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Reproductive and epigenetic parameters in sperm connected to the disruptive development and early embryo loss.

Reprodukční a epigenetické parametry spermií, spojené s aberantním embryonálním vývojem a časnou ztrátou embrya.

Bachelor Thesis

Supervisor: RnDr. Kateřina Dvořáková-Hortová, Ph.D.

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 2017

I would like to thank my supervisor, RnDr.Kateřina Hortová, Ph.D., for her advice, inspiring ideas and selfless kindness.

My thanks also go to my mother and grandmother for their unconditional support and empathy.

Abstrakt:

Globální metylace DNA, modifikace histonů a regulace nekódujícími RNA molekulami jsou epigenetické mechanismy, které mají zásadní vliv na regulaci embryonálního vývoje a gametogeneze. Epigenetické mechanismy mohou být negativně ovlivněny četnými vnějšími vlivy. Znečištěné životní prostředí a nezdravý životní styl, jsou dva hlavní faktory spojované s narušením epigenetických regulací, vedoucím ke snížení plodnosti a abnormálnímu embryonálnímu vývoji potomstva. U některých odchylek epigenetických značek může docházet k transgeneračnímu přenosu, avšak v jistých případech je možné negativní dopad na potomstvo odvrátit náležitým zásahem do životního stylu rodičů. Vzhledem k vysoké konzervovanosti epigenetických regulací a genů odpovědných za spermatogenezi, jsou studie založené na zkoumání zvířecích modelů zásadní pro porozumění mechanismů způsobujících zhoršení plodnosti a celkového zdraví u člověka.

Klíčová slova: Epigenetika, spermie, endokrinní disruptory, hypoxie, životní styl, transgenerační přenos.

Abstract:

DNA methylation, histone modifications and regulation by non-coding RNAs are considered to play vital role in embryonal development and gametogenesis. Epigenetic mechanisms are not only inwardly programmed, but are massively affected by numerous exogenous aspects. Environmetal pollution and unhealthy parental lifestyle are two major factors related to impaired fertility status, abberant embryonal development of progeny. Some altered epigenetic marks can be transmitted to offspring generations, however in some cases these aberrations may be reversed by adequate lifestyle interventions. Since epigenetic regulations and genes responsible for spermatogenesis are conserved among vertebrates, studies based on animal models are highly relevant for understanding mechanisms causing impaired fertility and overall health in humans.

Key words: Epigenetics, sperm, endocrine disruptors, hypoxia, lifestyle, transgenerational effect.

Abbreviations:

5-hmc	5- hydroxymethylcytosine
5-mc	5- methylcytosine
AR	Androgen receptor
ART	Assisted reproductive technology
BPA	Bisphenol A
BRDT	Bromodomain- containing protein
CpGs	Cytosine- phosphate-guanine residues
DES	Diethylstilbestrol
DMRs	Differentaly methylated regions
DNMT	DNA methyltransferase
DNMT1	DNA methyltransferase 1
DNMT3a	DNA methyltransferase 3a
DNMT3b	DNA methyltransferase 3b
DNMTL	DNA methyltransferase- like protein
E.X.X	Embryonal day X
EE2	17-α-ethynylestradiol
EHMT2	Histone-lysine methyltransferase 2
Endo-siRNA	Endogenous small interfering RNAs
ERs	Oestrogen receptors
FX	Filial generation X
H2A	Histone 2A
H2B	Histone 2B
H3	Histone H3

H4	Histone H4
HATs	Histone acetylases
HDACs	Histone deacetylases
HDM	Histone demethylase
HFD	High-fat diet
HMT	Histone methyltransferase
IVF	In vitro fertilization
LncRNAs	long non-coding RNAs
MiRNAs	Micro RNAs
Nc-RNA	Non-coding ribonucleic acid
P1	Protamine 1
P2	Protamine 2
PGCS	Primordial germ cells
PiRNA	PIWI-interacting RNAs
Prdm1	PR domain zinc finger 1 protein gene
Prdm14	PR domain zinc fonger 14 protein gene
rasRNA	Repeat- associated RNAs
ROS	Reactive oxygen species
SncRNA	Small non-coding RNAs
TET protein family	Ten-elevent tranclocation protein family
TH2B	Testis specific histone 2B
TP1	Transition protein 1
TP2	Transition protein 2

Table of Contents:

1 Introduction

The perception of the role of spermatozoa within and after conception has considerably changed. Many studies have shown that a sperm cell is not only ment to protect and transport precious cargo of DNA, but also plays crucial role in preimplantational period and early embryonal development, due to it´s epigenome.

Epigenetic modifications are caused by three principal mechanisms: 1. DNA methylation, 2. histone modification and 3. epigenetic regulations by non-coding RNAs (ncRNAs). Despite the fact that epigenetic alternations do not change the DNA sequence nor the copy number, they can be inherited multigenerationaly or even transgenerationaly and affect reproductive capability and other health aspects of offspring generations.

The sperm epigenom and fertilizing ability not only inwardly programmed but is massively affected by plenty of environmental agents such as hypoxia or exposure to endocrine-disrupting chemicals (EDCs), which are influental in impaired endocrine homeostasis.

Unhealthy parental lifestyle, including drug intake, unbalanced diet or exposure to excessive physical stress, is strongly related to impaired embryonal development, gametogenesis and overall health.

Although studies and experiments on human may be limited for ethic and practical reasons, we can rely on data collected on animal models as mice or zebra fish since the epigenetic regulations and genes responsible for spermatogenesis are highly consverved among vertebrates.

Main aim of this thesis is to give an overview of principal epigenetic features and how they could be affected by various factors as well as how these aberations may impact gamatogenesis and embryonic development.

2 Epigentic reprogramming

Althought multicellular organisms emerge from two cells only, their bodies are formed by many different tissues created by specialized cells. Every single cells in one individual carries the same DNA information, yet there is an obvious difference between astrocyte and muscle cell because of their epigenome.

Once a cell differentiate its epigenetic pattern is fixed, causing specific DNA chromatine structure and thereby regulates the specific genetic expresion of particular cell type.

Some cells udrego epigenetic reprogramming during disease, but more importantly two events of massive reprogramming also occur under physiological circumstitions and are essential for normal development.

The first reprogramming takes place upon fertilization as the future embryo erases parental marks and replaces them with marks important for early embryonic develompent, involved in pluri or

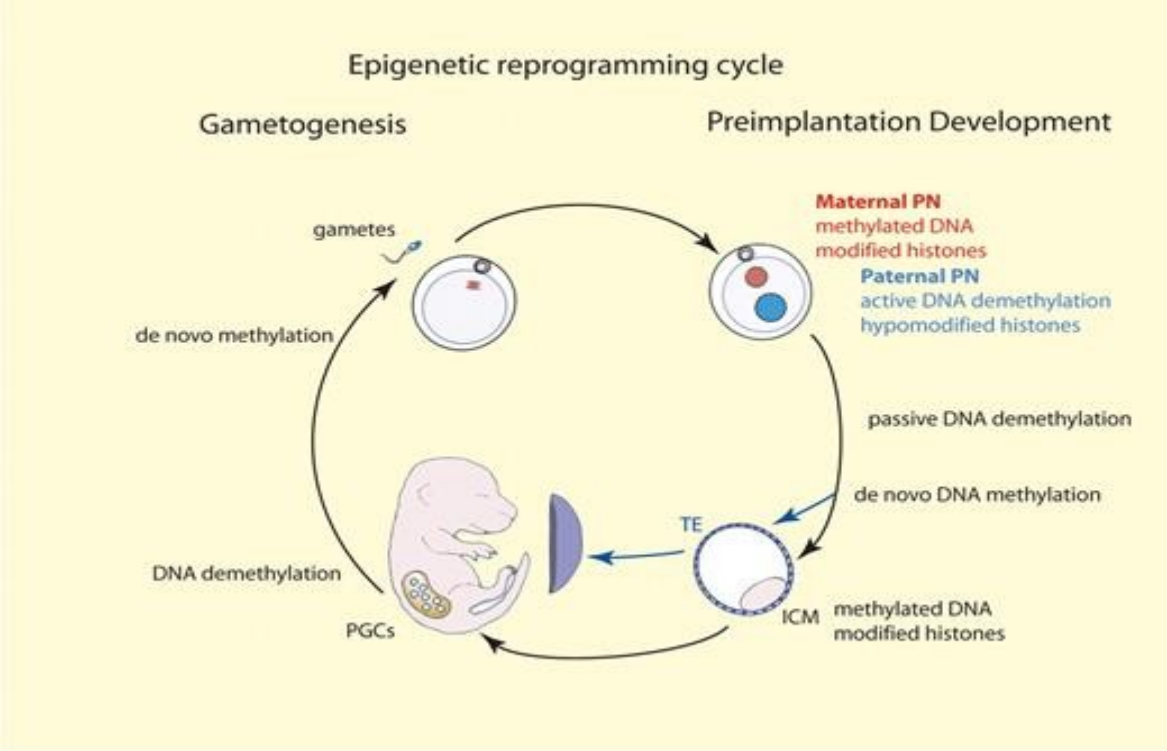

Figure 1: Scheme of epigenetic reprogramming events in mammalian development. From:(Morgan, Santos et al. 2005)

totipotency. Second event of epigentic reprogramming happens within primordial germ cells (PGCs), where parental imprinits are erased to allow sex-dependent de novo methylation.

2.1 Reprogramming in the early embryonic development

At the stage of fertilization, parental genomes differ a lot from each other. The DNA information in sperm is highly condensed and mostly packaged with protamines unlike the DNA of the oocyte coiled around histones.(Tanphaichitr, Sobhon et al. 1982)cit.after(Banerjee, Smallwood et al. 1995)

After fertilization, protamines in paternal chromatine are replaced with histones and the egg gets through meiosis.Key event in restoring totipotency is the active genome-wide paternal DNA demethylation (Oswald, Engemann et al. 2000), folowed by passive demethylation of chromosomes from both parents, however some areas remain methylated including imprinted genes(Rougier, Bourc'his et al. 1998).After erasure of parental marks, except those for parental imprinting, de novo methylation occurs to allow cell- specific differentation.

2.2 Reprogramming in the PGCs

Primordial germ cells arised from epiblast are precursors of gametes and their fate is determined by the sex of the developing embryo. The male requires production of sperm and female obviously production of oocytes, yet the precursor is the same.

Upon implantation germline and germ-cell specific genes are methylated de novo, methylational pattern of early PGCs resembles to pattern of somatic cells. To became a gamete, PGCs need to undergo dramatic changes in methylation patterns. PGCs specification, migration and division are under the control of many factors. Specification of PGCs goes along with repression of their somatic programme (Prmd 1) and reactivation of pluripotency (Prdm 14)(Ohinata, Payer et al. 2005, Kurimoto, Yamaji et al. 2008, Yamaji, Seki et al. 2008).

In mice, PGCs occur at around embyonal day 7 (E.7.0.) and enter gonads at around E.11.5. (Morgan, Santos et al. 2005). Between specification and arrival to desired destination of future gonads, PGCs undergo genome-wide epigenetic modifications that may be essencial for re-establishment of pluri and totipotency marks. In mouse, PGCs erase genome-wide DNA methylation and histone 3 lysine 9 di-methylation, at around E.8.0, whereas high levels of histone 3 lysine 27 tri-methylation is acquired at around E.9.0.(Seki, Hayashi et al. 2005).

Upon entry into genital ridge, PGCs temporarily increase H3K4 methylation and H3K9 acetylation, both related with active chromatine state (Seki, Hayashi et al. 2005, Seki, Yamaji et al. 2007). At the site of future gonads , other key events in connection with PGCs reprogramming take place between E.10.5. and E.12.5.. Around E.10.5. and E.11.5., PGCs display simmilar methylation level to sommatic cells and carry parental specific marks at DMRs of paternally and maternally methylated genes. At E.12.5. situation suddenly changes, when wide demethylation takes place.This event is identical in both sex embryos, therefore sex independent. However, some of the genes are freed from this process and thus alternations in these genes may be transmitted to offspring (Hajkova, Erhardt et al. 2002).

The next step of de novo methylation differs according to sex of the embryo (Kaneda, Okano et al. 2004).While de novo, parental specific methylation in sperm occurs prenataly and continues after birth(Oakes, La Salle et al. 2007), oocytes are reprogrammed much later at puberty and the reprogramming is nearly done at the time of ovulation(Gahurova, Tomizawa et al. 2017).

This hightly orchestrated procces of epigenetic reprogramming depends on many various factors (precise timing, expression of transkriptional factors) and its fragile balance can be easily disrupted by environmental agents.

3 Epigenetic features in sperm

Spermatozoa are highly specialised cells that developed unique morphology and chromatine packaging in order to protect DNA during passage through the reproductive tract and to succeed in challenging process of fertilization.

In the past, the contribution of the sperm cell to the embryonal development, apart from delivering set of chromosomes, was underestimated and thought to be neglectable compared to the impact of oocyte / egg, mainly for disproportional size of both gametes and indisputably unequal role in providing cellular oranelles for emerging individual.

Recently,many studies have ackowledged that spermatozoal epigenome is highly relevant for normal embryogenesis and future offspring health. Furthermore, finding that certain epigenetic marks are hereditable (Wang, Lau et al. 2016), which means that DNA sequence is not the one and only information passed on next generation, caused increased interest in mechanisms responsible for epigenetic alternations as well as how they can be affected by exogenous agents. Main areas of investigation are DNA methylation, histone-protamine replacement, various modifications of N-terminal tails of retained histones and the role of ncRNA´s.

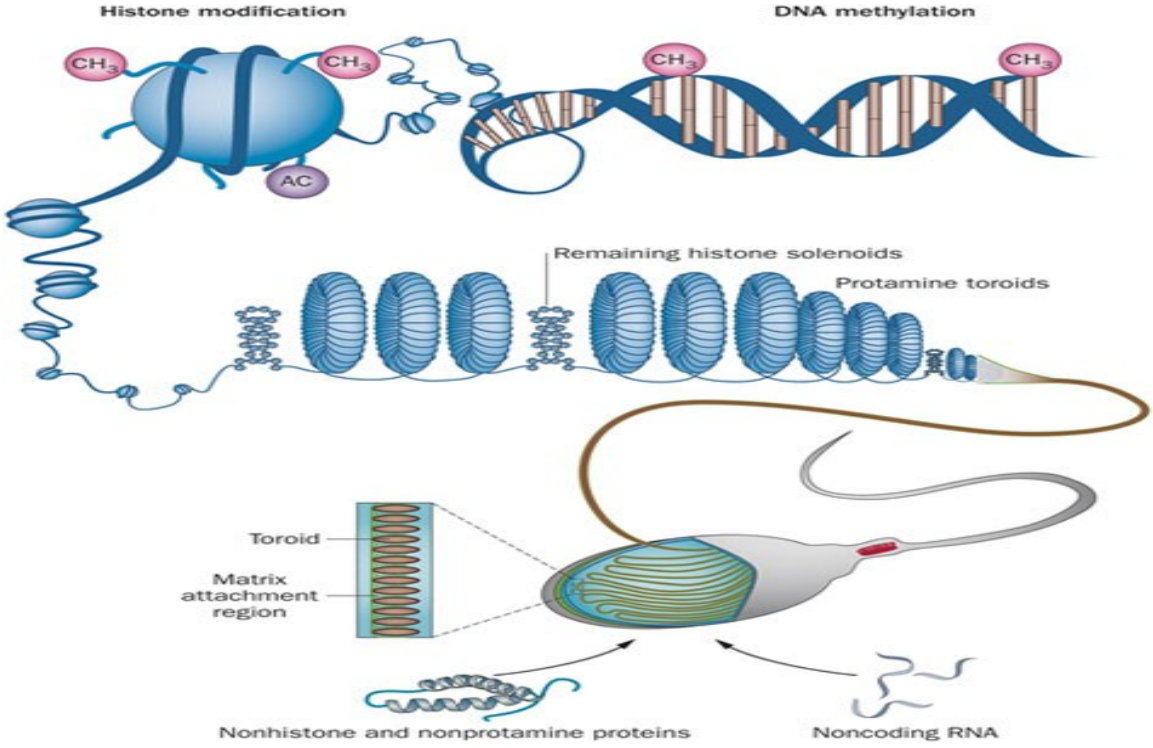

Figure 2: Epigenetic features within sperm. From: (Schagdarsurengin, Paradowska et al. 2012)

3.1 DNA methylation

DNA methylation is potent regulatory mark, found on the 5 carbon of cytosine residues (5 - mC) at cytosine-phospfate-guanine residudes (CpGs). Dna methylation is key feature in epigenomic reprogramming, essential for gene expression regulation and normal gametogenesis as wel as embryonal development. This epigenetic mark can activate or repress gene transkription by restricting or facilitating acces of transkriptional machinery. Effect of this regulation depends on methylation level, hypermethylation is connected to gene expression silencing while hypomethylation promotes transkription (Li, Bestor et al. 1992, Oakes, Kelly et al. 2007).

Demethylation may happen passively or actively via TET protein family. TET enzymes convert 5-mC to more stable intermediate 5-hydroxymethylcytosine (5-hmC) that may undergo full demethylation more quickly (Tahiliani, Koh et al. 2009). Gene promoters with enriched 5-hmC are easier to activate, enrichment patter was found at poised genes in the stem cell (Pastor, Pape et al. 2011).

DNA methyltransferase (DNMT) protein family is responsible for estamblishing new methylation marks as well as maintaing existing ones. New marks are layed down by de novo methytransferases DNMT3a and DNMT3b with support of DMNTL (Kato, Kaneda et al. 2007). DNMT1 maintains estamblished methylation marks during DNA replication and through cell division (Gruenbaum, Cedar et al. 1982).

Dnmt1 targeted mutation in mice resulted in global decrease of methylation level. These aberations go along with retarded gestational growth and inviable offspring (Li, Bestor et al. 1992).

Knock-out in both Dnmt3a and Dnmt3b genes resulted in embryonal lethality (Okano, Bell et al. 1999). Conditional knock-out of Dnmt3a in female mice results in intrauterine death of progeny. In male, same mutation causes significantly reduced testis weight and impaired spermatogenesis, causing infertility. Knock- outs in Dnmt3b dont differ from regular phenotype (Kaneda, Okano et al. 2004). Dnmt3L male knockouts demonstrate global hypomethylation, aberant gonadal development and significantly impaired spermatogenesis.(La Salle, Oakes et al. 2007) Impaired spermatogenesis caused by Dnmt3L knock-out results in infertility. Females are fertile, but offspring die in utero (Hata, Okano et al. 2002).

Exposure to cytidine analogs that can be incorporated to DNA: 5-azacytidine and 5-aza-2-deoxycitidine results in abberant DNA methylation state. Mice exposed to 5-azacytidine prior to mating had decreased fertility, increased intrauterine damage of embryos and newborn mortality (Seifertova, Veselý et al. 1976).

Male mice treated with 5-aza-2-deoxycitidine displayed decresed testes weight, sperm count, and increased preimplantation loss (Kelly, Li et al. 2003).

Transplacental exposure to 5-aza-2-deoxycitidine leads to altered pro-male ratio in progeny and affect sexual behavior in male offspring (Cisneros and Branch 2004).

3.2 Histone to protamine transition

One of the most unique epigenetic events is histone to protamine transition occuring in sperm. This multistep procces results in tightly compacted, transkriptionally silent chromatin structure preventing DNA from damage as mature sperm has no sufficient repair mechanism (Grunewald, Paasch et al. 2005).

Replacement of canonical histones by protamines is fundamental for normal sperm function and motility (Cho, Willis et al. 2001), though the same procces caused disbelief in paternal contribution to embryonic development as it erases an important layer of epigenetic regulation. However, not all of the histones are replaced and those remained are variously modified (Hazzouri, Pivot-Pajot et al. 2000). The amount of retained histones varies among species. In human sperm, more than 10% of DNA is remained packed by histones, in contrast to other species (e. g. bull, stallion, hamster, mice) where the presence of retained histones is much lower (Bench, Friz et al. 1996). The incompleteness of this replacement was found to be programmatic rather than outcome of insufficient machinery, seeing that nucleosome-bound DNA is retained at regions of hight importance as are

imprinted gene clusters, microRNA clusters and HOX gene clusters (Hammoud, Nix et al. 2009). The mechanism of this process is still not comletely undersood, but some particular events have been described.

In mammals, histone to protamine transition involves two principal steps: replacement of histone by transition proteins (TPs) and replacement of TPs by protamines.

Mature sperm contains canonical histones 2A and 2B (H2A, H2B), histone 3 (H3), histone 4 (H4) as well as testes-specific histone variants (Zalensky, Siino et al. 2002, Kwak and Dohmae 2016). In mice, depletion of testis specific histone 2B (TH2B) can be compensated by upregulation of H2B, permiting histone removal and normal spermiogenesis (Montellier, Boussouar et al. 2013).However, disruption of both Th2a and Th2a genes results in sterility, due to dramaticaly impaired spermiogenesis. Loss of both – Th2a and Th2b significantly affects histone replacement (Shinagawa, Huynh et al. 2015).

Replacement of some selected histones for specific variants goes along with increasement of acetylation of other histones. This hyperacetylation results in relaxed chromatin structure and seems to be the initiating step in cascade of events that results in histone to protamine replacement (Sonnack, Failing et al. 2002).

Testis-specific bromodomain-containing protein (BRDT) is considered to be crucial factor in the reorganization of chromatin as it intearcts with acetyated histones and induces differential retention of hyperacecetylated regions (Pivot-Pajot, Caron et al. 2003, Govin, Lestrat et al. 2006).
Male mice lacking Brdt gene are sterile, have delayed puberty and display decreased testosterone levels and testicular weight, complete spermatocyte maturation arrest and significant increase of cells exibiting DNA fragmentation. BRDT protein is also expressed in pituary gland tissue. Absence of Brdt gene lead to different expression of 54 genes, therefore may affect timing of puberty by disregulating pituary- gonad axis (Barda, Yogev et al. 2016).

Other fundamental actors are transition proteins 1 and 2 (TP1 and TP2).These proteins bind to to the DNA and faciliate removal of histones and they play fundamental role in following events in protamine compaction. In mice, mutation in tnp2 gene results in significant increase in sperm tail abnormalities, abnormal chromatine condensation, and decrease in litter size (Zhao, Shirley et al. 2001). Disruption in tnp1 gene leads to significantly decreased sperm motility, head abnormalities and reduced fertility (Yu, Zhang et al. 2000). However, mice lacking only TP1 or TP2 display simmilar protamine contribution as wild-type mice, therefore authors suggest that TPs may complement each other. Mice lacking both TPs have significantly abnormal spermatozoa with abberant chromatin condensation, resulting in sterility (Zhao, Shirley et al. 2004).

In the next step, transition proteins are replaced by protamines that vary among species (Oliva and Dixon 1991). In mammals, protamine 1 (P1) is expressed ubiquitously but protamine 2 (P2) occurs only in certain species. P2 conent varies greatly among species, but P1 to P2 ratio is highly conserved within genus (Corzett, Mazrimas et al. 2002). Mice lacking P1 or P2 are infertile (Cho,

Willis et al. 2001). In human, expression of both protamines is almost equal. Abberations in P1/P2 ratio result in reduced sperm concentration, motility, and abnormal morphology. DNA fragmentation is significantly increased in men with lower level of either P1 or P2. In men with regular ratio, increased DNA fragmentation occurs if both P1 and P2 are underexpressed (Aoki, Moskovtsev et al. 2005). Men with reduced P1 / P2 ratios have significantly decreased pregnancy rates (Aoki, Liu et al. 2006).

3.3 Histone modifications

Various, dynamicaly chaning modifications of histones are needed to allow different transkriptional status of genes during different stages of spermatogenesis and development. Post-translational modifications at 5´amino acid residues of N-terminal tails of retained histones were shown to be fundamental epigenetic regulation in sperm as well as in the developing embryo. Most common post-translational modifications are acetylation, methylation, deamination, ribosilation, phosporylation and ubiquitination. The regulatory effect of N-tail modification depends on character of the given mark(Strahl and Allis 2000).

Principal modification is acetylation of N-terminal lysine residues of histones. Proper acetylation is maintained by histone acetyl transeferases (HATs) and histone deacetylases (HDACs). Acetylation results in „looser“ chromatine structure, faciliating access of transkriptional machinery, while deacetylation leads to condesation of chromatine, therefore making it transkriptionaly silent (Davie 1998).

Mice treated with HDAC inhibitor trichostatin A had a dose-dependent decrease in testis weight and spermatogenesis (Fenic, Sonnack et al. 2004).

Aplication of trichostatin A resuls in decreased activity of HDAC, increased transkription of somatically expressed genes in the testis and programmed cell death of spermatocytes (Fenic, Hossain et al. 2008).

Treatment with HAT inhibitor curcumin results in decreased aktivity of HAT in a dose dependent manner.Impaired acetylation leads to histone replacement failure and abnormal nuclear condensation. Treated mice shown abnormal proliferation nad increased apoptosis of spermatids (Xia, Cai et al. 2012).

Other key modification regulatory for both activation and repression is methylation, regulated by histone methyltransferase (HMT) and histone demethylase (HDM). Methylation mainly occurs at lysine and arginine residues. Generaly, methylation on H3K4, H3K36 and H3K79 is connected with gene activation. On the other hand, di- and tri -methylation on lysines 9 and 27 of histone 3 (H3K9,H3K27) is linked with gene silencing. Regulatory effect of meethylation depends on degree of methylation as well as on localization of methylated histone (Martin and Zhang 2005).

3.4 Regulation by non-coding RNAs

NcRNAs include small non-cooding RNAs (sncRNAs) and long non-coding (lncRNAs), both crucial for proper regulation at many levels (e.g. transkription, mRNA degradation, splicing) (Romero, Meikar et al. 2011, Zhang, Gao et al. 2017).

Group of SncRNAs includes a number of different types : microRNAs (miRNAs) and repeat-associated RNAs (rasRNAs) including PIWI-interacting RNAs (piRNAs), and endogenous small interfering RNAs (endo-siRNAs). MiRNAs, endo-siRNA as well as piRNA are all essencial for PGCs prolifferation and proper spermiogenesis (Hayashi, Chuva de Sousa Lopes et al. 2008, Zheng and Wang 2012, Zimmermann, Romero et al. 2014).

DICER is conserved RNAIII endonuclease responsible for proccesing small RNAs, including miRNAs and endo-siRNAs. Depletion of Dicer 1 in germ cells resulted in reduction of testicular size and dramatic decrease in testicular weight. Muntant mice display normal sexual behavior, but are sterile due to higly abnormal testicular histology and almost no mature spermatozoa, due to meiotic arrest. Transition from round spermatids to spermatozoa is drasticaly impaired in mutant mice, reasulting in 99% decrease of mature spermatoza (Romero, Meikar et al. 2011).

Conditional knock-ou of DROSHA, RNAse III memeber responsible for cleaving primary miRNAs, in spermatogenic cells leads to infertility due to simmilar phenotype descibed above. However, disruption of testes development is more severe. While DICER is required for biogenesis of several RNA species, DROSHA is crucial for the canonical miRNA production (Wu, Song et al. 2012).

lncRNAs also play essencial role in developmental procceses. Well know is X-inactive specific transkript (Xist) expressed from paternal X chrmosome, essenciaal in silencing of X-cromosome (Borensztein, Syx et al. 2017).

4 Environmental factors

As metioned earlier, epigennetic regulations are not only developmentaly programmed but may undergo changes, due to many various factors. Environmetal agents such as irradiation, hypoxia and exposure to wide group of pollutants including pesticides (e.g. dichlordiphenyltrichloroethane (DDT)), fungicides (e.g. vinclozolin), plasticizers (e.g. phthalates), pharmaceutical agents (e.g. ethynylestradiol (EE)), and heavy metals (e.g. lead) are associated with abberant gonadal development, fertilizational ability and epigenetic status.

Many studies have demonstrated the relevance of parental lifestyle including smoking, alcohol intake, exposure to chemotherapy or stress. Parental diet is also significant subject of interest as high-fat diet, diet low on vitamins or even famine were shown to negativelly impact epigenomic programme. Assisted reproductive technology (ART), as widely used tool in solving human infertility, has been investigated for pottential epigennetic disturbances and influence in etiology of perinatal problems. The observation that some epigenetic alternations may lead to transgenerational transimission of specific marks to subseqent generations increased the interest in the field of

epigenetic.

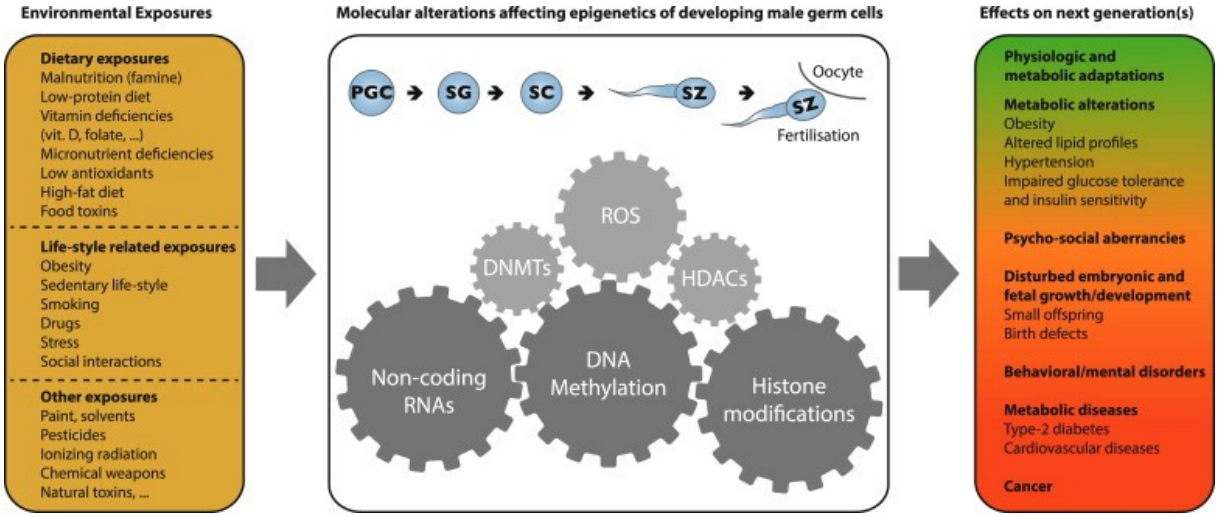

Figure 3: Summary of environmental factors connected to impaired epigenetic status. From:(Soubry 2015)

The multigenerational or transgenerational effect depends on whether the examined generation was directly exposed to endogenous agent or not.

Adult individuals that are exposed are considered F0. Offspring generation of F0 is F1. Because F1 generation is produced from exposed gametes of F0, the effect is multigenerational. F2 is the first generation not exposed to the environmental factor, therefore any alternations derived from F0 exposure are considered trangerational.

The case when the exposed individual is gestating female may also occur. In this context, the female corresponds to the F0 generation, developing embryo to the F1. Offspring generation of F1 is considered the F2 generation.Multigenerational effect extends to F2 generation regarding in utero exposure of F1 PGCs.In this case, transgenerational effect requires influence on the F3 generation.

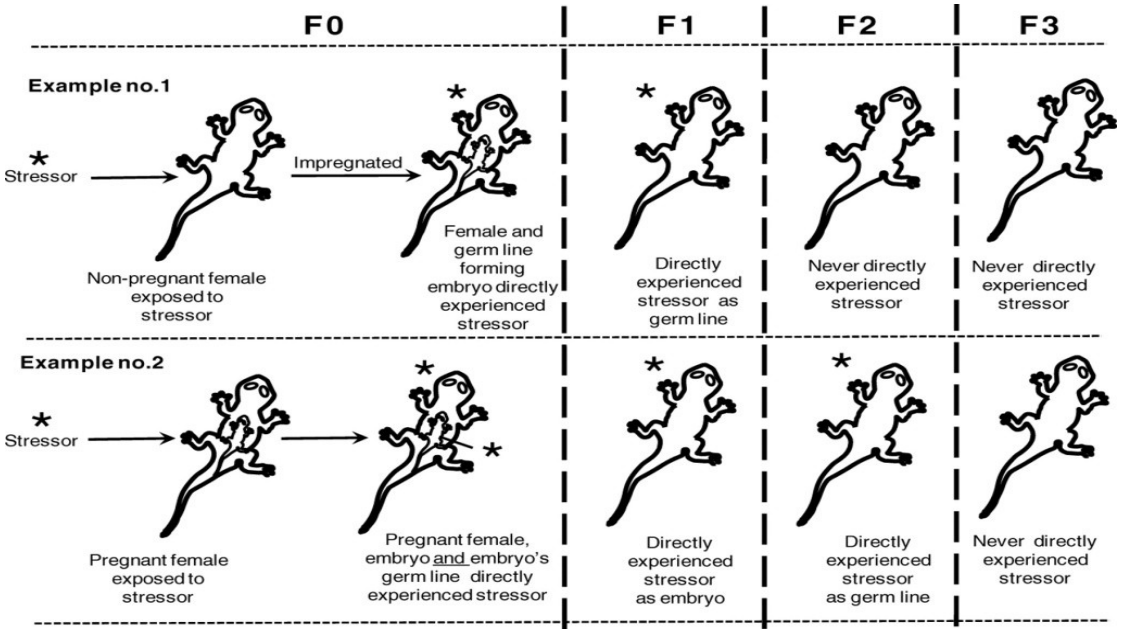

Figure 4: Schematic description of multi vs. Transgenerational phenomena From:(Ho and Burggren 2010)

4.1 Endocrine disrupting chemicals

Endocrine disrupting chemicals (EDCs) include wide range of man-made compounds as well as natural substances. EDCs may interfere with endocrine system, therefore negatively affect homeostasis and induce reproductive, developmental and other health impairments.

EDCs can mime endogenous hormones as they highly resemble in structure. In connection to reproduction, EDCs may act like estrogens, antiestrogens or antiandrogens - agonists or antagonists of endogenous steroidal sex hormones.These compounds differ in mechanism of action and cause various pathologies (e.g. abberant gonadal deelopment, sperm count and decrease,testicular cancer) but resemble in sence of deregulation of genetic expression.

4.1.1 Xenoestrogens

Oestrogens are steroidal hormones well known for their crucial role in maintaining balance of female menstrual cycle. However, oestrogens are also produced by males and their physiological level play essencial role in spermatogenesis, sperm capatitation, and acrosome reaction (Ded, Dostalova et al. 2010, Sebkova, Cerna et al. 2012, Dumasia, Kumar et al. 2017).

Production of oestrogens in males takes place mainly in testes, where aromatase converts testosterone into oestrogens. Mice affected by aromatase knock-out display abberant gonadal development, and impaired gametogenesis (Fisher, Graves et al. 1998, ROBERTSON, SIMPSON et al. 2001).

The oestrogen response is mediated through oestrogen receptors (ERs). Aside endogenous eoestrogens, these receptors can interact with EDCs. EDCs are able to bind to different types of ERs with different affinity, resulting in altered genomic and non-genomic activity, interaction with transkriptional factors and impaired activity of enzymes responsible for oestrogen metabolism. Three types of oestrogen receptors are described as many other are suggested. Two of them are intracellular and considered as classical, oestrogen receptor alpha (ERα) and oestrogen receptor beta (ERβ).The third receptor is GPER transmembrane protein.<sec.(Dostalova, Zatecka et al. 2017).

ER knock-outs display smaler testes with atrophic and degenerated seminiferous tubules, decreased sperm count and motility and are infertile (Eddy, Washburn et al. 1996). ERα and ERαβ knock-out mice were infertile, however some ERβ knock-outs remained fertile (Dupont, Krust et al. 2000).

As mentioned earlier, EDCs can bind to ERs and activate them, therefore they may be potent modulators of spermatogenesis and embryonal development. In male rats, overactivation of ERα and ERβ results in decreased sperm count as well as in increased rate of pre and post-implantation loss (Dumasia, Kumar et al. 2015).

Diethylstilbestrol was prescribed as a synthetic oestrogen for hormonal therapy to prevent misscariages from 1930s to 1970s. However, in 1971 it was shown to correlate with vaginal adenocarcinoma in daughters exposed in utero and other genital anomalies (Herbst, Ulfelder et al.

1971). Genital anomalies and impaired sperm parametres (e.g. hypoplastic testes, varicocele, cryptorchidism) were also seen in men exposed in utero (Whitehead and Leiter 1981).

In male mice, neonatal exposure to DES causes altered DNA methylation in epididymis. Exposure affected expression of DNA methyltransferases. Firstly, de novo methyltransferase Dnmt3 was significantly increased followed by increase of Dnmt1 and Dnmt3a (Sato, Fukata et al. 2006). Altered expression of methyltransferases was also detected in uterus of neonataly exposed female mice (Sato, Fukata et al. 2009). DES exposure was also linked with upregulation of gene containing promoters involved in synthesis of protamine 1 and 2 mRNAs (Matsuno, Adachi et al. 2004). Therefore, genital abnormalities may be associated with adverse expression of methyltransferases.

Bisphenol-A (BPA) is a widespread environmental contaminant used in production of platics. BPA negatively affects spermatozoal motility, velocity, capacitation as well as early development of offspring concieved with usage of exposed gametes (Rahman, Kwon et al. 2015). Studies performed in human placentas and breast cancer cells demonstrated that BPA deregulates the expression of numerous ncRNAs (Tilghman, Bratton et al. 2012, Bhan, Hussain et al. 2014, De Felice, Manfellotto et al. 2015). Exposure to BPA resulted in increased expresion of three memebers of let-7 miRNA family in placental cells(Avissar-Whiting, Veiga et al. 2010). BPA exposure of pregnant mice was also observed to reduce tyrosine phosporylation level in spermatozoa of progeny, thereby negatively affects functional parametres (Rahman, Kwon et al. 2017).

17-α-ethinylestradiol (EE2) is synthetic oestrogen used in contraceptive pills. EE2 is ubiquitnously spread water pollutant and many studies have demonstrated harmfull effect on fish populations, however is not ony fish endangered by EE2. Study based on guppies (Poecilia reticulata) demonstrated that EE2 exposure results in pro- female sex ratio, feminization of male fish. Decreased tests weight and sperm count, abnormal colorization and impaired sexual behavior results in significant decrease of fertility as exposed male are not able to succesfully compete with unexposed individuals(Kristensen, Baatrup et al. 2005) In zebra fish (Danio rerio) EE2 causes reduction in fertilizational succes of both sexes and is considered to transgenerally affect also non-mating behavior.(Volkova, Reyhanian Caspillo et al. 2015) Decreased number of fertilized eggs and elevated mortality at the period of late gastrulation and early organogenesis were also seen (Soares, Coimbra et al. 2009)In male rats, EE2 intake from drinking water causes significant decrease in body weight and production of smaller litters (Vosges, Braguer et al. 2008)In female rats EE2 in utero exposure causes malformations of external genitalia and abnormal histology off inner sexual organs, however these mice can be fertilized (Sawaki, Noda et al. 2003). In mice, F1 exposed as well as unexposed F2-F4 generations display simmilar pattern of sexual behaviour with reduced latencies of first mount and intromission, however averall intromission is increased in dose- dependent manner(Derouiche, Keller et al. 2015).

4.1.2 Anti-adrogens

AR is present in Sertoli cells, myoid cells and prespermatognia at the time of cord formation (Majdic, Millar et al. 1995). Therefore, deregulated expression or signaling of AR caused by EDCs exposure may lead to abberant morphological sex differentiation (Liu, Shen et al. 2015, Zheng, Armfield et al. 2015).

ARKO males have female-like body constitution, dramaticaly impaired development of external genitalia.Despite the fact that testes developed, their weigh is only about 20% of regular weigh and vas defferens, epididymis, seminal vesicle and prostate are not developed at all (Yeh, Tsai et al. 2002). Conditional knock-out of AR in Sertoli cells results in infertility, due to spermatogenic arrest at premeiotic stage and only fragments of spermatozoa in epididymis (Chang, Chen et al. 2004). Mice with conditional knock-out of AR in Leidig cells display decrease in testicular and epididymal weigh and testosterone levels. KO mice are infertile, due to azoospermia caused by spermatogenic arrest at second spermatocyte (Xu, Lin et al. 2007).

Vinclozolin is a fungicide, widely utilized in agriculture, that is used as a paradigmatic model for studying transgenerational efect of EDCs.

It was demonstrated that vinclozolin causes changes in DNA methylation that persist within subsequent generations and negatively affect their reproductive ability. In rat model, study on offspring of gestating mother treated with vinclozolin demonstrated impairment of sperm quality parametres and different testes morphology. More than two times increased spermatogenic cell apoptosis was seen in all F1-F4 male offsprings. More than 90% of all examined males displayed this phenotype with no decrease among generations (F1-F4). Around 8% of F1-F4 treated males older than 90 days developed infertility and about 20% developed decreased spermatogenesis (Anway and Skinner 2006). Other study demonstrated that vinclozolin is able to transgenerationally (F1-F3) disrupt regulation of RNAs involved in spermatogenesis, causing disbalance in Lin28/let-7/Prdm1 pathway. Significant decrease in PGCs and elevated apoptosis of seminiferous epithelium results in reduced fertility (Brieno-Enriquez, Garcia-Lopez et al. 2015).In zebra fish, exposure to vinclozolin cause pro-female sex ratio and increased mortality at juvenile age. Aberrant gonadal development in both sexes goes along with reduced fertility (Lor, Revak et al. 2015)

4.2 Hypoxia

Hypoxia is a potent endocrine disruptor occuring in aquatic environments as well as terrestrial systems in hight attitudes. Unlike chemicals, which may accumulate in tissues and germ cells, hypoxia can only affect exposed individuals as it can not be transmited to offspirng as residue.

Male mice exposed to intermittent chronic hypoxia mimicking obstructive sleep apnea (OSA) showed reduced fertility status due to significant reduction of progresive sperm motility. Decreased sperm motility and fertilization ability was linked with insufficient response to oxidative stress caused by impaired production and function of antioxidant enzymes glutathione peroxidase 1 (GPx1) and superoxid dismutase 1 (Sod1) (Torres, Laguna-Barraza et al. 2014).

In men,long time exposure(12 months) to hypoxia at hight altitude(5380 m above sea level) caused adverse effect on semen quality and sex hormones level, however these effects were shown to be reversible since quality parametres of ejaculate and hormone levels returned to regular level when examined men returned back to lower altitude(1400m) (He, Cui et al. 2015).

Study based on carp model (Cyprinus carpio) showed overall reproductive impairments in reproduction in both male and female fish exposed to hypoxia. Hypoxia resulted in decrease of sex hormone levels, inhibition of gonadal develompent as well as production of gametes, lower spawning and fertilization succes and decreased survival of larvae (Wu, Zhou et al. 2003).

In zebra fish (Danio Rerio), hypoxia negatively affected sex differentation with pro-male manner. This abnormality in sex ratio was linked with disturbed balance of testosterone (T) and oestradiol (E) levels, caused by deregulation of genes responsible for sex hormone synthesis (Shang, Yu et al. 2006).

Study with medaka fish (Orizias melasigma) as model, demonstrated negative transgenerational epigenetic actions of hypoxia on reproduction. Changes in DNA methylation,leading to different gene expresion were detected to F2 genearation, never exposed to hypoxia. In particular, promoter region of euchromatic hitone-lysine methyltransferase 2 (EHMT2) and promoter region of proten tyrosine kinase 2B (PTK2B) were hypomethylated, while forheadbox P2 (FOXP2) was hypomethylated. Testis weight, spermatid count and fertilization succes were decreased. The study demonstrated that the impairments in sperm parametres are probably driven by EHMT2 mediated histone modification, which may be essencial mechanism for transgenerational effect (Wang, Lau et al. 2016).

4.3 Lifestyle

It is widely known that parental lifestyle has an influence on children health, among other acpects, by setting an example. However, its not only the first generation of progeny that can be affected by our actions. Many studies have demonstrated transgenerational effect of widespread bad habits as are hight-fat diet, smoking or alcohol consumption, however these are only a fraction of lifestyle aspects that can have harmfull impact on offspring. It was demostrated that some of impairments caused by unhealthy lifestile can be reversed (Palmer NO 2012), therefore we may be awared of our actions, at least at preconceptional period.

4.3.1 Diet

Diet-induced obesity in mice resulted in significant impairment of sperm quality parametres. Male mice fed to hight-fat diet (HFD) had increased level of cholesterol and triglycerides compaired with males fed with control diet (CD). intracellular reactive oxygen species (ROS) and mitochondrial ROS levels were elevated as well as DNA damagage in sperm of HFD mice. The percentage of motile spermatozoa, non-capacitated spermatozoa, spermatozoa bound to oocyte, and percentage of fertilized oocytes was significantly decreased compared with controle (Bakos, Mitchell et al. 2011).

Other subsequent studies demonstrated how these perturbances can be reversed by lifestyle interventions. Male mice initialy fed with HFD were then divided into groups, one continuously kept on HFD(HH), and three groups where either diet(HC) and (CHCE) /or (HE) excercise intervention was applied.HC, HE and HCE males displayed improved glucose tolerance compared with HFD group. Interventions involving diet reduced serum cholesterole levels and triglyceride levels compared with HFD. Diet and / or excercise interventions increased sperm motility and decreased abnormal sperm morphology, diet alone (HC) increased sperm count to level comparable with CC. All intervetnions increased the percentage of sperm that had undergone capacitation and binding to the zona pellucida. All interventions reduced sperm DNA damage to normal levels. All intervetions reduced the percentage of sperm with hight mitochondrial membrane potential (MMP), correlating negatively with sperm motility (Palmer NO 2012).

Above mentioned interventions can improve embryonal development of offspring of initially HFD fed father. All interventions displayed advanced blastocyst development on day 4 and increased total blastocyst cell number on day 5 as well as total cell number and trophoectoderm cell number on day 6 compared with HFD. Diet and excercise alone lead to improvement of inner cell mass (ICM) and epiblast cell number. Only combined intervention (HCE) resulted in improvement of implantation rates compared with HFD. Any form of intervention increased fetal weights . Therefore, improved metabolic state of father positivelly effects embryonal development (McPherson, Bakos et al. 2013).

F1 male progeny of HFD fathers showed reduced sperm counts and progresive sperm motility. Diet intervention in founders increased the percentage of progressively motile sperm in F1, but exercise alone did not result in improvement of progressive sperm motility. No significant improvement to sperm count of F1 from diet and / or excersice intervention in father was detected.All interventions in founders increased the percentage of capacitated sperm in F1.Diet including interventions in founders increased sperm binding, whereas exercise alone did not improve sperm binging of F1. F1 male reproductive health strongly correlates with founder adiposity, metabolic and fertility status and may be modified by fathers actions (McPherson, Fullston et al. 2014).

High-fat diet reprograms epigenome and transgenerationally affects metabolism of offspring. F1 offspring from HFD fed father had decreased body weight. F2 generation displayed a similar phenotype. F1 females had decreased beta-cell mass. Both, F1 and F2 generation females were glucose intolerant and resistant to HFD induced weight gain. F2 females showed impairments in glucose tolerance in response to hight-fat diet and decreased insulin levels. In sperm from F0 and F1 male rats fed HFD, numerous differentialy methylated regions (DMR) were detected with enrichement of genes involved in cellular transport and metabolic procceses. HFD alters expression of miRNA let-7c in sperm of F0 and F1. Differential expression was passed to metabolic tissues of the offspring , altering expresion of targeted genes in adipose tissue. Sperm let-7c expression can be influenced by diet and its reprogramming may be essencial in mechanism altering metabolism of the offspring (de Castro Barbosa, Ingerslev et al. 2016).

Study on mice model demonstrated that low paternal dietary folate intake affects fertility and offspring health .B vitamins are coenzymes in metabolism of one carbon (C1), which is provided by methyl donor nutrients. Dietary folate intake can affect DNA methylation levels, thus affect gene expresion. Folate deficiency in male mice lead to impaired ability to impregnate females, increased post-implantation loss and various developmental malformations (e.g.hydrocephalus, limb defects, muscular dysplasia ,reduced ossification of skull). Sperm from mice exposed to folate deficiency displayed significantly reduced monomethylation of H3K4 and K9 as well as reduced trimethylation of H3K9. This modulation together with different DNA methylation is thought to be responsible for epigenetic transmission (Lambrot, Xu et al. 2013).

Undernourishment of gestating mother alters germline DNA methylation of F1 males. DMRs are enriched in regions with retained nucleosomes, thus may persist after early embry reprogramming and affect development. F2 offspring of father exposed to undernutrition in utero displayed reduced muscle mass, increased adiposity and were glucose intolerant. Increased lipid abundance was associed with icreased expression of genes involved in lipid oxidation and downregulation of genes involved in lipid sythesis (Radford, Ito et al. 2014).

In men,prenatal exposure to famine lead to persisting differental methylation of maternaly imprinted insulin growth factor 2 DMR (IGF2 DMR) in adult life. To compare methylation status of IGF2 DMR , individuals exposed to famine were compared to their unexposed same sex siblings. Lower methylation of IGF2 DMR was found in individuals exposed in early gestation, but was unchanged in individuals exposed in late stage of gestation (Heijmans, Tobi et al. 2008).

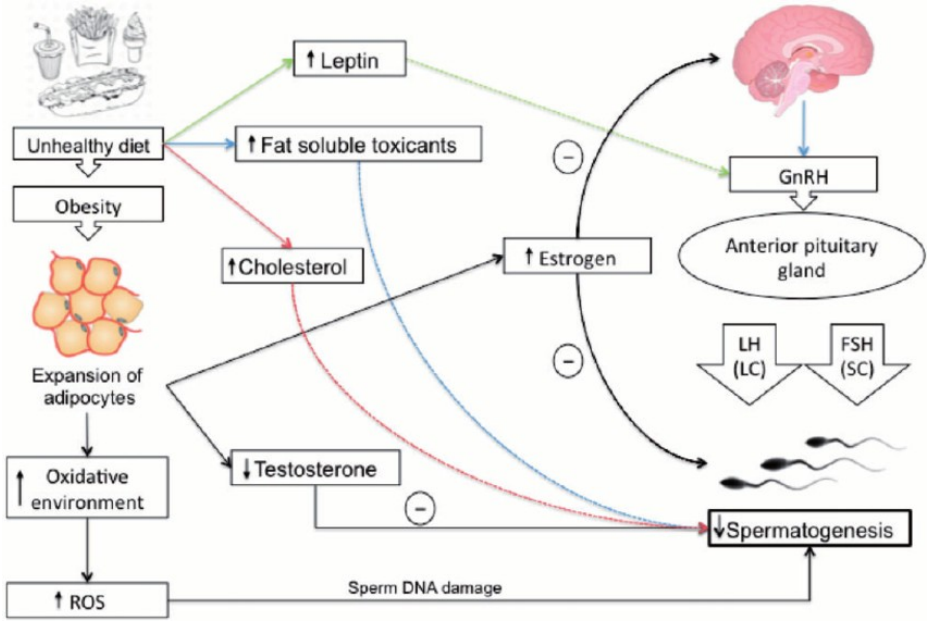

Figure 5: Scheme of actions of unhealthy diet From:(Giahi, Mohammadmoradi et al. 2016)

4.3.2 Smoking

Smokers have lower levels of total testosterone and follicle stimulating hormone (FSH) and display significantly decreased semen volume,sperm count, normal sperm morphology and

motility.There is a corelation between number of cigarettes smoked per day and semen pH, sperm count ,motility, viability and morphology.(Asare-Anane, Bannison et al. 2016)

In smokers, P2 levels are significantly decreased, while P1 / P2 ratio is increased. Low level of P2 correlates with increased oxidative stress.(Niederberger 2011, Hamad, Shelko et al. 2014)

Cigarette smoke was shown to alter spermatozoal miRNA content .Differentially expressed miRNAs in smokers were involved in mediating patways of cellular proliferation, differentiation and cell death.Numerous components of epigenetic machinery (e.g. DNMT3A, HDAC1) were shown to be targets of altered miRNAs.(Marczylo, Amoako et al. 2012)

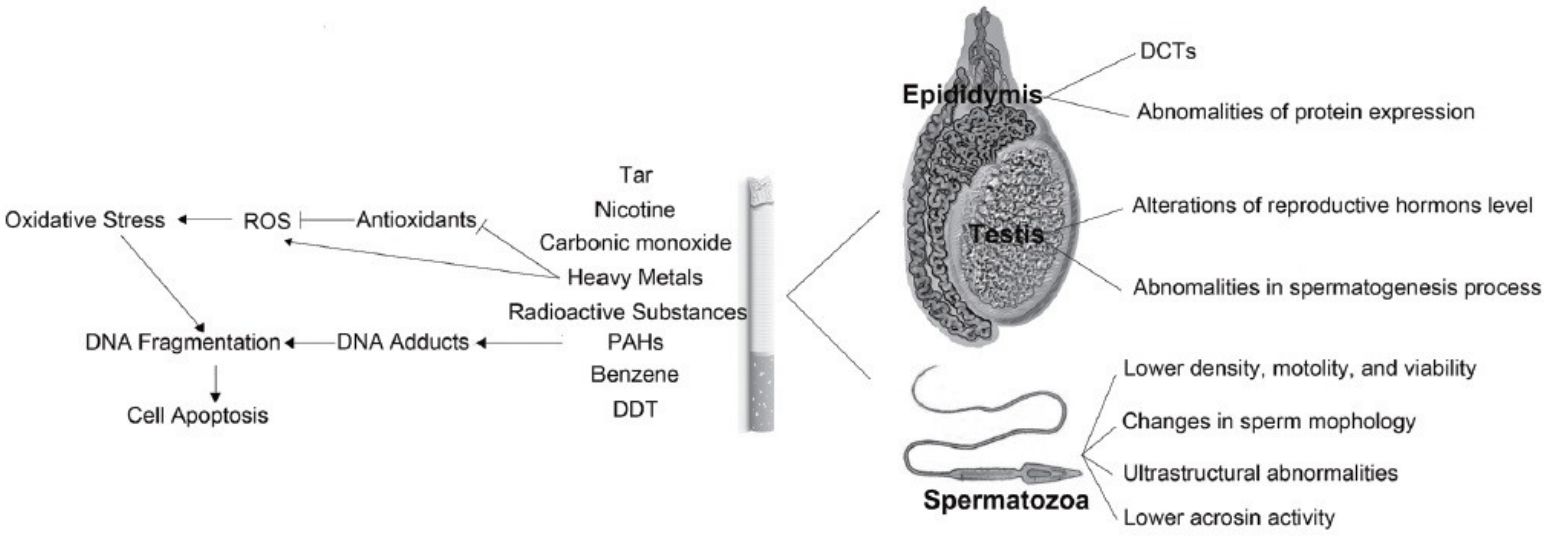

Figure 6: Scheme of effect of tabbacco smoke on gonads and spermatogenesis.From:(Dai, Wang et al. 2015)

Cigarette smoke exposure of gestating mouse causes male offspring ferrtility impairments. Male progeny has and bnormal testicular histology that goes along with germ cell depletion and apoptosis of spermatocytes. Sperm count is decreased with spermatozoa displaying several morphological anomalies leading to reduced fertility. These anomalies are linked with increased oxidative stress and disbalanced sex hormone metabolism (Sobinoff, Sutherland et al. 2014).

Paternal exposure to smoking decreases sperm motility, fertilization rate, clevage rate and blastocyst development.Impairments occur in dose-dependent manner (Kapawa, Giannakis et al. 2004)

In man, heavy smoking of pregnant mother is linked with cryptochorism, reduced gonocytes and spermatogonia count and increased risk of infertility (Thorup, Cortes et al. 2006).Smoking during pregnancy results in reduced numbers of germ and somatic cells in both, male and femal gonads(Mamsen, Lutterodt et al. 2010).

4.3.3 Alcohol

Chronic alkoholism disturbes metabolic patways in rat testes. Significant changes were seen in content of amino acids, cholesterol, and protein SH- groups. Alcohol adicted males also displayed highter DNA fragmentation. SDH enzymatic activity was decreased, on the other hand, LDH activity increased. Changes of amino acid pools may be taken as evidence for impaired metabolism of ATP and NADPH (Shayakhmetova, Bondarenko et al. 2014).

Inverse dose-response assosiation between alcohol intake and sperm paremetres was seen in cohort study of Dannish men. Habitual alcohol intake resulted in decreased sperm concentration, total sperm count and percentage of morphologicaly normal sperm. Recent alcohol intake (week preceding the testing)also negativelly affected semen quality, furthermore increased serum testosterone and decreased sex hormone binding globulin (SHBG) was detected. Men with a typical weekly intake above 40 units of alcohol (e.g. 40 beers) had a 33% reduction of sperm concetration in comparison to men with an intake of 1- 5 units (Jensen, Gottschau et al. 2014).

Slowly progressive impact of chronical alkoholism on semen quality was observed in male IVF patient followed up for 6 years. Chronical alcoholism firstly resulted in teratozoospermia, patient later (3 years) developed oligoasthenoteratospermia folowed by cryptozoospermia (10 spermatozoa in the whole ejaculate were present) and azoospermia. In this case,alcohol withdrawal for 3 moths resulted in absolutely normal semen parametres (Sermondade, Elloumi et al. 2010).

Alcohol exposure of pregnant mice resulted in decreased sperm concentration and number of methylated CpGs of H19 in F1 offspring sperm, but not somatic tissues (e.g. tail, liver, muscle, brain). CpGs 1, 2 and 6 were significantly different from control mice. Transgenerational effect of alcohol was seen in F2 brain, but not sperm, significant loss of CpGs H19 methylation was detected with CpGs 1,2, 3 and 6 different from control. Therefore, demethylational pettern of H19 was essencialy similar in both sperm of F1 and brain of F2 except of CpGs 3 (Stouder, Somm et al. 2011).

5 Conclusion

Main aim of this thesis was to give a an overview on plenteous epigenetic features that occur within gametogenesis and early development and to demonstrate how this mechanisms may be disrupted on many levels by various exogenous factors.

Since epigentic regulations are conserved among vertebrates data collected from animal models may elucidate potential risk four our species.

Assisted reproductive technology has become a widely used practise, however it has been considered to have handicaps since children concieved by ART display increased level of perinatal as well as later life problems with no obvious genetic backround. Hormonal treatment, maturation of gametes or embryo culture all represent dramatic irruption into higly orchestrated and fragile mechanism of naturaly occuring epigenetic programming. Therefore, gathered knowledge of

disturbing factors causing epigenetic impairments observed in animal models may be very helpfull in improving our IVF outcomes.

Epigenetic regulations that occur within early embryonal development highly resemble those in cancerous features not only in the meaning of establishing potent proliferating and high plasticity. New findings in the field of epigenetics may be mighty weapon to use in figth with cancer as well as other deseases.

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