CHARLES UNIVERSITY

Faculty of Science

Study program: Molecular and Cellular Biology, Genetics and Virology

The effects of epigenetic factor PRDM9 on the fertility of rodent females

Vlivy epigenetického faktoru PRDM9 na fertilitu samic hlodavců

Doctoral thesis

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Prague 2022

Declaration

A thesis submitted in fulfillment of requirements for the degree of Doctor of Philosophy at the Faculty of Science, Charles University in Prague. I hereby declare that this PhD dissertation represents my own work supervised by Dr. Zdeněk Trachtulec. I did my best to fully acknowledge all literature and people involved.

This thesis has not been submitted, in whole or in part, for the purpose of obtaining any other academic degree or equivalent at Charles University in Prague or any other University.

Prague, Signed,

June 2022 Srdjan Gašic

Prohlášení

Prohlašuji, že tato dizertační práce obsahuje výsledky mé vlastní samostatné práce získané pod vedením Ing. Zdeňka Trachtulce, PhD. Zároveň prohlašuji, že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena za účelem získání jiného nebo stejného akademického titulu na Karlově univerzitě v Praze, a ani na žádné jiné univerzitě.

Praha, Podpis,

Červen 2022 Srdjan Gašic

Acknowledgment

I would like to thank the following people, without whom I would not have been able to finish this thesis.

Special thanks to the Germ Cell development team at the Institute of Molecular Genetics, especially to my supervisor Dr. Zdenek Trachtulec for guiding me through the journey (and translating Abstract into Czech). To all members of the laboratory for their support and kindness.

My biggest thanks go to my family and friends for all love and support you have shown me, and without whom I would not make it so far.

Last but not least, I would like to thank my soulmate Tim, who never doubted me.

Abstract

The *Prdm9* gene encodes a histone-3-lysine-4,36-trimethyltransferase that specifies meiotic recombination sites and guides programmed double-strand breaks (DSBs) in mice, rats, and humans. Some vertebrates lost *Prdm9* but not fertility throughout evolution, while the removal of *Prdm9* caused sterility in some mouse strains, such as C57BL/6 (B6). The reasons for such species-specific fertility differences are unknown. To resolve these different requirements for PRDM9 in fertility, we produced *Prdm9* mutants in another mammalian species, *Rattus norvegicus*, strain SHR/OlaIpcv. The removal of *Prdm9* function did not completely abolish fertility in rats (as in B6 mice). Here I demonstrate that the loss of rat PRDM9 delayed female meiosis and caused synapsis and DSB repair defects that lead to a significant oocyte loss. However, unlike *Prdm9*-deficient B6 mouse oocytes, about 10-15% of pachytene-like mutant rat oocytes synapsed their chromosomes and repaired DSBs to the levels similar to controls. Because of this, female rats lacking PRDM9 maintained some oocytes until adulthood and yielded offspring, while B6 mice lost oocytes around the time of birth. Nevertheless, the adult rat mutant oocytes were exhausted earlier than the control adult rat oocytes. Therefore, PRDM9-lacking female rats suffered from premature ovarian failure (POF), a disorder described in humans. We hypothesized that longer meiotic prophase I might contribute to the increased fertility of SHR rats lacking PRDM9 versus B6 mice lacking PRDM9.

In contrast to B6 mutant oocytes, about 71% of PRDM9-lacking rat oocytes carried fewer persisting DSBs and non-homologous synapsis (NHS). In addition, the signals for DSB repair and for the checkpoint-control factor HORMAD2 were reduced on NHS chromosomes. We suggest that the NHS enabled some *Prdm9*-deficient rat oocytes to pass meiotic checkpoints; however, NHS chromosomes had a crossover rate below the level necessary to maintain the correct number of chromosomes (euploidy) after subsequent cell divisions, causing egg aneuploidy and pregnancy loss.

Some human *PRDM9* variants are associated with egg aneuploidy, and other variants with POF. Aneuploid pregnancies and POF co-occur in patients independent of their age, but no causal connection between aneuploidy and POF has been uncovered. Our rat model could provide one mechanistic explanation for the connection between POF and aneuploid

pregnancies in humans (via NHS), and it could apply to other cases of meiotic mutations causing partial synapsis and/or DSB repair defects.

Keywords: PRDM9, egg aneuploidy, POF, sterility, DSBs

Abstrakt

Gen *Prdm9* kóduje histon-3-lyzin-4,36-trimetyltransferázu, která specifikuje místa meiotické rekombinace a iniciuje programované dvouřetězcové zlomy (DSB) u myší, potkanů a lidí. Někteří obratlovci ztratili *Prdm9* během evoluce, ale neztratili plodnost, zatímco odstranění funkce *Prdm9* u C57BL/6 (B6) a dalších myší způsobuje sterilitu. Důvody pro tyto druhově specifické rozdíly v plodnosti nejsou známé. Abychom objasnili tyto různé požadavky na funkci *Prdm9* pro plodnost, vytvořili jsme mutanty *Prdm9* v jiném savčím druhu, *Rattus norvegicus*, kmeni SHR/OlaIpcv. Vyřazení funkce *Prdm9* nezrušilo kompletně plodnost u potkanů (jako u B6 myší). Demonstrovali jsme, že ztráta potkaního PRDM9 zpozdila samičí meiózu a způsobila synapsi a defekty opravy DSB, které vedly k významné ztrátě oocytů. Na rozdíl od B6 myších *Prdm9*-deficientních oocytů však více (cca 10-15%) mutantních potkaních pachytenních oocytů synapsovalo své chromozomy a opravovalo DSB - na úrovně podobné kontrolám. Proto si samice potkanů bez PRDM9 udržely některé oocyty až do dospělosti a porodily potomky, zatímco B6 myši ztratily oocyty v době kolem narození. Nicméně oocyty dospělých mutantních potkanů byly spotřebovány dříve než oocyty dospělých kontrolních potkanů. Potkaní samice tak v nepřítomnosti PRDM9 trpěly předčasným ovariálním selháním (POF), poruchou popsanou u lidí. Teoretizovali jsme, že delší meiotická profáze I by mohla přispívat ke zvýšené plodnosti SHR potkanů bez PRDM9 oproti B6 myším bez PRDM9.

Na rozdíl od mutantních B6 oocytů nesla většina (cca 71%) mutantních potkaních oocytů nehomologní synapse (NHS) a méně neopravených DSB. Navíc se NHS vyskytovala přímo v místech redukovaných signálů pro neopravené DSB a pro kontrolní faktor HORMAD2. Spekulovali jsme, že NHS umožnila některým *Prdm9*-deficientním oocytům projít meiotickými kontrolními body (checkpoints). Chromozomy s NHS však měly počty crossing-overů pod limitem nezbytným k udržení správného počtu chromozomů (euploidii) během následných buněčných dělení, což působí aneuploidii vajíček a ztrátu březosti.

Některé varianty lidského *PRDM9* asociují s aneuploidií a jiné varianty s POF. Aneuploidní těhotenství a POF se vyskytují současně u pacientů nezávisle na jejich věku, ale zatím nebyla odhalena žádná příčinná souvislost mezi aneuploidií a POF. Náš potkaní model nabízí jeden z mechanismů vysvětlujících souvislost mezi POF a aneuploidními těhotenstvími u lidí (via NHS), který by se mohl vztahovat i na další případy meiotických mutací způsobujících částečnou synapsi a/nebo defekty opravy DSB.

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Abbreviation

AS	Asynapsis (of meiotic chromosomes)
ATM	Ataxia-telangiectasia mutated kinase
ATR	Ataxia-telangiectasia and RAD3-related kinases
BRCA1	Breast type cancer 1 DNA repair associated
C57BL/6	B6 (mouse strain)
cAMP	Cyclic adenosine monophosphate
CE	Central elements
ChIP	Chromatin immunoprecipitation
CHK1, CHK2	Checkpoint kinase 1, Checkpoint kinase 2
COR	Crossover rate (COs per cell)
COs	Crossovers
dHJ	Double Holiday junction
DMC1	DNA meiotic recombinase 1
DNMT3L	DNA methyltransferase 3 like gene
DSBs	Double-stranded breaks
E	Embryonic day
EWSR1	Ewing sarcoma RNA binding protein 1
F1	First generation
FSH	Follicle-stimulating hormone
H3K36me3	Histone 3 trimethylation at lysine 36
H3K4me3	Histone 3 trimethylation at lysine 4
HELLS	Helicase, lymphoid specific
γH2AX	Phosphorylated serine 139 of histone 2AX
HORMADs	HORMA-domain proteins
HS	Homologous synapsis
KRAB	Kruppel-associated box
LE	Lateral elements
MEI1	Meiotic double-stranded break formation protein 1
MI	Metaphase one
MII	Metaphase two
MEI4	Meiotic double-stranded break formation protein 4
MRE11	MRE11 homolog, double-strand break repair nuclease
miRNA	Micro RNA
MLH1, and MLH3	MutL homolog proteins 1, and 3
MPF	Maturation promoting factor
MSCI	Meiotic sex chromosome inactivation
MSH	MutS homolog proteins
MSUC	Meiotic Silencing of Unsynapsed Chromatin
NBS1	Nijmegen breakage syndrome gene 1
NDRs	Nucleosome-depleted regions
NHS	Non-homologous synapsis
P	Postnatal day
PGCs	Primordial germ cells
POF	Premature ovarian failure
POI	Premature ovarian insufficiency

PRDM9	PR/SET domain-containing protein 9
RAD21L	RAD21L cohesin complex component
RAD50	RAD50 double-strand break repair protein
RAD51	RAD51 recombinase
REC8	REC8 meiotic recombination protein
REC114	meiotic recombination protein
RPA	Replication protein A
SAC	Spindle assembly checkpoint
SC	Synaptonemal complex
SDSA	Synthesis-dependent strand annealing
SHR	Spontaneously hypersensitive rat
ssDNA	Single-stranded DNA
SPO11	SPO11 initiator of meiotic double-strand breaks
SRY	Sex-determining region of Y-chromosome
STAG3	Stromal antigen 3
STRA8	Stimulated by retinoic acid gene 8
SYCE1, SYCE2, and SYCE3	Synaptonemal complex central element protein 1, 2, and 3
SYCP1, SYCP2, and SYCP3	Synaptonemal complex protein 1, 2, and 3
ZCWPW1	Zinc Finger CW-Type and PWWP Domain Containing 1
ZnF	Zinc finger

1. Introduction

1.1. Oogenesis

1.1.1. Primordial germ cells and initiation of meiosis

Oogenesis is the process essential for the fertility of female animals. The final products of oogenesis are functional eggs that have the potential to be fertilized and grow into a new organism. The process of oogenesis is accompanied by meiosis, a process that divides a single cell into four gametes with haploid DNA content (Zuccotti et al. 2011).

Primordial germ cells (PGCs) are formed during perinatal development, and they represent the cells that differentiate over time into haploid sperm or egg. The precursors of PGCs are observed in the proximal epiblast. Driven by the signals from the surrounding cells (e.g., bone morphogenic proteins), PGCs migrate to the genital ridge (the somatic precursor of gonads), where they proliferate and differentiate into male or female germ cells based on the presence or the lack of functional *Sry* locus on the Y chromosome, respectively. XY male germ cells, called prospermatogonia, become arrested and remain in the quiescent (G_0) cell cycle phase. Male germ cells commit to meiosis only at puberty (reviewed in Bowles and Koopman 2010). In contrast, XX female germ cells, called oogonia, initiate meiosis on E13.5 in mice (Speed 1982) or on E18.5 in rats (Li and Clagett-Dame 2009).

Meiosis is initiated by retinoic acid, a vitamin A derivative, through activation of *Stra8* (*Stimulated by retinoic acid gene 8*) transcript (Koubova et al. 2005). The expression of *Stra8* is required for premeiotic DNA replication, chromosome condensation, and the expression of genes essential for meiotic processes (Baltus et al. 2006).

1.1.2. Meiotic prophase I

Meiosis involves two cell divisions of germ cells of sexually-reproducing eukaryotic organisms. These cell divisions (meiosis I and meiosis II) reduce chromosome number by half. Therefore, meiosis results in the formation of gametes that carry one set of chromosomes (haploid): eggs and spermatids (reviewed in Marston and Amon 2004).

Following premeiotic DNA replication, oogonia semisynchronously enter meiosis. Prophase I is the longest stage of meiosis, and it is divided into substages: leptotene, zygotene,

pachytene, diplotene, and diakinesis. The hallmark of the leptotene substage is the formation of programmed double-strand breaks (DSBs) that co-occurs with the assembly of axial elements between sister chromatids of single chromosomes. Axial elements are composed of cohesins (e.g., REC8, STAG3, RAD21L) and synaptonemal complex (SC) proteins (Cohen et al. 2006). The SC proteins are zipper-like structural proteins that tether homologous chromosomes and thus facilitate meiotic recombination. Lateral elements (LE) and central elements (CE) of SC, the two parts of SC, are responsible for the recognition and pairing of homologous chromosomes. In the leptotene substage, subunits of LE, synaptonemal complex protein 2 (SYCP2) and SYCP3, form fragments that link sister chromatids (Fig. 1). The length of LE fragments increases as oocytes progress to the next substage, zygotene. The feature of the zygotene substage is chromosome condensation and elongation, and the onset of synapsis between duplicated homologous chromosome pairs is driven by central elements (CE) of SC. Some of the main CE components are transverse filaments SYCP1, synaptonemal complex central element protein 1 (SYCE1), SYCE2, SYCE3 (Cohen et al. 2006; Hernández-Hernández et al. 2016). The CE assembles between LE of homologous chromosomes and connects them as the repair of programmed DSBs proceeds. Complete synapsed homologous pairs are a hallmark of the following substage, named pachytene. In the subsequent substage, diplotene, homologous pairs desynapse, and SC disassemble.

Meiotic recombination is a type of DNA repair initiated by the formation of programmed DSB, which leads to a reciprocal exchange of genetic materials between parental chromosomes, known as crossovers (COs), as well as to non-crossovers (reviewed in Bolcun-Filas and Schimenti 2012). The formation of DSBs in leptotene is catalyzed by the topoisomerase-subunit-like protein SPO11 (Neale et al. 2005). This process facilities chromosome pairing since *Spo11*-deficient mice lack programmed DSBs, resulting in sterility in both sexes (Baudat et al. 2000). Assembly of “pre-DSB complex” is necessary to form and repair programmed DSBs successfully. Some components of the “pre-DSB complex” (e.g., HORMAD1, MEI4, MEI1, REC114) have a role in linking the DSB machinery with the

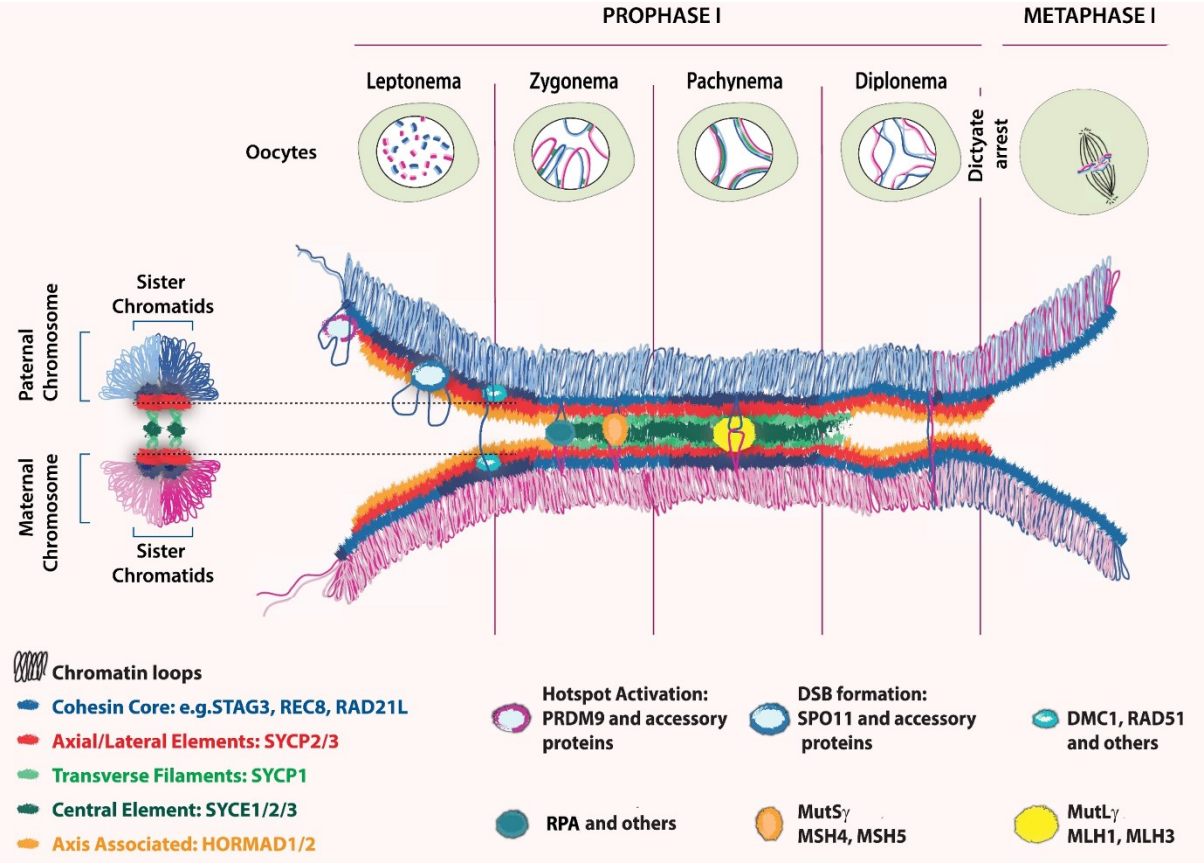

Figure 1. Schematic illustration of chromosome axis and meiotic recombination machinery in a mammalian oocyte. The progress of female meiosis is presented from left to right. Meiotic recombination occurs simultaneously with the assembly of the structural proteins necessary for chromosome synapsis and DSB repair. RN, recombination nodules. Adapted from Bolcun-Filas and Handel 2018

chromosome axis and providing the optimal environment for the DSB formation (Dereli et al. 2021). Programmed DSBs induce the recruitment of homologous recombination proteins. Among the first ones to be recruited is the MRX (MRE11, RAD50, and NBS1) complex, which is responsible for the removal of SPO11-oligonucleotides, a short-lived species of SPO11 covalently liked to an oligonucleotide (Mimitou PE 2010). MRX complex also resects the 5' end of the breaks that results in 3' single-stranded DNA (ssDNA) (Mimitou PE 2010). The following step includes the binding of replication protein A (RPA), RAD51 recombinase, and meiosis-specific recombinase DMC1 to 3' ssDNA overhangs (Fig. 2). RPA keeps ssDNA unwounded and prevents it from forming secondary structures, while RAD51 and DMC1 recombinase promote strand invasion and homology search (Masson and West 2001; Zou et al. 2006). It has been implied that the role of meiosis-specific recombinase DMC1 is to promote CO and non-CO formation over sister-chromatid recombination (Schwacha and Kleckner 1997; Shinohara et al. 1997). *Dmc1*$^{-/-}$ mice are defective in chromosome synapsis

and DSB repair, resulting in sterility of both sexes (Bishop et al. 1992; Pittman et al. 1998). RPA/RAD51/DMC1 nucleoprotein filament inserts itself into the homologous double-strand DNA (dsDNA) and looks for a complementary sequence. Upon finding the homologous sequence within dsDNA, the triple-stranded DNA termed 'D-loop' is formed (Masson and West 2001). The following DSB repair involves synthesis-dependent strand annealing (SDSA) or a formation of a double Holiday junction (dHJ).

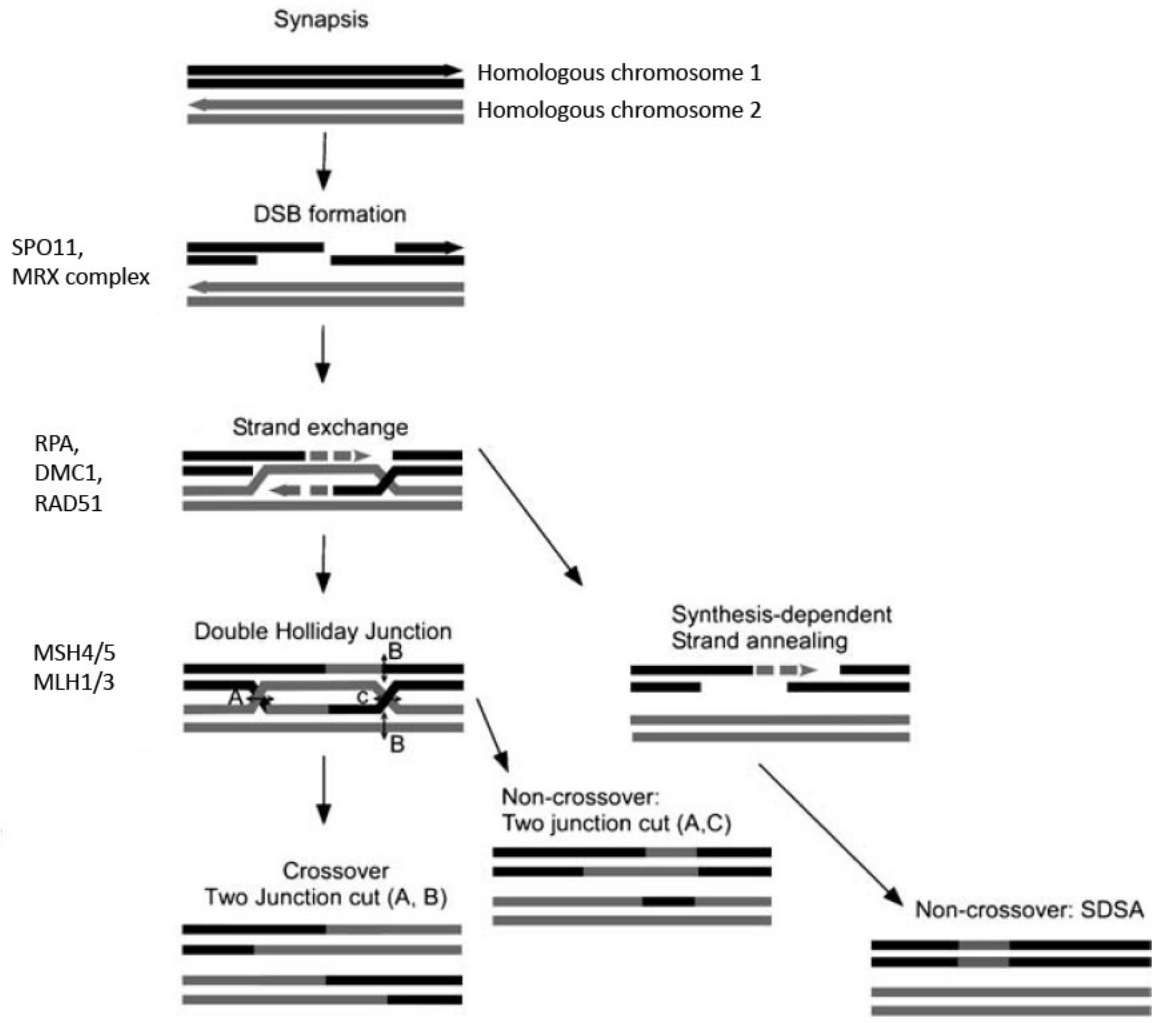

Figure 2. Pathways of programmed DSB repair. Meiotic recombination is initiated by SPO11. Then, 3' single-strand overhangs are produced by the MRX resection. RPA, DMC1, and RAD51 are involved in invading complementary DNA sequences and forming a "D-loop" in the other homologous chromosome. The following step includes the formation of CO or non-CO. DSBs are repaired by synthesis-dependent strand annealing (SDSA) if 3' overhangs dissociate and anneal. SDSA produced only non-COs. If a double Holiday junction (dHJ) is formed, its resolution dictates whether COs will be produced. An asymmetrical resolution of the dHJ will lead to the formation of COs, while symmetrical to non-COs. Adapted from Cohen and Holloway 2015.

The SDSA is a more frequent choice of homologous recombination, where 3' ssDNA overhangs are extended within D-loop by DNA polymerase in a process called "branch migrating." The following steps include dissociating extended 3' ssDNA from homologous dsDNA and annealing of extended 3' ssDNA into its original dsDNA (McMahill et al. 2007). SDSA is a type of DSB repair that does not produce COs, so there is no exchange of genetic materials between homologous chromosomes. The other path of DSB repair includes the formation of dHJ after 3' ssDNA overhangs capture the other side of the break. The dHJ is a recombination intermediate made from four connected DNA molecules. An asymmetric resolution of dHJ results in chromosomes with CO, while a symmetric resolution of dHJ results in chromosomes with non-CO (Youds and Boulton 2011). It is estimated that 90% of DSBs are repaired as non-COs (Bishop and Zickler 2004). At the zygotene substage, the MSH4-MSH5 complex (encompassing MutS homolog proteins) localizes and stabilizes dHJ (Fig. 2). Mice deficient in MSH4, and MSH5 gene are sterile and without COs (Edelmann et al. 1999; Kneitz et al. 2000). During the pachytene substage, MSH4-MSH5 heterodimer recruits MLH1 and MLH3 (MutL homolog proteins), which ensures the commitment of dHJ to COs. The formation of COs is necessary, not only as means to higher genetic variability, but also to ensure the correct segregation of homologous chromosomes and thereby euploidy (Bolcun-Filas and Schimenti 2012).

1.1.3. Defects in chromosome synapsis and meiotic recombination

Gene knock-out technologies revealed common defects in chromosome synapsis and meiotic recombination of mutant meiocytes deficient in essential meiotic genes. Some examples are mice deficient for meiotic DSB repair genes such as *Dmc1* (Bishop et al. 1992; Pittman et al. 1998)*, Msh5* (Edelmann et al. 1999), and mice deficient for chromosome synapsis genes such as *Sycp1* (De Vries et al. 2005)*, Syce1* (Bolcun-Filas et al. 2009). The unique feature of these mouse mutants is a failure of synapsis between homologous chromosomes, resulting in chromosome asynapsis (AS) that carries unrepaired DSBs. Mice lacking the genes mentioned above are not able to repair DSBs and/or complete meiotic synapsis resulting in early oocyte loss, spermatocyte arrest, and sterility.

The analysis of chromosome synapsis revealed that some recombination mutants (e.g., *Spo11, Msh5, Hormad1*) could partially compensate AS for synapsis among non-homologous chromosomes, i.e., non-homologous synapsis (NHS) (De Vries et al. 1999; Baudat et al. 2000; Petukhova et al. 2003). The appearance of NHS (also known as heterologous synapsis or ectopic synapsis) is not specific just to recombination mutants; it has been reported in other unrelated mouse models, such as hybrid mouse males (Bhattacharyya et al. 2013) and mice with chromosome rearrangements (Moses et al. 1982; Ashley and Cacheiro 1990). Moses et al. 1982 analyzed meiotic synapsis in mice with chromosome rearrangements and discovered that homologous synapsis (HS) is based on chromosome homology, and in the absence of the homology, chromosomes are driven to the NHS in a process called "synaptic adjustment". Although the role of NHS is not entirely understood, some authors imply that NHS could increase the likelihood of meiocyte survival (Speed 1982; Moses et al. 1982; Saadallah and Hulten 1986).

1.1.4. Folliculogenesis and oocyte maturation

Folliculogenesis is a process of the formation and development of ovarian follicles. Mammalian ovarian follicles represent a functional unit of every ovary. Ovarian follicles consist of an oocyte surrounded by one or more layers of follicular (granulosa) cells and an outer layer of theca cells. These follicular cells support the growth and development of an oocyte. Endocrine and paracrine factors regulate folliculogenesis to select the best follicle, which will release a mature oocyte programmed for fertilization (reviewed in McGee & Hsueh, 2000).

Folliculogenesis starts with the formation of primordial follicles. Following meiotic recombination, oocytes arrest at the prolonged diplotene substage of prophase I, named the dictyotene. Dictyotene-arrested oocytes get surrounded by a single layer of flattened granulosa cells and form primordial follicles. Once established, the primordial follicles are the only source of oocytes; their number represents the reproductive capacity of a female mammal. Primordial follicles are established in the first postnatal days in mice (Pepling and Spradling 2001) and rats (Rajah et al. 1992). After their formation, primordial follicles develop into growing stages; primary, secondary, and antral stages. This follicle recruitment lasts

throughout reproductive life until the reserve of primordial follicles is depleted. The features of primary follicles are oocyte growth, formation of *zona pellucida* (a protective glycoprotein layer surrounding an oocyte), and differentiation of flattened granulosa cells to cuboid-shaped cells. The proliferation of granulosa cells into multiple layers surrounding the oocyte is a mark of a secondary follicle. The development of follicles from the primordial stage to the secondary stage is hormone-independent and has only two directions: ovulation or apoptosis (Da Silva Bitecourt et al. 2018). Gonadotropic hormones synthesized by the pituitary gland during puberty will support further growth and development of secondary follicles. Under the influence of gonadotropin FSH (follicle-stimulating hormone), granulosa cells produce estrogens that form a follicular antrum, a space within the follicle filled with follicular fluid. The formation of a follicular antrum and thus an increased size of the follicle is a hallmark of antral follicles. In every reproductive (estrous) cycle, which lasts 4-5 days in rats (Hirshfield 1991) or ~28 days in humans (Gougeon 1996), increased synthesis of FSH recruits and supports a group of secondary follicles that develop into antral follicles; however, only dominant (De Graafian) follicles are selected to ovulate. Ovulation is a critical process that leads to the resumption of oocyte meiosis, the rupture of its follicle wall, and the release of the mature fertilizable oocyte into fallopian tubes. After ovulation, the ruptured follicle transforms into a luteal body, a structure that has a role in supporting pregnancy. The luteal body degrades if fertilization does not occur and a new reproductive cycle starts (Hirshfield 1991; Gougeon 1996).

Oocytes are kept in prophase I arrest throughout folliculogenesis by a high level of cyclic adenosine monophosphate (cAMP) (Conti et al. 2012). This diplotene arrest may be maintained for several months or over 50 years depending on the mammalian species (Huber and Fieder 2018). The reduction in cAMP levels causes oocyte maturation, i.e., the resumption of meiosis (Conti et al. 2012). The high level of cAMP maintains an inactive state of the maturation promoting factor (MPF), a complex responsible for the prophase arrest of oocytes. MPF, consisting of cyclin-dependent kinase 1 and cyclin B, drives oocyte meiosis by regulating crucial proteins necessary for meiotic progress. It is assumed that the factors transported from granulosa cells through nexuses into oocytes maintain the high level of cAMP and thus keep MPF inactivated. During ovulation, the connection between the oocyte and granulosa cells is ceased and thus ends the maintaince of the high level of cAMP in

oocytes (Norris et al. 2009; Zhang et al. 2010). Without the inhibitory effect, MPF is activated and causes nuclear membrane breakdown, spindle formation, chromosome condensation, and alignment at the metaphase plate, the extrusion of the first polar body, and meiotic progress to the metaphase stage of meiosis II. Following ovulation and maturation, oocytes are again kept in arrest, this time at MII stage, until the fertilization stimulates exit from meiosis II and the formation of zygotes (Schmidt et al. 2006).

1.1.5. Surveillance system

A strict control of meiosis is necessary to ensure that only the healthy gametes are produced, while defective gametes are removed from a population. If not eliminated, defective gametes can lead to congenital disabilities, miscarriages, and inheritance of mutations or chromosomal aberrations in the next generation (Hunt and Hassold 2008).

In rats (Beaumont et al. 1962), mice (Borum 1961), and humans (Baker 1963), a drastic oocyte loss occurs shortly around birth. This oocyte loss is preceded by the meiotic recombination and followed by the formation of primordial follicles. For example, from the onset of meiosis in rats, two-thirds of oocytes are eliminated by P02 (Beaumont et al. 1962). One explanation for such oocyte loss is a checkpoint system(s) triggered by the defects that arise during meiotic recombination, such as a failure of chromosome synapsis (asynapsis) or unrepaired DSBs (Di Giacomo et al. 2005; Rinaldi et al. 2017).

Defective chromosome synapsis and/or unrepaired DSBs can trigger programmed cell death (apoptosis) or arrest at the pachytene substage. Therefore, this checkpoint is often called a “pachytene” checkpoint because it prevents further meiotic progress unless chromosome synapsis and DNA recombination are completed (Roeder and Bailis 2000). In mammals, the pachytene checkpoint has been categorized into two separate checkpoints: DNA damage checkpoint activated by unrepaired DSBs, and synapsis checkpoint activated by asynapsis. However, it has been speculated that the pathways of these two checkpoints overlap since asynapsed chromosomes are often rich in unrepaired DSBs (Rinaldi et al. 2017). Additionally, the studies of mice deficient in several meiotic genes revealed that oocytes (unlike spermatocytes) with chromosome asynapsis and/or unrepaired DSBs could progress beyond the pachytene substage (Table 1). Therefore, it appears that the pachytene checkpoint is less strict in females (Morelli and Cohen 2005; Jagarlamudi et al. 2010).

Table 1. Sex-specific phenotypes in mice with meiotic gene mutations

Gene (Allele)	Function	Phenotype in Male Mice	Phenotype in Female Mice
Sycp3$^{-/-}$	SC formation	Sterile: early pachytene arrest	Sterile: inviable embryos
Atm$^{-/-}$	Programmed DSB levels control	Sterile: early pachytene arrest	Sterile: reach or pass pachytene progress, oocyte depletion around birth
Mlh1$^{-/-}$	CO formation	Sterile: arrest at metaphase I	Sterile: 2-cell embryos arrest
Mlh3$^{-/-}$	CO formation	Sterile: arrest at metaphase I	Sterile: 2-cell embryos arrest
Msh4$^{-/-}$	Stabilization of dHJ	Sterile: early pachytene arrest	Sterile: reach or pass pachytene progress, oocyte depletion around birth
Msh5$^{-/-}$	Stabilization of dHJ	Sterile: early pachytene arrest	Sterile: reach or pass pachytene progress, oocyte depletion around birth
Rec8$^{mei8/mei8}$	Sister chromatid cohesion	Sterile: early pachytene arrest	Sterile: reach or pass pachytene progress, oocyte depletion around birth
Dmc1$^{-/-}$	Strand invasion	Sterile: early pachytene arrest	Sterile: reach or pass pachytene progress, oocyte depletion around birth
Spo11$^{-/-}$	DSB formation	Sterile: early pachytene arrest	Sterile: reach or pass pachytene progress, oocyte depletion around birth
Brca1$^{-/-}$*p53*$^{-/-}$	DSB repair	Sterile: early pachytene arrest	Sterile: formation of primary follicles
Brca2$^{-/-;Tg+}$ (hypomorphic)	DSB repair	Sterile: early pachytene arrest	Sterile: inviable embryos
H2fax$^{-/-}$	Chromatin modification	Sterile: early pachytene arrest	Sterile: reduced litter size

Adapted from Morelli and Cohen 2005; Jagarlamudi et al. 2010

A surveillance system(s) closely monitors the formation and the repair of programmed DSBs during meiosis to prevent genome instability (Rinaldi et al. 2017). The core machineries that

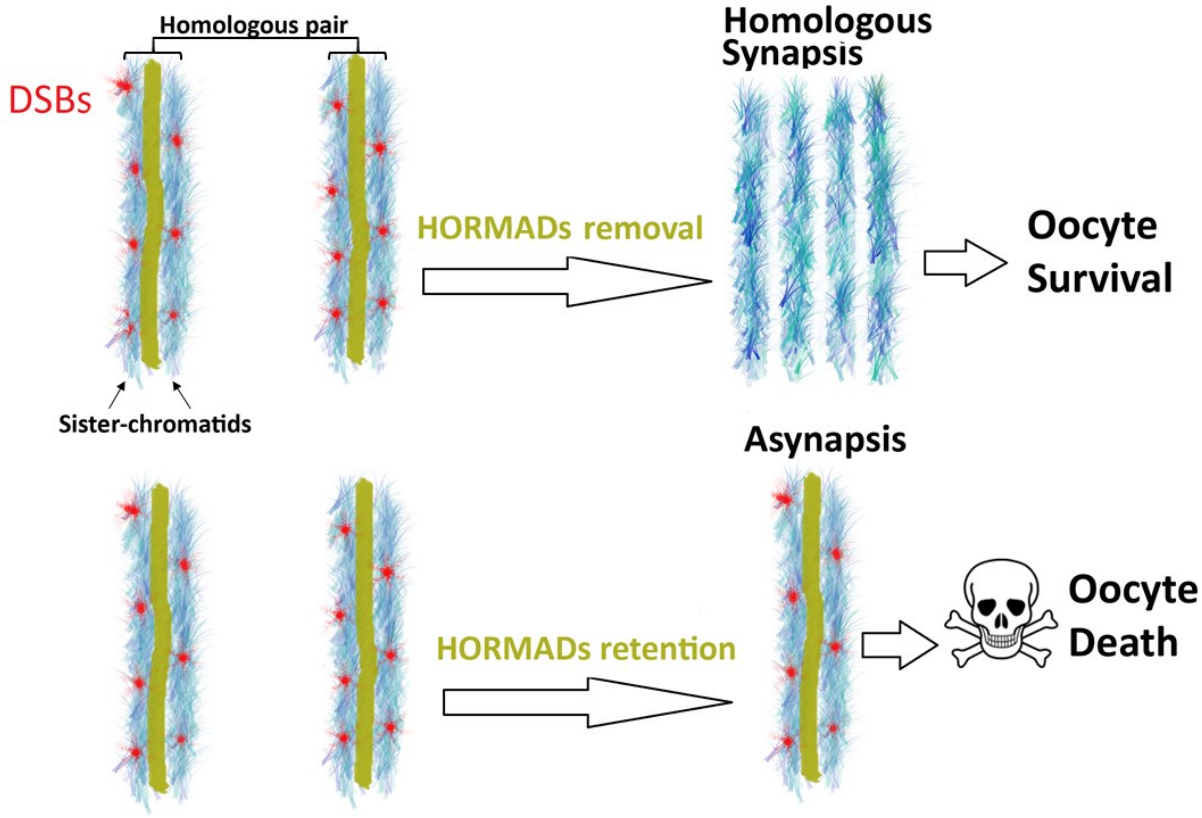

Figure 3. HORMADs are involved in the surveillance of programmed DSB repair and chromosome synapsis in oocytes. HORMADs are located on the axis between sister-chromatids of unsynapsed homologous chromosomes. HORMADs block sister-chromatid recombination. Synapsis of homologous chromosomes leads to the removal of HORMADs, which permits sister-chromatid recombination and CO and non-CO formation. The removal of HORMADs allows oocytes to fulfill the criteria to pass the pachytene checkpoint (upper). Conversely, chromosomes that fail to synapse (asynapsis) maintain HORMADs that block sister-chromatid recombination. As a consequence of persisted DSBs, and of gene inactivation by MSUC (Meiotic Silencing of Unsynapsed Chromatin), defective oocytes are recognized by the pachytene checkpoint that causes their apoptosis.

sense meiotic DNA breaks and regulates their repair are ATM (ataxia-telangiectasia mutated) and ATR (ataxia-telangiectasia and RAD3-related) kinases. The functions of ATM and ATR have been extensively researched in somatic cells (Maréchal and Zou 2013). Studies on somatic cells showed that ATM is activated by DSBs, while ATR is activated by single-strand breaks. Canonically, ATR primarily phosphorylates CHK2 (checkpoint kinase 2), while ATR phosphorylates CHK1 (Maréchal and Zou 2013). ATM and ATR carry distinct roles in meiosis. Both ATM and ATR are found along the axis of synapsed chromosomes, while ATR also marks asynapsed chromosomes. Mouse deficient for the *Atm* gene (*Atm*$^{-/-}$) carried an increased number of programmed DSBs, suggesting that this kinase negatively regulates the levels of

DSBs (Lange et al. 2011). *Atm*$^{-/-}$ oocytes are eliminated in the CHK2-dependent pathway, indicating that ATM might have a role in controlling DSB formation and repair than in DNA damage checkpoint. ATR has been proposed to activate oocyte apoptosis because, unlike ATM, it localizes to unrepaired DSBs and asynapsed chromosomes (Garcia-Cruz et al. 2009; Bolcun-Filas et al. 2014).

Mouse HORMA (Hop1, Rev7, Mad2)-domain proteins (HORMADs: HORMAD1, HORMAD2) are in charge of recruiting ATR on unrepaired DSBs and asynapsed chromosomes. HORMADs bind the axis of unsynapsed chromosomes during the leptotene and zygotene substage, and act as a barrier by blocking sister-chromatid recombination, and therefore promoting CO formation (Wojtasz et al. 2009; Wojtasz et al. 2012; Rinaldi et al. 2017). Synapsis between homologous chromosomes leads to depletion of HORMADs from the axis by late pachytene substage, which sequentially permits both sister-chromatid recombination and inter-homologous recombination (Fig. 3). In other words, HORMADs are removed from completely synapsed chromosomes but retained on asynapsed chromosomes where DSB repair is inhibited. In this way, HORMADs maintain DSBs unrepaired, which triggers apoptosis (Rinaldi et al. 2017; Rinaldi et al. 2020). Furthermore, HORMADs, ATR and BRCA1 are involved in "sensing" asynapsis and promoting the process known as Meiotic Silencing of Unsynapsed Chromatin (MSUC). Silencing is mediated by phosphorylation of the histone H2AX on serine 139 (γH2AX) along asynapsed chromosomes leading to heterochromatinization, which disables RNA polymerase activity (Garcia-Cruz et al. 2009). This process of transcriptional silencing of chromatin on asynapsed chromosomes also normally occurs on unsynapsed regions of sex chromosomes in males (Meiotic; Sex Chromosome Inactivation; MSCI). However, MSCI is necessary for meiotic progress in males, while MSUC is involved in eliminating AS meiocytes in both sexes by silencing genes critical for gametogenesis (i.e., germ cell survival) (Turner 2007).

Oocytes that pass the pachytene checkpoint and the follicle selection get an opportunity to ovulate and resume meiosis. The oocyte maturation includes the formation of the tubulin spindle and chromosome segregation, a critical step necessary for euploidy (a normal chromosome number in a cell or an organism) (Touati and Wassmann 2016). A proper attachment of spindle microtubules to kinetochores of chromosomes provides the tension across the tubulin spindle that is essential for the correct chromosome segregation. The

surveillance system called spindle assembly checkpoint (SAC) controls tubulin-to-kinetochore attachments, and operates both in mitotic and meiotic eukaryotic cells (Touati and Wassmann 2016). In meiosis I, sister chromatids of a single chromosome are mono-orientated leading to the separation of bivalents to opposite poles. The tension across the tubulin spindle is provided by COs (chiasmata) that link homologous chromosomes. In mitosis and meiosis II, sister-chromatids are bi-oriented prior to their segregation, and the tension is provided by the cohesion ring that holds together sister chromatids (Watanabe and Nurse 1999; Hauf and Watanabe 2004). Microtubule attachments to kinetochores of chromosomes are an "on/off" process until spindle SAC is satisfied. When stable attachment of microtubules-kinetochores and spindle tension are formed, SAC signals cell cycle machinery to promote metaphase-anaphase transition (Touati and Wassmann 2016).

1.1.6. Egg aneuploidy

A potential missegregation of chromosomes during meiosis or mitosis can generate cells with the wrong number of chromosomes, a condition named aneuploidy. The frequency of aneuploidy in somatic cells is lower than in meiotic cells. Male meiosis is less prone to aneuploidy (1-5% in humans) than female meiosis, as the rate of human oocytes with the wrong number of chromosomes is remarkable 20-30% (Hassold and Hunt 2001). Many miscarriages, stillbirths, and congenital disabilities are caused by the maternal origin of aneuploidy (Hassold and Hunt 2001). In contrast, aneuploidy in mouse oocytes and spermatocytes is 2-4%; it has been shown that the mouse genetic background can influence the rate of chromosome missegregation (Pyle and Handel 2003; Danylevska et al. 2014).

Age-related processes, such as deterioration of cohesion, are accountable for increased aneuploidies in human and mouse oocytes (Pan et al. 2008; Tsutsumi et al. 2014). In addition to age-related processes, reduced COR possesses a high risk for producing aneuploid eggs (Hassold and Hunt 2001; Hassold et. 2021). The number and distribution of COs is essential for chromosome segregation and euploidy (Hassold and Hunt 2001). Several human gene variations have been linked to aneuploidy (Chernus et al. 2019), and some of them were confirmed in mice as well, such as *Mlh3* (Lipkin et al. 2002; Singh et al. 2021) and *Sycp3* (Yuan et al. 2002; Bolor et al. 2009).

Table 2. Variants of meiotic genes found in POF patients and conformed as sterile in mutant mice.

Gene	Function	Phenotype in humans	Phenotype in mutant mice
STAG3	Subunit of cohesin	Primary amenorrhea Secondary amenorrhea	Follicles exhausted by P42
SYCE1	Central element of SC	Primary amenorrhea Secondary amenorrhea	Oocyte loss before reproductive age
SMC1B	Subunit of cohesin	Primary amenorrhea	Loss of oocytes after P28
REC8	Subunit of cohesin	Primary amenorrhea	Gradual follicle decrease from P28 to P56
DMC1	Subunit of cohesin	Primary amenorrhea	Complete lack of oocytes at P05
FANCU	Strand invasion	Primary amenorrhea	Oocytes exhausted in adult ovaries
PSMC3IP	Strand invasion	Primary amenorrhea	Gradual oocyte loss from P21 to P180
BRCA2	Strand invasion	Primary amenorrhea	Absence of follicles in knock-out
MEIOB	Meiotic DSB repair	Primary amenorrhea	Absence of follicles in adult ovary
MSH4	Meiotic DSB repair	Secondary amenorrhea	Complete lack of oocytes at P02

Adapted from (Huang et al. 2021)

1.1.7. Premature ovarian failure

Many female mouse models with defective meiotic recombination suffer from complete oocyte loss (Table 1; Jagarlamudi et al. 2010), a condition that resembles human syndrome called premature ovarian failure (POF, also known as premature ovarian insufficiency - POI). POF is an infertility disorder characterized by depletion of ovarian function, and it affects 1-5% of women younger than 40 years, and 0.01% of women younger than 20 years (Beck-Peccoz and Persani 2006; Yuan and Wang 2010). Clinically, POF is present in patients as primary amenorrhea (absence of first menstruation) or as depletion of ovarian follicles and secondary amenorrhea (cessation of menstruation before the age of 40). POF associated with primary amenorrhea is characterized by prepubertal events, such as an ovarian dysgenesis, leading to a lack of sexual maturation and reduced growth. Most POF cases are due to post-pubertal depletion of ovarian follicles; POF associated with secondary amenorrhea is clinically similar to precocious menopause (Beck-Peccoz and Persani 2006; Yuan and Wang 2010). The etiology of POF is heterogeneous, including autoimmune diseases, chemotherapy, genetic factors, and infections; however, the causality has not been known in many cases (idiopathic POF). Approximately 20-25% of POF are caused by genetic factors such as monosomic mutations and chromosome abnormalities (e.g., X monosomies). Over 75 mutated genes have been identified in POF patients (Jagarlamudi et al. 2010), many of which are involved in meiotic recombination (Table 2). Some of these genes have been validated as causative using mouse models, because the mutant animals displayed a similar phenotype (Huang et al. 2021).

Although POF and aneuploid oocytes are different conditions affecting the women's fertility and health, an association between the reduced oocyte number and aneuploidy has been reported in mothers with trisomic pregnancies (Haadsma et al. 2010). Additionally, earlier age at menopause was linked with trisomic pregnancies as well (Kline et al. 2000).

1.2. PRDM9

1.2.1. Importance of PRDM9 for meiosis

The genomic sites with elevated frequency of programmed DSBs, which initiate meiotic recombination, were named hotspots. In most eukaryotes, these sites occur within nucleosome-depleted regions that are enriched by "open" chromatin epigenetic marks, suggesting that local chromatin accessibility contributes to the localization and formation of DSBs (Walker et al. 2015). The meiotic hotspots in budding yeast (Gerton et al. 2000), plants (Muyt et al. 2009), and birds (Singhal et al. 2015) are located in H3K4me3 sites of gene promotors without identifiable common *cis* sequences. In humans (Pratto et al. 2014) and mice (Myers et al. 2010; Baudat et al. 2010; Parvanov et al. 2010), the meiotic hotspots occur in H3K4me3 sites that are mostly located away from promotors. These hotspots are characterized by specific *cis* sequences that are recognized by protein PRDM9 (PR/SET domain-containing protein 9) (Fig. 4).

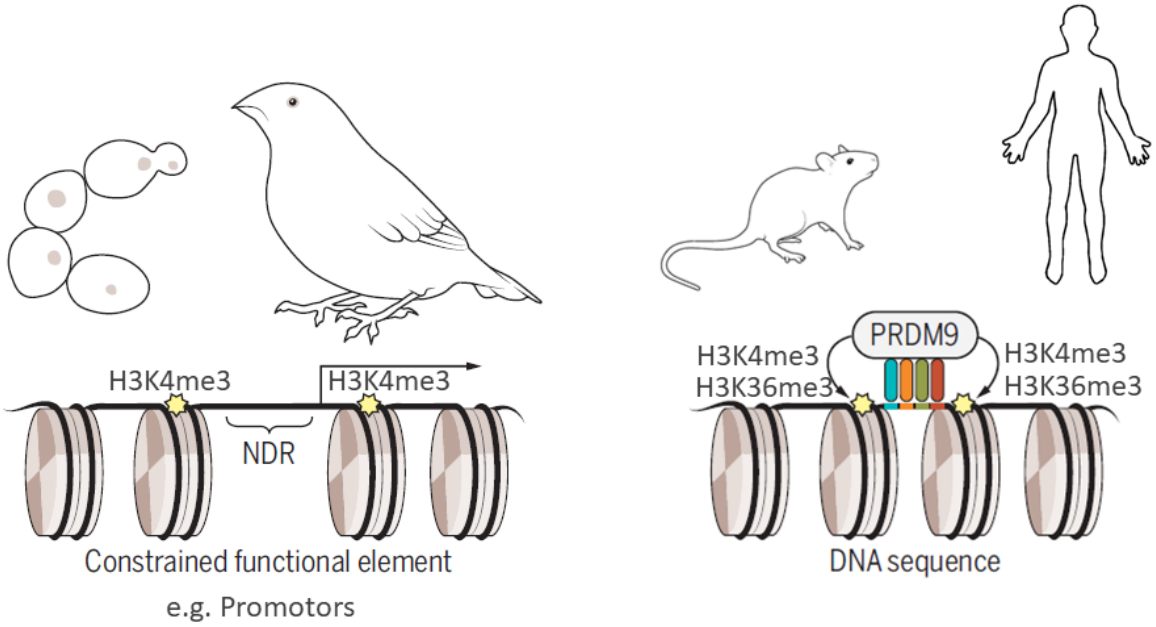

Figure 4. The existence of at least two pathways of meiotic hotspot selection. In many mammals, meiotic hotspots are defined by PRDM9 that binds specific sequences (PRDM9-binding sites), leading to histone modification (H3K4me3 and H3K36me3) and recruitment of DSB machinery at PRDM9-binding sites. Birds, yeasts, and other eukaryotes without PRDM9 form programmed DSBs at the default H3K4me3, such as in promoters. Adapted from Lichten 2015

PRDM9 is a histone methyltransferase that catalyzes the trimethylation of histone 3 at lysine 4 (H3K4me3) (Grey et al. 2011; Diagouraga et al. 2018) and lysine 36 (H3K36me3) (Powers et al. 2016). Thus, the PRDM9-defined hotspots are marked by a unique dual epigenetic mark. The mouse *Prdm9* gene was described in 2005 under the name *Meisetz* (Hayashi et al. 2005). In normal conditions, *Prdm9* is specific for germ cells. Its expression is detected in oocytes and spermatocytes during leptotene and zygotene substages of prophase one, the time of meiotic recombination (Hayashi et al. 2005; Sun et al. 2016). The deletion of *Prdm9* in the classical laboratory mice resulted in the sterility of both sexes due to pachytene arrest in males or oocyte depletion at birth in females (Hayashi et al. 2005). In 2010, three independent groups discovered the role of PRDM9 in the determination of the programmed DSB sites, revealing the crucial role of this gene in the regulation of the meiotic recombination (Baudat et al., 2010; Myers et al., 2010; Parvanov et al., 2010).

Before the discovery of its role in the positioning of meiotic recombination, *Prdm9* was identified as the first vertebrate hybrid sterility gene (Mihola et al. 2009). Hybrid sterility refers to a situation where fertile parents produce sterile offspring. Hybridization of closely related (sub)species can lead to epistatic interactions between genes that have diverged in their parental populations. These interactions may involve two or more loci, decreasing hybrid offspring's reproductive capacity, referred to as hybrid sterility (Dobzhansky 1936; Muller 1942). *Prdm9*-controlled hybrid sterility affects the first generation (F1) of only male mice derived from intersubspecific crosses of female PWD/PhJ mouse strain (subspecies *Mus musculus musculus*) and female C57BL/6 (i.e., B6) mouse strain (subspecies *Mus musculus domesticus*). The sterility of these hybrid mice was rescued by transgenes that carried the *Prdm9* gene (Mihola et al. 2009). Although the (PWD x B6)F1 male hybrid mice are viable, they suffer from sterility caused by pachytene arrest of spermatocytes that carry asynapsed chromosomes (Mihola et al. 2009; Flachs et al. 2012; Flachs et al. 2014). Fertile (PWD x B6)F1 female mice obey Haldane's rule that states when in the F1 offspring is one sex sterile, it is always the heterozygous sex (Haldane 1922; Bhattacharyya et al. 2013). *Prdm9* is involved not only in the complete sterility of the F1 male offspring of the PWD female and B6 male, (PWD x B6)F1, but also in the semifertility of the F1 males born from matings of B6 female with PWD male, (B6 x PWD)F1 (Flachs et al. 2012).

1.2.2. Domain structure of PRDM9 and its interactors

PRDM9 contains three main domains shown in Fig. 5. The N-terminal KRAB (Kruppel-associated box) domain is involved in protein-protein interactions (Imai et al. 2017; Parvanov et al. 2017). The middle PR/SET domain with histone methyltransferase activity catalyzes trimethylation of H3K4 (Diagouraga et al. 2018) and H3K36 (Powers et al. 2016). The C-terminal ZnF (Zinc finger) domain consists of a tandem array of C_2H_2 ZnFs that bind and recognize DNA (Sun et al. 2016). Studies of the mouse *Prdm9* gene revealed that an undisturbed activity of all three main domains of PRDM9 is necessary for successful oogenesis and spermatogenesis (Hayashi et al. 2005; Sun et al. 2016; Parvanov et al. 2017).

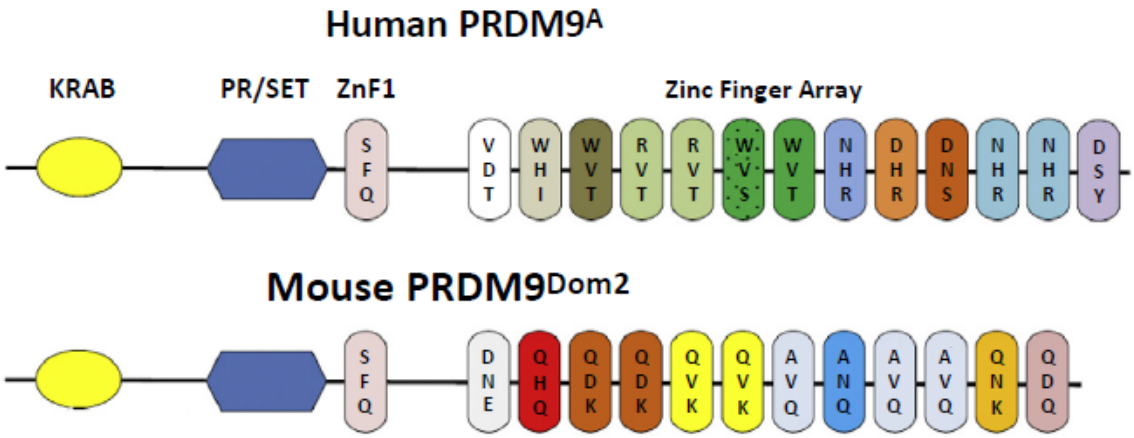

Figure 5. The image illustrates the structure of PRDM9 protein from humans (variant A) and mice (variant Dom2). PRDM9 consists of three main domains: KRAB domain (on the left), PR/SET domain (in the middle), and Zinc Finger Array (on the right, letters stand for DNA-binding amino-acid residues). Adapted from Paigen and Petkov 2018

Although proteins with KRAB or SET domains are involved in transcriptional regulation, the RNA sequencing of *Prdm9*-deficient mouse testes showed no changes in the mRNA or miRNA levels other than unspecific ones caused by different cellularity (Thibault-Sennett et al. 2018). *In vivo* studies of mouse spermatocytes revealed that the KRAB domain of PRDM9 interacts with several proteins involved in meiotic processes. Through interactions with PRDM9, cohesin components STAG3 and REC8 (Bhattacharyya et al. 2019) and SC components SYCP3 and SYCP1 (Imai et al. 2017; Parvanov et al. 2017) facilitate hotspot activation. These axis elements arguably serve as a "scaffold" for DSB-formation proteins at hotspots. In addition,

EWSR1 (Ewing sarcoma RNA binding protein 1) promotes PRDM9-induced histone methylation (Tian et al. 2021). PRDM9 also interacts with EHMT2 (euchromatic histone methyltransferase 2) and CDYL (chromodomain-containing Y chromosome-like); although the nature of these interactions is not clear yet, protein-protein interactions of PRDM9 might be necessary to properly direct DSB machinery at hotspots (Imai et al. 2017; Parvanov et al. 2017).

The ZnF domain of PRDM9 binds specific DNA sequences (i.e., PRDM9-binding sites) and thereby determines the sites of meiotic DSBs. PRDM9 likely forms a trimer via ZnF; however, a function of this multimerization is not yet known (Baker et al., 2015; Petkova, et al., 2015; Schwarz et al., 2019). The main hallmark of ZnF array is its rapid evolution making *Prdm9* highly polymorphic among and within species (Buard et al. 2014). Although all zinc-fingers from the ZnF array bind DNA, only four to six fingers provide specificity (Patel et al. 2016). The number of these fingers and variability of amino acids within the fingers result in a diversity of PRDM9 alleles and therefore of meiotic hotspots. PRDM9 allelic diversity was reported in mice (Mihola et al. 2009; Parvanov et al. 2010; Baker et al. 2015; Mukaj et al. 2020), humans (Berg et al. 2010), and chimpanzees (Auton et al. 2012), and explains a variation of the recombination landscape even within the same species. The positive selection of *Prdm9* ZnF domain could be attributed to the phenomenon named “hotspot erosion” due to biased gene conversion induced by DSBs and centered within *Prdm9*-binding sites. This means that “stronger” hotspots recognized by PRDM9 and DSB machinery are, over time, replaced by “weaker” hotspots used as templates (Baker et al., 2015). The “hotspot erosion” hypothesis may be accountable for the hybrid sterility of (PWD x B6)F1 male mice, because these hybrids carry homologous chromosomes from two mouse subspecies with differential “hotspot erosion” that may cause asymmetry of PRDM9 binding and thus DSB sites (Davies et al. 2016; Gregorova et al. 2018). The introduction of *Prdm9* alleles naive to the B6 and PWD backgrounds (e.g., C3H/Hej mouse allele or human allele A) increased synapsis rate and rescued hybrid sterility by recognizing non-eroded sequences on both homologs and thereby symmetric binding of PRDM9 (Mihola et al. 2009; Davies et al. 2016). The genetic manipulations that resulted in symmetric binding of PRDM9 on both homologous chromosomes also partially rescued male sterility of hybrids between distant mouse species *Mus musculus* and *Mus spretus* (Davies et al. 2021).

1.2.3. Mechanism and role of PRDM9 in meiotic recombination

The gene *Prdm9* has been extensively analyzed in mouse and human spermatocytes. The development of chromatin immunoprecipitation techniques coupled with sequencing (ChIP-seq) allowed analyzing meiotic recombination sites on a genome-wide level. This technique enabled the mapping of PRDM9-binding sites via PRDM9-ChIP-seq (Walker et al. 2015; Grey et al. 2017), mapping meiotic DSB sites with single-stranded DNA DMC1-ChIP-seq (Smagulova et al. 2011; Grey et al. 2017), and sequencing of SPO11-oligonucleotides released from DSBs (Lange et al. 2016). The epigenetic environment associated with DSB and PRDM9-binding sites was determined by ChIP-seq of histone modifications (Baker et al. 2014; Powers et al. 2016; Mihola et al. 2019).

PRDM9 appears during preleptotene and leptotene substages and disappears by the zygotene substage (Hayashi et al. 2005; Parvanov et al. 2017). PRDM9-dependent histone modification leads to nucleosome-depleted regions and provides accessibility for DSB machinery (SPO11 and auxiliary proteins). PRDM9 likely works with chromatin remodeler HELLS to open the chromatin (Spruce et al. 2020; Imai et al. 2020). From ~16 000 possible binding sites present in mouse spermatocytes, PRDM9 binds only ~4 700 of them in every mouse spermatocyte (Baker et al., 2014). Only 200-300 of these sites are utilized for the initiation of programmed DSBs, from which 10% are repaired as COs (Bishop and Zickler 2004). The affinity of PRDM9 to its DNA binding sites and hotpots activation is greatly influenced by surrounding chromatin modifications prior to binding of PRDM9. For example, epigenetic marks of the “closed” state of chromatin, such as H3K9me2/3, could represent a barrier to PRDM9 binding (Walker et al. 2015). Additionally, the binding affinity of PRDM9 is associated with the structure of the ZnF domain, making some PRDM9 alleles more or less dominant (Walker et al. 2015).

PRDM9 might be one of the factors that influence the probability of DSB repair as COs or non-COs; DSBs are more likely be repaired as COs if PRDM9 is also bound in the corresponding sites of uncut homologous chromosomes (Hinch et al. 2019). The migration of dHJ from the sites of DSB is limited within the nucleosome-depleted region. This means that COs are restricted to the regions marked by PRDM9-specified H3K4me3 (Baker et al., 2014). In this way, the PRDM9-primed epigenetic environment determines not only the location of DSB hotspots (Baudat et al., 2010; Myers et al., 2010; Parvanov et al., 2010) but also the

boundaries of dHJ migration (Baker et al., 2014) and possibly CO commitment as well (Hinch et al. 2019; Chen et al. 2020).

1.2.4. Recombination initiation and fertility with and without *Prdm9*

Prdm9 is not specific to mammals. Its homologs are present in some bony fishes (Osteichthyes), lizards, and snakes. *Prdm9* probably first evolved in the most primitive vertebrates (Agnathans; jawless fish); however, it has been lost in birds, amphibians, and several lineages of fish and reptiles (Baker et al., 2017), as well as in canines including dogs (Muñoz-Fuentes et al. 2011).

Deletion of *Prdm9* in mouse strains derived from *Mus musculus domesticus* (B6 and C3H) causes sterility (Hayashi et al. 2005; Baker et al. 2015b). Germ cells of these mice carry chromosome asynapsis and unrepaired DSBs leading to early pachytene arrest of spermatocytes, azoospermia (i.e., no sperm in an ejaculate), and oocyte depletion at birth (Hayashi et al. 2005; Mihola et al. 2019). However, it has been shown that mouse background and sex can influence the tolerance of *Prdm9* deficiency. Deletion of *Prdm9* in PWD mouse strain (PWD$^{tm/tm}$; derived from *Mus m. musculus*) also caused sterility in both sexes; however, unlike azoospermic B6-*Prdm9*$^{tm/tm}$, sterile PWD-*Prdm9*$^{tm/tm}$ males carry low sperm count, indicating that some spermatocytes were able to progress beyond the pachytene substage and develop into spermatozoa (Mihola et al. 2019). *Prdm9*-deficient (C3H x PWD)F1 hybrid males produced offspring. The proposed explanation for the variable fertility in mutant rodent males was a positive correlation between the presence of sperm and number of COs per meiotic cell (COR). The COR of (C3H x PWD)F1-*Prdm9*$^{tm/tm}$ males, and sperm carrying PWD$^{tm/tm}$ males is higher than of B6 and C3H mouse males that are sterile and azoospermic in the absence of *Prdm9* (Mihola et al. 2019; Mihola et al. 2021). It was hypothesized that increased COR might elevate the chances of COs between chromosome pairs that could stabilize synapsis, required for the formation of HS pachytene spermatocytes. Thus elevated COR could increase a chance of developing sperm cells and restoring fertility in the absence of *Prdm9* (Mihola et al. 2019).

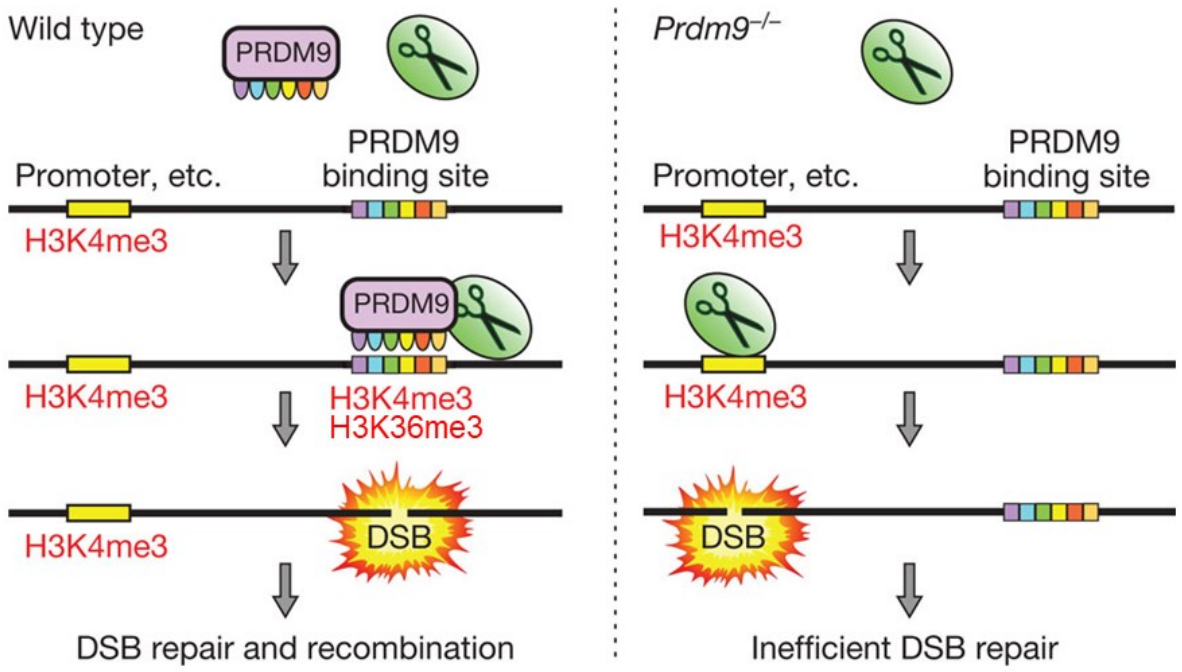

Figure 6. Role of PRDM9. On the left, germ cells of laboratory mice that contain functional PRDM9. PRDM9 binds DNA, catalyzes H3K4me3 and H3K36me3 formation, and directs programmed DSBs (scissors) to the PRDM9 binding site. On the right, germ cells of laboratory mice that are knocked-out for *Prdm9* have their programmed DSBs formed at the other H3K4me3 (PRDM9-independent) sites, such as promotors. It is assumed that these DSBs at PRDM9-independent sites are difficult to repair, leading to germ cell death and sterility. Adapted from Brick et al. 2012

Studies in male B6 mice lacking PRDM9 revealed that the formation of programmed DSBs is relocated from PRDM9-specified sites to the PRDM9-independent H3K4me3 ("default") sites, such as promotors (Brick et al. 2012; Mihola et al. 2019). This demonstrated that PRDM9 is essential for determining of DSB sites but not for DSB formation. DSBs at "default" sites are not repaired efficiently in B6, resulting in extensive chromosome asynapsis, germ cell death, and sterility in both sexes (Hayashi et al. 2005; Brick et al. 2012; Mihola et al. 2019). The epigenetic environment of H3K4me3 "default" sites lacks the presence of H3K36me3 marks (Fig. 6). The H3K4/H3K36me3 dual marks co-occur mainly at meiotic hotspots of germ cells (Powers et al. 2016). In somatic cells, H3K36me3 marks are required for DSB repair through homologous recombination (Pfister et al. 2014). Therefore, it has been proposed that H3K36me3 mark could be essential for the repair of meiotic DSBs (Powers et al. 2016). However, dogs are fertile despite having a nonfunctional *Prdm9*, and one case of a homozygous null *PRDM9* mutation in a woman with children was reported (Narasimhan et al. 2016).

Rare gene variants of *PRDM9* have been reported in human infertility conditions: in several cases of azoospermia (Miyamoto et al. 2008; Irie et al. 2009) and POF with secondary amenorrhea (Wang et al. 2021). In addition, Down syndrome children were associated with a lack of COs and increased nondisjunction on chromosome 21 during maternal meiosis I (Oliver et al. 2016; Chernus et al. 2019).

2. Aims of the study

Prdm9 is one of the most investigated meiotic genes. However, most studies on the impact of *Prdm9* loss have been analyzed in male mouse models. This study aims to shed light on the requirements of *Prdm9* for rat oogenesis, because the same mutation of *Prdm9*, which entirely abolishes female fertility in some mouse strains, has a different effect on rat fertility. Therefore, the main goals of this thesis are:

1. **Understanding why *Prdm9*-deficient rats can produce offspring while mouse strains are sterile:** Studying and comparing the oogenesis of semi-fertile rats and sterile mouse strains with the same mutation of *Prdm9*.

2. **Elucidating the impact of the *Prdm9* loss on female rat fertility:** Phenotyping rat oocytes throughout all stages of oogenesis, identifying the consequences of *Prdm9* loss on the quality of oocytes.

3. **Exploring possible mechanisms allowing rat oocytes to adapt to the *Prdm9* deficiency:** Analyzing *Prdm9*-deficient and control oocytes at the time of meiosis when the surveillance systems are activated (i.e., pachytene and metaphase stages)

4. **Discovering the significance of studying *Prdm9*-deficient female rats for reproductive medicine.**

3. List of publications

The articles discussed in thesis and published by me and my colleagues in peer reviewed journals. These relevant publications are incorporated in the end of the thesis (as a attachment to the thesis):

1) Mihola O, Landa V, Pratto F, Brick K, Kobets T, Kusari F, **Gasic S**, Smagulova F, Grey C, Flachs P, Gergelits V, Tresnak K, Silhavy J, Mlejnek P, Camerini-Otero RD, Pravenec M, Petukhova G V., Trachtulec Z (2021) Rat PRDM9 shapes recombination landscapes,duration of meiosis, gametogenesis, and age of fertility. BMC Biol 19:86. https://doi.org/10.1186/s12915-021-01017-0

2) **Gasic S**, Mihola O, Trachtulec Z (2022) *Prdm9* deficiency of rat oocytes causes synapsis among non-homologous chromosomes and aneuploidy. Mamm Genome. https://doi.org/10.1007/s00335-022-09954-z

Publications not related to the thesis:

Mihola O, Kobets T, Krivankova K, Linhartova E, **Gasic S**, Schimenti JC, Trachtulec Z (2020) Copy-number variation introduced by long transgenes compromises mouse male fertility independently of pachytene checkpoints. Chromosoma 129:69–82.

3.1. Summary of the publications

Please refer to the attached publications in the Supplement for more in-depth understanding of the thesis.

Publication 1

Mihola O, Landa V, Pratto F, Brick K, Kobets T, Kusari F, **Gasic S**, Smagulova F, Grey C, Flachs P, Gergelits V, Tresnak K, Silhavy J, Mlejnek P, Camerini-Otero RD, Pravenec M, Petukhova G V., Trachtulec Z (2021) Rat PRDM9 shapes recombination landscapes, duration of meiosis, gametogenesis, and age of fertility. BMC Biol 19:86.

In this study, we investigated whether the deletion of *Prdm9* influences the recombination landscape and abolishes fertility as in some *Prdm9*-deficient laboratory mouse strains. We found the relocation of meiotic hotspots into functional elements (e.g., promotors) in male rats. These rat meiotic hotspot shifts resemble those in *Prdm9*-deficient sterile male mice. However, unlike sterile mice, the *Prdm9*-lacking rats were able to produce offspring suggesting that PRDM9 is not necessary for the fertility of both sexes. We also found that rat *Prdm9* loss delays meiosis in both sexes and influences post-meiotic processes in males. *Prdm9*-deficient rat females suffered from premature ovarian failure (POF), a disorder described in humans. The loss of *Prdm9* decreased but did not abolish the fertility of rat. We hypothesized that a longer meiotic prophase I in the absence of PRDM9 might contribute to the increased fertility of rats versus mice.

My contribution to this publication was designing the experiments of the oocyte quantification throughout rat embryonic and postnatal development, generation of the oocyte quantification data, and the oocyte quantification data analysis. I also participated in writing a part of the discussion and commenting the entire manuscript.

Publication 2

Gasic S, Mihola O, Trachtulec Z (2022) *Prdm9* deficiency of rat oocytes causes synapsis among non-homologous chromosomes and aneuploidy. Mamm Genome.

This paper follows up on Mihola et al. 2021, which revealed that PRDM9-lacking female rats develop POF. We expanded the knowledge of the influence of PRDM9 loss on rodent female fertility to rats. Besides POF, *Prdm9*-deficient rats suffer from aneuploid eggs and pregnancy loss. Interestingly, rare variants of *PRDM9* were found in POF patients and mothers of Down syndrome children. Since POF and aneuploid pregnancies co-occur in humans independent of their age, we used our rat model to find the reason for this link. Unlike some mouse *Prdm9*-deficient strains that lose most follicles after birth, female rats kept some follicles throughout adulthood, but suffered from POF. The majority of *Prdm9*-deficient pachytene-like rat oocytes (71%) but the minority of mouse pachytene-like oocytes (19-21%) manifested non-homologous synapsis (NHS). The NHS chromosomes carried reduced level of DSB repaired foci and checkpoint-control factor HORMAD2. We argued that NHS allowed some defective oocytes to overcome meiotic checkpoints; however, crossover levels were reduced on NHS chromosomes below the number necessary to maintain euploidy. Our findings offer a mechanistic explanation for the link between POF and aneuploid pregnancies in humans that could apply to other cases of meiotic mutations.

My contribution was "co-designed the study, performed most experiments, analyzed the data, and drafted the manuscript", as stated in the Author contribution *section of this publication*.

4. Discussion

This thesis summarizes my contribution to the studies of *Prdm9* deficiency on female fertility, using a *Rattus norvegicus*; spontaneously hypersensitive rat (SHR) strain as an animal model. Here, I have provided evidence that the same deletion of *Prdm9* in SHR females (SHR-*Prdm9*$^{KO/KO}$) does not lead to complete female sterility as in all investigated strains derived from two mouse subspecies, *Mus musculus domestics* (strain B6; B6$^{tm/tm}$) and *Mus musculus musculus* (PWD/PhJ; PWD$^{tm/tm}$). The removal of *Prdm9* in the SHR strain decreases but does not entirely abolish female fertility; 44% of SHR-*Prdm9*$^{KO/KO}$ animals were able to produce small litters; however, the cost of rat *Prdm9* loss in females is POF, egg aneuploidy, and subsequent embryo death. Here, I point out plausible mechanisms that could allow rat oocytes to tolerate the absence of *Prdm9*, and the consequences of the *Prdm9* deletion on the reproductive fitness of rats. These findings have implications for reproductive medicine since the *PRDM9* human variants have been associated with offspring aneuploidy and POF.

4.1. Unlike PWD and B6 mice, SHR rat females escape complete sterility caused by *Prdm9* deletion

The shared consequence of the *Prdm9* deletion in SHR rat and B6 and PWD mouse oocytes is a failure to form HS pachytene oocytes; instead, zygotene oocytes progress into pachytene-like oocytes that bear chromosome asynapsis (AS) and unrepaired DSBs. B6$^{tm/tm}$ and PWD$^{tm/tm}$ oocytes rarely bear complete HS chromosomes. The AS pachytene-like mouse oocytes probably do not fulfill the requirements that allow them to pass the surveillance system causing oocyte depletion around birth (Hayashi et al., 2005; Mihola et al., 2019). Deficiency of PRDM9 delays rat pachytene substage, which could give some oocytes time to complete HS and repair DSBs. Eventually, 10% to 15% of SHR-*Prdm9*$^{KO/KO}$ rat oocytes succeeded to completely synapse their homologous chromosomes. In this way, a small population of HS pachytene oocytes could fulfill pachytene checkpoint requirements to allow further meiotic progress and development into healthy eggs. Delayed meiosis might be a response from the pachytene checkpoint system that modulates pachytene progress to accommodate defects of pachytene-like oocytes (Crichton et al. 2018). In addition to delayed meiosis of SHR-*Prdm9*$^{KO/KO}$ rat oocytes, the species-specific differences in the duration of meiosis may

influence the increased chance of HS and thus semi-fertility of SHR-*Prdm9*$^{KO/KO}$ females. Early prophase I (counted from leptotene substage to onset of primordial follicle formation) in the female rat lasts longer (8 to 11 days; Beaumont et al. 1962) than in the mouse (7 days; Borum 1961; McClellan et al. 2003). Early prophase I also takes longer in female dogs (17 days; Andersen and Simpson 1973), which are fertile despite having a non-functional *Prdm9* gene (Muñoz-Fuentes et al. 2011), than in female mice. Male meiosis also takes longer in rats and dogs than in mice (Adler 1996; Soares et al. 2009). Therefore, longer meiosis could provide more time for meiocytes to synapse their chromosomes and repair DSBs, and thus overcome the critical threshold that would allow them to pass the checkpoint system(s).

Studies in sperm-producing mice lacking *Prdm9* provided correlations suggesting that a higher COR stabilizes chromosome synapsis and thus increases the likelihood of HS pachytene spermatocytes that could develop into sperm cells and restore fertility in the absence of *Prdm9* (Mihola et al. 2019). A correspondingly high COR were observed in sperm-carrying SHR-*Prdm9*$^{KO/KO}$ males (Mihola et al. 2021). However, this was not the case in female rodents. The mean COR of B6 female mice, which are sterile in the absence of *Prdm9*, was similar to the fertile *Prdm9*-deficient male mice (Balcova et al. 2016; Mihola et al. 2019). Additionally, the mean COR of B6 female mice was higher than in SHR-*Prdm9*$^{KO/KO}$ female rats that produce offspring (Balcova et al. 2016; Mihola et al. 2019). Therefore, unlike in male rodents, a positive correlation between COR and fertility is not observed in female rodents, and it cannot explain the fertility of SHR-*Prdm9*$^{KO/KO}$ female rats. Maybe a modifier responsible for the higher COR in fertile *Prdm9*-deficient male mice is not expressed or sufficient to increase the likelihood of HS pachytene oocytes. Perhaps another modifier dependent on genetic background or sex is accountable for the fertility of SHR-*Prdm9*$^{KO/KO}$ female rats. Recent studies of *Prdm9* deficiency in another mouse subspecies, *Mus m. castaneus* (strain: CAST/EiJ), also revealed the sexual dimorphism in the requirement for PRDM9; male mice are sterile, while females kept fertility parameters of wild-types (Powers et al. 2020). As a potential modifier accountable for the fertility of CAST.*Prdm9*$^{-/-}$ mouse females, authors identified a proapoptotic gene, checkpoint kinase 2 (*Chk2*), involved in DNA damage surveillance in oocytes. Indeed, the deletion of *Chk2* (*Chk2*$^{-/-}$) rescued fertility in *Prdm9*-deficient B6 females (henceforth, B6$^{tm/tm}$*Chk2*$^{-/-}$) but not in males. These B6$^{tm/tm}$-*Chk2*$^{-/-}$ mouse females shared some phenotypes with SHR-*Prdm9*$^{KO/KO}$ rat females, such as univalents in metaphase I oocytes

and embryo death (Powers et al. 2020). However, future investigations are needed to validate the *Chk2* gene as a gene modifier of SHR-*Prdm9*$^{KO/KO}$ female fertility.

Prdm9-deficient rats exhibit sex-differences in fertility; 95% SHR males but 44% SHR females with the same mutation produced offspring. This difference in fertility between sexes, besides possible sex-specific modifiers, could be attributed to the self-renewing male germ stem cells, spermatogonia, which secure more germ cells and extend reproductive life in males compared to females (Morelli and Cohen 2005).

4.2. The deficiency of rat *Prdm9* causes the prevalence of the NHS oocytes over AS and HS oocytes

The main feature distinguishing semi-fertile SHR-*Prdm9*$^{KO/KO}$ female rats from sterile B6-*Prdm9*$^{tm/tm}$ and PWD-*Prdm9*$^{tm/tm}$ mouse females is the progress of AS pachytene-like oocytes into NHS pachytene-like oocytes. The percentage of pachytene-like oocytes with the synapsis among non-homologous chromosomes was up to 71% in SHR-*Prdm9*$^{KO/KO}$ female rats, while B6-*Prdm9*$^{tm/tm}$ and PWD-*Prdm9*$^{tm/tm}$ female mice carried fewer NHS oocytes (21% and 19%). Along with the proposed influence of delayed and longer meiosis on chromosome synapsis, the SHR rat genetic background could be permissive for NHS, while some mouse strain backgrounds could restrict NHS. The evidence of the NHS variation between two mouse strains, BALB/c and CBA, supports the view that genetic background could influence the chances of NHS formation (Spangenberg et al. 2021). In addition, the effect of mouse genetic backgrounds on chromosome synapsis and COR is well documented in some mouse strains (Balcova et al. 2016; Mihola et al. 2019).

In contrast to rat females, SHR-*Prdm9*$^{KO/KO}$ male rats carried only 16% of spermatocytes with NHS. Although the factor(s) responsible for the prevalence of NHS in SHR-*Prdm9*$^{KO/KO}$ rat females is yet unknown, the sexual dimorphisms of SC at the time of meiotic recombination might be held accountable. Rodent oocytes have wider and longer SC than spermatocytes (Martinez-Flores et al. 2003; Agostinho et al. 2018). In addition, it has been proposed that rodent oocytes might have different assembly and post-translation modification of SC as compared to rodent spermatocytes (Martinez-Flores et al. 2003; Agostinho et al. 2018).

4.3. NHS may indirectly cause egg aneuploidy of *Prdm9*-deficient rats

The *Prdm9* deficiency does not influence the mouse recombination rate (COR) (Mihola et al. 2019; Powers et al. 2020). Correspondingly, the removal of rat *Prdm9* did not change the recombination rate in neither sex when estimated by COR (i.e., counts of non-telomeric CDK2 nodules on chromosome spreads). However, this COR was analyzed using only HS chromosomes. When the COR was measured per NHS chromosome configurations (trivalents and quadrivalents), a significant COR decrease was observed as compared to HS chromosomes. The NHS was reported to be linked with CO suppression bearing chromosome rearrangements in mice and yeasts (Ashley and Cacheiro 1990; Dresser et al. 1994), and in allopolyploid plants (Park et al. 2020). At least one CO per chromosomal pair is necessary for euploidy (Touati and Wassmann 2016), and as SHR-*Prdm9*$^{KO/KO}$ females produce aneuploid eggs, it seems reasonable to propose that NHS contributes indirectly to aneuploidy by reducing COR. To be responsible for chromosome missegregation, NHS oocytes with decreased COR must first pass the surveillance system. Therefore, the question I raised is: "how would NHS oocytes bypass the pachytene checkpoint?" Studies in *C. elegans* showed that NHS could allow sister-chromatid recombination (Almanzar et al. 2021; Liu et al. 2021). In my thesis, I demonstrate that the "surveillance" protein HORMAD2 was retained on AS chromosomes, thus blocking sister-chromatid recombination (i.e., DSB repair on asynapsed chromosomes), and possibly activating the checkpoint mechanism to eliminate AS oocytes. However, HORMAD2 was successfully removed from NHS and HS chromosomes, suggesting that NHS could permit COs, non-COs, and sister-chromatid recombination (Rinaldi et al. 2017). DSBs on NHS chromosomes were more likely to be repaired by sister-chromatid recombination, since there were fewer CO nodules on NHS chromosomes than on HS chromosomes. The NHS could permit DSB repair via sister-chromatid recombination, and satisfy the pachytene checkpoint.

The next question I raised is: "Which NHS oocytes could bypass the pachytene checkpoint?". NHS pachytene-like oocytes represent the major group of all oocyte types in PRDM9-lacking females (up to 71%). I arbitrarily characterized three types of NHS pachytene-like oocytes based on their synapsis defects. From all described oocyte types, the NHS Type I oocytes and AS oocytes carried the highest counts of foci marking DSBs under repair (DMC1/RAD51) suggesting that these cells struggle to repair DSBs. On the other hand, NHS Type II carried

fewer DMC1/RAD51 foci than NHS Type I and AS oocytes, while NHS Type III oocytes had similar DMC1/RAD51 foci counts as wild-type HS oocytes. In addition, the synapsis defects of NHS oocytes correlated with the high level of unrepaired DSBs and accumulation of HORMAD2. Therefore, NHS Type I and AS oocytes would be at the highest risk of being eliminated by the pachytene checkpoint system(s). This view is supported by a decreased number of NHS Type I and AS oocytes in favor of NHS Type II, NHS Type III, and HS oocytes during the delayed pachytene substage. The number of AS oocytes was decreasing in controls during pachytene substage, and the lack of postnatal aneuploid oocytes in controls suggests that meiotic surveillance eliminates AS oocytes efficiently. Because of unrepaired DSBs and defects in chromosome synapsis, the majority of SHR-*Prdm9*$^{KO/KO}$ oocytes does not pass the pachytene checkpoint leading to a drastic oocyte loss. Only 25% of SHR-*Prdm9*$^{KO/KO}$ oocytes, as compared to controls, manage to pass the pachytene checkpoint and form primordial follicles. Consequently, SHR-*Prdm9*$^{KO/KO}$ females suffer from POF, a syndrome that has been described in humans (Coulam et al. 1986). NHS Type II and NHS Type III pachytene-like oocytes might have a better chance of survival, because they carried lower counts of DMC1/RAD51 foci and fewer synapsis defects than NHS Type I and AS oocytes. However, the best chance would have NHS Type III oocytes because they had similar counts of DMC1/RAD51 foci counts as control HS oocytes, as well as “absent” or “low” accumulation of HORMAD2.

If some NHS oocytes with fewer COs had evaded the pachytene checkpoint system, they would be checked by SAC. Meiotic SAC can recognize the lack of CO-provided tension that stabilizes tubulin-kinetochore attachments, which are essential for the chromosome segregation at metaphase I. In this way, meiotic SAC would arrest the metaphase-anaphase progress of oocytes and thus prevent transmission of aneuploidy into a new genome (Touati and Wassmann 2016). The *in vitro* maturation analysis suggested that meiotic SAC arrested 45% of SHR-*Prdm9*$^{KO/KO}$ oocytes. However, the subsequent immunofluorescent analysis of tubulin and chromosomes revealed that 67% of SHR-*Prdm9*$^{KO/KO}$ oocytes carried abnormalities in chromosome alignments, validating the insufficiency of the meiotic SAC system suggested previously (LeMaire-Adkins et al. 1997). Presence of separated univalent chromosomes (chromatids) in MII oocytes of SHR-*Prdm9*$^{KO/KO}$ could result from the bi-orientation of univalent chromosomes during metaphase I. In this way, oocytes with

univalents could satisfy meiotic SAC at metaphase I and progress to metaphase II (Lane and Kauppi 2019).

I found out that PWD- and B6-deficient females also carry NHS (19% and 21%), and that the phenotypes of the B6$^{tm/tm}$*Chk2*$^{-/-}$ females (Powers et al. 2020) partially resemble those in SHR-*Prdm9*$^{KO/KO}$ (i.e., metaphase I univalent, and pregnancy loss). It would be interesting to investigate whether the NHS rate differs between sterile B6$^{tm/tm}$ and sub-fertile B6$^{tm/tm}$*Chk2*$^{-/-}$ females. Other animal models with egg aneuploidy (Sheppard et al. 2012) could be assessed for NHS to obtain a correlative evidence or refute the hypothesis.

In my thesis, I provided a link between NHS, decreased COR, and aneuploidy in SHR-*Prdm9*$^{KO/KO}$ oocytes. However, SC proteins used as a marker to recognize NHS oocytes disassemble at the diplotene/dictyotene substage (Paredes et al. 2005). Therefore, there is no direct evidence that NHS pachytene-like oocytes could develop into aneuploid eggs. Identification of a marker (such as an antibody or FISH probe) that recognizes both NHS pachytene-like oocytes and postnatal eggs would be the critical component in future research on the impact of NHS on postnatal egg production.

4.4. The analysis of SHR-*Prdm9*$^{KO/KO}$ rat females might offer a link between POF and aneuploid pregnancies in humans

In 2000, Kline et al. reported that menopause occurs earlier in women who have had trisomic pregnancies and miscarriages than in women who have had normal pregnancies, indicating an association between declined follicle number and increased risk of aneuploid pregnancies. A similar conclusion was reached by several other authors who reported the incidence of trisomic pregnancies with the reduced follicle number in women, independent of their age (Haadsma et al. 2010; Kline et al. 2011; Kushnir et al. 2013; Shim et al. 2015; Jaswa et al. 2021). Although these studies showed a relationship between aneuploidy and diminishing oocyte number, they did not explain this association. Similar to these patients, *Prdm9*-deficient female rats suffer from reduced follicle number (i.e., POF), egg aneuploidy, and pregnancy loss. The NHS could explain the relationship between these disorders. In addition, rare human *PRDM9* variants have been reported in POF cases with the secondary amenorrhea (Wang et al. 2021) and mothers of children with Down syndrome (Oliver et al. 2016; Chernus et al.

2019). Therefore, I propose that partially compromised meiotic recombination and chromosome synapsis (e.g., due to meiotic gene mutations) could lead to NHS and be responsible for one of the links between POF and aneuploid pregnancy in humans.

The mouse POF models used are chemotherapy-induced and gene mutants with early or late follicle loss (Jagarlamudi et al. 2010; Lee et al. 2018). *Prdm9*-deficient rat females keep some follicles in adulthood, resemble the POF with the secondary amenorrhea, and are thus a valuable model for developing therapeutics and studying POF.

4.5. Meiotic recombination initiation is less studied in females than in males

Nearly all studies of the *Prdm9* role have been focused on the male mouse model, likely due to difficulties examining female meiosis, which onsets during fetal development. In 2018, the first female genome-wide map of recombination-initiating sites was generated in a wild-type mouse. The study revealed that most recombination hotspots are shared by both sexes, but sex-biased strength of hotspots is detected. This means that some hotspots are more activate in females, while others in male (Brick et al. 2018). The authors reported that the sex-biased strength of hotspots results from the different epigenetic landscapes of oocytes and spermatocytes at the time of meiotic recombination; oocytes have hypomethylated genome, while spermatocytes have established *de novo* methylation. The influence of DNA methylation on the meiotic recombination was supported by the genome-wide analysis of the hypomethylated mouse spermatocytes that lacked the *Dnmt3l* (DNA methyltransferase 3 like) gene; the hotspot strength of *Dnmt3l*$^{-/-}$ spermatocytes changed from male-biased to female-biased (Brick et al. 2018). It would be noteworthy to investigate other influences of the genetic background on the female meiotic recombination, such as the impact of active retrotransposons (Malki et al. 2019).

It seems that some taxa or species that lost *Prdm9,* including genetically modified rodents, adapted the ancestral pathway of recombination that existed before the evolution of *Prdm9*, such as the one in yeast that forms DSBs hotspots preferentially at promotors (Borde et al. 2009; Baker et al. 2017). A hypothesis states that *Prdm9* evolved to direct potential mutagenic recombination away from functional elements (e.g., promotors) into intergenic regions (Brick et al. 2012). In addition PRDM9 may prevent the recombination competition with the transcription (Schwarzkopf and Cornejo 2022). If so, why some taxa or species lost *Prdm9*

function while some others maintained it? Furthermore, if some species overcome the necessity of *Prdm9* in determining the DSB sites, how did they overcome the *Prdm9* requirement for the DSB repair? Recent studies analyzed genes that co-evolved with *Prdm9.* These studies discovered a histone-code reader ZCWPW1 (Zinc Finger CW-Type and PWWP Domain Containing 1) that recognizes PRDM9´s dual marks and it is essential for hotspot selection and efficient meiosis. Deletion of *Zcwpw1* in B6 mice also showed sex-specific differences; while males were sterile, females produced offspring but suffered from POF (Li et al. 2019). Interestingly, the analysis of 241 genes across 189 species revealed losses of *Zcwpw1*, and a few other meiotic genes, to co-occur with the lack of functional *Prdm9* in seven taxa/species (Li et al. 2019; Mahgoub et al. 2020; Wells et al. 2020; Cavassim et al. 2021). Further investigation of these genes in *Prdm9*-deficient animals and the production of mutants for the genes co-evolved with *Prdm9* could provide a great insight into species and sex differences in requirement for *Prdm9*.

The deletion of *Prdm9* in SHR males led to the relocation of programmed DSBs from meiotic hotspots to the functional H3K4me3 sites (e.g., promotors). This result is in agreement with the previously published maps of recombination-initiating sites in sterile azoospermic B6 males and sterile yet sperm-producing PWD males (Brick et al. 2012; Mihola et al. 2019). Although the ChIP-seq technologies revealed the effects of *Prdm9* loss on genome-wide meiotic recombination in male rodents, it is still a question whether it would be the same in female rodents. It would be of particular interest to discover whether the female meiotic hotspots would, in the absence of PRDM9, relocate to “default” sites like in males, but that is yet to be determined.

5. Conclusion

In this thesis:

1. We hypothesized that a longer, and delayed meiosis might be responsible, at least in part, for rat fertility versus mouse infertility in the absence of *Prdm9* function. A longer and delayed meiosis might provide more time for *Prdm9*-deficient rat oocytes to synapse homologous chromosomes and repair DSBs.
2. By comparing two sterile mouse models with semi-fertile rats carrying the same *Prdm9* mutation, I uncovered that the main difference in the female meiotic prophase I is the prevalence of NHS in pachytene-like oocytes of rat mutants. In addition, *Prdm9* affected metaphase progress and egg euploidy. A high number of oocytes failed to form primordial follicles (likely underwent apoptosis), leading to POF.
3. I pointed out the putative mechanism that could, via NHS, permit the repair of some DSBs in the *Prdm9*-deficient oocytes and thus let them pass the pachytene checkpoint system. The result of this possible evading the checkpoint using NHS could be increased egg aneuploidy and embryonic lethality of the offspring.
4. *Prdm9*-deficient female rats could provide one of the plausible mechanistic elucidations connecting reduced human oocyte number with aneuploid pregnancies. Rare *PRDM9* mutations are associated with egg aneuploidy and POF with secondary amenorrhea, and the SHR-*Prdm9*$^{KO/KO}$ phenotype resembles human POF; therefore, *Prdm9*-deficient rats are an important model for infertility studies.

6. References

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