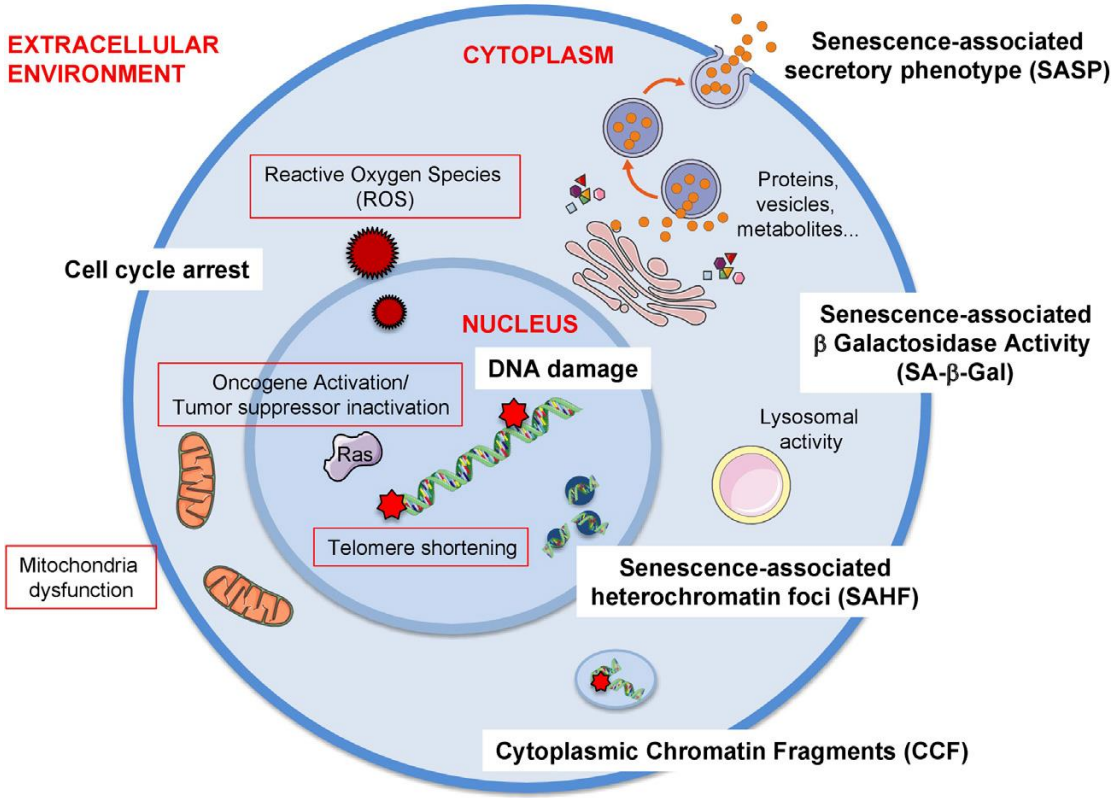


Sikder et al, 2022

Cellular Senescence and Ageing

Co-funded by the Erasmus+ Programme of the European Union

There are several stimuli or triggers that activate cellular senescence (red outline). Some of these are depicted in the figure such as the formation of **Reactive Oxygen Species (ROS) both from external factors or internal such as mitochondrial dysfunction**. Others include the expression of certain oncogenes, e.g., RAS (Rat sarcoma virus) or the loss of tumour suppressor genes, e.g., PTEN (Phosphatase And Tensin Homolog). The shortening of telomeres due to the lack of telomerase enzyme also elicits cellular senescence. Additionally, mitochondrial dysfunction, which can be due to mitochondrial malfunction, increase in mitochondrial size or mass, mitochondrial fusion or mitochondrial fragmentation can also induce senescence. As there is no gold standard biomarker of senescence, a combination of several biomarkers are used to identify this cellular phenotype both in vitro and in vivo. Some of these biomarkers are the release of a senescence-specific secretome, the senescence-associated phenotype (SASP) formed by proteins, vesicles, metabolites. Other biomarkers the presence of DNA damage and the establishment of a stable cell cycle arrest. Furthermore, chromatin alterations such as heterochromatin foci (senescence-associated heterochromatin foci, SAHF) or the presence of chromatin in the cytoplasm (cytoplasmic chromatin fragments, CCF) are also present during senescence. Finally, the most extensively used biomarker of senescence is the presence of senescence-associated -β- galactosidase activity (SA-β-Gal) which is due to an increase in lysosomal activity, although it is important to take into account that this feature is not exclusive of senescent cells.



Mylonas & O'Loghlen, 2022

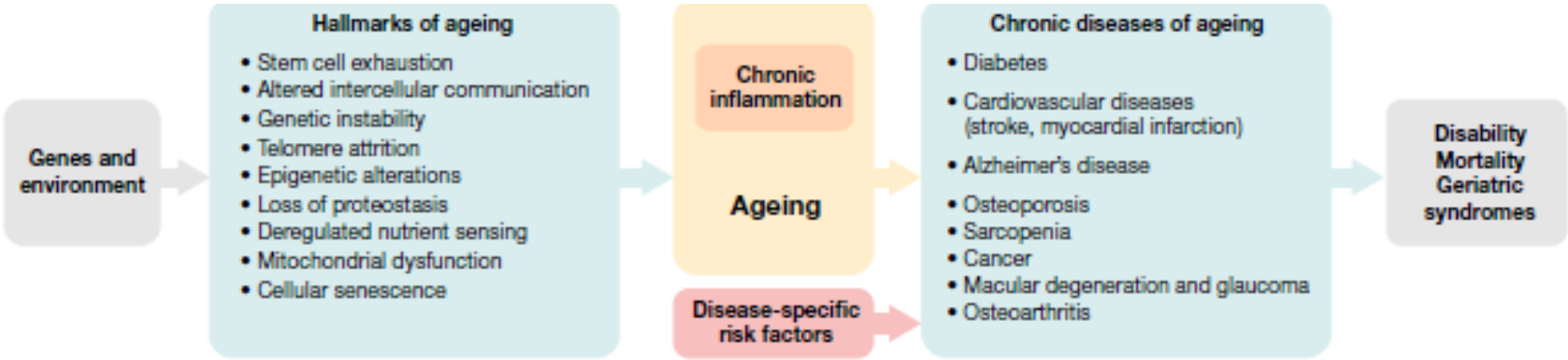
From discoveries in ageing research to therapeutics for healthy ageing

Co-funded by the Erasmus+ Programme of the European Union

Table 1 | Interventions to increase healthspan and/or lifespan

Intervention	Target or process	Major effects
Rapamycin	mTOR	Geroprotective effects in mice and dogs. Human clinical trials with rapamycin and rapalogs are underway.
Senolytics	Cellular senescence	Protective against age-related disease in mice. Ongoing clinical trials in human diseases, including arthritis and eye degeneration.
NAD precursors	NAD metabolism	Geroprotective in animal models. Supplements available for human consumption, but no clinical trials have been reported yet.
Sirtuin-activating compounds	Sirtuins	Geroprotective in rodents and non-human primates but mixed results in humans; SRT2104 may have effects beyond mitigating some age-associated conditions.
Metformin	Mitochondrial respiration	Associated with increased lifespan in human patients with diabetes and decreased risk of cancer. TAME trial is planned to test effects in individuals without diabetes.
Exercise	Unknown	Associated with reduced risk of age-related disease, improved quality of life and increased lifespan in humans.
Calorie restriction	Several targets, including mTOR and sirtuins	Enhanced lifespan and protection from disease in worms, flies, mice, rats and non-human primates. Associated with decreased risk factors for disease in humans.

Several key interventions that are currently under investigation in human trials for their potential to increase healthspan and lifespan are described (see text for further details).



Campisi et al, 2019

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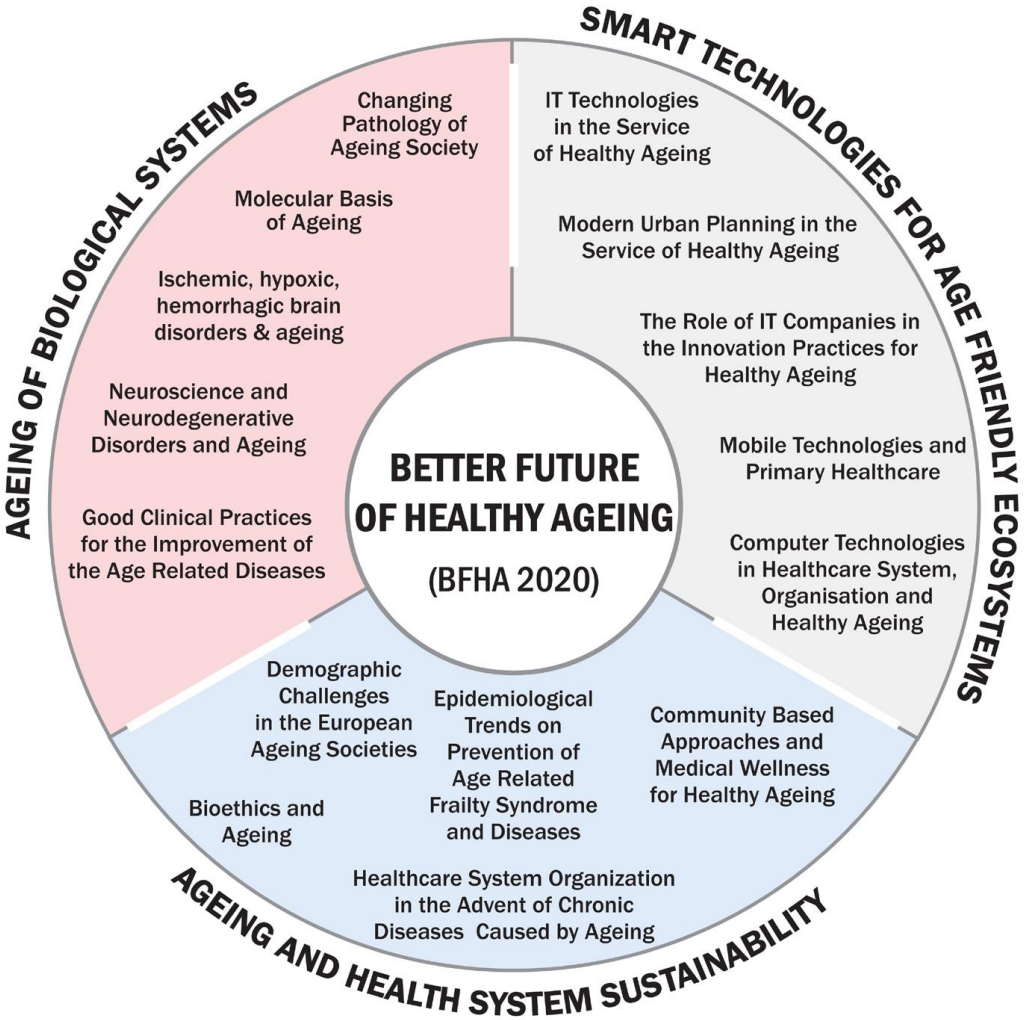
Better Future of Healthy Aging 2020 Conference topics

Co-funded by the Erasmus+ Programme of the European Union

- All people, regardless of their origin, should be granted the opportunity to live a long and healthy life.
- Yet, the environments in which we live can promote health or degrade it. Environment and spatial epidemiology play an important role in defining the health risks of a population.
- An other important factor is the accessibility to quality health regardless of social status and other sources of deprivation. Population aging makes these issues increasingly more visible.
- Healthy aging is about creating the social climate and opportunities that enable people to thrive throughout their lives.
- The COVID-19 pandemic has shown the importance of every person's involvement in creating a healthy environment, healthy relationships, building solidarity, and social awareness of health needs. Let us use this newly obtained attitude to improve the care for the elderly and lead the world to a better future of aging.



Kujundžić Tiljak et al, 2020

Sex and gender differences across the life span

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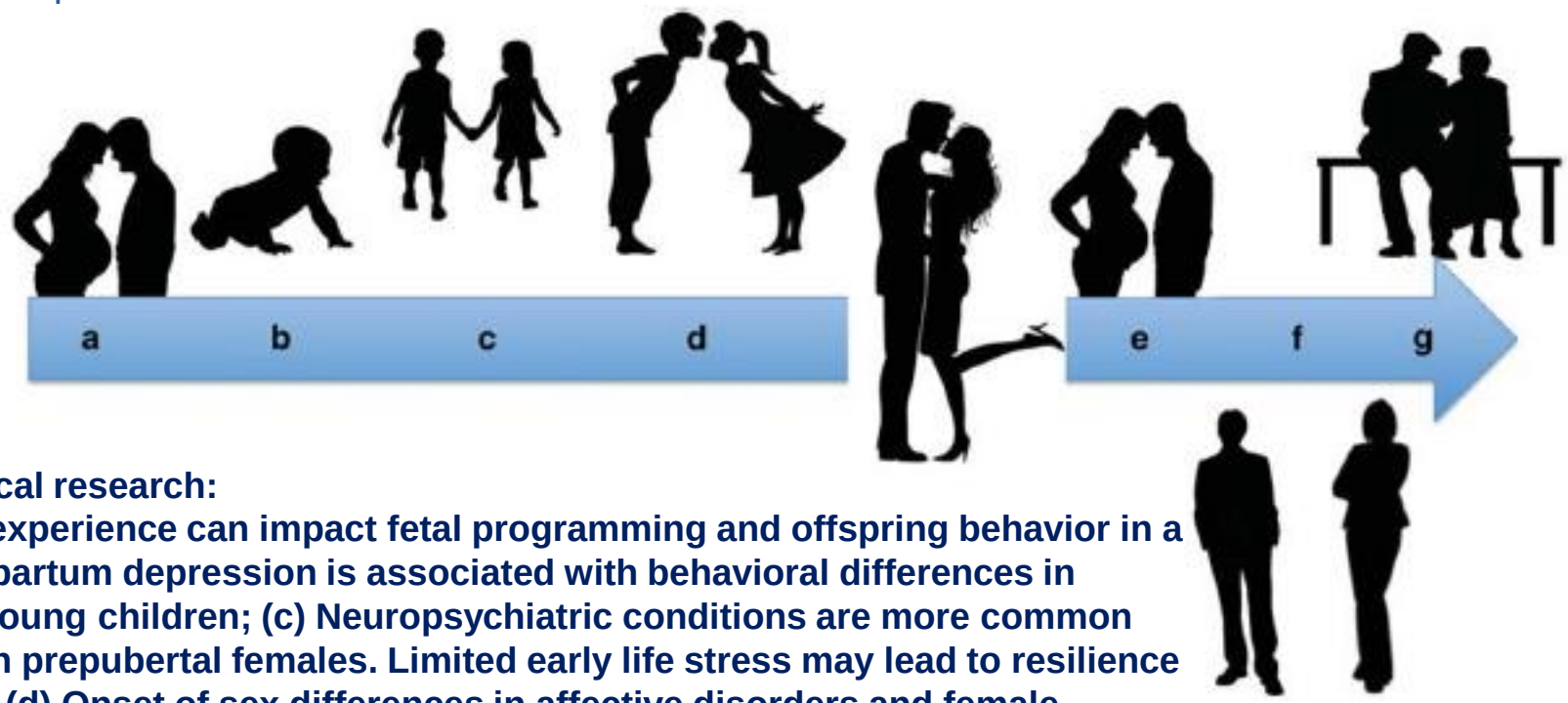


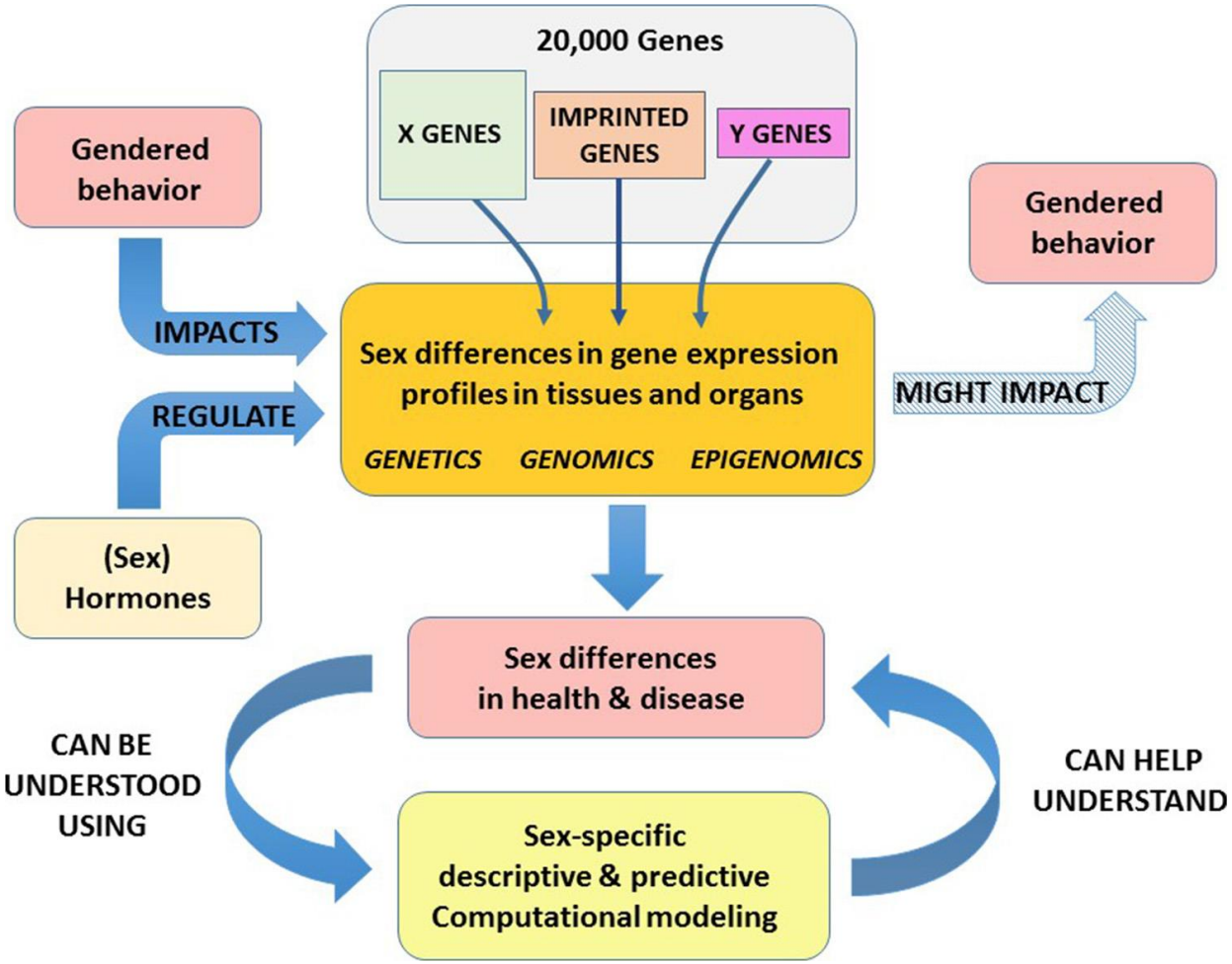
Based on preclinical and clinical research:
(a) maternal and paternal life experience can impact fetal programming and offspring behavior in a sex-specific manner; (b) Postpartum depression is associated with behavioral differences in female and male infants and young children; (c) Neuropsychiatric conditions are more common among prepubertal males than prepubertal females. Limited early life stress may lead to resilience to depression among women. (d) Onset of sex differences in affective disorders and female-specific mood disorders at puberty. Ovarian hormones modulate brain neurochemistry, structure, and (e) Prenatal stress contributes to risk for diseases that exhibit sex differences across the life span (f) Mid-life is associated with marked hormonal shifts for women, but not men. Estradiol effects on stress responses varies in pre- vs post-menopausal women; (g) Females are at greater risk of dementia and adverse effects of many pharmacologic agents used in the treatment of adult disorders.

Bale & Neill Epperson, 2017

The impact of sex differences on genomic research

Co-funded by the Erasmus+ Programme of the European Union

- The detectable sex differences in genetics, genomics and epigenomics have multiple origins. Sex-specific and imprinted autosomes contribute, as well as (sex) hormones and the consequences of gendered behavior (such as e.g. different exposures to risk factors, health behavior, stress exposure etc.).
- Sex differences in genomics and epigenomics might have an impact on gendered behavior, but currently this cannot be postulated. The identified sex differences in health and disease can be better understood using a sex-specific focus in their analysis.



Oertelt-Prigione & Mariman, 2020

Recommendations towards sex-sensitive genomics research

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Table 1
Definitions.

Sex – sex is a biological concept used to define differences between female and male organisms. Some individuals do not conform to the binary female-male categorization. These individuals have been historically called "intersex" individuals. Other definitions, such as individuals with "differences of sexual development" (DSD) are more inclusive. In the medical field DSD is often translated into "disorders of sex development"

Gender – gender is a multidimensional construct, which encompasses at least three different levels: the subjective perception of an individual, the perception through others and the interaction between the two. Different scholars might use different terminologies to describe similar concepts.

- ***gender identity*** (subjective perception) is the subjective knowledge of one´s identity as woman, man, queer, non-binary etc.
- ***gender roles*** or ***gender norms*** (perception through others) are the perceptions of one´s role in society based on one´s gender
- ***gendered behaviors*** or ***gender relations*** (interaction between the two) describe how the interaction between individuals is affected by gender

1 In formulating the research question, consider the potential role of sex differences
- Review the literature
- Consider biological plausibility
- Justify, when inclusion of both sexes is not needed (e.g. when investigating diseases that only affect one sex such as prostate cancer)

2 Identify the sex of the analyzed biological material
- Some commercially available cell lines are only male (e.g. fibroblasts derived from neonatal foreskin)
- Ascertain the stability of karyotype (e.g. culture passage and type of cell might lead to alterations over time)
- Ascertain that medium, staining and collection technique do not impact the cells in a sex-specific manner

3 Report details on all participants
- Report sex-disaggregated overall participant numbers and numbers in all potential sub-populations
- Include information about all samples from all tissues disaggregated by sex
- If multiple tests are performed on the same individual, consider reporting data on hormonal status and variations thereof (e.g. phase of the menstrual cycle in fertile women, menopause, andropause, hormonal therapy)

4 Report aspects that could induce sex-specific bias if animal samples are being used (e.g. animal chow, bedding, number of animals in cages, use of antibiotics etc.)

5 Report aspects that could induce sex-specific bias if human samples are being analyzed (e.g. pharmacotherapy, parity, exposure to hormonal and genetic modifiers etc.)

6 Conduct every analysis in sex-disaggregated manner
- Consider potential interactions between sex and other factors such as age or hormonal status

7 Report all analysis results in sex-disaggregated manner
- Sex has to be an integral part of genomics experiment annotations in public databases

8 Discuss the identified sex differences and identify future research needs

Oertelt-Prigione & Mariman, 2020

Do not forget to do research in transgender health: inclusion for a better care

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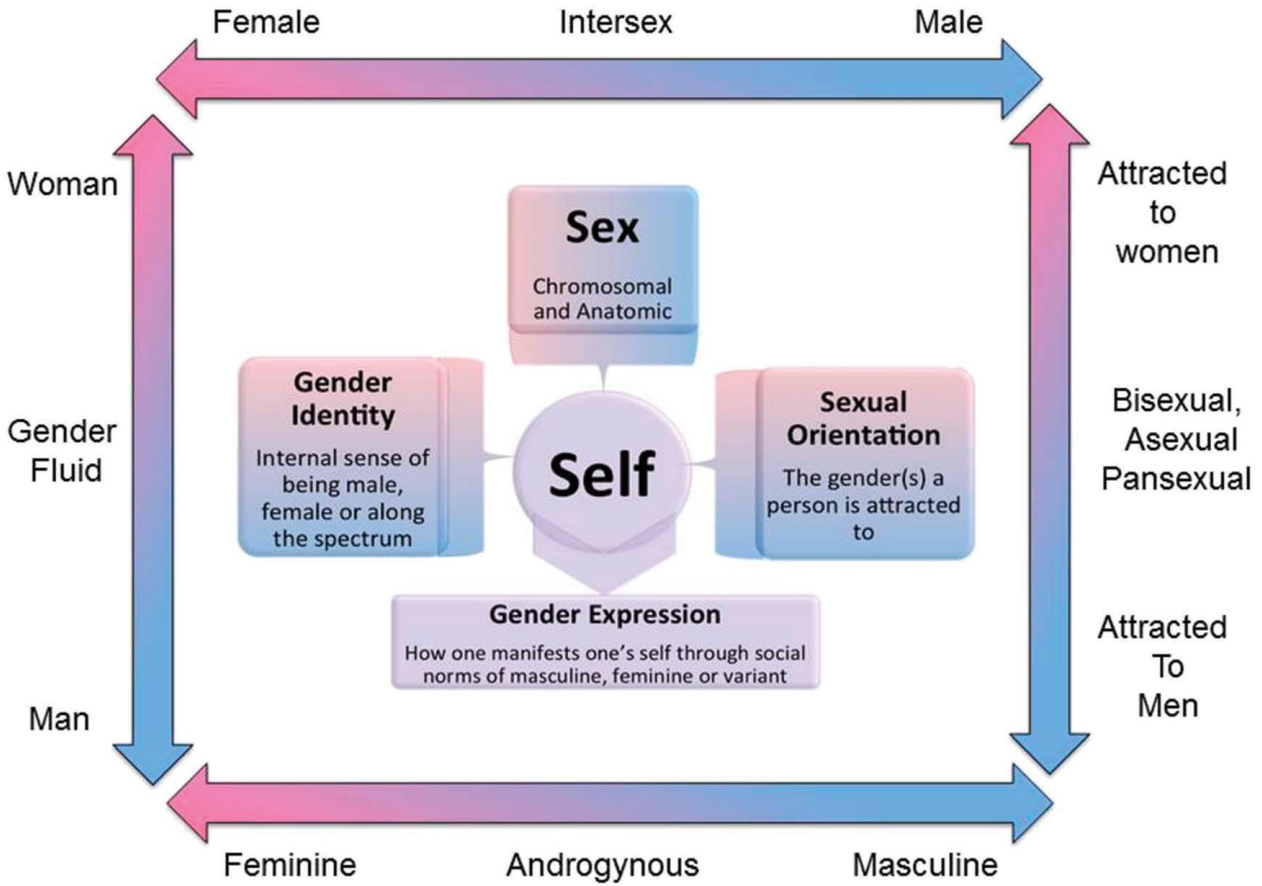

- **To reduce the inequities experienced by the transgender community, the provision of inclusive health care is essential.**

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Thank You!

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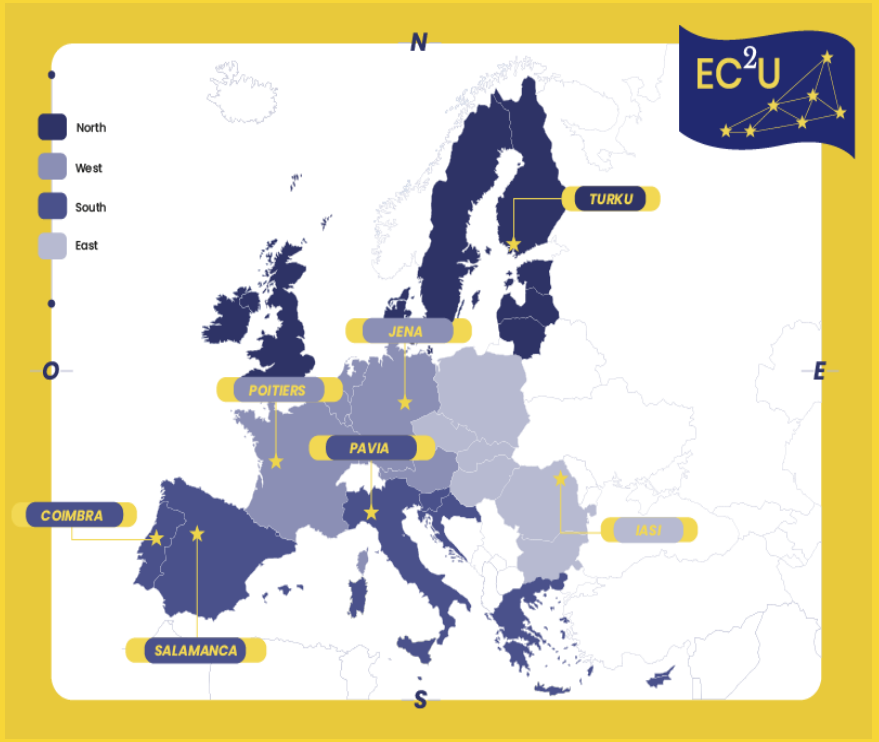

Thank you !

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